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Emails:
editor@sciencepub.net
sciencepub@gmail.com

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Life Science Journal

Acta Zhengzhou University Oversea Version

(*Life Sci J*)

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CONTENTS

The Impact of English Language Development (ELD) on English learning (Case Study in Tehran language schools)

Seyed majid abolhassani

Professor at Shahrood Payame Noor University, Semnan, Iran
S.M.Abolhassani@gmail.com

Abstract: The English Language Development (ELD) program provides services to students who require assistance in gaining English proficiency. Program activities include instruction in learning English, content area classroom support, curriculum development, assessment, staff training, bilingual communication with families, and parent involvement. The purpose of systematic English Language Development (ELD) is to develop a solid English language foundation. The content of ELD follows scope and sequence of language skills in functional contexts. It is a state-mandated program based on English language proficiency levels. ELD is a separate graded class in which students are grouped by proficiency levels. ELD is assessed using the statewide English Language Proficiency Assessment. ELD is usually delivered by an endorsed ESOL teacher. However, there are times when a classroom teacher, trained in ELD, can be expected to teach the subject. In this paper we examine the role of ELD in increasing the score of students in Tehran in language examinations and we prove that ELD has an effective role in the students' language score.

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Keyword: English Language Development (ELD), English learning

1. Introduction

Acquiring another language is a developmental process. Consequently, the standards display patterns of language development which range from the student's initial contact with formal instruction in English to the point at which his or her use of English is comparable to that of native English-speaking peers. In order to be aligned with what is known about the acquisition of a second language and to underscore the developmental nature of this process, the standards are organized by language proficiency level, then by grade span. Because limited English proficient (LEP) students enter iran schools and begin the process of learning English at any grade level, there are distinctions in the activities indicative of ELD progress according to grade spans. The goal of the ELD Program is to develop the English language proficiency of eligible English Language Learners (ELLs) so that they can become socially and academically successful in Spokane Public Schools or The ELD Standards are designed to develop communicative competence in English. Note that each proficiency level description includes many aspects of language proficiency (such as grammar, fluency, function, pronunciation, and syntax), which contribute to overall language proficiency.

2. What is English Language Development (ELD)

English Language Development (ELD) is a specialized English language program for English Language Learner (ELL) students who have progressed in their language skills and are identified as proficient in English, but are working towards greater fluency. This more advanced English language skills program allows students proficient in English to build upon recently acquired skills in listening, speaking, reading, and writing. The English-language development (ELD) standards are designed to supplement the English language arts content standards. efficiency required for an English learner to move through the levels of English-language development. The standards are designed to move all students, regardless of their instructional program, into the main-stream English–language arts curriculum. The levels of proficiency in a second lan-guage have been well documented through research, and the ELD standards were designed around those levels to provide teachers in all types of programs with clear benchmarks of pr ogress. The ELD standards provide different academic pathways, which reflect critical developmental differences, for students who enter school at various grade levels. The ELD standards are written as path-ways to, or benchmarks of, the English– language arts standards. At the early profi-ciency levels, one ELD standard may be a pathway to attain several English–language arts standards. At the more advanced levels, the skills in the ELD standards begin to resemble those in the English–language arts standards and represent the standards at which an English learner has attained

academic proficiency in English. The ELD standards integrate listening, speaking, reading, and writing and create a distinct pathway to reading in English rather than delaying the introduction of English reading.

The ELD standards are designed to assist classroom teachers in assessing the progress of English learners toward attaining full fluency in English. The strategies used to help students attain proficiency in English differ according to the age at which a stu-dent begins learning English; therefore, the standards include outcomes for students who begin learning English in kindergarten through grade two, grades three through five, grades six through eight, and grades nine through twelve. The standards in those grade ranges were developed to help teach-ers move English learners to full fluency in English and to proficiency in the English– language arts standards. English learners at the advanced level of the ELD standards are to demonstrate proficiency in all standards detailed in this document and all language arts standards for the grades in which they are enrolled. English learners at the intermediate level of these ELD standards should be able to demonstrate proficiency in the language arts standards for all prior grade levels. Teachers will need to work concur-rently with this document and the English– Language Arts Content Standards for California Public Schools, Kindergarten Through Grade Twelve (1998) to ensure that English learners achieve proficiency. The ELD standards are comprehensive, with more detailed proficiency levels than were included in the Executive Summary. This refinement is needed so that teachers can better assess the progress of their stu-dents. The proficiency levels are as follows: Beginning, Early intermediate, Intermediate, Early advanced and Advanced.

3. Why is Systematic ELD needed?

The English Language Development (ELD) Program is designed to assist limited and non-English proficient students to develop their English language skills while they continue to gain academic skills in core subject areas. Placement in the program is based on initial assessment, ELD scores and progress through the ELD sequence of courses along with recommendations made by the LRT (Language Review Team). A coherent approach for developing proficiency in English is essential for increasing the academic achievement of English learners. This must include explicit language support for literacy and content instruction taught in English, as well as a plan for providing instruction in English as its own subject of study: Systematic English Language Development.

3.1. What is the LRT (Language Review Team)?

The teachers of each English Learner along with our ELD Counselor, the bilingual instructional aides and Assistant Principal of Curriculum make-up the Language Review Team. Language Review Teams meet twice a year in the fall and spring to discuss the student's progress in their classes and acquisition of the English language.

General Info: ELD

- ELD courses count towards fulfilling the English graduation requirement.
- Through the LRT process, students with qualifying CELDT and CST scores may be moved up a level at the semester.
- At times it may be necessary for students to stay at a level more than 1 year in which case the student will continue to earn credit towards the 4-year English graduation requirement with a passing grade of a D- or higher in the class.
- Be advised that only 1 year of ELD 4/Transition fulfills a-g requirements. Students entering at lower ELD levels who wish to attend a 4-year college immediately after high school will need to enroll in additional a-g English classes as they progress through high school to be eligible.

3.2. What is Systematic ELD?

Systematic ELD instruction is part of a comprehensive program for English Learners. The purpose of dedicated ELD instruction is to develop a solid foundation in the English language and to increase students' ability to communicate for a range of purposes. Effective ELD instruction supports achievement in other content areas by teaching students the language skills to successfully engage in content learning. It helps equip students with the language needed to express the sophistication of their thinking.

Systematic ELD is taught regularly, during time specifically dedicated to teaching English. It follows a developmental scope and sequence of language skills that includes substantive practice to ensure students develop fluency and accuracy. For this part of the instructional day, students are taught at their assessed level of English proficiency to ensure they develop a solid English language foundation and are continually challenged to stretch their ability to use language flexibly.

Systematic English Language instruction:

- Explicitly teaches language by assessed proficiency level
- Emphasizes oral language development (listening and speaking) through carefully

structured, purposeful and engaging interactions
- Lays out a scope and sequence of grammatical forms and sentence structures needed to communicate for a range of purposes (functions)
- Teaches vocabulary for social and academic purposes moving from general to increasingly precise words
- Provides ample oral and written practice for application of newly taught language in authentic contexts
- Does not replace literacy or other content instruction, but rather equips English Learners with the language they:
  - Are not likely to learn outside of school,
  - Will not be taught in any other content area, and
  - Are expected to use every day for academic and real life purposes.

4. The Importance of Practice for learning English

Which one of 4 key skills is the "Odd-One-Out"? Which one of these is different from the other three? The answer is speaking. The other three you can do alone, on your own, without anyone else. You can listen to the radio alone. You can read a book alone. You can write a letter alone. But you can't really speak alone! Speaking to yourself can be "dangerous" because men in white coats may come and take you away!!

That is why you should make every effort possible to find somebody to speak with. Where can you find people who can speak English with you? And how can you practice speaking when you are alone? At School: If you go to a language school, you should use the opportunity to speak to your teachers and other students. When you go home, you can still practice listening, reading and writing, but you probably can't practise speaking. If your teacher asks you a question, take the opportunity to answer. Try to say as much as possible. If your teacher asks you to speak in pairs or groups with other students, try to say as much as possible. Don't worry about your mistakes. Just speak! Conversation Clubs: Many cities around the world have conversation clubs where people can exchange one language for another. Look in your local newspaper to find a conversation club near you. They are usually free although some may charge a small entrance fee. Shopping: If you are living in an English-speaking country, you have a wonderful opportunity. Practice speaking to the local people such as shop assistants or taxi drivers. Even if you don't want to buy anything, you can ask questions about products that interest you in a shop. "How much does this cost?" "Can I pay by cheque ?" "Which do you recommend?" Often you can start a real conversation - and it costs you nothing! Even if you don't live in an English-speaking country, there are often American, British, Irish and Australian pubs in many large cities. If you can find one of these restaurants, you'll probably meet many people speaking English as a first or second language. Language is all around You: Everywhere you go you find language. Shop names, street names, advertisements, notices on buses and trains... Even if you are not in an English-speaking country, there are often a lot of English words you can see when walking in the street, especially in big cities. And there are always numbers. Car numbers, telephone numbers, house numbers... How can this help you? When you walk down the street, practice reading the words and numbers that you see. Say them to yourself. It's not exactly a conversation, but it will help you to "think" in English. For example, if you walk along a line of parked cars, say the number on each car quickly as you pass it. Test yourself, to see how fast you can walk and still say each number. But don't speak too loud! Songs and Video: Listen to the words of an English-language song that you like. Then repeat them to yourself and try to sing with the music. Repeat the words as many times as possible until they become automatic. Soon you'll be singing the whole song. Or listen to one of your favorite actors on video and repeat one or two sentences that you like. Do it until it becomes automatic. It's good practice for your memory and for the mouth muscles that you need for English.

Above all, don't be afraid to speak. You must try to speak, even if you make mistakes. You cannot learn without mistakes. There is a saying: "The person who never made a mistake never made anything." So think of your mistakes as something positive and useful.

Speak as much as possible! Make as many mistakes as possible! When you know that you have made a mistake, you know that you have made progress.

4.1.Some Creative Writing Ideas for English Students

As English learners begin to develop language skills in listening, speaking, and reading, they also need to develop writing skills. Linguistic studies note that English learners will transfer language skills from their primary language to English (Odlin 1989), especially if similarities between English and the primary language exist and if students are substantially literate in their primary language. Research also indicates that integrating the four language skills (reading, writing, speaking, and listening) is crucial for English learners to develop the

ability to write effectively (Mangeldorf 1989). Reading is particularly important because it provides English learners with opportuni-ties to acquire grammar, expand vocabulary, gain increasing fluency with written texts, and improve speaking skills (Interactive Approaches to Second Language Reading 1988). Reading provides students with model sentence patterns and linguistic structures. However, improved writing does not neces-sarily follow from reading. For English learners to apply their knowledge of sen-tence patterns and linguistic structures, they must put into practice what they observe from reading by engaging in various types of writing. If these students are to become successful users of English, their integrated instructional program must include numer-ous opportunities to develop writing skills. Because English learners working at the advanced level of the ELD standards are also expected to demonstrate proficiency in the language arts standards, it is essential for teachers to use the two standards documents concurrently and to monitor students' progress on both sets of standards. There are many ways for English language students to practice using the English that they are learning in a useful and creative way. One fun way is through the various practices of creative writing. Creative writing allows an English language student to practice the English that they already know by actually using it to write a story. Here are some fun creative writing activities for you to enjoy.

Group Story

One really fun creative writing activity is actually done with a group of people. The people can either be in a room together or writing together via email or even by letters sent through traditional mail. Find some other English language students and a native English speaker who can read the story you and the other students are going to write. The students can make a list of possible story beginnings and vote on their favorite beginning to a story. Choose a certain amount of minutes for each person to write part of the story before handing the document to the next person to continue writing. For instance, one person begins writing a story that is "Once upon a time, " and continues writing for ten minutes. After ten minutes, the person passes the story to the next person. The next person continues the story however they choose and then passes the story to the next person ten minutes later. This continues until everyone has written or until an agreed upon amount of minutes. After the story is written, each student can take turns reading the part of the story they have written aloud if the people are in the same room. Another option is to ask the native English speaker to read it. Another option is to give each person a copy of the completed story to read silently. A story written in this way can be very fun, because each person may write a special kind of way. For instance, one person may start a story about flowers, the next person might change the scene to a spaceship, and another person might change the scene to a romantic story taking place on a beach.

Write a news diary.

Another daily writing task that can work for people who would be bored by writing about their own routines in a diary is to write about the news that you read and listen to everyday. If you include your predictions for how you think the story will develop (e.g. "I think Hillary will become president"), this can give you a good reason to read old entries another time, at which time you can also correct and mistakes you have made and generally improve what you have written.

Online chat.

The closest thing to speaking for people who don't have the chance to speak English is online chat, as you have to think and respond quickly, and the language is short and informal just like speech.

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4.2. Reading

"Reading" is the process of looking at a series of written symbols and getting meaning from them. When we read, we use our eyes to receive written symbols (letters, punctuation marks and spaces) and we use our brain to convert them into words, sentences and paragraphs that communicate something to us. Reading can be silent (in our head) or aloud (so that other people can hear). Reading is a *receptive* skill - through it we *receive* information. But the complex process of reading also requires the skill of speaking, so that we can pronounce the words that we read. In this sense, reading is also a productive skill in that we are both receiving information and transmitting it (even if only to ourselves).

For all students, developing skills in reading English begins with a solid under-standing of the relationships between En-glish sounds and letters.the relationships between the spoken and written language. For the English learner those concepts are first developed through the recognition and production of English sounds. Students need to learn first those sounds that exist and then those that do not exist in their first language Students then are taught to transfer this knowledge to the printed language. As students develop knowledge of the corre-spondence between sounds and printed symbols, they also develop skills to deal with English morphemes (e.g., prefixes, suffixes, root words). Those word-analysis skills are

some of the building blocks stu-dents need to develop fluency in English and literacy skills. Native speakers of English are expected to recognize and produce all the English sounds by no later than first grade. This knowledge is then used in phonics instruc-tion when children learn to match the En-glish sounds with printed letters and use this knowledge to decode and encode words. English learners in kindergarten through grade two are to demonstrate proficiency in those English language arts standards pertaining to phonemic awareness, concepts about print, and decoding standards appropriate for their grade levels by the time they reach the advanced level of the ELD standards. Because the English–language arts stan-dards are essential for all students learning to read in English, English learners in grades three through twelve should be proficient in those standards related to phonemic aware-ness, concepts about print, and decoding no later than at the early intermediate level. Except where it is necessary for instruction to use nonsense words for teaching and assessing students, such as in phonemic awareness and early decoding instruction, care should be taken to ensure that students work with vocabulary and concepts that are meaningful and understandable to them. For kindergarten through grade two, the English–language arts standards pertaining to phonemic awareness, concepts about print, and decoding/word recognition have been incorporated into the ELD standards. Those language arts standards serve as signs of whether English learners are making appropriate progress toward becoming proficient readers. The ELD standards indicate the grade span in which students are to demonstrate proficiency, the language arts seastrand, and the number of the targeted language arts standard. Nonreaders of any age must move through the same sequence of skills when learning to read. Therefore, the instructional sequence for kindergarten through grade two should be used as a guide for English-language devel-opment and reading instruction at all grade levels. The instructional sequence for teaching phonemic awareness, concepts about print, and decoding skills is more specific in the kindergarten-through-grade-two span because the language arts standards for those grades focus primarily on developing literacy fluency. In grades three through twelve, students must greatly increase their content knowledge while learning English literacy skills. Older students with properly sequenced instruction may achieve literacy more rapidly than very young children do. In the ELD standards pathways are provided that enable students of all ages to build literacy skills. The language arts standards for grades three through twelve have linking ELD standards in each grade span that are designed to help students achieve proficiency in their grade-level language arts standards by the time they reach the advanced level of the ELD stan-dards. Students at the advanced level in ELD are expected to demonstrate proficiency in the language arts standards for their own grade and for all prior grades. One reason for incorporating the language arts standards for kindergarten through grade two into ELD standards is to clarify a point: Kindergarten and first-grade students at the advanced level in the ELD standards are also expected to be proficient in the language arts standards for their grade level. No limited-English-proficient student is expected to learn the language arts standards beyond his or her grade level

As the English learner recognizes and produces the sounds of English, the student is simultaneously building vocabulary. Learning new labels for concepts, objects, and actions is a key building block for the integration of the language. The pathways in the English-language development (ELD) standards lead to the achievement of fluent oral and silent reading. Those pathways are created by building vocabulary and are demonstrated through actions and spoken words, phrases, and sentences and by transferring this understanding to reading. The successful learning of a second language requires that the instruction of students be highly integrated to include all language skills and challenging activities that focus on subject-matter content (Brinton, Snow, and Wesche 1989). Therefore, at the higher proficiency levels, the student is asked to apply knowledge of vocabulary to literature and subject-matter texts and achieve an appropriate level of independent reading. At the lower ELD proficiency levels, reading materials should be at the student's developmental level. Grade-level reading materials should be used with students working at the advanced level. In addition to demonstrating proficiency in the ELD standards, students at the advanced level must also demonstrate proficiency in the English–language arts standards at their own grade level and at all prior grade levels.

Some Creative reading Ideas For English Students

- **Read English language magazines**. Like books, if you can read two versions of the same magazine (Newsweek in your language and in English, for example), that could make understanding it much easier.
- **Read English language entertainment guides**. Nowadays most big cities in the world have an English language magazine and/ or online guide to the movies, plays, exhibitions that are on in the city that week. Reading this in English is not only good value, but it could also

guide you to places that English speakers are interested in and where you might hear some English spoken around you.

- **Start your own English language blog**. Even for people who don't have to write in English, writing can be a great way of properly learning the kind of vocabulary you need to describe your own life and interests, and of thinking about how to stop making grammar mistakes. The problem most people have is that they don't know what to write about. One traditional way to make sure you write every day in English is to write an English diary (journal), and a more up to date way of doing this is to write a blog. Popular topics include your language learning experience, your experience studying abroad, your local area, your language, or translations of your local news into English.
- **Read the whole thing with no help**. Although using a dictionary has been shown to help with both short term and long term learning of vocabulary, the fact that using it slows reading down can stop some people reading in English at all. Reading a whole book quickly through just for pleasure from time to time will help you remember how fun reading in another language can be.
- **Read and learn everything**. At the opposite extreme, it can be hard work but very satisfying to get to the end of a book knowing that you have learnt every word in it. See other tips on this page to make sure it is a book that is easy enough to do this with and to ensure that the vocabulary you learn is useful.

4.3. Listening

Everyone knows that there are four skills in learning a language, namely listening, speaking, reading and writing. They are always related in terms of usage, and speaking is viewed by learners as the most desirable skill in face-to-face communication in the globalization era. However, what is the answer to the following questions?

- What do you have to do before you can speak?
- What does a child learn before he talks?
- What do we do before chatting?

Naturally, children begin listening to their parents when they are babies. They are often greeted, spoken to and admired without any response expected. Though nobody knows if the baby understands the spoken words, the process continues. Children automatically acquire such language over some time, and later on gradually produce it through actual experience. The production may be incomplete at first, but successful at last. That leads to speaking skill which is quite applicable to daily conversation.
In learning English, listening can help improve speaking considerably. Although it is the first of all skills, it is neither the easiest nor the most meaningless. We need to hear various types of English repeatedly and continuously if we want to communicate properly, meaningfully and naturally.

Why is listening good?

1. When listening, we are reviewing a lot of English usage such as vocabulary, grammatical structures, intonation, accent and our own interpretation.
2. We can learn new words and expressions by hearing them frequently.
3. Besides the English revision, general knowledge from news, features, or even advertising spots is certainly beneficial for regular listeners.
4. We can imitate what we hear and apply it with great confidence.
5. Listening can be a good "hobby" while we do other things such as cooking, ironing, exercising, relaxing etc. In other words, we have no wasted time at all.
6. Listening is also a great way to train our attention.

How can we listen to English?

Nowadays, radio cassette recorders are household appliances, but we often overlook their radio function. We can experience English language radio programs almost anywhere in the world. They are usually picked up on FM bands and aired particularly for foreigners. Short wave radio programs are another option. Two of the most easily found English language broadcasters are the BBC and Voice of America. Today, you can even access them by internet. You'll find some useful links for listening to the radio by internet, including "News in Easy English"

Some Creative listening Ideas for English Students

- **Sign up for a regular English tip**. Some websites offer a weekly or even daily short English lesson sent to your email account. If your mobile phone has an e-mail address, it is also possible to have the tips sent to your phone to read on the way to work or school. Please note, however, that such services are not usually graded very well to the levels of different students, and they should be used as a little added extra or revision in your English studies rather than as a replacement for

something you or your teacher have chosen more carefully as what you need to learn.

- **Follow your intensive course up with an extensive course**. The more time you can spend studying English the better, but studying periodic intensive courses with a few hours of study a week in between is probably better value for money than any other system as it gives your brain time to subconsciously learn and start using the new language you have learnt before you introduce the next new "chunk" of language.
- **Listen to MP3s**. Although buying music on the internet is becoming more popular in many countries, not so many people know that you can download speech radio such as audio books (an actor reading out a novel) and speech radio. Not only is this better practice for your English than listening to English music, from sources like Scientific American, BBC and Australia's ABC Radio it is also free.
- **Listen to English music**. Even listening to music while doing something else can help a little for things like getting used to the natural rhythm and tone of English speech, although the more time and attention you give to a song the more you will learn from listening to it again in the future.
- **Watch English films with subtitles in your language**. Again, this is not as good practice as English language films with English subtitles, but is more relaxing, can be easier to find suitable DVDs for, and is also possible with VHS.
- **Record your own voice**. For people who don't have much or any correction of pronunciation from a teacher, recording yourself and listening back makes it easier to hear whether you are really making the English sounds that you are trying to or not.
- **Buy a speaking electronic dictionary**. Although most electronic dictionaries are not as good as paper ones for the amount of information they give you about each word, some of them have the very useful function of saying the word with the correct pronunciation.
- **Learn your electronic dictionary vocabulary list**. Most electronic dictionaries also have a button which you can push to see the last 30 or more words you looked up. By deleting words you decide are useless or you have already learnt from this list, you can use it as a "to do list" of words to learn that you can look at several times a day in the train etc.
- **witch operating system to English**. Changing the operating language of your mobile phone, video recorder etc. to English can be an easy way of making sure you use the language everyday.

Strategies and Applications for listening and speaking

The listening and speaking standards for English learners identify a student's competency to understand the English language and to produce the language orally. Students must be prepared to use English effectively in social and academic settings. Listening and speaking skills provide one of the most important building blocks for the foundation of second-language acquisition and are essential for developing reading and writing skills in English. To develop proficiency in listening, speaking, reading, and writing, students must receive instruction in reading and writing while developing fluency in oral English. Teachers must use both the ELD and the English language arts standards to ensure that English learners develop proficiency in listening and speaking and acquire the concepts in the English language arts standards. English learners achieving at the advanced level of the ELD standards should demonstrate proficiency in the language arts standards at their own grade level and at all prior grade levels. This expectation means that by the early advanced ELD level, all prerequisite skills needed to achieve the level of skills in the English language arts standards must have been learned. English learners must develop both fluency in English and proficiency in the language arts standards. Teachers must ensure that En-glish learners receive instruction in listening and speaking that will enable them to meet the speaking applications standards of the language arts standards.

4.4. Some Creative speaking Ideas For English Students

To motivate students, teachers should include many activities and strategies that attract students' attention and make them interested in the lesson. As Peck (1978), cited in Celce-Murcia (2001), states "Activities need to be child centered and communication should be authentic. This means that children are listening or speaking about something that interests them, for their own reasons, and not merely because a teacher has asked them to". Also, Peck (1978), cited in Celce- Murcia, outlines some points that the teacher should consider in the activities: a focus on meaning and value, not correctness; a focus on collaboration and social development; the provision of a rich context, and teaching the four skills through a variety of activities.

A superior teacher encourages her/his students to speak English as much as possible inside and outside the classroom.

5. Method

This research has been done to develop knowledge on The Impact of English Language Development (ELD) on English learning. The purpose of this research is an applied research. How to collect data from the research project, is a descriptive Survey. It aims to assess the awareness about English Language Development (ELD).

6. Sample

The statistical is community that in which are observed English Language Development (ELD) and teaching is in compliance with these standards. With a random sample of 200 individuals were selected to answer questions. from this number 159 questionnaires were completed and analyzed.

7. Data collection and Analysis tool

Using data collection tools in the investigation is different, because the data collection tool to the subject, purpose and research design depends. Basis points in the method of research tools are such as: interviews, library studies and questionnaires were used for data collection. A questionnaire to identify the score of listening, speaking, reading, and writing is designed with Likert Scale that the Likert Scale is a five point scale that by SPSS software has been analyzed. In this study, has been used statistical analysis of a specialized SPSS software And Independent sample Test was used to check Hypothesis.

7.1.Validity of questionnaires

Validity means that we are measuring what we want to measure. There are a number of types of validity including: **1.** Face Validity - whether at face value, the questions appear to be measuring the construct. This is largely a "common-sense" assessment, but also relies on knowledge of the way people respond to survey questions and common pitfalls in questionnaire design; **2.** Content Validity - whether all important aspects of the construct are covered. Clear definitions of the construct and its components come in useful here. **3**. Criterion Validity/Predictive Validity - whether scores on the questionnaire successfully predict a specific criterion. For example, does the questionnaire used in selecting executives predict the success of those executives once they have been appointed; and**. 4.** Concurrent Validity - whether results of a new questionnaire are consistent with results of established measures.

To increase the validity of research were reviewed the research literature from the library of theses and research papers and several books. After interviews with managers and experts, research variables are identified and questionnaire was prepared. Finally questionnaire was reformed with faculty advisors consultation. We Ensure that respondents understand the questions in the questionnaire does not have a problem with the final questionnaire it was distributed.

7.2. Reliability of estimates of questionnaire

Reliability means the consistency or repeatability of the measure. This is especially important if the measure is to be used on an on-going basis to detect change. There are several forms of reliability, including:**1.**Test-retest reliability - whether repeating the test/questionnaire under the same conditions produces the same results; and**. 2.**Reliability within a scale - that all the questions designed to measure a particular trait are indeed measuring the same trait.

Questionnaire reliability is measured using Cronbach's alpha. value 0.83 has acceptable.

8.Analysis of data

To check hypothesis, is used the hypothesis H_0 and H_1 hypothesis that expression as follows

$H_0: \mu_f = \mu_m$

$H_1: \mu_f \neq \mu_m$

H_0 :There is no significant difference between the mean of scores the students.

H_1: There is significant difference between the mean of scores the students.

This table has two columns of data. one column is for equal variance and one column is for unequal variance. If Sig for Levene's Test for Equality of Variances is more than the value 0.05 we conclude that the variances are equal. So, we should use the column that we assume equal variances and vice versa. For The study of compared mean in two groups, if the sig foe Independent Samples Test is less than 0.5, indicating that is significant differences in the two groups. And we can conclude that Classes with ELD standards are a positive impact on student scores in English test.

9.Evaluation hypothesis

9.1. Evaluation The first hypothesis

H_0 :There is no significant difference between the mean of scores in listening.

H_1: There is significant difference between the mean of scores in listening.

Table 1: Independent Samples Test for listening

			listening	
			Equal variances assumed	Equal variances not assumed
Levene's Test for Equality of Variances	F		.096	
	Sig.		.757	
t-test for Equality of Means	t		-.574	-.583
	df		158	85.966
	Sig. (2-tailed)		.047	.041
	95% Confidence Interval of the Difference	Lower	-.26414	-.26241
		Upper	.14512	.14339

According to the test table, sig for Independent Samples Test is less than 0.05, and then we can conclude that there is significant difference between the mean of scores in speaking and we can conclude that Classes with ELD standards are a positive impact on student listening scores.

9.2. Evaluation The second hypothesis

H_0 :There is no significant difference between the mean of scores in speaking.

H_1: There is significant difference between the mean of scores in speaking.

Table 2: Independent Samples Test for speaking

			speaking	
			Equal variances assumed	Equal variances not assumed
Levene's Test for Equality of Variances	F		.096	
	Sig.		.757	
t-test for Equality of Means	t		-.574	-.583
	df		158	85.966
	Sig. (2-tailed)		.037	.041
	95% Confidence Interval of the Difference	Lower	.16414	.16241
		Upper	.24512	.24339

According to the test table, sig for Independent Samples Test is less than 0.05, and then we can conclude that there is significant difference between the mean of scores in speaking and we can conclude that Classes with ELD standards are a positive impact on student speaking scores.

9.3.Evaluation The third hypothesis

H_0 :There is no significant difference between the mean of scores in reading.

H_1: There is significant difference between the mean of scores in reading.

Table 3: Independent Samples Test for reading

			reading	
			Equal variances assumed	Equal variances not assumed
Levene's Test for Equality of Variances	F		.096	
	Sig.		.757	
t-test for Equality of Means	t		-.574	-.583
	df		158	85.966
	Sig. (2-tailed)		.027	.033
	95% Confidence Interval of the Difference	Lower	.26414	.26241
		Upper	.28512	.34349

According to the test table, sig for Independent Samples Test is less than 0.05, and then we can conclude that there is significant difference between the mean of scores in speaking and we can conclude that Classes with ELD standards are a positive impact on student reading scores.

9.4.Evaluation The fourth hypothesis

H_0 :There is no significant difference between the mean of scores in writing.

H_1: There is significant difference between the mean of scores in writing.

Table 4: Independent Samples Test for writing

			writing	
			Equal variances assumed	Equal variances not assumed
Levene's Test for Equality of Variances	F		.096	
	Sig.		.757	
t-test for Equality of Means	t		-.574	-.583
	df		158	85.966
	Sig. (2-tailed)		.013	.0251
	95% Confidence Interval of the Difference	Lower	.14414	.11241
		Upper	.15662	.13445

According to the test table, sig for Independent Samples Test is less than 0.05, and then we can conclude that there is significant difference between the mean of scores in speaking and we can conclude that Classes with ELD standards are a positive impact on student writing scores.

Conclusion

To ensure each student's success, schools must offer instruction leading to proficiency in the language arts standards. Instruction must begin as early as possible within the framework of the ELD standards. To ensure that all English learners achieve proficiency in the language arts standards, teachers must concurrently use both documents: the English–language arts standards and the ELD standards. When English learners reach the advanced level of the ELD standards, they must also be able to demonstrate proficiency in the language arts standards for their current grade level and all prior grade levels. Students at the advanced level of the ELD standards must use grade-level texts; however, students working at lower levels should use reading materials appropriate for their developmental levels. To ensure e that English learners become proficient in both the ELD and the language arts standards, teachers must use the two standards docu-ments concurrently and provide instruction leading to proficiency in the language arts standards at a level no later than the inter-mediate level of the ELD standards .Through this research, I learnt about many strategies that help to promote speaking in the young language learners' classroom, and it gave me an opportunity to implement the use of songs and puppets to enhance students' speaking skills. The data collected seem to support my assumptions that using songs and puppets would have a positive impact on students' spoken production, and would increase students' confidence in acquiring the language, and would improve their speaking skills.

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The Effect of Nursing Intervention on Eliminating Feeding Problems induced by Deficit Oral-Motor function among Children with Severe Head Injury

Fawzia El Sayed Abusaad [1] and Mohammed Ali Kassem[2]

[1]Department of Pediatric Nursing, Faculty of Nursing, Mansoura University, Egypt
[2]Department of Neurosurgery, Faculty of Medicine, Mansoura University, Egypt
dr.fawzia_abusaad@yahoo.com

Abstract: Addressing feeding problems induced by deficit oral motor activities is important for preventing or eliminating nutritional concerns among children with severe head injury. The aim of this study was to investigate the effect of nursing intervention on Eliminating Feeding Problems induced by oral-motor deficit among traumatic head injury of children. An experimental design (pre- post intervention) was used in a sample of 60 children admitted to Emergency hospital and Mansoura International hospital with severe head injury that were randomly selected. Interview questionnaire sheet including Pediatric feeding evaluation checklist (pre- post format) was used to collect the data. A serial of nursing intervention including modification of the manner of feeding, positioning and posture change for safe swallowing, oral-motor exercises and controlling of drooling were done by the researcher to correct the most evident feeding problems in spoon feeding, biting, chewing, swallowing and drooling induced by deficit oral-motor function. With the exception of biting skill (t= 1.07, *p* 0.2) a significant improvement are founded in the feeding domains of spoon feeding, chewing, cup drinking and drooling in the intervention group (t= 3.66,3.52, 1.34, 3.43 and *p* .001, .001, 0.18, .001, respectively). It is concluded that nursing intervention for severe head injury children using a behavior modification program combining education and exercises has been shown to eliminate feeding problems and enhance oral-motor functions.
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Key words: Feeding problems, Oral motor deficit, Children, Severe head injury.

1. Introduction

Traumatic head injury is a major cause of disability, death and economic cost to our society. Children with severe head injury are often at high risk for several problems (**Mckinlay *et al.*, 2010**). About (100.000 to 200.000) pediatric head injuries occur per year in United States , males are more affected than females (**DeMatteo, 2008**). In a head injury the brain may hit the inside of the skull, this force can occur as a result of a car accident or a fall, a blow to the head can also cause it (**Morgan *et al.*, 2001**). The impact of head injury is presenting a variety of possible cognitive, communication, physical and behavioral changes (**Blisset &Harris, 2007**). In Egypt the pediatric population suffering from significant disability as a result of head trauma is about 2-3% of the population (**WHO, 2003**). Children with head injury appear to have similar physiologic disorders of feeding and swallowing problems.

Most children with severe head injury will have feeding difficulties in the early stages of recovery, this feeding difficulties result from an oral motor deficit that affect the musculature of the mouth including the lips, tongue, and jaw. Weakness of these muscles has a greatest impact on the ability to manipulate food in the mouth, including chewing and swallowing (**Sliverman, 2010**). Oral-motor problems in children are easy to recognize when the child coughs and chokes while eating. The major factor of this problem is in the pathogenesis of under nutrition that usually correlates with the severity of motor impairment (**Cecilia *et al.*, 2005**). However, the majority of feeding problems present initially in more subtle ways, such as difficulty introducing spoon feedings or advancing texture, or limited variety of foods associated with absent or limited sensation. Children may present with inadequate lip closure, drooling and persistent tongue thrust, resulting in food loss through spillage (**Morgan *et al*., 2004**).

Traumatic brain injury increases the metabolic response of body, therefore nutritional support due to hyper metabolism and increased protein catabolism are essential (**Marchand & Motil, 2006**). Issues of nutrition and hydration are particularly significant for the pediatric population. Adequate nutrition is extremely important for children to ensure sufficient growth and development of all body systems. Consequently, the presence of dysphagia in the pediatric population may have a negative impact upon physical and intellectual development (**Arvedson &Brodsky, 2002**). As the growth and maturation of oral-motor skills and swallowing parallel general neurological maturation, any disruption of neurological functioning, can occur subsequent to

traumatic brain injury and may compromise oral-motor or feeding development in a child (**Arvedson, 2008**)

According to the recommendations of the American Academy of Pediatrics, screening for nutrition risks and problems is an expected part of routine preventive health services **(Rockvile, 2010)**. Comprehensive assessment of children with dysphasia and feeding disorders involves considerations of the broad environment, parent-child interactions, parental concerns and health status of the child. All of those factors must be taken into account by professionals in order to make optimal management decisions for every child to ensure that nutrition and hydration needs are met for adequate growth. Nurse must have adequate knowledge and skills about associated health conditions and specific feeding/swallowing issues (Ratanalert *et al*., 2007)**.** Safety is a major concern because the child may be at risk of choking or aspiration. An oral – motor assessment looking at the skill of the jaw, lips, tongue, and coordination of these muscles is very important to manage food **(Carnby *et al*., 2006).** A specific intervention will vary according to the needs of the child, so that interdisciplinary management can enhance the lives of children**.(Greer *et al*., 2008).** The aim of this study was to investigate the effect of nursing intervention on eliminating feeding problems induced by oral-motor deficit among severe head injury in children.

Research hypotheses

Children with severe head injury in the intervention group will have improvement in their feeding problems induced by oral-motor deficit function post nursing intervention than in the control group who receive routine hospital care.

2. Subjects and Methods:

Design

An experimental design (pre- post intervention) was used in this study.

Setting

The study was conducted in Emergency Hospital and Mansoura International Hospital, Neurosurgery department in Mansoura city**,** Al-Dakahleia governorate, Egypt **.** The first setting receive all types of emergency accidents throughout the week except two days of the week for the second setting**.** Permission to carry out the study was obtained from the director of the mentioned settings after explaining the purpose and significance of the study.

Subjects:

A sample of 60 children admitted with severe head injury was included in the study and were randomly divided into intervention group and control group after taking a consent from their mothers to participate in the study. The intervention group receives the specific intervention while the control group receives the routine hospital care. All the participants selected through the following criteria: - All children presented with severe Traumatic Brain Injury (TBI) their Glasgow Coma Scale [GCS] (4–8) as diagnosed by a neurosurgeon on admission. Permission for subject inclusion was obtained from the supervising medical consultant and the child's parent(s)/guardian. Discharged from Intensive Care Unit (ICU) to ward level as their clinical condition were stable. Presence or deficit gag and swallowing reflex. Physician order to begin oral feeding. And their CT scan shows improvement with follow up with no complications or deterioration clinically.

Exclusion Criteria:

Children had a history of significant premorbid neurological, developmental, sensory, or structural deficits, or a significant history of nonspecific feeding /swallowing disorder. Children admitted with facial trauma or mandible fractures. and children with fractured cervical spine.

Tool of data collection:

Interview questionnaire sheet was used to collect data that contain two parts:

Part I:

Socio demographic characteristics of the child that was designed by the researcher include information about age, sex, causes of head injury, score of GCS on admission. Socio- demographic characteristics of the mothers such as education, occupation and residence.

Part II:

Pediatric Feeding Evaluation Checklist (pre-post format): Adopted from **Abou-El saad & Abdelatif (2008)** that was modified by the researcher to assess children neuromuscular response during eating in relation to spoon feeding, biting, chewing; cup drinking and drooling . The neuromuscular response ranging from adequate (good muscle control during eating and drinking with absent food and fluid loss),to poor(week muscle with inadequate neuromuscular response during feeding and drinking) and absent (unable to open, hold food or fluid with presence loss of food and fluids) . Regarding degree of drooling, it ranges from no drooling to mild drooling (to lips only), moderate drooling (lip and chin) and severe drooling (clothing soiled).Each neuromuscular response tack a score of 2, 1, 0 respectively, while drooling score were 3,2,1,0 respectively. The tool was tested for content validity by five specialists in pediatric nursing and neurosurgery and the reliability was assured using Cronbach's coefficient alpha 0.83

Procedure

Informed consent was obtained from each mother\ guardian for participation in the study after explaining the aim of the study assure confidentiality of data and their right's to accept or refuse participation in the study.

Acceptance was obtained from ethical committee from Faculty of Nursing at Mansoura University to carry out the research.

Each child with his or her mother were interviewed individually after discharging from ICU to neurosurgical ward to start oral feeding, the child oral motor feeding capabilities was assessed using pediatric feeding evaluation checklist to assess spoon feeding ability, neuromuscular eating response (biting, chewing), neuromuscular cup drinking response and drooling degree .

Each child in the intervention group was enrolled in a serial of intervention to correct the most evident breakdown of his\her feeding problems. This intervention conducted by the researcher 5 days weekly, each session consumed 30 minutes that provided regularly for a month. The mother\ guardian was included in the intervention cessions to do it for their child through the reset days of the week, that include:

1- Modification of the manner of feeding through: Scheduling of meal time at constant time; Pacing during meal times for regulating the time interval between bites and swallow; Nature of food: concerning volume, starting with small amount and increased gradually. And regarding consistency: pureeing of solid foods, such as vegetables, Yogurt, bananas and biscuits can also used; Adaptive feeding utensils: verities of specially adapted utensils were used according to the skills needed; And environmental modification (verbal, tactile) by reducing or increasing visual and auditory stimulation such as using colored articles, loud voice or calm environment

2- Positioning and posture changes for safe swallowing using either; adapted chairs, the optimal body position used was an upright 90-degree sitting position with hips, knee and ankles flexed at a 90-degree angle and feet flat on a surface. The chin was slightly flexed with arms and hands near midline of the body. Or sitting the child on the mother lap, a mother usually feeds a child who is very young by making him sit on her lap and place her arm around his neck so that his head is kept straight and facing forward.

3- Oral- motor exercises: a type of exercise done by the researcher after reviewing the related literature and assessment of jaw stability, movement, tone and movement of the lips, cheeks and tongue to facilitate chewing, biting and swallowing(**Bowen, 2005** and **Cecilia *et al.*, 2005**) sensorimotor exercise session lasted 7-10 min/day that include: Massaging of the cheeks and lips gently in a circular movement using middle and index fingers to enhance relaxation of the facial musculature; Tapping and quick stretch of the jaw by gentle pushing his chin upward to improve the function and increase the tone; Tongue stimulation by making pressure on the middle of the tongue or lateral sides of tongue can facilitate intrinsic and extrinsic lingual muscles; And stroke the child throat gently with the fingers and keeping head bent slightly forward to help him swallow.

4- Controlling of drooling through : Optimizing head control and body posture by keeping child's head either upright or slightly tilted back; Enhancing lip closure by keeping teeth and jaw's in good position; Facilitating active swallowing by stroke child's throat; Improve tongue control through pressure .And increase self awareness of drooling.

- The control group receive routine hospital care in the form of given prescribed medication, follow-up, and feeding child either parentral or enteral feeding.
- Children (both in intervention and control group) were reassessed after intervention for feeding domain using the same pre intervention format. Data were collected through one year starting from January 2010 to February 2011.

Limitations of the study:

The limitation of this study was that the subject's pre intervention not has the same responses regarding deficit neuromuscular feeding behavior. The control group were had poor neuromuscular behavior than the intervention group in spite of both groups were selected randomly.

Statistical Analysis:

Data entry and analyses were performed using SPSS statistical package version 10 (SPSS, Inc., Chicago, IL, USA). Quantitative variables were presented as mean and standard deviation. Student t-test and One-Way ANOVA procedure were conducted to assess the effects of socio-demographic criteria on feeding parameters for the intervention and control group at pre and post intervention assessments. The chi-square ($\chi 2$) was used to compare the intervention and control groups regarding the condition of each feeding parameter in

pre and post intervention. The P value of < 0.05and <0.001 indicate significant and highly significant results respectively at confidence interval 95%.

3. Results

Table (1)shows that the ages of children included in the study were between 2 years and 11 years with mean age 6.33± 2.38 in the intervention group and 6.23 ± 2.90 in the control group and more than half of them (53.3% in the intervention group and 60% in the control group) are female. While their GCS were 3-8 in the intervention group and 3-8 in control group. The majority of them(83.3% in the intervention, 86.6% in the control group) not have Ryle feeding and more than half (53.3%) their cause of injury was road traffic accident and the minority (16.7%, 3.3%) being struck by object. Regarding mother education (46.7%) in the intervention and (56.7%) in the control group were moderately educated, while (66.7%) of mothers in the intervention group were employed and the same percentage were not employed in the control group. 65.7% of mothers in the intervention group were from urban areas and half of mothers in the control group were from urban and other half from rural areas.

Table(2) shows comparisons between intervention and control groups regarding mean difference of improvement in feeding domains post nursing intervention, it is clear that the mean score of spoon feeding ability, chewing, cup drinking , drooling in the intervention group were 4.70 ±1.49, 4.30 1±.12, 5.83 ±1.78, 1.73 ± 0.52 respectively, while the mean score in the control group were 3.43± 1.17, 2.57± 1.04, 3.23 ±1.22 respectively and the total mean score are 19.47 ± 4.75 in the intervention group and 15.03± 5.25 in the control group with a significant statistical improvement in intervention group (t=3.66, 3.52, 1.34,3.43 and *p* .001, .001, 0.l8,.001). Except in biting domain there this no statistical significant improvement(t=1.07, *p* 0.2).

Table (3) Shows subjects demographic criteria and its relation to improvement in feeding domains. It is obvious that the improvement among male children in the intervention group regarding biting, cup drinking and drooling is significantly high (t= 2.47, 3.45, 3.1 and *p* 0.02, 0.002, 0.004 respectively) than female children. Concerning subject's residence the only statistical improvement are founded in the cup drinking domain among urban subject's in the intervention group (t= 2.19, *p* 0.03). Also the high educated mothers associated with statistical significant improvement regarding their children spoon feeding abilities , chewing and drooling in the intervention group (ANOVA 3.1, 3.4, 2.3 and *p* 0.02 ,0.04 ,0.01) compared to the control group. Except that there is no significant variation with Sociodemographic criteria of the subject's as well as their mean difference of improvement in feeding domains.

Figure (1) show that the improvement in spoon feeding ability regarding upper/ lower lip movement and food loss after nursing intervention are significantly higher in intervention group compared to control group post nursing intervention(X^2 27.1, 21.6, 26,04 and *p* 0.000)

Figure (2) shows a highly statistical differences of improvement in intervention group compared to control group post nursing intervention regarding neuromuscular eating response (biting and chewing) of sustained control bite, circular rotatory pattern of jaw, lip closure and food loss(X^2 26.7, 32.4, 18.09, 28.1 respectively ,*p* 0.000)

Figure(3**)** shows a significant improvement in intervention group than control group regarding neuromuscular cup drinking response to lip seal around cup, jaw position, sips sequence, cup holding and liquid loss post nursing intervention (X^2 24.3, 20.37, 11.27, 25.6, 25.7 and *p* 0.000, 0.002).

Figure (4) shows that there is a highly significant improvement in intervention group compared to control group regarding degree of drooling post nursing intervention (X^2 38.7, *p* 0.000).

4. Discussion

Nurses play a critical role in the management of traumatic head injury among children. Pediatric head injury ranks among the primary causes of mortality and severe morbidity in this age group, moreover the aspect inherent to pediatric intensive care demand professional expertise to optimize management and improve outcome (**Andrew & Sulivan, 2010**).

The finding of the current study indicated that there are significant improvement regarding the mean score of feeding domains in the intervention group post intervention when compared to pre intervention except biting domain show no significant variation (Table 2). These findings agreed with a study made by **Abu Elsaad & Abdelateif (2008)** on children with cerebral palsy that demonstrated significant improvements in feeding domains in end-test when compared to pre-test except biting which demonstrated non-significant difference. Also this finding is contradicting with the results of a study done by **Gangil *et al.* (2001)** that stated limited improvement observed in spoon feeding, biting and chewing skills but not in drinking skills.

Studies provide evidence that early nursing intervention improved motor recovery of head injury children. Oral stimulation can be an effective adjunct to feeding treatment, it provide the child with the necessary sensory and movement input to adequately prepare the child for controlled practice with food

using suitable spoon(**Kumin *et al*., 2009**). Others emphasize that exercises and positioning may help improve a child's ability to chew and swallow, the use of varying foods a day is the key to increase the frequency of positive practice and thus increasing the acceptance of variety and volume of foods consumed in children exhibiting oral –motor difficulties (**Baily and Angell, 2005**) . Also a study on oral motor therapy which was designed to increase tongue lateralization, lip control, and vigor of chewing that delivered before the lunch meal 5-7 minutes/day,3 days/week for 20 weeks were associated with improved spoon feeding, biting and chewing (**Haberfellner *et al*., 2001**).Moreover other studies done by **Gisel *et al.* (2003)** and **DeMatteo (2003)** demonstrated that using different foods possess a wide array of sensory qualities with respect to temperature, texture and taste, these sensory qualities in combination stimulate oral movements. As well as changing food texture can make a difference in a child's ability to manage food in his/her mouth and swallow. Several researches have designed behavioral interventions to improve oral- motor problems such as swallowing dysfunction and failure of cup drinking; the technique assumes competent oral-motor tongue and jaw movements, the children had increased swallow frequency, adequate jaw position and improved skill of cup drinking (**Kerwin &Eicher,2004 and Gerek &Ciyiltepe, 2005**). All these researches emphasized the implemented nursing intervention and are agreed with the results of the current study as showing in (Figures 1,2,3) that indicated significant improvement in neuromuscular response to spoon feeding ability, chewing, biting and cup drinking response among intervention group post nursing intervention .

Table (1)Socio demographic criteria of the intervention and control groups in percentage distribution:

Items	intervention group No=30	Control group No=30	X^2	*p*-value
Child age(year)				
< 6	17(56.7%)	16(53.3%)	.351	.684
>6	13(43.3%)	14(46.7%)		
Mean ± SD	6.33± 2.38	6.23± 2.90		
Child sex				
Mal	14 (46.7 %)	12 (40 %)	.271	.795
Female	16 (53.3 %)	18 (60 %)		
Presence of Ryle				
Yes	5 (16.7 %)	4 (13.3 %)	.131	1.00
No	25 (83.3 %)	26 (86.7 %)		
Mother education				
Illiterate	0 (0 %)	5 (16.7 %)	8.262	.041
Read/write	6 (20 %)	4 (13.3 %)		
Moderate education	14 (46.7 %)	17 (56.7 %)		
High education	10 (33.3 %)	4 (23.3 %)		
Mother occupation				
Employed	20 (66.7 %)	10 (33.3 %)	6.667	.019
Housewife	10 (33.3 %)	20 (66.7 %)		
Residence				
Rural	13 (43.3 %)	15 (50 %)	.268	.796
Urban	17 (56.7 %)	15 (50 %)		
Causes of injury				
Root Traffic Accident	16 (53.3 %)	16 (53.3 %)	3.394	.183
Fall from high	9 (30 %)	13 (43.3 %)		
Being struck by object	5 (16.7 %)	1 (3.3 %)		

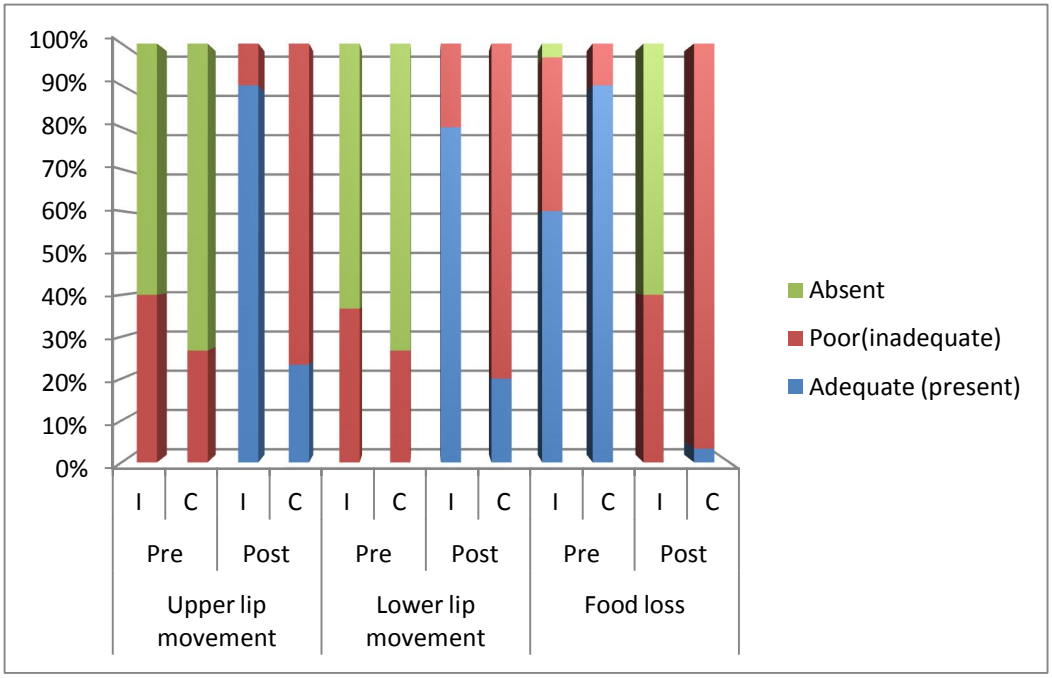
Table (2) comparisons of intervention and control groups regarding Mean Difference of Improvement in Feeding Domains:

Feeding Domains	Mean ± SD		t-test	*P* value
	intervention group/ n=30	Control group/ n=30		
Spoon Feeding	4.70± 1.49	3.43±1.17	3.66	.001
Biting	2.90±1.35	2.57±1.04	1.07	0.2
Chewing	4.30±1.12	3.23±1.22	3.52	.001
Cup drinking	5.83±1.78	5.17±2.04	1.34	0.18
Drooling	1.73±.52	0.90±.71	3.43	.001
Total	19.47±4.75	15.03±5.25	5.17	0.00

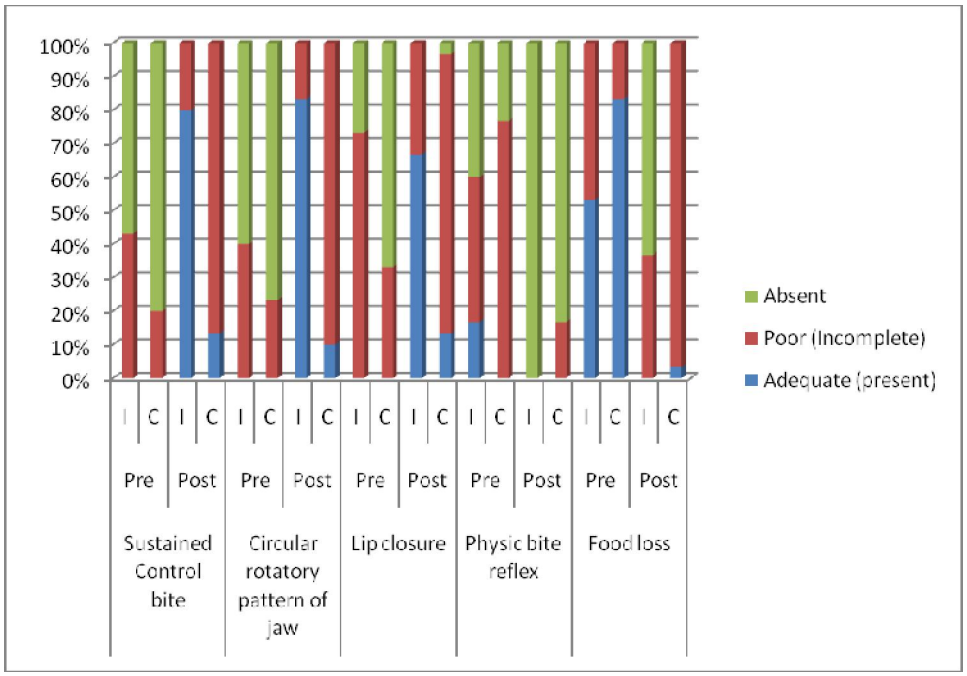
Table (3): Subject's Socio-demographic Criteria and Their Improvement in Feeding Domain.

Socio-demographic criteria	Mean ± SD									
	Spoon feeding		Biting		Chewing		Cup drinking		Drooling	
	I[a]	C[b]	I[a]	C[b]	I[a]	C[b]	I[a]	C[b]	I[a]	C[b]
1-**Age (years)**										
▪ < 6	4.94±1.48	3.06±1.12	3.00±1.58	2.3±0.79	4.47±1.18	3.06±1.12	6.24±2.05	5.06±2.17	1.82±0.53	1.00±0.73
▪ > 6	4.38±1.50	3.86±1.10	2.77±1.01	2.86±1.23	4.08±1.04	3.43±1.34	5.31±1.25	5.29±1.94	1.62±0.51	0.79±0.70
t-test	1.013	-1.9	0.458	-1.4	0.970	-.80	1.43	-.29	1.08	0.818
P- value	0.32	0.06	0.65	0.15	0.34	0.42	0.16	0.77	0.28	0.42
2-**Sex**										
▪ Male	5.07±1.77	3.33±0.89	3.50±1.16	2.58±0.90	4.50±1.22	3.17±0.72	6.86±2.07	5.58±1.93	1.33±0.65	1.86±o.53
▪ Female	4.38±1.15	3.50±1.34	2.37±1.31	2.56±1.15	4.13±1.02	3.28±1.49	4.93±0.78	4.89±2.11	0.61±0.61	1.63±0.05
t- test	1.25	-.41	2.47	0.07	0.90	-.27	3.45	.913	3.1	1.22
P-value	0.22	0.69	0.02	0.94	0.38	0.79	0.002	0.37	0.004	0.29
3-**Residance**										
▪ Rural	4.85±1.52	3.06±1.39	2.46±1.57	2.40±1.18	4.38±1.39	3.13±1.35	4.40±2.11	6.00±2.19	1.62±0.65	0.73±0.70
▪ Urban	4.59±1.51	3.80±0.77	3.24±1.09	2.74±0.88	4.23±0.90	3.33±1.11	5.93±1.58	5.71±1.45	1.82±0.39	1.07±0.70
t- test	0.46	-1.7	-1.5	-.87	0.33	-.44	-2.1	0.44	-1.08	-1.2
P-value	0.65	0.08	0.12	0.39	0.74	0.66	0.03	0.66	0.29	0.20
4**M.education**										
▪ Illiterate	-	4.20±1.48	-	2.60±1.82	-	3.20±2.28	-	5.60±1.82	-	0.60±0.54
▪ Read / write	3.17±2.04	3.25±1.70	2.83±1.94	2.25±0.95	3.60±1.03	3.75±0.50	5.33±1.03	4.75±3.20	1.83±0.40	0.75±0.95
▪ Middle	4.30±0.98	3.17±1.01	3.21±1.25	2.53±0.87	4.64±1.16	3.11±1.11	6.28±2.16	3.11±1.76	2.71±0.61	0.94±0.65
▪ High	5.21±1.63	3.75±0.50	2.50±1.08	3.00±0.82	4.67±0.84	3.25±0.50	5.50±1.51	5.25±2.87	4.70±0.48	1.25±0.95
ANOVA	3.1	1.14	0.81	0.33	3.4	0.26	0.85	0.12	2.3	0.67
P-value	0.02	0.35	0.45	0.79	0.04	0.84	0.43	0.94	0.01	0.58
5**M.Occupation**										
▪ Employed	4.80±1.28	3.40±0.61	2.90±1.29	2.50±1.17	4.30±1.17	3.10±0.87	6.20±1.99	5.00±2.05	1.70±0.57	1.00±0.82
▪ House wife	4.50±1.90	3.45±1.36	2.90±1.52	2.60±0.99	4.30±1.05	3.30±1.38	5.10±0.99	5.25±2.07	1.80±0.42	0.85±0.67
t- test	0.45	-.13	.000	-.24	.000	-.48	1.6	-.31	-.48	.53
P-value	0.66	0.89	1.00	0.80	1.00	0.63	0.11	0.75	0.63	0.59

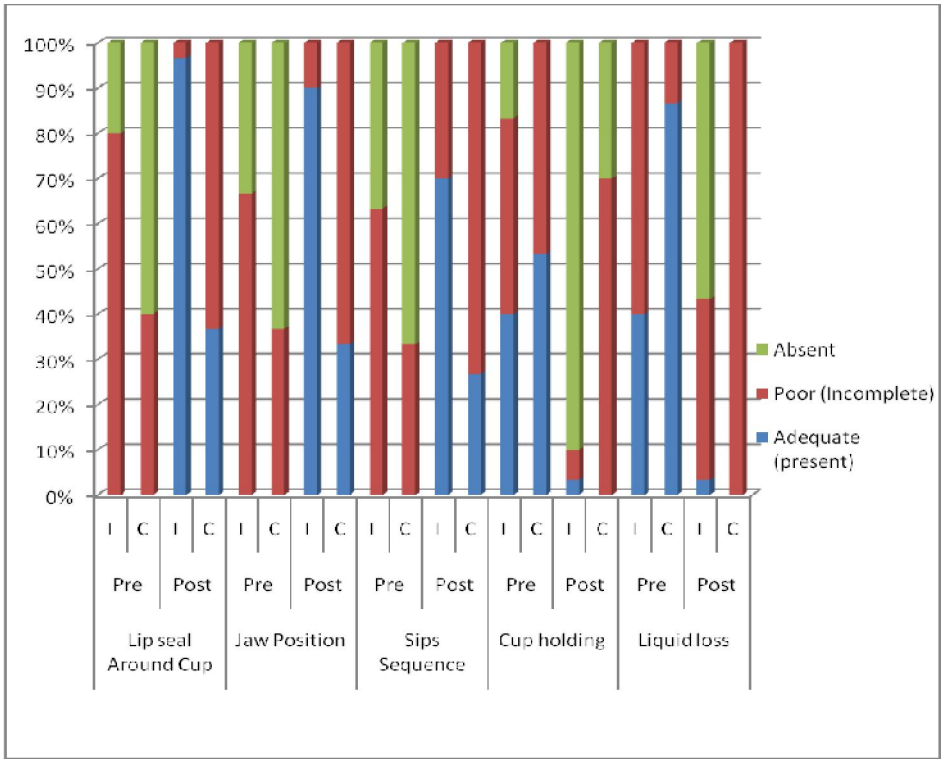
[a] Intervention group, [b] Control group.



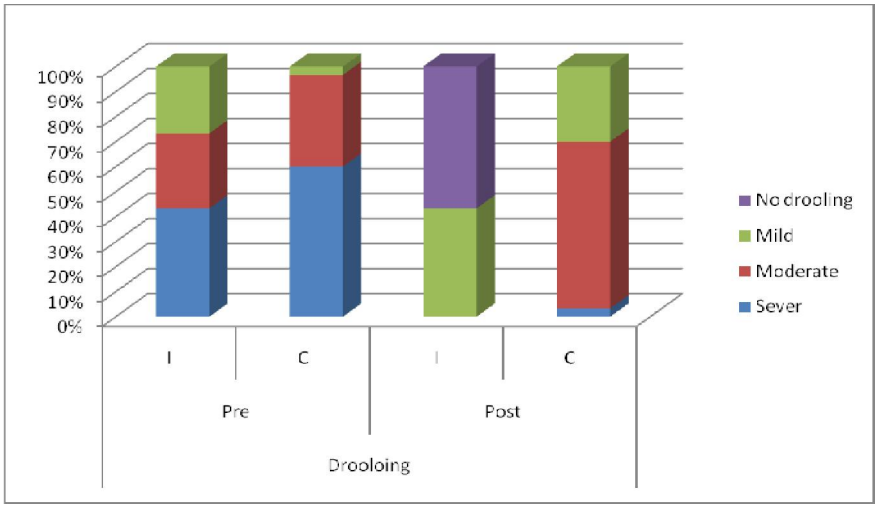
Figure(1) :Comparison between intervention(n=30) and control group(n=30) regarding spoon feeding ability pre and post intervention



Figure(2) Comparison between intervention(n=30) and control group(n=30) regarding neuromuscular eating response pre and post intervention



Figure(3) Comparison between intervention(n=30) and control group(n=30) regarding neuromuscular cup drinking response pre and post interventions



Figure(4) comparison between intervention(n=30) and control group (n=30)regarding degree of drooling pre and post intervention

Mothers/ guardian has a primary role in the given nursing care through their participation in teaching their child the various skills of daily living. In our study the improvement rate of drooling post intervention in the intervention group are obvious and has statistical significant variation , this resulting from education and stimulation of intervention children to optimize head control, enhancing lip closure, improve tongue control through pressure and Increase self awareness of drooling. These measures implemented by the researcher in combination with the mothers that are trained by the researcher (Figure 4).

The current study revealed that the improvement rate in neuromuscular feeding skills are significantly higher between male children than female, this may because of our society culture and tradition in protecting, supporting and taking care of male better than female. In addition to this the improvement in feeding problems concerned with spoon feeding ability, chewing and drooling among children of highly educated mothers has a significant difference of improvement than among children of moderated to illiterate mothers (Table 3). This emphasize the importance of mothers educational level that help in efficient implementation of the given instructions. **Mueller, *et al.* (2003), Najdowski *et al*.,(2003) and Lewis and Kritzinger (2004)** utilized parent training packages to implement pediatric feeding protocols, the components evaluated included verbal instructions plus modeling, verbal instructions plus rehearsal, and verbal instructions alone. Results indicated parents could be trained to implement intervention procedures for their children's feeding difficulties.

Conclusion:

This study concluded that feeding problems in children who have acquired severe head injury could be eliminated by specific nursing intervention using a behavior modification program combining education and exercises to enhance oral-motor functions. Also mother education could help in eliminating children feeding problems induced by oral-motor deficit.

Recommendation:

According to the observed results the recommendation are, a carefully planned and subsequent feeding program designed on evidence-based to be compatible with the child's needs and abilities should be developed, the planning and implementation of these programs should involve the child's family especially primary caregivers. Also nursing care research must be conducted to achieve high quality nursing management of feeding problems induced by oral-motor difficulties.

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On the Contribution of Shear Reinforcement in Shear Strength of Shallow Wide Beams

Mohamed M. Hanafy, Hatem M. Mohamed and Nabil A.B. Yehia

Structural Engineering Department, Faculty of Engineering Cairo University, Egypt
Hatem_amn@yahoo.com , Hatem_amn@hotmail.com

Abstract: One of the common floor structural systems used in the Middle East is reinforced concrete hollow block slab with shallow wide beams (hidden beams). Most of the building codes in the middle east; the current Egyptian Code of practice (ECP 203-2007) for example, require that the applied one way shear stress in the shallow wide beams be less than the concrete shear strength without any shear reinforcement contribution, and the shear strength provided by concrete equals two thirds of concrete shear strength of shallow slender beams. As a consequence; a large cross-sectional areas of concrete shall be provided for these members to resist one-way shear demands which results in a conservative uneconomic design provision. The above mentioned requirements by some building codes in the Middle East were not found in most of other recognized international codes or standards. An experimental program was carried out to investigate the contribution of web shear reinforcement to shear strength of shallow wide beams. The main parameters considered in this investigation were: concrete compressive strengths and vertical stirrups; with varying amount, configuration and spacing. The experimental program consisted of twelve simply-supported reinforced concrete wide beams subjected to two concentrated loads at third points. The specimens were divided into 5 groups. All specimens were typically proportioned so that shear failure would preclude flexural failure. Shear strengths at failure recorded in this experimental program are compared to the analytical strengths calculated according to some international codes. Test results clearly demonstrate the significance of the web reinforcement in improving the shear capacity the ductility of the shallow wide beams which is consistent with the recognized international codes and standards provisions.

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Keywords: shear strength, shallow wide beams, stirrups, normal strength concrete, high strength concrete, modified compression field theory.

1. Introduction

In design of buildings, modern architectural constraints are pushing the designers to provide longer clear spans at a reasonable cost. At the same time, there is a need to minimize the overall structural slab depth to achieve more floor clear height, which can be achieved through the use of either shallow wide beams (Hidden Beams) or flat plate slabs.

According to the majority of building code in the middle east; Egyptian Code of practice (ECP 203-2007) [1] for example; the shear stress in shallow wide beams must be less than the concrete shear strength with no consideration of the contribution of shear reinforcement. Moreover, according to the same code; the shear strength provided by concrete for shallow wide beams equals 67% of the concrete shear strength for shallow slender beams. As a consequence, large cross-sectional areas of concrete shall be provided to meet one-way shear demands. In other words, while the code neglects the web reinforcement contribution in shear strength, it persists on providing specified minimum web reinforcement, and moreover, reduces the concrete shear strength. These three conjugate requirements of the code lead to a very conservative, yet uneconomic, shear design of shallow wide beams. In the same stream, the code requires the stirrups to be arranged so that the distance between stirrup branches across the beam section not to exceed 250 mm.

High-strength concrete has gained an increased interest in reinforced concrete structures in last ten years as it generally leads to the design of smaller sections. This in turn reduces the dead weight, allowing longer spans and more usable area of building. However an increase in the concrete strength produces an increase in its brittleness and smoothness of shear failure surfaces, leading to some concerns about the application of high strength concrete. In the last few years the development of concrete technology and practice has led to a significant change of what high strength concrete is, and subsequently, the definition of high strength concrete has changed over the time. For instance in the 1950s, concrete with compressive strength ($f_c^{'}$) of 35 N/mm^2 was considered to be high strength concrete. Currently, a number of construction projects have used concrete with 28-day compressive strengths ($f_c^{'}$) in the range of 65 to70 N/mm^2. American Concrete Institute ACI 363 [2] defines the high-strength concrete as a concrete with a minimum 28-day cylinder compressive strength ($f_c^{'}$) of 41 N/mm^2.

Recently, few experimental and analytical investigators directed their attention to study the shear behavior of shallow wide beams. Most of the current

shear procedures are based on tests carried out on beams with a concrete compressive strength (f_c') lower than 70 N/mm^2. In addition, the mechanism of shear failure is not fully understood due to the lack of research in high-strength concrete shallow wide beam.

Khalil,[3] carried out an experimental study to investigate the shear behavior of hidden beams (wide shallow beams) in hollow block slabs. His experimental investigation included nine medium-scales simply supported hidden beams and five full-scale hollow block one way slabs with normal concrete strength. The results showed that the capacity of specimens with shear reinforcement reached as high as 300% of those without shear reinforcement. Lubell *et al.,* [4] carried out an experimental study to investigate the shear behavior of the wide beams and thick slabs as well as the influence of member width. In their study they tested five specimens of normal strength concrete with a nominal thickness of 470 mm and varied in width from 250 to 3005 mm. The study demonstrated that the failure shear stresses of narrow beams, wide beams, and slabs are all very similar. It is worth mentioning that the basic expression for one-way shear in ACI 318-02 [5] is the same for narrow beams and wide beams. Dino Angelakos, *et al.,* [6] investigated the effect of concrete strength and minimum stirrups on shear strength of large members in more details. They conducted an experimental program of twelve 1000 mm deep beams with concrete strengths (f_c') varying from 21 to 80 N/mm^2. The beams were loaded by a point load applied at the middle of a 5400 mm simply supported span. Their tests revealed that even large lightly reinforced members containing minimum level of stirrups can fail at approximately 70% of the ACI 318-02 [5] predicted shear strength. They also concluded that changing the concrete strength by a factor of 4 had almost no influence on the shear strength of these large beams while changing the longitudinal reinforcement ratio from 0.5 to 2.09% increased the observed shear strength by 62%. James and James [7] investigated the shear behavior of reinforced concrete exterior wide beam-column-slab connections subjected to lateral earthquake loading. An experimental program of three reinforced concrete exterior wide beam-column-slab specimens were designed, constructed, instrumented and tested. The three specimens were all two-thirds scale, and had 300 mm deep wide beam. The width of the wide beams varied from 865 to 940 mm. The wide beams were constructed with concrete strengths varying from 29 to 34.5 N/mm^2. Upon examining the beams after failure, they observed that the wide beams never exhibited any inclined cracking that could be characterized as related to shear. Observed cracks were narrow, vertical flexural cracks that opened very little. Stirrups strain gages never measured strains in the stirrups vertical legs greater than one-third of the yield strain, hence, they concluded that the wide beams performed well in the shear. Lubell *et al.,* [8] investigated the influence of the shear reinforcement spacing on the one-way shear capacity of wide reinforced concrete members. A series of 13 normal strength concrete specimens were designed and tested. Shear reinforcement spacing was a primary test variable. The specimens contained web reinforcement ratios close to ACI 318-02 [5] minimum requirements. The study concluded that the effectiveness of the shear reinforcement decreases as the spacing of web reinforcement legs across the width of a member increases, the use of few web reinforcement legs, even when widely spaced up to a distance of approximately 2d, has been shown to decrease the brittleness of the failure mode compared with a geometrically similar member without web reinforcement. To ensure that the shear capacity of all members with web reinforcement are adequate when designed according to ACI 318-02 [5], the study recommended that the transverse spacing of web reinforcement should be limited to the lesser of both the effective member depth and 600 mm.

The objective of this research program is to determine the effect of the following parameters on the shallow wide beam shear resistance: (i) concrete compressive strength, (ii) existence of vertical stirrups as web reinforcement, (iii) volumetric ratio of vertical stirrups (iv) spacing between vertical stirrups, and (v) number of vertical stirrups branches in section . A comparison between test results and the prediction of different building codes such as (ECP 203-2007) [1], ACI 318-02 [5], EN1992 [9], ASHTO-LRFD [10] and CSA 2004 [11] is also presented. A similar comparison is made between the experimental test results and analytical results obtained through the application of the windows based computer program *"Response 2000"* which employs the modified compression field theory (MCFT) [12]. CSA 2004 [10] prediction was obtained using the computer program "*Response 2000*" [13] since the modified compression field theory forms much of the basis of the Canadian design code.

Codes' Review For Shear Of Shallow Wide Beams

Egyptian Code of practice (ECP 203-2007) [1]

The current Egyptian Code of practice (ECP 203-2007) determines the shear resistance of shallow wide beams as following:

$$q_u \le q_{cu} \quad (1)$$

$$q_{cu} = 0.16\sqrt{\frac{f_{cu}}{\gamma_c}}\, b_w d \quad (2)$$

Where q_{cu} is the concrete shear capacity (N/mm^2), f_{cu} is the concrete characteristic cube strength (N/mm^2), γ_c is concrete partial safety factor equals 1.50, b_w is the width of the web (mm) and d is the effective depth of the section (mm). The code neglects the web reinforcement contribution in shear strength of shallow

wide beams, while stressing the need to provide specified minimum web reinforcement, and at the same time reduces the concrete shear strength for shallow wide beams.

American Concrete Institute (ACI 318-02) [5]

According to ACI 318-02; the nominal shear strength, V_n, of non-prestressed members is the sum of the concrete contribution; V_c, and shear reinforcement contribution; V_s. Thus,

$\phi V_n \geq V_u$ (3)

$V_n = V_c + V_s$ (4)

Where V_u is the factored shear force at the section, the concrete contribution term, V_c , can be calculated by either of the following two equations:

$$V_c = 0.17\sqrt{f_c'}\, b_w d \quad (5)$$

$$V_c = \left[0.16\sqrt{f'_c} + 17\rho_w \frac{V_u d}{M_u}\right] b_w d \leq 0.3\sqrt{f_c'}\, b_w d \quad (6)$$

When the factored shear force V_u exceeds the shear strength provided by concrete; ϕV_c, shear reinforcement must be provided to carry the excess shear and its contribution is calculated as:

$$V_s = \frac{A_v f_y d}{s} \leq 0.66\sqrt{f_c'}\, b_w d \quad (7)$$

Where: V_u = factored shear force at the section (N), V_c = nominal shear strength provided by concrete (N), V_s = nominal shear strength provided by shear reinforcement (N), V_n = nominal shear strength (N), M_u = factored flexural moment at section (N.mm), ϕ = strength reduction factor = 0.75, $\rho_w = A_s/b_w d$, As = area of longitudinal reinforcement (mm^2), A_v = area of shear reinforcement (mm^2), b_w= web width of section (mm), d= distance from the extreme compression fiber to the centroidal axis of the longitudinal reinforcement (mm), s = spacing of the transverse reinforcement (mm), f_c' = concrete compressive cylinder strength (MPa), f_y = yield strength of the transverse reinforcement (MPa).

The ACI prediction gives un-conservative results for large lightly reinforced members without shear reinforcement. A minimum area of shear reinforcement, $A_{v,min}$, shall be provided in all reinforced concrete flexural members where V_u exceeds $0.5\varphi Vc$, except beams with h not greater than the largest of 250mm, 2.5 times thickness of flange, or 0.5 the width of web (i.e. shallow wide beams) because there is a possibility of load sharing between weak and strong areas.

Eurocode (EN1992) [9]

Members Not Requiring Shear Reinforcement

The design value for the shear resistance $V_{Rd,c}$ is given by:

$$V_{Rd,c} = [(0.18/\gamma_c)k(100\rho f_{ck})^{1/3}]b_w d \quad (8)$$

$$k = 1 + \sqrt{\frac{0.20}{d}} \leq 2.0 \quad (9)$$

$$\rho_l = \frac{A_{sl}}{b_w d} \leq 0.02 \quad (10)$$

Where f_{ck} = characteristic concrete cube strength (MPa), A_{sl} =the area of the tensile reinforcement (mm^2), b_w = the smallest width of the cross-section in the tensile area (mm), γ_c is concrete partial safety factor equals *1.50*.

Members Requiring Shear Reinforcement

The code neglects the concrete contribution in this case $V_{Rd,c}$= *0*. The design of members with shear reinforcement is based on a truss model, whereby the values for the angle θ of the inclined struts in the web are limited as follows:

$1 \leq cot\theta \leq 2.5$ (11)

For members with vertical shear reinforcement, the shear resistance, $V_{Rd,s}$, is given by:

$V_{Rd,s} = (A_{sw}/s)zf_{ywd}\, cot\theta$ (12)

Where:

A_{sw} = cross-sectional area of the shear reinforcement (mm^2), s = spacing of the stirrups (mm), f_{ywd} = yield strength of the shear reinforcement (MPa), θ = the angle between inclined concrete struts and the main tension chord, z = the inner lever arm for a member with constant depth (mm)

The Eurocode EN1992 [9] is applicable up to concrete strengths of f_{ck} =90 MPa, which corresponds to f_c'=91.6 MPa. The characteristic value f_{ck} for the cylinder strength is defined as a 5% fractile. By contrast f_c' is a 9% fractile, and the relation between the two quantities is $f_{ck} = f_c' - 1.6$ (MPa).

AASHTO LRFD Bridge Design Specifications (2005) [10]

AASHTO LRFD Section Design Model for Shear is a hand-based shear design procedure derived from the Modified Compression Field Theory (MCFT). The nominal shear resistance; *Vn*, can be computed by:

$V_n = V_c + V_s$ (13)

$$V_C = 0.083\beta\sqrt{f_c'}\, b_v d_v \quad (14)$$

$$V_s = \frac{A_v f_y d_v (\cot\theta + \cot\alpha)\sin\alpha}{s} \quad (15)$$

Where:

b_v = effective web width, taken as the minimum web width within the depth (mm), d_v = effective shear depth = the greater of (*0.9d* or *0.72h*) (mm), β = factor indicating the ability of diagonally cracked concrete to transmit tension, θ = Angle of inclination of the diagonal compressive struts, α = angle of inclined stirrups to longitudinal axis. All other notation are

identical to those indicated before.

Canadian Standards Association (CSA A23.3-04) [11]

The MCFT is the basis for the general shear provisions of the CSA [11]. In order to overcome concerns by practicing engineers over difficulties in using the LRFD [10] specifications, the CSA [11] specifications presented below were developed to provide a simpler way to obtain θ and β . In this proposed method, for design purposes θ is taken equal to 30° for evaluating the demand of shear on the longitudinal reinforcement. In this approach, the nominal strength is defined as:

$$V_n = V_c + V_s \leq 0.25 f_c' b_w d_v \quad (16)$$

$$V_C = 0.083\beta \sqrt{f_c'}\, b_w d_v \quad (17)$$

$$V_s = \frac{A_v f_y d_v (\cot\theta + \cot\alpha)\sin\alpha}{s} \quad (18)$$

Where: $b_w = b_v$

It should be noted that ACI-318-02, the AASHTO LRFD and CSA do not permit the use of a concrete compressive strength; f_c', greater than 70 MPa for shear strength calculations.

2. EXPERIMENTAL WORK:

In order to investigate effect of the above mentioned parameters on the shear resistance of the shallow wide beams, an experimental program was carried out to test twelve simply-supported reinforced concrete beams, six beams are made of normal concrete compressive strength of f_{cu}=40 N/mm^2, and the remaining six are made of high concrete compressive strength of f_{cu} =90 N/mm^2. Detailed description of the specimens, the material properties, test set-up, instrumentation, test procedure, and measurements are presented in this section.

Test Specimens:

In the experimental program, tests were carried out on twelve concrete beams named (NB1 to NB6) and (HB1 to HB6) where "NB" refers to normal strength concrete beams and "HB" refers to high strength concrete beams. The width/depth ratio is limited to 2 in all specimens.

All tested beams are 500mm x 250mm in cross-section with 800mm flange width along a length of 1100 mm centered in span to ensure that shear failure would preclude flexural failure. All tested beams have 2000mm clear span and the same flexural longitudinal top and bottom reinforcement (6T25+5T22 Bottom and 6T12 Top). The beams were simply supported and subjected to two concentrated static loads (four-point bending). The details of the tested beams are shown in table (1) and Figs. 1, 2 and 3. The test specimens were divided into 5 groups.

Group No. (1): This group consists of two specimens (NB1) and (HB1), each specimen represents the reference specimen for normal and high strength concrete respectively with no web shear reinforcement.

Groups No. (2 & 4): Each group consists of three specimens: (NB2, NB3 and NB4) in group (2) and (HB2, HB3 and HB4) in group (4). All specimens in these two groups are reinforced with Minimum web shear reinforcement ratio (□ = 0.167%) according to ECP203-2007 [1].

Groups No. (3 & 5): Each group consists of two specimens; (NB5 and NB6) in group (3) and (HB5 and HB6) in group (5), all specimens in these two groups are reinforced with (0.46%) web shear reinforcement ratio (more than minimum stirrups).

Materials:

Trial mixes were conducted in the Concrete Research Laboratory at Cairo University to reach the target cubic compressive strength of 40 N/mm^2 and 90 N/mm^2 after 28 days. Table (2) shows mix proportions by weight of the quantities needed for one cubic meter of concrete to achieve the target cube compressive strength

Test Procedure:

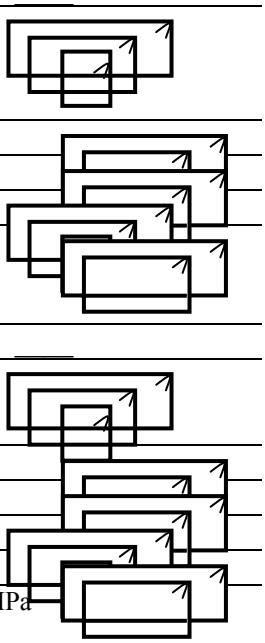
The specimens were placed in the testing machine between the jack head and the steel frame and supported on two hinged supports. The strain gages, load cell and linear voltage displacement transducer (LVDT) are all connected to the data acquisition system attached to the computer.

All beams were subjected to two concentrated loads; each load was applied at 750 mm from the support where shear span-to-depth ratio (a/d) = 3.57. The load was monitored by a load cell of 2500 KN capacity and transmitted to the reinforced concrete beam through two transversal steel I-beams resting on steel pads to provide uniform bearing surfaces. Figs. (4) and (5) show the testing setup. The load was applied gradually with constant rate of loading, about 50 KN load increments, during the test.

The data acquisition system continuously recorded readings of the electrical load cell; the two LVTDs that measure the beam deflection at mid span and the stirrups strain gages.

Table 1 : Tested beams details

Group	*Specimen*	f_{cu} *(N/mm²)*	*Longitudinal RFT**		*Web Shear RFT.* (Vertical Stirrups)*
			Bottom	*Top*	
G1	NB1	40	6T25 + 5T22	6T12	3Y6@200
G2	NB2				
	NB3				Y8+Y6@200
	NB4				2Y6@135
G3	NB5				3T10@200
	NB6				2T10@135
G1	HB1	90	6T25 + 5T22	6T12	3Y6@200
G4	HB2				
	HB3				Y8+Y6@200
	HB4				2Y6@135
G5	HB5				3T10@200
	HB6				2T10@135



*T: High Strength steel reinforcement; f_y =420MPa, Y: Mild steel reinforcement; f_y =300MPa

Table 2 Mix Design of Normal and High Strength Concrete

	Compressive target strength (N/mm²)	*Cement (KN)*	*Silica Fume (KN)*	*Crushed Dolomite (KN)*	*Sand (KN)*	*Water (liter)*	*Super-plasticizer (liter)*
NSC	40	4.00	----	12.8	6.4	200	2.5
HSC	90	5.60	1.20	11.20	5.60	145	20

Table 3 Summary of experimental results.

Normal Strength Concrete; F_{cu}=40 MPa					High Strength Concrete; F_{cu}=90 MPa				
Group/ Specimen	Test Results (KN)			Failure Mode**	Group/ Specimen	Test Results (KN)			Failure Mode**
	Cracking Load*		Failure Load			Cracking Load*		Failure Load	
	Flexural	Shear				Flexural	Shear		
G1/NB1	270.0	450.0	490.0	SC	G1/HB1	200.0	500.0	590.0	SC
G2/NB2	100.0	450.0	700.0	ST	G4/HB2	150.0	600.0	795.0	ST
G2/NB3	200.0	500.0	600.0	SC	G4/HB3	200.0	500.0	680.0	ST
G2/NB4	150.0	550.0	610.0	ST	G4/HB4	150.0	600.0	700.0	SC
G3/NB5	150.0	550.0	990.0	SC	G5/HB5	150.0	550.0	1190.0	ST
G3/NB6	100.0	500.0	1100.0	SC	G5/HB6	200.0	600.0	1220.0	ST

* cracking load values are approximate values with ± 10.00 KN tolerance.
** Failure mode; SC: shear-compression & ST: shear tension.

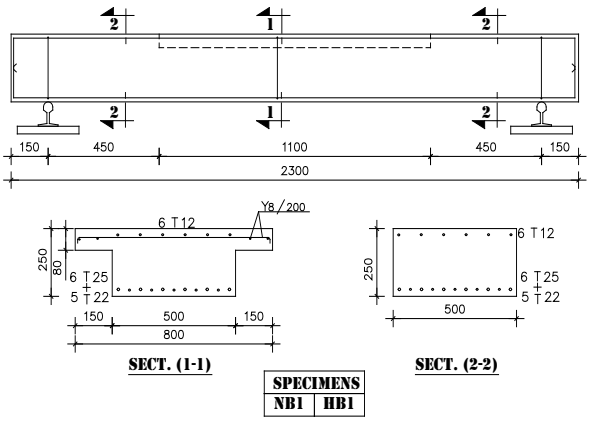


Fig. 1, Details of group No. (1)

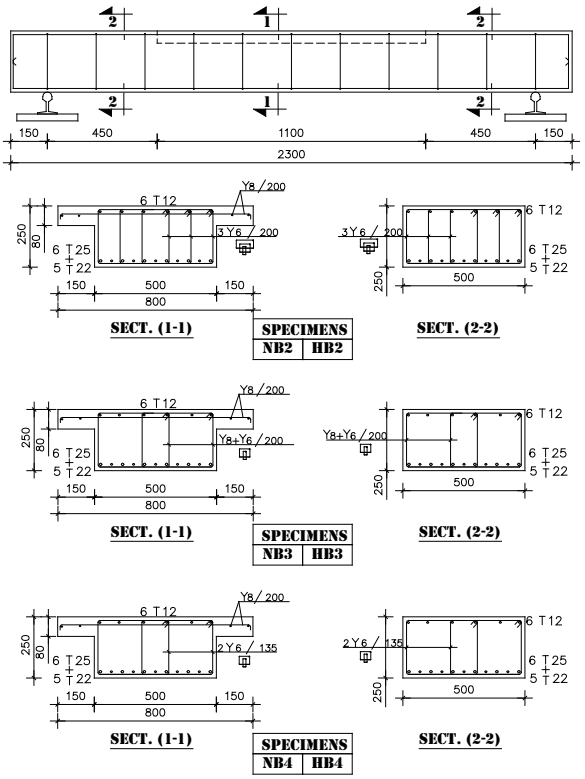


Fig. 2, Details of groups No. (2&4)

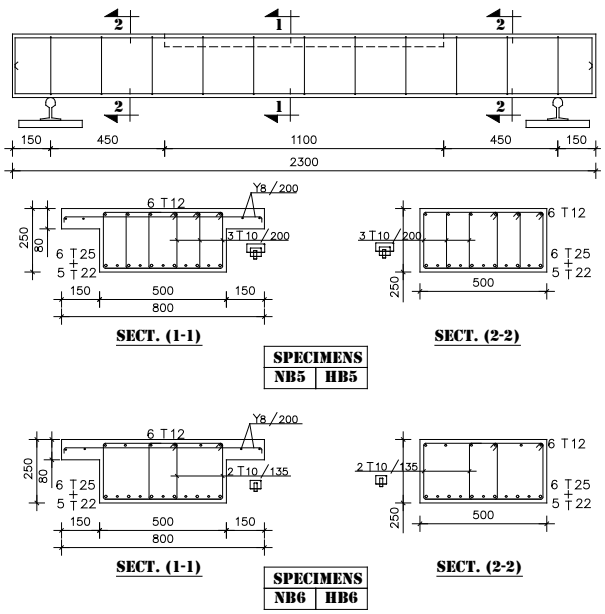


Fig. 3, Details of groups No. (3&5)

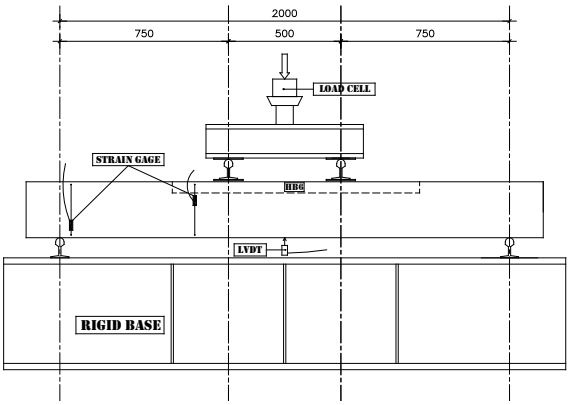


Fig. 4, Schematic Test Arrangement

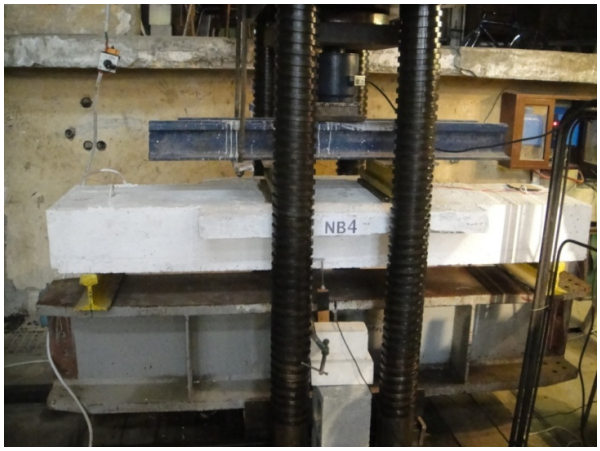


Fig. 5, Test Arrangement

Test Result:

Experimental test results of the twelve specimens are concluded in cracking pattern, load - deflection, and load - stirrups strains for each test specimen.

The windows-based computer program "Response 2000" [13] was used in the current investigation to predict the response of the tested specimens using modified compression field theory (MCFT) [12].The program outputs the ultimate load at failure, the load deflection graphs and the failure crack pattern. A comparison between test results and Response 2000 [13] outputs is also presented in this section.

A comparison between test results of the failure load and the prediction using different building codes such as ECP 203-2007 [1], ACI 318-02 [5], EN1992 [9], ASHTO-LRFD [10] and CSA 2004 [11] is also presented in this section.

Cracking Pattern and Mode of Failure:

For all specimens, the first crack development, crack propagation, and plane of failure were observed during the test. As stated before; all tested specimens were designed to fail in one way shear. This presumption was investigated for all tested specimens.

The general behavior of all tested specimens was relatively similar and the crack development followed a similar pattern in all tested specimens. All beam specimens failed in shear and shear cracks crossed the compression zone of beam section.

It was observed that the first batch of cracks was vertical flexural cracks occurred in the specimens mid span and near mid span section. No crack has been witnessed at ends of beam along 450 mm of each side - outside flange zone. Upon increasing the applied load, new series of flexural cracks was formed at the bottom in the shear span region and gradually propagated towards the compression flange then rotated towards the two loading points while no crack had been witnessed at beam ends. By increasing the applied load and at intermediate loading stages, a new series of flexural cracks was formed in the shear span region then rotated to form flexural – shear cracks; joining the loading and supporting points.

During subsequent loading stages, additional diagonal shear cracks appeared and developed through a substantial depth of the specimen section, and propagated towards the top compression flange. Cracks continued propagating horizontally just underneath the flanges towards the loading points as shown in Fig. (6).

Generally, there were two phases after flexural cracking, starting from the first shear crack up to the shear failure: the first phase is the cracking formation phase; in which new shear cracks occur, and the second phase is the stabilizing cracking phase; in which only the shear cracks widen until reaching shear failure due to significant widening of shear cracks.

Table 3 summarizes the results of the twelve tested beam specimens. The table gives the main characteristics of each specimen, its flexural cracking load, shear cracking load, and the failure shear load.

Fig. 6, Shear Cracks propagation

Figs. 7 and 8 show the experimental and the predicted failure cracking patterns for all specimens. It should be noted that in experimental results; the load is recorded along cracks to show crack propagation history. However, the failure cracking pattern as output by *Response 2000* program shows the crack width (mm) along the crack at failure stage. So, the comparison between the two crack patterns; the experimental and the analytical, can be carried out only on the context of the general distribution and extension of cracks.

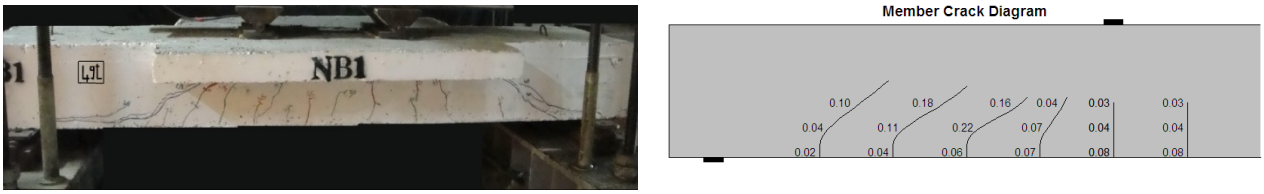


Fig. 7a, Experimental (left) and Analytical (right) crack pattern for Specimen NB1.

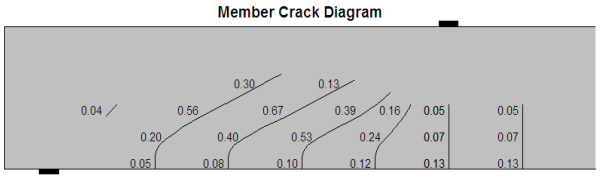
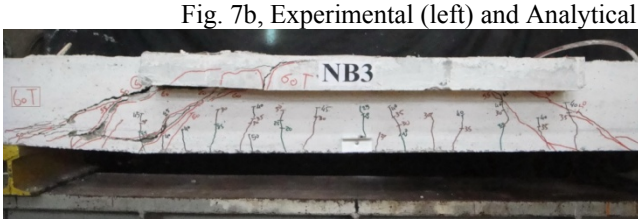


Fig. 7b, Experimental (left) and Analytical (right) crack pattern for Specimen NB2.



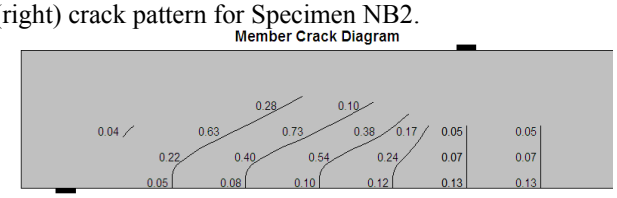
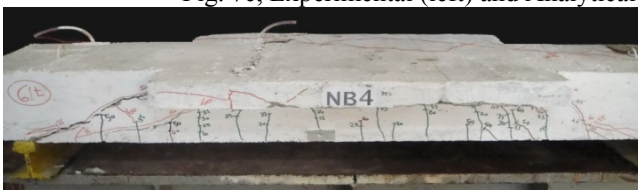


Fig. 7c, Experimental (left) and Analytical (right) crack pattern for Specimen NB3.



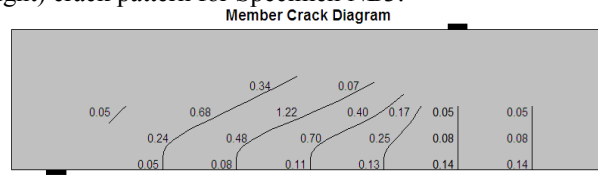
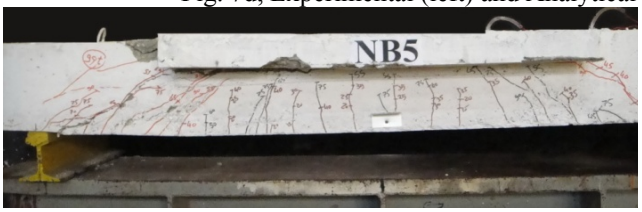


Fig. 7d, Experimental (left) and Analytical (right) crack pattern for Specimen NB4.



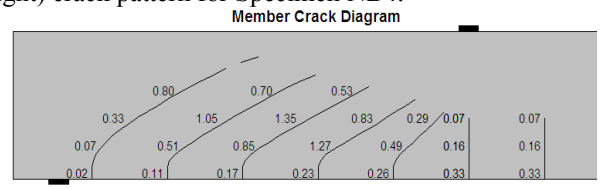
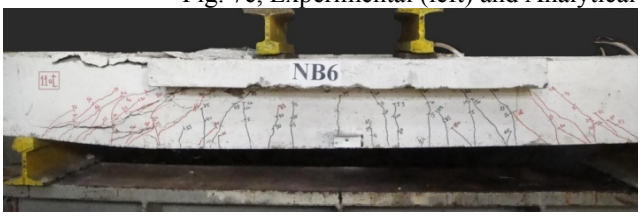


Fig. 7e, Experimental (left) and Analytical (right) crack pattern for Specimen NB5.



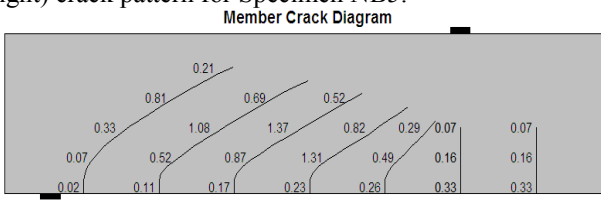


Fig. 7f, Experimental (left) and Analytical (right) crack pattern for Specimen NB6.

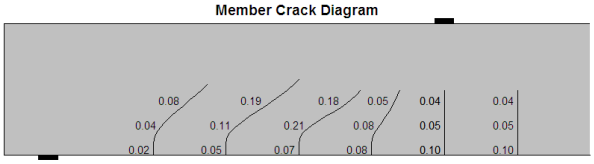
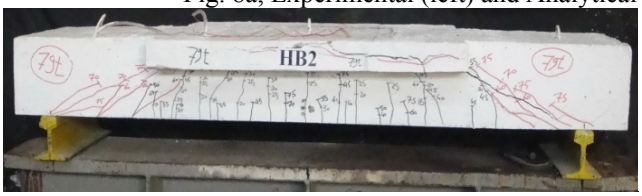


Fig. 8a, Experimental (left) and Analytical (right) crack pattern for Specimen HB1.



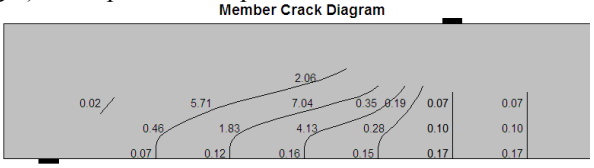


Fig. 8b, Experimental (left) and Analytical (right) crack pattern for Specimen HB2.

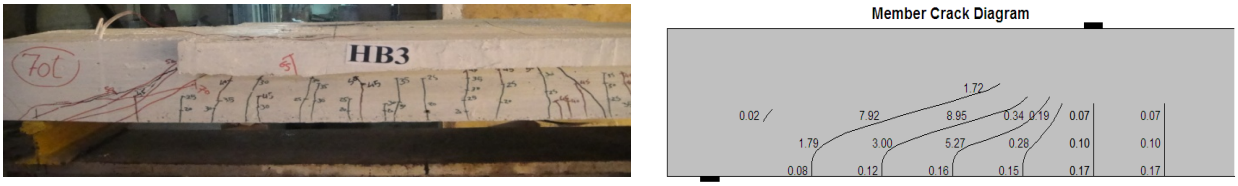


Fig. 8c, Experimental (left) and Analytical (right) crack pattern for Specimen HB3.

Fig. 8d, Experimental (left) and Analytical (right) crack pattern for Specimen HB5.

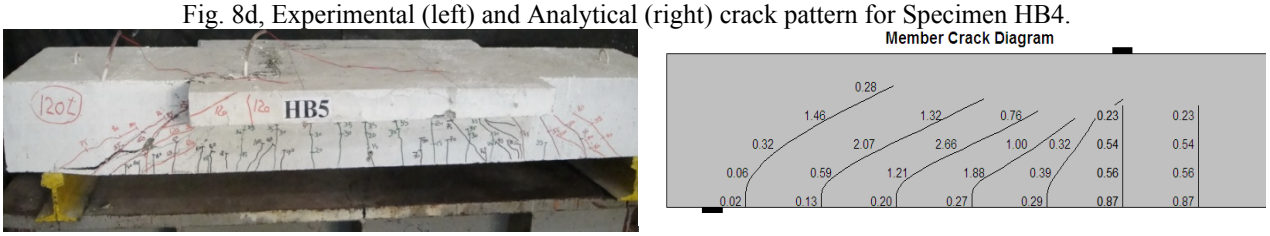


Fig. 8e, Experimental (left) and Analytical (right) crack pattern for Specimen HB6.

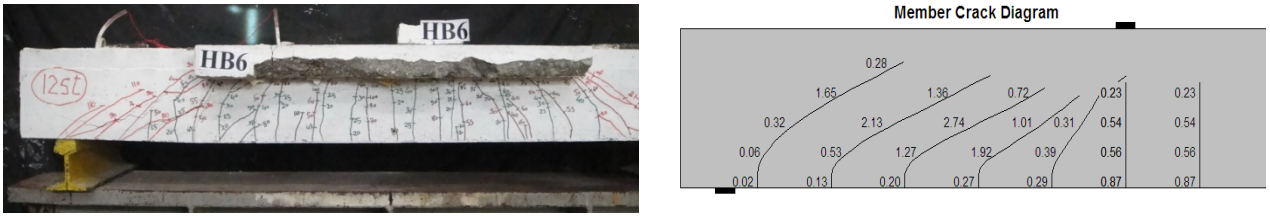


Fig. 8f, Experimental (left) and Analytical (right) crack pattern for Specimen HB6.

Load-Deflection Relationship:

Fig. 9 shows the load versus mid span deflection for the five tested groups. The curves show that the specimens exhibit three stages of behavior which are marked by a significant change in the slope of the load deflection curve.

Stage (1) which is the pre-cracking stage, starts from zero loading till the first cracking load. The behavior in this stage is characterized by the uncracked behavior where the maximum tensile stress is less than concrete flexural tensile strength (concrete modulus of rupture f_r). This is presented through the steep slope of the load deflection line where the deflection almost increased linearly with loading. The pre-cracking stage ends at the initiation of the first crack.

Stage (2) which is the post-cracking stage, begins with the first cracking in the mid span, the specimens behaves with a reduced stiffness compared to the slope of the load deflection line in the first stage where there were slight change in slope of the load deflection curve due to cracking. In this stage, the specimens developed a stable cracking in distribution and width. After cracking, deflections increased linearly with the load again.

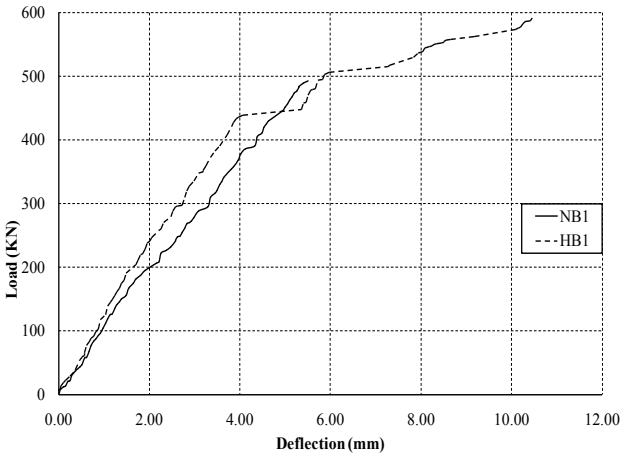
Stage (3) which is the post-serviceability stage (steel yields), specimens in this stage behaved with significantly reduced flexural stiffness compared with the previous stages. This is presented through the near horizontal to horizontal load deflection curve in this stage due to substantial loss in stiffness of the specimens section, deeper and wider extensive cracks take placed till failure.

The load-deflection curves of the Normal Strength Concrete specimens show that shear reinforcement had no significant impact on the deflection values at any loading stage where all N.S.C

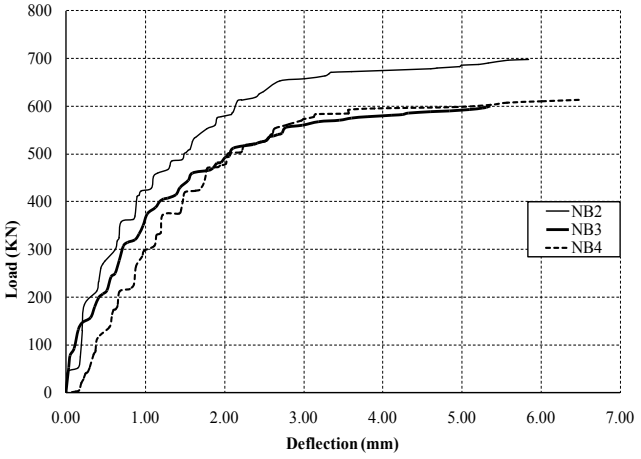
specimens had approximately equal deflection value in different loading stage (maximum deflection value ranged from 5.5 mm to 7.5 mm) except specimen NB6 which developed about 13.60 mm deflection at failure. Similar observation was recorded for H.S.C specimens. They had approximately equal deflection value in different loading stage (maximum deflection value ranged from 4.00 mm to 6.50 mm) except specimens HB1 and HB2 had approximately 10.00 mm deflection at failure. Table 4 summarizes the recorded deflection values at flexural cracking stage and at failure for all specimens. Fig. 10 shows a comparison between analytical and the experimental deflection of the five tested groups.

Table 4 Summary of recorded deflection values

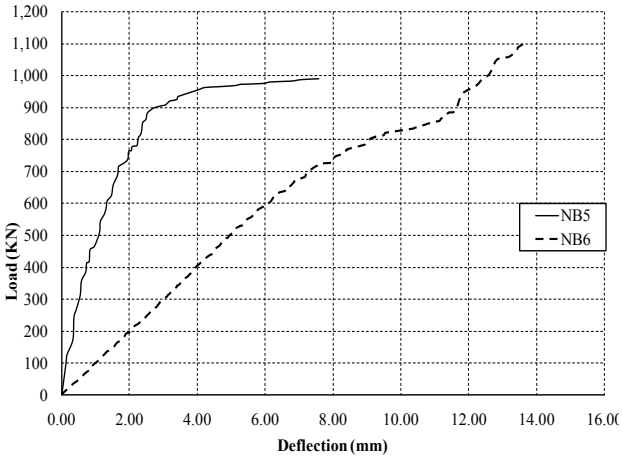
Normal Strength Concrete Specimens			*High Strength Concrete Specimens*		
Specimen	$\Delta_{cracking}$ (mm)	$\Delta_{failure}$ (mm)	Specimen	$\Delta_{cracking}$ (mm)	$\Delta_{failure}$ (mm)
NB1	3.00	5.50	HB1	1.70	10.50
NB2	3.70	5.85	HB2	8.00	9.63
NB3	4.00	5.35	HB3	2.85	4.40
NB4	4.70	6.50	HB4	4.00	5.50
NB5	4.70	7.50	HB5	4.50	6.50
NB6	1.00	13.60	HB6	4.60	6.30



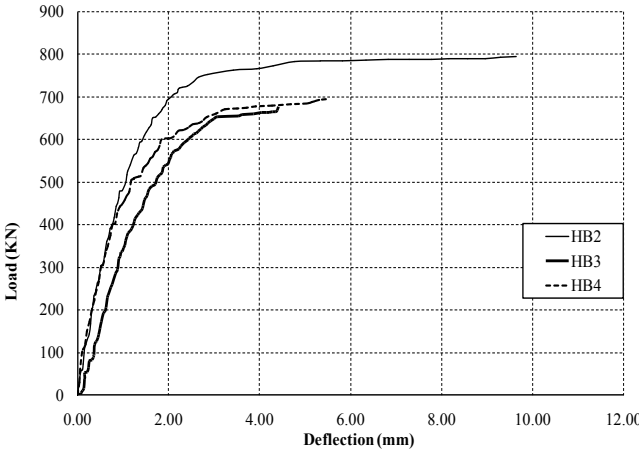
a) Group 1 specimens



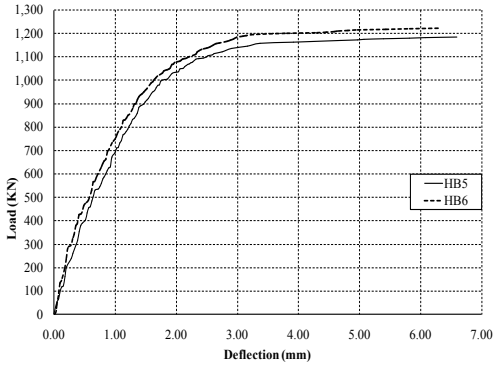
b) Group 2 specimens



c) Group 3 specimens

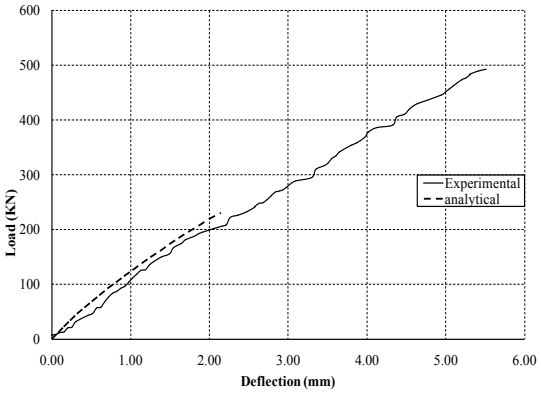


d) Group 4 specimens

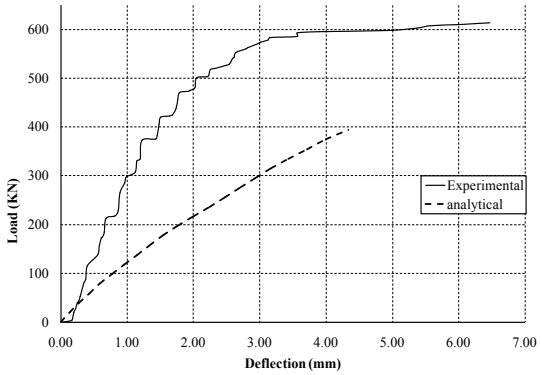


e) Group 5 specimens

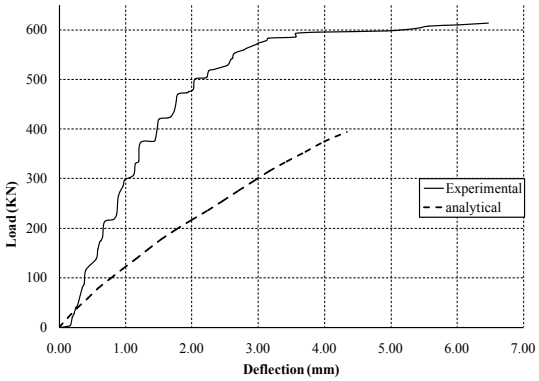
Fig. 9, Load Deflection Curves



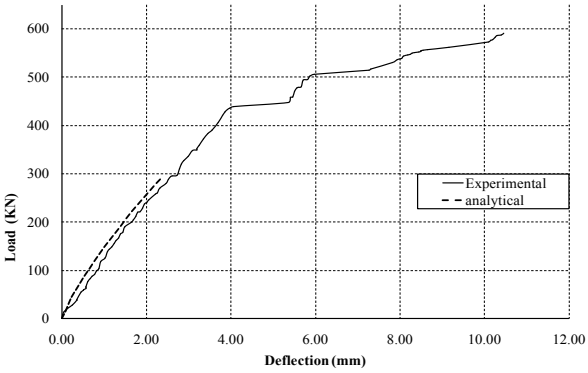
a) N.S.C specimen "NB1"



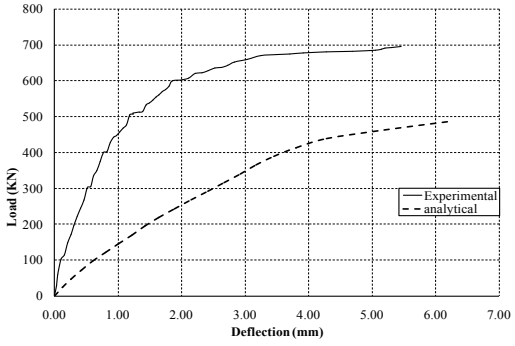
b) N.S.C specimen "NB4"



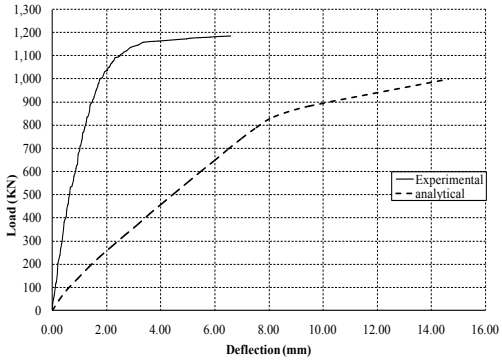
c) N.S.C specimen "NB5"



d) H.S.C specimen "HB1"



e) H.S.C specimen "HB4"



f) H.S.C specimen "HB5"

Fig. 10, Analytical versus the experimental deflection

The ductility can either be represented in terms of the ratio of maximum displacement to the yield displacement; both measured at mid span, or in terms of the stain energy consumed by the specimen during the test measured as the area under the load displacement curve. Since the flexure mode of failure has been secured for all specimens to allow for shear mode of failure, it is found more appropriate to use the second measure of ductility. Figs. 11&12 show ductility measure for all tested specimens. It easily be seen that the increase in web reinforcement generally increases the ductility for both N.S.C. and H.S.C. As the web reinforcement increases beyond the minimum ratio, its effect becomes more pronounced lower concrete strength. However, with no web reinforcement, the ductility increases as concrete strength increases.

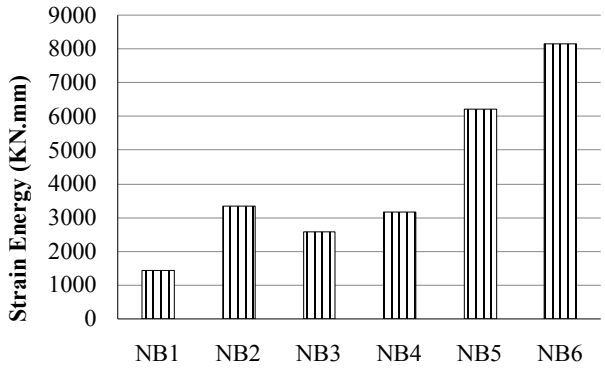


Fig. 11, Ductility measure of NSC specimens

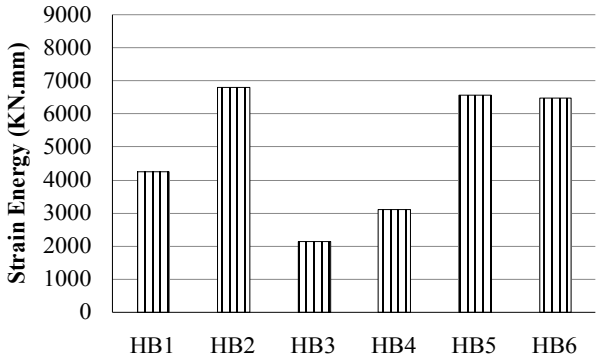


Fig. 12, Ductility measure of HSC specimens

Effect of web reinforcement

The effect in shear capacity due to the presence of the web reinforcement can be concluded as shown in Table 5. It can seen that the increase is evident for both normal and high strength concrete specimens. It is also evident that with minimum web reinforcement, the effect is more pronounced with normal strength concrete. However, as volume of web reinforcement increase, concrete strength has almost no significance.

Table 5. Increase in failure load due to the presence on web reinforcement.

Normal Strength Concrete Specimens			*High Strength Concrete Specimens*		
Specimen	*Experimental Failure Load (KN)*	*% increase Failure load*	*Specimen*	*Experimental Failure Load (KN)*	*% increase Failure load*
NB1	490.0	0	HB1	590.0	0
NB2	700.0	43	HB2	795.0	35
NB3	600.0	22	HB3	680.0	15
NB4	610.0	24	HB4	700.0	19
NB5	990.0	102	HB5	1190.0	102
NB6	1100.0	124	HB6	1220.0	107

Strains in Stirrups

Two electrical strain gages were attached to stirrups vertical branches per specimen, one strain gage was fixed closer to loading point and the other strain gage was fixed closer to support .Only specimens NB3 and HB3 had only one strain gage closer to support. Curves of load–maximum tensile strain in stirrups showed that there are two stages of behavior common between all specimens: Stage (1) is before shear crack load is reached. The strains were compression with small values (less than 100 micro - strain). Compression strains resulted from applying the load on the top surface of the specimens. Stage (2) starts after shear crack load is reached. The stirrups developed tensile strains, thus indicating that the stirrups were successful in resisting the shear stresses in test specimens. The rate of strain increase was small just after formation of first shear crack and increased rapidly when specimens approached failure load. Table 6 summarizes the recorded stirrups strain values as a percentage from stirrups yielding strain for shear tension failure and shear compression failure specimens.

Table 6 Summary of recorded stirrups strain values

Shear- Tension Failure		*Shear- Compression Failure*	
Specimen	$\varepsilon_{failure}$ *	*Specimen*	$\varepsilon_{failure}$ *
NB2	600%ε_{yield}	NB3	15.50%ε_{yield}
NB4	150%ε_{yield}	NB5	52.50%ε_{yield}
HB2	145%ε_{yield}	NB6	40.00%ε_{yield}
HB3	200%ε_{yield}	HB4	87.50%ε_{yield}
HB5	140%ε_{yield}		
HB6	105%ε_{yield}		

* ε_{yield} = 1600 macro strain for mild steel
ε_{yield} = 2000 macro strain for high grade steel

Fig. 13 plots the load strain relationships in the stirrups vertical branches for shear tension failure specimens, the highest recorded strains per specimen are plotted in the figure. No Load-strain curve was plotted for shear compression failure specimens.

Comparison between Test Results and Code Prediction for Shear Strength

Tables (7) and (8) summarize the shear capacity predictions for normal strength concrete and high strength concrete specimens respectively using the above mentioned international codes and also provide the shear capacities obtained from experimental testing. It should be noted that the experimental shear capacity $V_{exp.}$ shown in theses tables is half the failure load of the specimen shown in table 3.

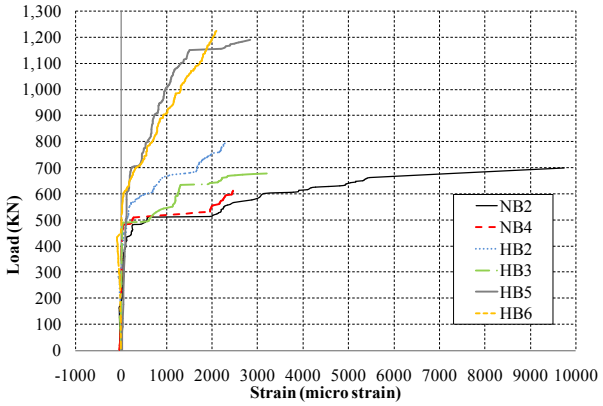


Fig. 13, Load strain in stirrup

Table 7 Summary of codes' prediction of shear capacity for N.S.C specimens.

		Predicted Shear Capacity "$V_{predicted}$" (KN)					*Experimental Shear Capacity "$V_{exp.}$" (KN*
Group	*Specimen*	*ECP 203-2007*	*ACI 318-02*	*EN1992*	*ASHTO LRFD*	*CSA 2004*	
G1	NB1	88.50	128.00	97.50	84.50	115.00	246.50
G2	NB2	88.50	173.20	107.00	180.00	187.50	350.00
	NB3	88.50	173.20	107.00	180.00	183.50	300.00
	NB4	88.50	173.20	107.00	180.00	197.00	306.50
G3	NB5	88.50	278.00	360.00	305.00	395.00	495.00
	NB6	88.50	278.00	360.00	305.00	395.00	550.00

Table 8 Summary of codes' predictions of shear capacity for H.S.C specimens.

		Predicted Shear Capacity "$V_{predicted}$" (KN)					*Experimental Shear Capacity "$V_{exp.}$" (KN)*
Group	*Specimen*	*ECP 203-2007*	*ACI 318-02*	*EN1992*	*ASHTO LRFD***	*CSA 2004***	
G1	HB1	132.50	157.50**	129.30	125.00	148.00	296.00
G4	HB2	132.50	205.00	107.00	230.00	248.00	398.00
	HB3	132.50	205.00	107.00	230.00	244.00	339.00
	HB4	132.50	205.00	107.00	230.00	243.00	350.00
G5	HB5	132.50	310.00	360.00	335.00	500.00	593.00
	HB6	132.50	310.00	360.00	335.00	500.00	612.00

** Value was calculated based on f_c =70 MPa

Tables (9) and (10) summarize the comparison between the experimental shear capacity and predictions using the current international codes; "$V_{exp.}/V_{predicted}$", for normal strength concrete specimens and high strength concrete specimens respectively.

Table 9 :Experimental shear capacity versus codes' prediction; "$V_{exp.}/V_{predicted}$", for N.S.C specimens

		"$V_{exp.}/V_{predicted}$"				
Group	*Specimen*	*ECP 203-2007*	*ACI 318-02*	*EN1992*	*ASHTO LRFD*	*CSA 2004*
G1	NB1	2.79	1.93	2.53	2.92	2.14
G2	NB2	3.95	2.02	3.27	1.94	1.87
	NB3	3.39	1.73	2.80	1.67	1.63
	NB4	3.46	1.77	2.86	1.70	1.56
G3	NB5	5.59	1.78	1.38	1.62	1.25
	NB6	6.21	1.98	1.53	1.80	1.39

Table 10: Experimental shear capacity versus codes' prediction; "$V_{exp.}/V_{predicted}$", for H.S.C specimens

		"$V_{exp.}/V_{predicted}$"				
Group	*Specimen*	*ECP 203-2007*	*ACI 318-02*	*EN1992*	*ASHTO LRFD***	*CSA 2004***
G1	HB1	2.23	1.88**	2.29	2.37	2.00
G4	HB2	3.00	1.94	3.72	1.73	1.60
	HB3	2.56	1.65	3.17	1.47	1.39
	HB4	2.64	1.71	3.27	1.52	1.44
G5	HB5	4.48	1.91	1.65	1.77	1.19
	HB6	4.62	1.97	1.70	1.83	1.22

** Value was calculated based on f_c'=70 MPa

The predictions made by Canadian code CSA 2004 [11] –calculated by *Response 2000* program - correlate much better with the experimental results than various results given by the other codes.

For the six normal strength concrete specimens, the average "$V_{exp.}/V_{predicted}$" ratio is 4.25 for ECP 203-2007 [1], 1.85 for ACI 318-02 [5], 2.40 for EN1992 [9] , 1.95 for ASHTO-LRFD [10] and 1.65 for CSA 2004 [11] "*Response 2000* program [13]". For the six high strength concrete specimens, the average "$V_{exp.}/V_{predicted}$" ratio is 3.25 for ECP 203-2007 [1], 1.85 for ACI318-02 [5], 2.65 for EN1992 [9] , 1.80 for ASHTO-LRFD [10] and 1.45 for CSA 2004 [11] "*Response 2000* program [13]".

One can see that ECP 203-2007 [1] had the highest average value of "$V_{exp.}/V_{predicted}$" ratio in both normal and high strength concrete specimens while CSA 2004 [11] had the lowest average value of "$V_{exp.}/V_{predicted}$" ratio in both normal and high strength concrete specimens. This conclusion confirms the fact that the contribution of web reinforcement in enhancing shear capacity of shallow wide beams cannot be ignored especially for normal strength concrete.

It can easily be noticed that (ECP 203-2007) [1], ASHTO-LRFD [10] and CSA 2004 [11] achieved a higher average value of "$V_{exp.}/V_{predicted}$" ratio in normal strength concrete specimens compared to the average value in high strength concrete specimens. On the contrary, EN1992 [9] achieved a higher average value of "$V_{exp.}/V_{predicted}$" ratio in high strength concrete specimens compared to the value in normal strength concrete specimens, while ACI318-02 [5] had the same average value of "$V_{exp.}/V_{predicted}$" ratio in both normal and high strength concrete specimens.

Fig. 14 shows the comparison between "$V_{exp.}/V_{predicted}$" ratios of groups 1 (NB1), 2 and 3 specimens. One can see that the predictions made by (ECP 203-2007) [1] correlate much better with members without stirrups than with members with stirrups. That is due to the fact that (ECP 203-2007) [1] totally discard the shear reinforcement contribution in shallow wide beam shear resistance. On the contrary the predictions made by CSA 2004 [11] "*Response 2000* program [13] " correlate much better with members with stirrups amount more than the minimum, than with members without stirrups due to the fact that CSA 2004 [11] totally acknowledge such contribution. EN1992 [9] achieved the highest value of "$V_{exp.}/V_{predicted}$" ratio in group 2 (specimens with minimum stirrups) compared to the values in group 1(NB1) and 3 which can be attributed to the fact that EN1992 [9] discard the concrete contribution in beams shear resistance. Fig. 15 shows that similar conclusion can be drawn for High Strength Concrete specimens.

CONCLUSION

Based on the experimental results and the observed behavior, the following conclusions may be made:

1. Contribution of web reinforcement- in form of vertical stirrups- in shear strength of shallow wide beams cannot be ignored.
2. H.S.C shallow wide beams without web reinforcement presented a more ductile behavior compared to N.S.C beams. On the other hand, H.S.C beams with stirrups, twice as much as the minimum web reinforcement, exhibited a less ductile behavior.
3. For shallow wide beams without web reinforcement, the shear strength generally increases as the concrete compressive strength

increased.

4. The effect of web reinforcement on improving shear strength is more pronounced at lower compressive strength of concrete and low web reinforcement ratio.
5. The influence of stirrups amount on shear strength does not vary according to concrete compressive strength.
6. The spacing between vertical stirrups and branches number of stirrups in cross section have a less effect in improving shear capacity as concrete strength increases.
7. The shear reinforcement significantly enhances the ductility of the shallow wide beams with normal strength concrete. This effect is less pronounced with high strength concrete.
8. The predictions made by Canadian code CSA based on MCFT – calculated by *Response 2000* program - correlate much better with the experimental results than the various results given by the other codes while still provide an average factor of safety around 1.5 for H.S.C. and 1.68 for N.S.C.

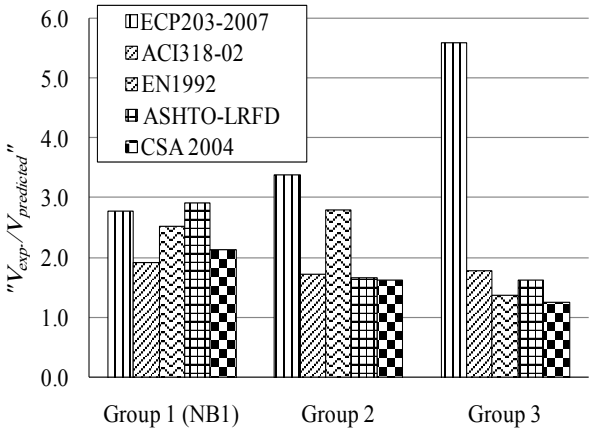


Fig. 14 "$V_{exp.}/V_{predicted}$" Ratio for Normal Strength Concrete

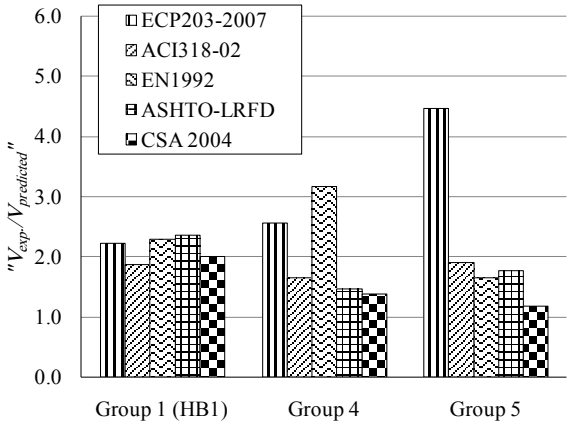


Fig. 15 "$V_{exp.}/V_{predicted}$" Ratio for Normal Strength Concrete

Corresponding author
Hatem M. Mohamed
Structural Engineering Department, Faculty of Engineering Cairo University, Egypt
Hatem_amn@yahoo.com , Hatem_amn@hotmail.com

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6/6/2012

PLEURAL CYFRA 21-1 AND CA 15-3 IN DIFFERENTIATION OF MALIGNANT FROM BENIGN PLEURAL EFFUSIONS

Dalia H. Farag[1], Eman El.Hadidi[1], Mohamed O. El Maraghy[1] and Maha M. Hussein[2]

Departments of Clinical Pathology[1] and Internal Medicine[2], Faculty of Medicine, Ain Shams University
maraghy@yahoo.com

Abstract:Objective: The aim of this study was to evaluate the individual and combined diagnostic values of CYFRA 21-1 and CA15-3 in pleural fluid for differentiation between malignant and benign pleural effusions. **Subjects and Methods:** Twenty patients with malignant pleural effusion (17 with primary lung cancer and 3 with breast cancer) were included, in addition to 20 diseased controls with benign pleural effusion (10 with congestive heart failure, 7 with parapneumonic effusion and 3 with tuberculosis). Following radiological investigations, thoracocentesis and pleural fluid examination, pleural CA 15-3 was assessed by chemiluminescence immune assay and pleural CYFRA 21-1 by enzyme-linked immunosorbent assay. **Results:** Results of the present study revealed a high sensitivity 95% and specificity 90% of CYFRA 21-1 for diagnosis of malignant pleural effusion. Combining CYFRA 21-1 and CA 15-3 did not improve diagnostic performance than that of CYFRA 21-1 used individually. **Conclusion:** CYFRA 21-1 is a non-invasive reliable marker for differentiating pleural effusions of malignant from benign causes. Its high diagnostic performance will help detections of cases possibly missed by routine cytology. This high performance did not benefit from the adjuvant use of CA 15-3.

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Keywords: CYFRA 21-1, CA 15-3, pleural effusion.

1. Introduction

Pleural effusion is a vexing problem in clinical practice, especially in terms of differentiation between malignant (MPE) from benign pleural effusion (BPE), due to the significant difference in the treatment and prognosis involved. Most common causes of transudative effusions include congestive heart failure and hypoalbuminemic states, while those of exudative effusions involve malignancy, infection, and tuberculosis. Malignant pleural effusion accounts for 42 to 77% of cases[(1)].The majority of neoplasms can cause pleural effusion during their progression. Lung cancer accounts for up to 30% of all cases of malig-nant pleural effusion followed by breast cancer and lymphomas[(2)].Although most malignant effusions occur among patients with known cancers, effusion can be the first indication for the presence of malignancy in third of patients. This explains the importance of diagnosis of MPE[(3)]. Pleural fluid cytology findings are positive only in 60% of cases, while thoracoscopy will establish the diagnosis in approximately 95% of cases. The latter, however, is an expensive, invasive and potentially traumatizing interventional procedure, hence the necessity for less invasive discriminatory markers with comparable diagnostic performance[(4)].

CYFRA 21-1 is a cytokeratin-19 fragment, an acid-type cytoplasmic protein, with a molecular weight of 40 kD, expressed in epithelial cells. Following cell death, it is released in serum in the form of soluble fragments. CYFRA 21-1 is a potential diagnostic marker for MPE as it is found not only in serum but also in the pleural fluid[(5)]. In the course of searching for diagnostic tools for MPE, several combinations of markers have been studied including neuron specific enolase, CYFRA21-1, CA15-3,CA19-9 and CA125[(6)]. Since CYFRA21-1 and CA 15-3 have a high diagnostic performances for lung and breast cancers, respectively,their combined assay in pleural fluid provides a promising combination for diagnosis of MPE[(7)].

2. Subjects and Methods:

I- Subjects:

This study was conducted on 20 patients with MPE (group A) and 20 patients with BPE (group B) as disease controls. They were selected from the Oncology and Chest Departments, Ain Shams University Hospitals. Group A MPE were secondary to primary lung cancer (n = 17) and breast cancer (n = 3).These included 13 males and 7 females. Their ages ranged from 35 to 69 years, with median and interquartile range (IQR)of 55.5 (48-59). The effusions were considered to be malignant when malignant cells were encountered on cytological examination of the pleural fluid or in pleural biopsy. Group B included 15 males and 5 females, aged 25 to 70 years, with a median and IQR of 51.5 (41- 62.25) years. Causes of BPE included congestive heart failure (n = 10), parapneumonic pleural effusion (n= 7) and

tuberculosis (n= 3). BPE was chiefly diagnosed by clinical manifestations and laboratory examinations. Tuberculous effusion was diagnosed if one of the following criteria were met: (i) radiological and clinical evidence of tuberculous effusion with positive acid-fast bacilli (AFB) in sputum; (ii) identification of AFB in pleural fluid or biopsy specimen cultures and (iii) presence of caseous granulomas in pleural biopsy tissue. Parapneumonic pleural effusion was diagnosed by the presence of acute fever with purulent sputum, pulmonary infiltrate, leucocytosis, neutrophilia and identification of microorganisms in the pleural fluid. Congestive heart failure was determined by cardio-megaly and pulmonary venous congestion on the radiograph, peripheral edema, hepatomegaly, bilateral pleural transudate and findings on echocardiograph. Informed consent was taken from all participants in this study.

All studied individuals were submitted to thorough history taking, proper clinical examination, chest X-ray and thoracocentesis, in addition to physical, chemical, bacteriological and cytological examination of pleural fluid.Pleural CA 15-3 was assessed by chemiluminescence immune assay and pleural CYFRA 21-1 by enzyme-linked immuno-sorbent assay (ELISA).

II- Samples:

Pleural fluid specimens were obtained by thoracocentesis with aseptic technique. Supernatant of the pleural fluid obtained by centrifugation at 3000g for 10 min was aliquoted and stored frozen at -20°C prior to assay.

III- Methods:

A- Assay of CA 15-3 by Chemiluminescence Immune Assay:

This was done on fully automated Immulite 2000 (Siemens Healthcare Diagnostics, USA) using instrument manufacturer's reagent. This is a two-step sequential chemiluminescent immunoassay using two monoclonal mouse antibodies, 115D8 and DF3 specific for CA 15-3, for capture and detection, respectively, and chemiluminescence as the detection signal.

B-Assay of CYFRA 21-1 by ELISA:

The assay was performed using the DRG CYFRA 21-1 ELISA Kit supplied by DRG International (DRG International, New York, USA). It consists of a solid phase ELISA based on the sandwich principle. The microtiter wells are coated with a monoclonal antibody directed towards a unique antigenic site on a CYFRA 21-1 molecule. An aliquot of patient sample containing endogenous CYFRA 21-1 is incubated in the coated well with enzyme conjugate, which is an anti-CYFRA 21-1 monoclonal antibody conjugated with horseradish peroxidase. Following incubation, the unbound conjugate is washed off. The amount of bound peroxidase is proportional to the concentration of CYFRA 21-1 in the sample. Having added the substrate solution, the intensity of color developed is proportional to the concentration of CYFRA 21-1 in the patient sample, and is deduced from a calibration curve drawn from standard results obtained in the same run.

IV- Statistical Analysis:

Statistical analysis was done using SPSS software package (version 15.0, 2006, Echosoft Corporation, USA). Data were expressed descriptively as percentages for qualitative data and median and IQR for quantitative non- parametric data. Comparison between groups was done using Mann Whitney U test for quantitative non-parametric data. $p< 0.05$ was considered significant and $p< 0.01$ was considered highly significant. Ranked Spearman correlation coefficient was used in correlatingnon-parametric variables. The diagnostic performance of CA 15-3 and CYFRA 21-1 was evaluated in terms of diagnostic sensitivity, specificity, positive (PPV) and negative predictive values(NPV), and efficacy. The best possible cutoff was selected from the receiver operating characteristics (ROC) curve.

3. Results:

The descriptive data of the studied MPE patients (group A) and BPE patients (group B) are shown in Table (1) and Figures (1 and 2). Comparison of groups A and B(Table 2 and Figure 3) revealed a significantly higher CA15-3 ($z = -3.088$, $p< 0.01$) and CYFRA 21-1 ($z = -5.309$, $p< 0.001$)levels in group A than B. Correlation between CA 15-3 and CYFRA 21-1 (Figure 4)among group A subjects revealed a positive significant correlation ($r= 0.664$, $p<0.001$).

Study of the diagnostic performance of CA 15-3 and CYFRA 21-1 for differentiating MPE from BPE (Table 3 and Figure5), revealed that a best cutoff for CA15-3 level of 35 U/mL yielded a sensitivity, specificity, PPV, NPV and efficiency of 80%, 65%, 69.6%, 76.5% and 72.5% and a best cutoff for CYFRA 21-1 level of 45 ng /mL yielded 95 %,90 %, 90.5%, 94.7 %, and 92.5%.Combination of CYFRA 21-1 and CA 15-3 positivity (cases considered positive if either CYFRA 21-1 or CA 15-3 were positive) did not result in an additional benefit over the diagnostic performance of CYFRA 21-1 used individually.

4. Discussion:

Pleural effusions are common complications of a wide varietyof diseases.Thoracoscopy is the MPE gold diagnostic standard with a diagnostic sensitivity of 93–97%.A wide range of markers have been proposed for the detection of MPE, with no existing one yet having sufficient diagnostic accuracy in discriminating

MPE from BPE. One of the promising tumor markers is CYFRA 21-1[4].

In our study, the median value of CA 15-3 in pleural fluid was significantly higher among patients with MPE than patients with BPE which agrees with **David *et al.* (2005)**[8] and **Li *et al.* (2007)**[6]. These results could be explained by CA 15-3 over-expression on the cell surfaces of malignant glandular cells, and increasing amounts being shed into pleural fluid[9].As regards CYFRA21-1,our study revealed that its median value in pleural fluid was significantly higher among patients with MPE those with BPE. Many studies, as **David *et al.* (2005)**[8], **Li *et al.* (2007)**[6]**,Liang *et al.*(2008)**[7]and **Huang *et al.*(2010)**[10]**,**supported our results. This finding could be attributed to increased cytokeratin solubility which results frommodification at the amino and carboxyl terminals of keratin (phosphorylation, glycosylation andtransglutamination) occurring during transformation of normalcells into malignant cells. Furthermore, higher CYFRA 21-1 levels in MPE may be due to proteolytic degradation ofkeratin during cell lysis, abnormal mitosis and tumor necrosis. Thus, CYFRA 21-1 spills over from cells undergoing proliferation and apoptosis[10].Moreover, both markers (CA 15-3 and CYFRA 21-1) were highly significantly correlated in MPEgroup ($r = 0.664$, $p < 0.001$). This may be explained by common mechanism of release by spilling over from proliferating cells. This correlation was also proved in the study of **Li *et al.* (2007)**[6].

CA 15-3 showed a diagnostic accuracy of 72.5% for differentiating MPE from BPE with sensitivity and specificity of 80% and 65%, respectively. The resultsof **Li and his colleagues (2007)**[6](n= 62) showed a sensitivity of 62.5% and specificity of 73.3 %and results of **Antonangelo*et al.* (2009)**[11](n=78) revealed a sensitivity of 64.4% and specificity of 89.5% of CA 15-3 in diagnosing MPE. The lower specificity reported in our study was due to the overlap in CA 15-3 results between MPE and BPE groups, possibly underlined by our fewer number of study subjects.As regards CYFRA 21-1, asensitivity of 95%, specificity of 90%, and accuracy of 92.5% were demonstrated in our study for diagnosis of MPE. This is in accordance with most published values as those in the studies of **Alatas*et al.*(2001)**[12](n= 74), **Li *et al.*(2007)**[6](n = 62) and **Huang *et al.*(2010)**[10](n =134) which revealed a sensitivity and specificity of 91% and 90%,84.4 and 90.0% and 80.5% and 92.5%, respectively, for diagnosis of MPE. However, other studies reported lower sensitivities of CYFRA 21-1 for diagnosing MPE. **David et al (2005)**[8] (n=116) reported a sensitivity of 59.1% and specificity of 80.5%. At a cutoff of 70 ng/mL, **Antonangelo*et al.* (2010)**[9]**(**n=175)reported a sensitivity of 46% and specificity of 94% for differentiating MPE from BPE. This lower sensitivity can be explained either by their high chosen cutoff level which achieved higher specificity at the expense of sensitivity or by the variation in the types of primary tumors causing MPE included in their studies. The etiologies of MPE in their studies were lung, breast, colorectal, ovarian, renal, lymphoproliferative and prostatic cancers.

Comparing the diagnostic performance of the two markers studied individually at their best cutoff level, CYFRA 21-1 gave a much better diagnostic accuracy than CA 15-3 (92.5% versus 72.5%). These findings are in accordance with **Li and his coworkers(2007)**[6] who conducted their research on 32 patients with MPE from advanced lung cancer and 30 patients with BPE with five tumor markersmeasured in the pleural fluidincluding CYFRA 21-1, CA15-3, CA19-9, neuron-specific enolase and CA125. They concluded that CYFRA 21-1 was the tumor marker with the highest sensitivity (84.4%), specificity (90.9%), and accuracy (87.1%). The combined use of both of CYFRA 21-1 and CA 15-3 in their study did not add to the performance of CYFRA 21-1 used alone. However, **Alatas and his colleagues (2001)**[12]reported that when CA 15-3 and CYFRA 21-1 were combined, the sensitivity increased to 100% and specificity decreased to 83%. Comparable results could be achieved in our study by using CYFRA 21-1 alone at a lower cutoff value of 35 ng/mL instead of our chosen 45 ng/mL, where the sensitivity, specificity and accuracy would have been 100%, 85% and 92.5%.

In conclusion, our data suggest that CYFRA 21-1 is a non-invasive reliable marker for differentiating MPE from BPE. The use of CYFRA21-1 as a single marker had the same performance as its combination with CA15-3. Addition of CYFRA 21-1 to the current standard tests for diagnosis of MPE could be helpful for identification of MPE patients that might have been missed by routine cytology. Based on very high detection rates of CYFRA 21-1, negative CYFRA21-1 patients mightbe alleviated from proceeding to unnecessary thoracoscopy. The benefit of combining tumor markers for increasing diagnostic performance needs further large-scale studies in clinical practice with trial of different markers. Further studies are needed to determine the value of tumor markers in pleural fluid for judging prognosis and efficacy of therapy.

Table (1): Descriptive Statistics of the Demographic and Pleural Malignant (group A) and Benign Examination Data of the Studied (group B) Pleural Effusion Patients

	Group A (n = 20)	**Group B (n = 20)**
Sex:		
Males	13 (65%)	15 (75%)
Females	7 (35%)	5 (25%)
Age (years)*	55.5 (48-59)	51.5 (41-62)
Causes of effusion	Lung cancer 17 (85%) Breast cancer 3 (15%)	CHF 10 (50%) Parapneumonic 7 (35%) Tuberculosis 3 (15%)
Pleural fluid appearance:		
Bloody	10 (50%)	0 (0%)
Clear straw colored	0 (0%)	15 (75%)
Turbid	10 (50%)	5 (25%)
Gram stain positive	0 (0%)	4 (20%)
ZN stain positive	0 (0%)	1 (5%)
Positive Culture	0 (0%)	6 (30%)
Protein (fluid/serum ratio)*	0.74 (0.65-0.83)	0.35 (0.27-0.47)
LDH (fluid/serum ratio)*	1.25 (0-1.5)	0.45 (0.3-0.88)
Glucose (mg/dL)*	37.5 (33.5-42.3)	102.5 (42.3-167)
TLC / mL*	3000 (2000-4650)	490 (350-570)
Predominant cells:		
Mononuclear cells	20 (100%)	16 (80%)
Polymorphs	0 (0%)	4 (20%)
CA 15-3 (U/mL)*	75.5 (35-100)	30 (25-51.3)
CYFRA 21-1 (ng/mL)*	185 (122.5-237.5)	17.5 (10-28.8)

* Median (interquartile range)
CHF: congestive heart failure; ZN: ZeihlNeelsen stain.

Table (2): Statistical Comparison of CA 15-3 and CYFRA 21-1 Levels between MPE patients (group A) and BPE patients (group B) using Mann Whitney U test

	Group A	Group B	Z	P
CA 15-3 (U/mL)	75.5 (35-100)	30 (25-51.3)	-3.088	< 0.01
CYFRA 21-1 (ng/mL)	185 (122.5-237.5)	17.5 (10-28.8)	-5.309	< 0.001

p< 0.01and p< 0.001: Highly significant.

Table (3): Diagnostic Performance of CA 15-3 and CYFRA 21-1 in discrimination of malignant from benign pleural effusions

Best cutoff	Sensitivity	Specificity	PPV	NPV	Efficiency
CA 15-3 (35 U/mL)	80%	65%	69.6%	76.5%	72.5%
CYFRA 21-1 (45 ng/mL)	95%	90%	90.5%	94.7%	92.5%
CYFRA 21-1 and CA 15-3	95%	90%	90.5%	94.7%	92.5%

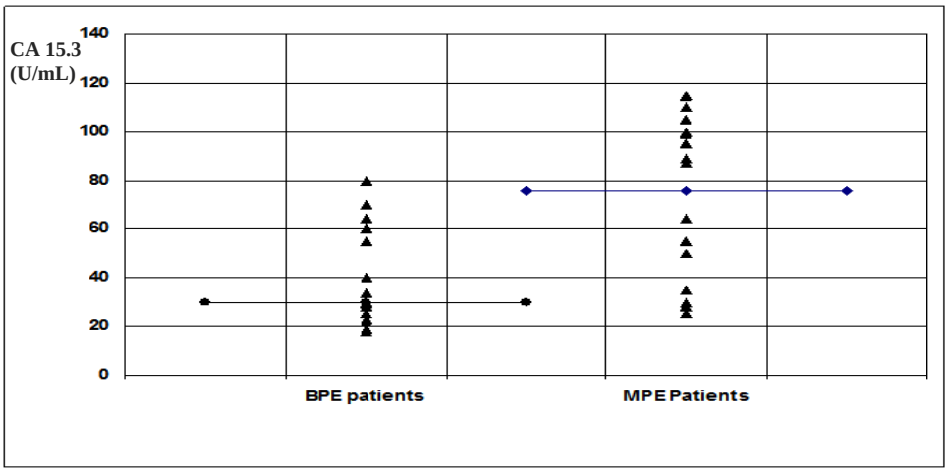

Figure (1): Scatterdiagram showing the distribution of results of CA15-3 among patients with benign (BPE) and malignant (MPE) effusions.

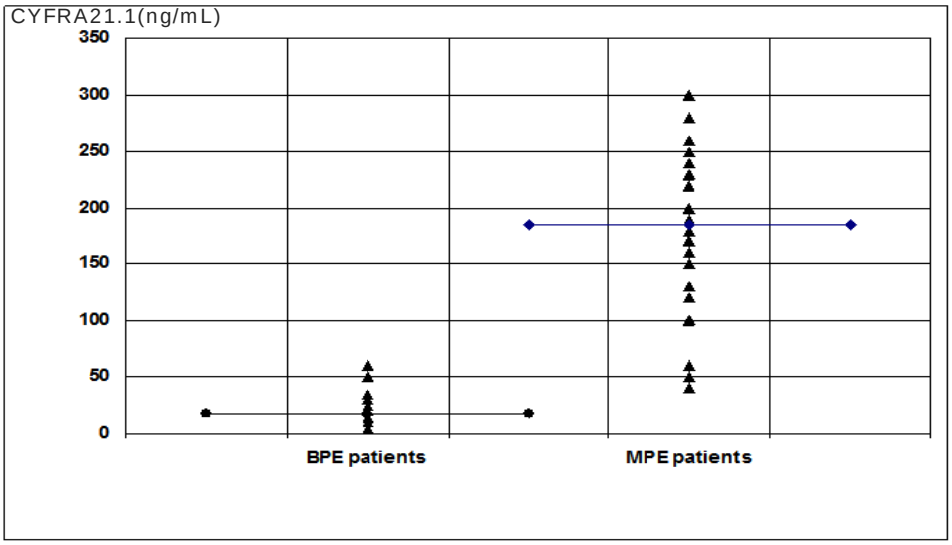

Figure (2): Scatterdiagram showing the distribution of results of CYFRA21-1 among patients with benign (BPE) and malignant (MPE) effusions.

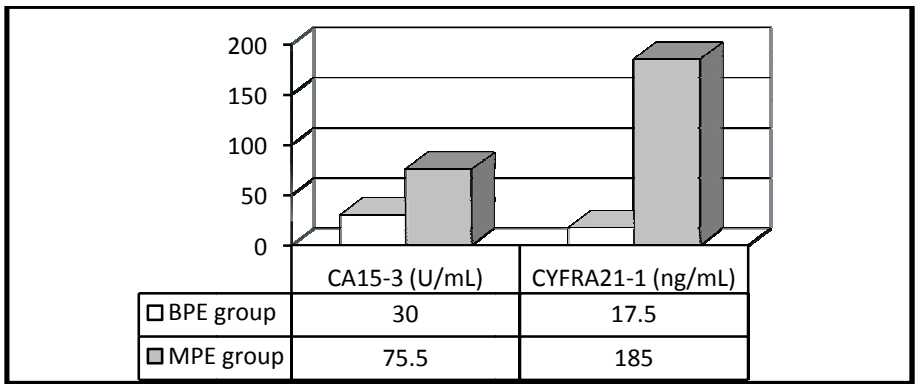

Figure (3): Comparison between benign (BPE) and malignant (MPE) effusion groups as regards median values of CA15-3 and CYFRA21-1.

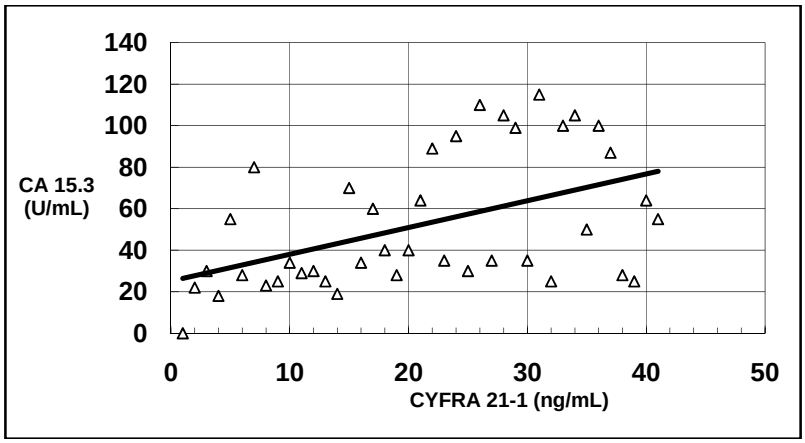

Figure (4): Correlation between CA 15-3 and CYFRA 21-1in malignant pleural effusion group (r= 0.664, *p*<0.001).

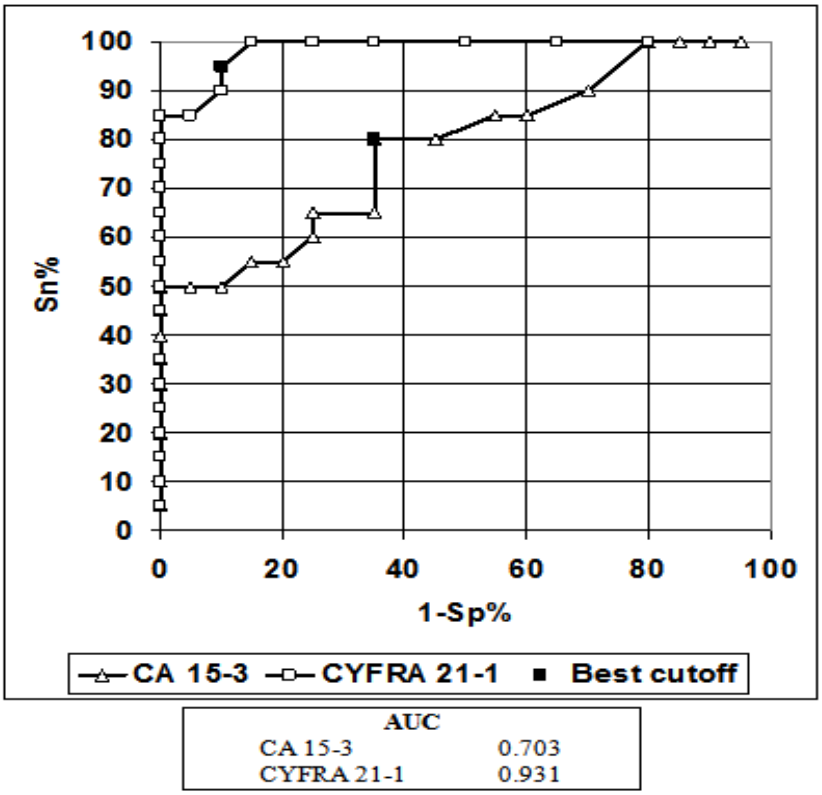

Figure (5):ROC curve analysis showing the diagnostic performance of CA 15-3 and CYFRA 21-1 for discriminating MPE from BPE.

Corresponding author
Mohamed O. El Maraghy
Department of Clinical Pathology, Faculty of Medicine, Ain Shams University
maraghy@yahoo.com

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Studies on Prevailing Cestodiasis in Wild African Catfish *Clarias Gariepinus* at Kafr El-Sheikh Governorate

Eissa, I. A. M.[1]; Viola, H. Zaki[2] , Nadia, G. M. Ali [3] and Mona S. Zaki[4]

[1]Dept. of Fish Diseases and Management, Fac. of Vet. Med. Suez Canal Univ.
[2]Dept. of Fish Diseases and Management, Fac. of Vet. Med., Mansoura Univ.
3Veterinary Division, Kafr-Elsheikh Governorate
4 Dept. of Hydrobiology, National Research Center.
Eissavet29@yahoo.com

Abstract: A total number of 200 fish (50 fish in each season) were collected randomly and examined for presence of cestodes. Two species of cestodes were recovered as *Polyonchobothrium clarias* and Monobothria sp. with infestation rate of 50.5 % (101out of 200) and 14.5 % (29 out of 200) respectively. Seasonally, *P clarias* was prevalent in spring and summer while Monobothria sp. was prevalent in spring, autumn and winter with no record in summer. There was significant decrease in the total serum proteins, albumin and globulin of infested fish comparatively with non-infested fish. The histopathological alterations were manifested as destruction, desquamation and sloughing of affected tissue mucosa with presence of degenerative changes.
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Keywords: Cestodes, Clarias gariepinus, Polyonchobothrium clarias, Monobothria sp., histopathology, serum protein, prevalence.

1. Introduction

Fish is important as a source of protein with low cholesterol level in the diets of the human and economically as a source of subsistence income **(Aken'ova, 2000).** Fish not only provide food for immediate consumption but people rely directly or indirectly on fishing for their economic survival and a source of job. In Egypt, parasitic diseases represent about 80 % of fish diseases **(Eissa, 2006).** Parasitic infections in fish cause decreased production and economic losses through direct fish mortality, reduction in fish growth, fecundity and stamina, increase susceptibility of fish to other diseases and high cost of treatment **(Cowx, 1992).** Under natural conditions 50 – 90 % of freshwater fishes harbor at least one species of parasites **(Sineszko, 1979).**

The present study was designed to investigate the prevalent diseases caused by cestodes in wild African catfish *Clarias gariepinus* at Kafr El-Sheikh governorate. Besides, determination of total and seasonal prevalence, histopathological alterations and serum proteins were discussed.

2. Materials and Methods

Fish:

A total number of 200 *Clarias gariepinus* ranged between 45 to 315 g in body weight and from 18 to 39 cm in total length were collected randomly alive from river Nile at Kafr El – Sheikh Governorate during 2011 as 50 fish seasonally. Fish were kept in glass aquaria and supplied with chlorine free tap water with continuous aeration and filtration according to Innes (1966).

Clinical picture:

Alive fish were examined for clinical signs and postmortem lesions as described by Austin and Austin (1987).

Blood sampling:

Blood samples were collected from the caudal blood vessels and serum was obtained by centrifugation of collected blood at 5000 rpm according to Rowley (1990).

Parasitological examination:

The gastrointestinal tract was separated from the other internal organs then the stomach was separated from the intestine and each part examined. In clean Petri dish, stomach was opened and intestinal mucosa was stripped off by scalpel and washed with normal saline in another clean dry Petri dish. Gall bladder was separated, opened and examined. Cestodes were collected and preserved in alcohol formalin acetic acid and stained with Semichon's acetocarmine stain then the whole mount of collected cestodes was done according to Woodland, (2006). The collected cestodes were identified according to the identification key of Yamaguti (1958, 1959 and 1961).

Serum analysis:

In 20 fish (10 infested and 10 non-infested), serum total proteins were determined according to the method described by Peters *et al*. (1982), serum albumin was determined according to Peters (1970) and serum globulin was calculated by subtraction of albumin value from total protein value as described by Doumas and Biggs (1972). The data of serum protein analysis were statistically analyzed for variance (ANOVA) and least significant difference as described by Snedecor and Cochran (1989) using and (Med Calc. version 11, 2010) computer statistical software. Data were evaluated as significant at $P \leq 0.05$.

Histopathological examination:

The histopathological examinations of affected tissue (intestines, stomach and gallbladder) were performed as described by Drury and Wallington (1980).

3. Results and Discussion

The clinical signs appeared on the infested fish were weakness, severe emaciation, anemia, imbalanced swimming, some infested fish showed sluggish movement, loss of condition with paler coloration (Plate, 1) which was in agreement with that described by **Islam and Woo (1991), Hassen (2002), Eissa (2002)**, **Nadia Ali (2007)** and **Sabri *et al* (2010).**

Monobothrium sp. was isolated from the intestine that appeared hemorrhagic and congested leading to intestinal obstruction (plate 1). Monobothria was white in color, elongated. It has large rounded or triangular scolex Plate 2 (d). The body length ranged between 7 – 40 mm and body width ranged between 2.5 – 4.5 mm. The testis located laterally, appeared as oval follicles, the ovary laterally located in the posterior part of the worm and occupying the two lateral sides (Plate 2). The male genital pore opens slightly anterior to the female one. The female genital pore occurred in the middle of the worm. The egg was rounded in shape; these descriptions were similar to that described by **Nadia Mahfouz (1991), Mwita and Nkwengulila (2004), Oniye *et al.* (2004)** and **Oofintoye (2006)** who isolated Monobothrium sp. from the intestine of *Clarias gariepinus*.

Polyonchobothrium clarias was isolated from the gall bladder that appeared enlarged with thickened bile duct and containing pale colored watery bile. It was long ranged from 60 - 100 mm in length, 0.5 – 1 mm in width. The scolex was elongated, triangular in shape and carries one raw of hooks and bears laterally two shallow bothria Plate 1 (h), segmentation begin directly after the scolex with immature stages then mature stages. The ovary is rounded to oval in shape and centrally located in the segment Plate 2 (a).The eggs are spherical and containing a mass of rounded cells. The worm attached mainly inside the gall bladder near the neck of bile duct. These findings agree with that recorded by **Wabuke – Bunoti (1980)** who isolated *Polyonchobothrium clarias* from the gall bladder of naturally infested *Clarias mossambicus*.

P clarias was isolated also from the glandular stomach which appeared congested. The parasites were attached mainly at the junction between muscular and glandular stomach. Also, it was attached near the opening of bile duct in the glandular stomach that was in agreement with the results described by **Shotter and Medaiyedu (1977)** as they found *P clarias* concentrated in the spiral valve in the area close to the entry of the bile duct. Moreover, **Nadia Mahfouz (1991)** and **Moyo *et al.* (2009)** isolated *P clarias* from the stomach of *Clarias gariepinus*.

The seasonal prevalence of cestode infestation was peaked during spring (96 %) followed by summer (80) then winter (46) and reach the lowest in autumn (38 %), also, **Noor Eldin (1981)** and **Negm Eldin (1987)** also recorded the highest prevalence with cestode infestation was during spring and summer.

Concerning, *P clarias* was isolated from the gall bladder only during spring and season while it was isolated from the glandular stomach allover the year with a total prevalence of 50.5 % that was near that described by **Imam (1971)** who recorded, that the infestation rate with *P clarias* was 41% in *Clarias lazera* collected from the Nile while, **Sahlab (1982)** recorded that the infestation rate with *P clarias* was 22.22% in ***Clarias*** *lazera* from Manzala. This variation may be attributed to the difference in locality, time of collection, water temperature and size of fish.

The seasonal prevalence of *P clarias* was peaked during spring (82 %) followed by summer (80 %) then winter (32 %) and reached the lowest infestation in autumn (8 %) (Table 1 & 2). These results were nearly similar with that recorded by **Abd Elaal (1996)** who recorded the highest prevalence in spring and low prevalence in autumn. Also, was nearly similar to **Aml Atwa (2006)** who recorded the highest prevalence in spring and the lowest prevalence occur in winter and **Sahlab (1982)** who recorded increase prevalence of cestodes in spring and summer. On the other side, **Nadia Mahfouz (1991)** recorded the highest seasonal prevalence in winter and the lowest prevalence occurred in autumn in cultured *C gariepinus*. This may be explained to that our fish are wild.

Monobothria sp was isolated from the intestine of *C gariepinus* with a total prevalence of (14.5 %) which was higher than that described by other researchers as **Negm Eldin (1987)** , **Khattab (1990)** and **Nadia Mahfouz (1991)** as they isolated it with an infestation rate as 6.33 , 4.82 and 1.5 % respectively. This may be attributed to the difference in locality and breeding. The highest seasonal prevalence of monobothria sp was recorded in autumn (30 %), spring, winter (14 %) and lowest prevalence occurred in summer (0 %) Table (2).

Table (3) shows that the serum total proteins, albumin and globulins were significantly decreased in heavily infested fish in comparison with non-infested fish which was similar to that described by **Steinhagen *et al.* (1997)** and **Hamouda (2011).** This decrease may be as a result of consumption of nutrient material by the parasite, also can be resulted from destruction occurred in intestinal mucosa that allow leakage of plasma protein and destruction of intestinal villi which are responsible for absorption of nutrients and protein from food materials. These findings may act as immunodepressants and open the gate to secondary infection.

Concerning histopathological examination of the intestine of monobothria sp. infested *Clarias gariepinus* revealed presence of atrophy of the intestinal villi that became shorten and compressed under the pressure caused by the parasite that completely occupying the intestinal lumen. The glandular stomach infested with *Polyonchobothrium clarias* showing presence of hyperplasia and sloughing of gastric mucosa with presence of sub mucosal inflammation and mononuclear cell infiltration. There was observed desquamation of lining gastric mucosa with presence of transverse section in gastric lumen near the site of attachment of the parasite while, multiple longitudinal and cross sections of the parasite was observed in the gall bladder that showed degeneration and sloughing of the lining mucosa, as well as mucinous degeneration demonstrating goblet cell hyperplasia with lymphocytic infiltration. The mucosa of gall bladder showed multifocal thickening of the lining epithelium giving a feature of squamous like epithelium as a result of parasite attachment. These descriptions were nearly similar to the description given by **Nadia Mahfouz (1991)** and **Eissa *et al.* (2010).**

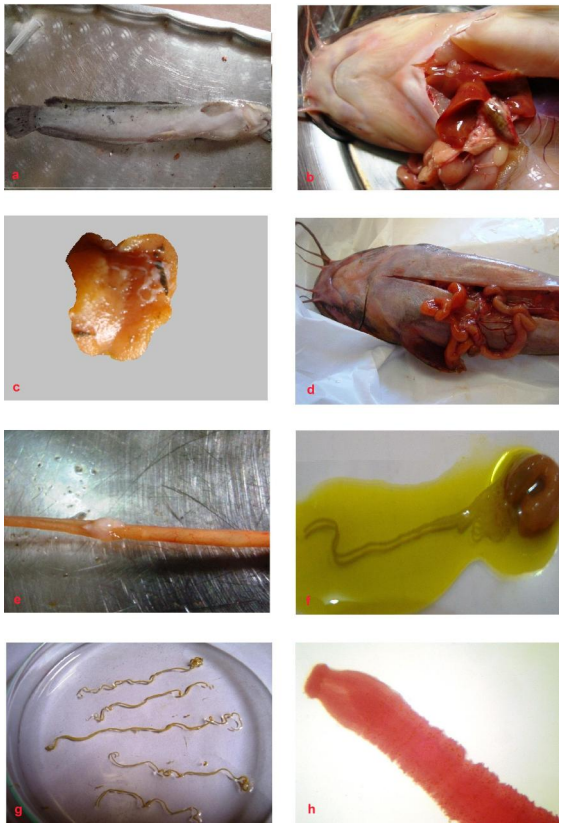

Plate 1

a Heavily infested fish showing fading coloration.
b Enlarged distended gall bladder containing *Polyonchobothrium clarias.*
c Glandular stomach containing *Polyonchobothrium clarias.*
d Congested intestine of infested fish with Monobothria sp.
e Intestine of heavily infested fish occluded with great number of Monobothria sp.
f Gall bladder containing great number of *Polyonchobothrium clarias.*
g five *Polyonchobothrium clarias* collected from one gall bladder of heavily infested fish
h Scolex of *Polyonchobothrium clarias* isolated from gall bladder and stained with carmine stain.

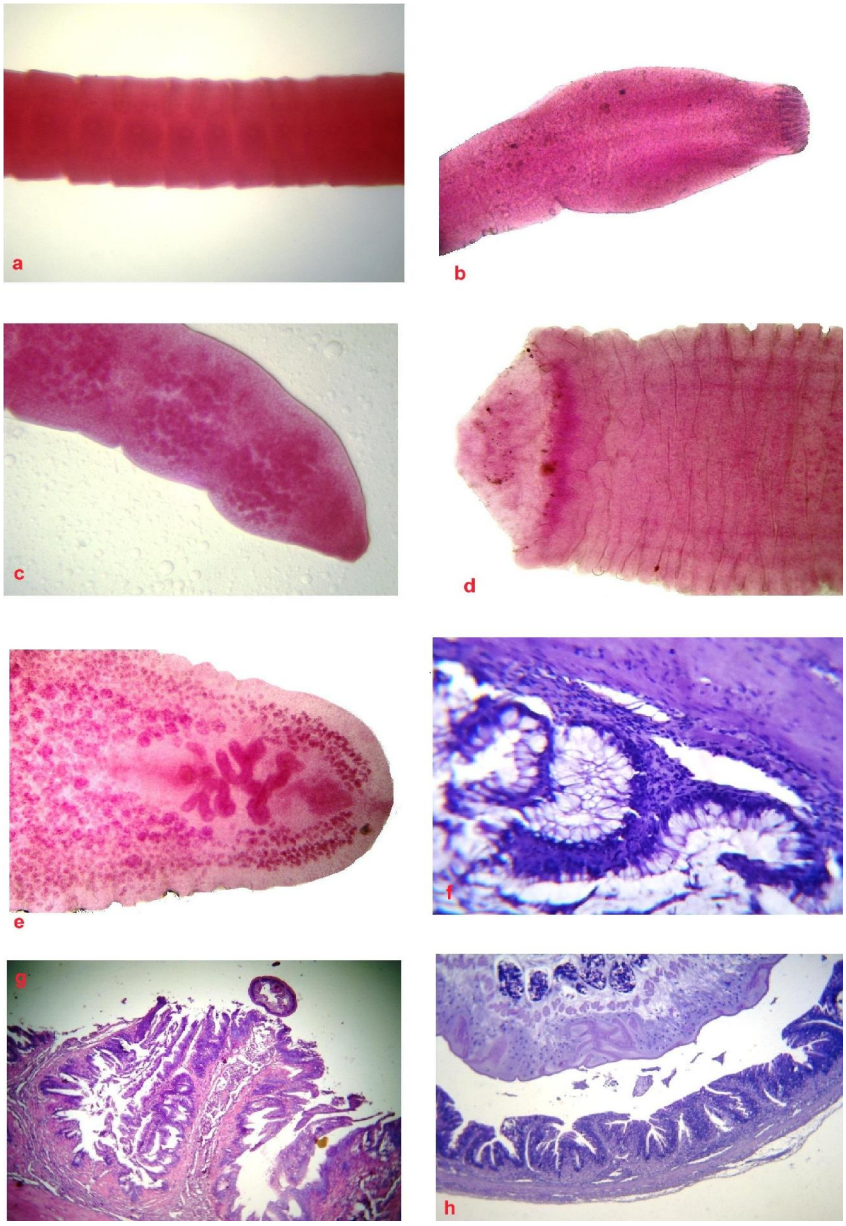

Plate 2

a **Immature segments of *Polyonchobothrium clarias* isolated from gall bladder.**

b **Scolex of *Polyonchobothrium clarias* isolated from glandular stomach.**

c **Posterior part of *Polyonchobothrium clarias* isolated from glandular stomach.**

d **Anterior end of monobothria sp. isolated from intestine of infested fish.**

e **Posterior end of monobothria sp. isolated from intestine of infested fish.**

f **Mucinous degeneration demonstrating goblet cell hyperplasia with lymphocytic infiltration observed in the gall bladder of infested fish.**

g **Hyperplasia and sloughing of gastric mucosa of glandular stomach with presence of sub mucosal inflammation and mononuclear cell infiltration.**

h **Atrophy of the intestinal villi that became shorten and compressed under the pressure caused monobothria parasite.**

Table (1): Showing the seasonal prevalence of cestode infestation in *C gariepinus*.

Season	No. of examined fish	No. of infested fish	Infestation %
Spring	50	48	96
Summer	50	40	80
Autumn	50	19	38
Winter	50	23	46
Total	200	130	65

Table (2): Showing the seasonal prevalence of *Polyonchobothrium clarias* and Monobothria sp.

Cestode type		*Polyonchobothrium clarias*		Monobothria sp.	
Season	No. of examined fish	No. of infested fish	%	No. of infested fish	%
Spring	50	41	82	7	14
Summer	50	40	80	0	0
Autumn	50	4	8	15	30
Winter	50	16	32	7	14
Total No.	200	101	50.5	29	14.5

Table (3): Showing serum protein analysis of non-infested and infested *Clarias gariepinus* (No. 10 fish).

Parameter (g / dl)	Non-infested	Infested
Total protein	3.82 ± 0.25	3.28 ± 0.13
Albumin	1.72 ± 0.08	1.52 ± 0.13
Globulin	2.1 ± 0.27	1.76 ± 0.18

Corresponding author
Eissa, I. A. M
Dept. of Fish Diseases and Management, Fac. of Vet. Med. Suez Canal Univ.
Eissavet29@yahoo.com

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Studies on Crustacean Diseases of Seabass, *Morone Labrax*, in Suez Canal, Ismailia Governorate

Eissa I A M, Maather El-Lamie & Mona Zakai*

Dept. of Fish Diseases & Management, Fac. of Vet Med., Suez Canal Univ.
* Hydrobiology Dept., National Research Center, Dokki.
Eissavet29@yahoo.com

Abstract: The present study was carried out to detect the parasitic crustaceans affecting marine seabass *Morone labrax* that collected seasonally from different areas of Suez Canal (Ismailia province). There were no pathognomic signs in infested fish. Some revealed signs and P.M. lesions as bulging of opercula, hemorrhages, abrasions and ulcers on skin, sluggish movement and emaciation. The crustacean parasites were identified as copepods of *Lernanthropus psciaenae* and *Caligus carangis.* The total prevalence was (47%) and the summer displayed the highest seasonal prevalence. The relation between fish body weights, lengths and infestation rate were also studied.

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Key words: Seabass, Lernanthropus psciaenae, Caligus carangis, Prevalence

1. Introduction:

Marine fishes are preferable than freshwater fishes as the former are rich in trace elements as phosphorous and iodine, which are essential for cell metabolism. Fishes are generally rich in unsaturated fatty acids which preferred by some diseased people especially with heart and circulatory disorders ***(Hisk, 1987)***.

Parasitic infestations represent the majority of the known infectious diseases affecting fish (***Eissa, 2002; Ragias et al., 2004; Timi and Lanfranchi, 2006; Woo, 2006 and Noga, 2010).*** Most studies of fish parasitic diseases in Egypt have been conducted on freshwater fishes especially in Suez Canal area.

The present study was directed towards further understanding of marine seabass fish in Suez Canal region (Ismailia). The objectives were decided to throw the light on the clinical picture, total & seasonal prevalence of the crustacean parasitic diseases affecting such fish. Besides, the infestation rates in relation to body weights & lengths.

2. Material & Methods

Fish

A total of 100 marine fish *Morone labrax* were collected from Suez Canal area (Ismailia Province). Their body weights and lengths were ranged from less than 50 up to 300 gm and 15 to 35 cm, respectively. The fish were obtained seasonally (each 25 fish) by the aid of fishermen and fishing gears, then transported to the laboratory alive in polyethylene bags containing 1/3 of its volume water where the remaining volume was filled with air.

Aquaria

Fully prepared glass aquaria (100 x 50 X 50 cm) were used for holding fish. They were supplied with sea water from Suez Canal; continuous aeration was maintained in each aquarium using an air pump (Elmassy, Model EM-148). Thermostatic heaters (Type CMI, Germany) were used along the course of the study.

Clinical picture:

First, body weight and body length of the examined fish were recorded and then clinical examination was done on the live fish or freshly dead ones. Fish specimens under investigation were grossly examined for determination of any clinical abnormalities and any external parasite. For demonstration of the internal abnormalities, the postmortem examination was performed on all fish according to ***Amlacker (1970).***

Parasitological examination:

1. Macroscopic examination:

Macroscopic examination was done for detection of any abnormalities in different parts of fish body by nacked eyes and hand lens. Skin, fins, gills, eyes and opercula were dissected and examined for presence of parasitic crustaceans.

2. Microscopic examinations:

Freshly sacrificed fish was scraped with a scalpel blade from just behind the operculum to the tip of the tail fin. Scales and mucus were transferred to slides with a drop of marine water and cover slip to prevent drying and examined microscopically *(Lucky, 1977).*

3- Permanent slides, smear preparations and staining:

The attached crustaceans to the gills, skin and buccal cavity of fish were collected. They could be recovered, detached by a dissecting needle and a fine brush, put in small vial and washed with distilled water, preserved in equal amount of 70% alcohol-5% glycerin in test tube and permanent amounts were prepared by passage in descending grades of alcohol (70, 50 and 30%), cleared in glycerin and mounted in glycerin-gelatin according to *Lucky (1977)* then examined

microscopically. Crustacean parasites were identified according to Badawy (1994).

3. Results

Clinical picture:

The clinical signs in the naturally infested fishes (*Morone labrax*) revealed no pathognomonic clinical abnormalities. Infested fish showed hemorrhagic areas on gill cover, abdomen and on the bases of fins , abrasions and ulcers on the body surface with rubbing the body against objects and sides of aquaria, sluggish movement, abdominal distension and somewhat emaciation. The examination was performed on the freshly dead fish and it revealed a marbling appearance with excessive mucus secretion. Gill tips were sticking with grayish coloration. Black lines and black spots between the gill filaments were seen (Plates, 1-3).

Results of parasitological examination:

A crustacean species was collected from the gills of *M. labrax*. The male body is slender in shape and measuring 1.8 mm. in length and 0.6 mm. in width. The mandible is slender and has 7 denticles. The first maxilla consists of 3 segments; the terminal is conical, and the basal segment has 2 distal broad spines. The terminal segment of second maxilla is provided of 2 rows of blunt teeth and blunt spines on the inner margins. The third segment has a single distal spine. The exopod has 5 short distal spines, while the endopod has slender bristled seta. The caudal rami are short. The female is somewhat cylindrical and measured 3.2 mm and 0.9 mm in width at the middle of the body. The head separated by a constriction from the rest of the body. The first thoracic leg is biramous, the exopod of the first segment bear blunt distal spines, while, the endopod bear an elongated distal spine. A tiny papilla-like process is located at the base of the endopod. The egg strings are elongated and uniseriate, strongly flattened eggs (Plates, 4&5). Based on the morphological characters, such crustaceans are belonged to Lernanthropidae, *Lernanthropus psciaenae* Badawy, 1994.

Another crustacean parasite was collected from the gills, buccal cavity and skin of *M. labrax*. The body length of the female measures 2.6 mm and the greatest width measures 1.02 mm. The cephalothorax is nearly as long as wide. The abdomen has one segment and is nearly 3 times longer than broader. The caudal rami are longer than wide. The second antenna has a recurved claw. Female characterized by long bar-shaped egg pouches or strings (Plate, 6). Based on the morphological characters, these crustaceans are related to Calgidae, *Caligus carangis* Badawy, 1994

Prevalence of crustacean infestation in seabass fish:

Table (1) shows total and seasonal prevalence of crustacean parasites in examined *M. labrax* fish. Tables (2&3) show the prevalence in relation to body weights and lengths.

Table (1): Seasonal prevalence of crustacean infestations among seabass *M. labrax*

Season	No of examined fish	No. of infested fish with Caligus carangis	No. of infested fish with Lernanthropus piscianae	No. (%) of infested fish
Autumn	25	10	7	17 (68)
Winter	25	3	1	4 (16)
Spring	25	5	2	7 (28)
Summer	25	11	8	19 (76)
Total	100	29 (29)	18 (18)	47 (47)

Table (2): Prevalence of the recorded crustacean infestations in relation to length among *M. labrax*

Body length (cm)	No. examined	Crustacean infestation	
		No. infected	%
10-15	20	0	0
15- 20	20	8	40
20- 25	20	10	50
25- 30	20	9	45
30- 35	20	20	100
Total	100	47	47

Table (3): Prevalence of the recorded crustacean infestations in relation to body weight among *M. labrax*

Fish body weight (g)	No. examined	Crustacean infestation	
		No. Infected	%
< 50	21	6	28.57
50- 100	19	9	47.36
100-150	18	13	72.22
150- 200	22	9	40.90
200- 300	20	10	50

Total	100	47	47

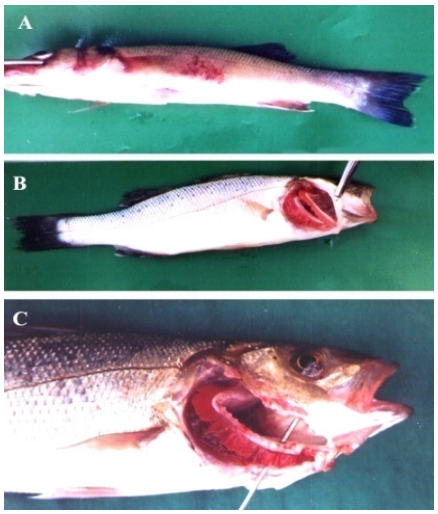

Plate (1): A. Haemorrhages allover the body surface and at the bases of fins of Morone labrax, B. & C. Showing gills of Morone labrax with sticking of the gills.

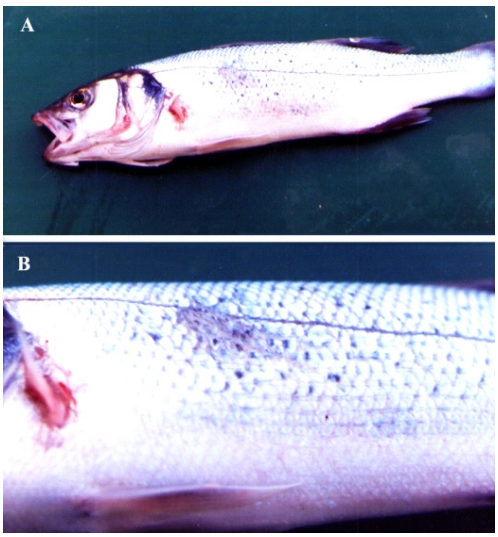

Plate (2): A. & B. Abrasions and ulcers on the body surface of *Morone labrax*.

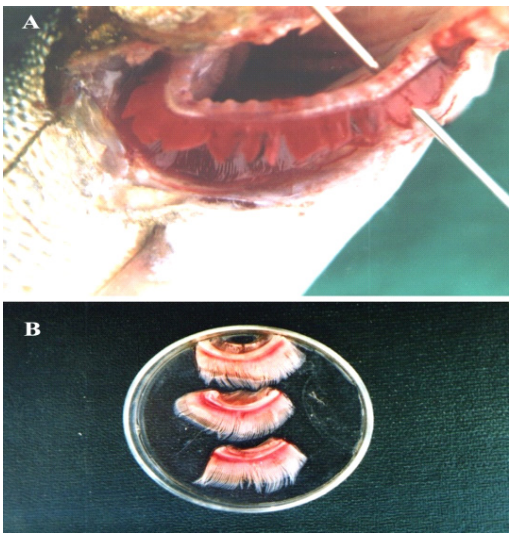

Plate (3): A. & B. Gills of *Morone labrax* with mosaic appearance, sticking of the gills and grayish coloration.

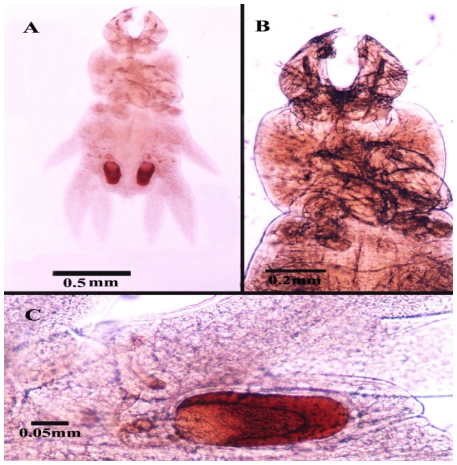

Plate (4): Light photomicrograph of male *Lernanthropus pscianae* A. Whole copepode, B. Anterior end showing

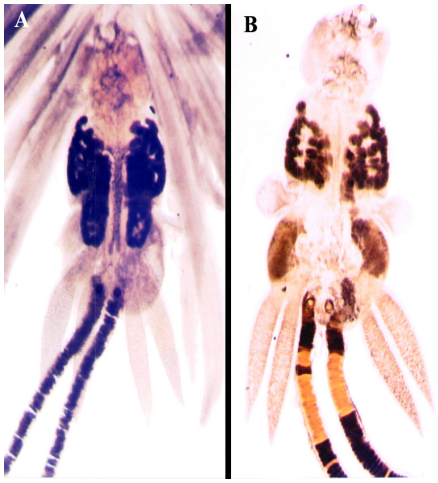

Plate (5): Light photomicrograph of female *Lernanthropus pscianae*: A. female attacking gills of *Morone labrax* forming black lines between gill filaments, B. Whole copepode

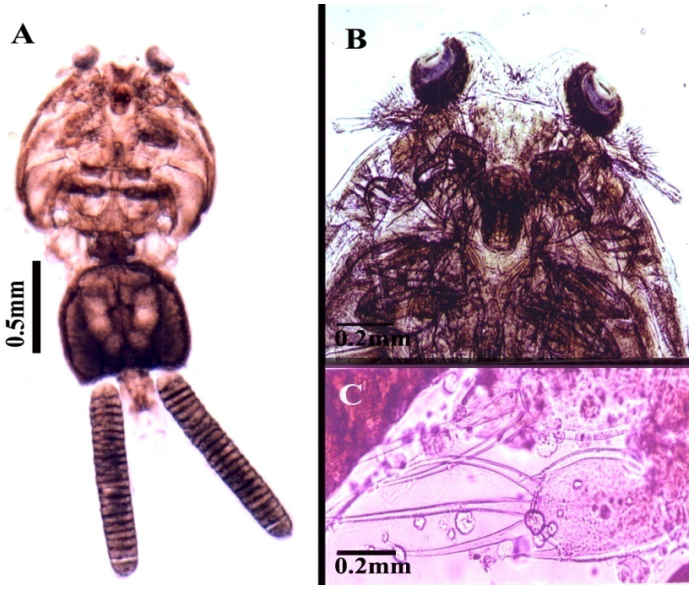

Plate (6): Light photomicrograph of female *Caligus carangis*: **A.** (Whole copepod), **B.** Anterior end showing two eyes, first & second maxilla and Claws, **C.** Caudal ramai.

4. Discussion:

The main clinical signs observed in infested *M. labrax* with crustacean infestations were excessive mucus production, sluggishness and rubbing the body against hard objects and sides of aquaria to get rid the irritation induced by the parasites. Opercula were bulging. Fish gathered at water surface (surface breathing) with gulping the atmospheric air. These results are in agreement with those reported by ***Andrews et al. (1988) Poynton et al. (1997)***, ***Ragias et al. (2004) and Osman (2005).*** Any slight structural damage of gills can render a fish very vulnerable to osmoregulation as well as respiratory difficulties as fish gills are responsible for regulating the exchange of salt and water and play a major role in excretion of nitrogenous waste products. These results may be attributed to the low respired oxygen of destructed gill epithelium which caused by feeding activity, attachment, fixation and locomotion of crustaceans

causing massive destruction of respiratory epithelial cells ***(Eissa, 2002).***

This study showed that skin, gill cover and bases of fins appeared with hemorrhagic areas, abrasions and ulceration on the body surface, these signs agreed with those obtained by ***Ragias et al. (2004)*** and may attributed to irritation caused by *Caligus carnages*, they often remains attached when host is removed from the water. In which the female isolated from skin and external surface ***(Kabata, 1988***; ***Santos, 1996; Karlsbakk et al., 2001 and Noga, 2010).*** Sea lice feed on surface tissues of their hosts, which can lead to many problems especially for small juvenile fish ***(Price et al., 2011)***. Sea lice at the peak of infection were associated with the severity of eye damage; this may be because lice graze on the cornea or because tuna injure their eyes when flashing rubbing ***(Hayward et al., 2008)***.

Emaciation was recorded in *M. labrax* may be due to crustacean infestation which reduce fish appetite and became off food, this agreed with ***Tavares and Luque (2001) and Nagasawa (2004).*** Crustaceans reduce growth rates and, in sufficient numbers, result in host death ***(Morton et al., 2005; Krkosek et al., 2006 and Costello, 2009).***

Regarding the postmortem examination, it was revealed areas of congestion and paler (Marbling) of gills with excessive mucus secretions and sticking of the gill tips and grayish discoloration. This result agreed with ***Andrews et al. (1988), Nahla (1993) and Osman (2005).*** Lernanthropus is known to cause some pathological effects on its host. It attaches to the gill filaments using antennae and third legs leading to pathological effects such as erosion, desquamation, necrosis in branchial epithelial tissue, increase of mucus secretion, narrowing in capillary veins have been reported **(*Manera and Dezfuli, 2003 and Tosken et al., 2008)***. These lesions may be attributed to the severe irritation caused by movement, feeding activity and fixation of such crustaceans which result in asphyxia and then death. Also, it may be due to the hard fixation of crustacean parasites with their claws activity. The excessive mucus secretion, may be to dilute the irritation and act as a defense mechanism against the infestation.

The parasite under discussion isolated from gills of *M. labrax*. This agree with ***Akmrza (2003); Tosken et al. (2008) and Henery et al., (2009)*** who isolated the same genus from the same host and site. ***Badawy (1994)*** who obtained the same species from gills of *Sciana umbra* and disagree with ***Roubal (1986), Luque et al. (1989)*** who obtained the same genus from *Acanthopagrus australis* and *Paralonchuri peruanus*, *Seriolella violacea* and *Anisotremus scapularis*, respectively. The site of infestation was in accordance to that mentioned by ***Kensely and Grundley (1973); Badawy (1994).*** However the host and locality varied from those mentioned before. The parasite measurements and morphological characters are nearly similar to that obtained by ***Badawy (1994)*** so it was identified as *Lernanthropus psciaenae*, Badawy, 1994.

The second parasite under discussion isolated from gills, oral cavity and skin and this is agreed with ***Kabata (1988)*** who isolated it from skin and with ***Oldwage (1990)*** who collected female parasite from the buccal cavity of *Arthron hipidus* and ***Cressy (1991)*** and ***Badawy (1994)*** who isolated it from gills of *Caranx sem and* ***Maran et al. (2009)*** *who isolated the same genus from gill cavities and body surface of marine fish*. Comparing the present data with the other previous data, it is clear that the parasite has all morphological characters and measurements of *Caligus carnages*, Badawy,1994.

Regarding Crustacean infestation (Copepodiasis), the total prevalence was 47% . This result is higher than that obtained by ***Abd El-Aal (2003)*** as it was (10.43%) while it is much higher than that obtained by ***Badawy (2001)*** as the rate was (2.25%). This difference may be attributed to the locality from which fish samples obtained and the difference in fish species. Also, the prevalence was disagree with that obtained by ***Badawy (2001)*** who found no infestation in *M. labrax* but with a rate of (0.61%) in *Siganus canaliculatus*, ***Abd El-Aal (2003)*** who detected *Caligus elongates* (8.75%) in *S. commerson*, ***Manera and Dezfuli (2003)*** with *Lernanthropus kroyeri* (35%) among *Dicentrarchus labrax* and ***Vagianou et al. (2004)*** with copepode infestation rate (13.6%).

Regarding the seasonal prevalence of crustacean infestation, the peak was highest in summer 76%, followed by autumn 68% then Spring 28% and Winter 16%. This agrees with results obtained by ***Badawy (1994)*** in which he recorded the summer season as the season of the highest infestation rate and the lowest was the winter season. Increasing mean sea temperatures are likely to increase infestation pressure with sea lice on farms and wild fish as well as affecting the geographical distribution of hosts and parasites.

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Haemostatic Changes Associated with Thrombosis in Long Term Hemodialysis Treatment

Hanan G. Abd El-Azeem[1], Eman Nasr Eldin[1], Adel HM Mekawy[2], Ahmad F. Thabet[2], Nahla Mohamed Elsherbiny[3].

Departments of [1]Clinical Pathology; [2]Internal Medicine and [3]Medical Microbiology and Immunology, Faculty of Medicine, Assiut University, Egypt
emannasr2000@yahoo.com.

Abstract: In end-stage renal disease, particularly when treated with haemodialysis, the function of platelets, coagulation and fibrinolytic systems can be disturbed; those patients may show both thrombotic complications and bleeding abnormalities. Thus, it is essential to investigate haemostatic alterations in patients on hemodialysis so that adequate regime for anticoagulant therapy could be implemented. Haemostatic changes in patients on hemodialysis may result from alterations in vessel wall integrity and platelet function, and reduced blood flow in the native arteriovenous fistula. We study the haemostatic abnormalities associated with thrombosis in long term hemodialytic patients to determine whether coagulation and fibrinolysis are enhanced or not in 42 uremia patients on chronic regular hemodialysis treatment (20 of them had history of thrombotic events "group I" and the remaining 22 patients showed no history of thrombosis" group II") and 20 apparently health control group. Plasma levels of some blood coagulation-fibrinolysis parameters were measured including platelet count, prothrombin time/concentration (PT/PC), activated partial thromboplastin time (aPTT), thrombin time (TT), fibrinogen and D-Dimer, platelet aggregation (induced by adenosine diphosphate, collagen, Ristocetin, and Arachedonic acid), and the levels of natural anticoagulant protein C, protein S and antithrombin-III (AT-III). The mean platelet count was normal in all studied groups, while higher mean value of platelet count was observed among patients in group I than group II. Prolonged PT/sec., aPTT/sec and TT in patients groups were observed; those differences were statistically highly significant in comparison with healthy controls ($p < 0.001$). The mean plasma fibrinogen (g/l) concentration was normal in all groups although levels above normal limits were noted in group I, fibrinogen level was significantly higher ($p < 0.05$) in group I patients than in normal controls. The mean value of D-dimer (ng/ml) was significantly higher in group I than group II and in comparison with control group ($p < 0.001$). We did not find differences between group I patients and control group as regard platelet aggregation induced with all agents, while there were statistically significant difference were observed between group II and control except for collagen. In contrast, the level of natural anticoagulants (protein C, protein S and AT III) were significantly reduced in patients groups than control and they were statistically significant, and the levels were lower in group I than group II. In conclusion, our results showed that the long term haemodialysis procedure affects the haemostatic process and may contribute to a thrombotic tendency. Careful weighing of risks and benefits of pharmacological prevention of thrombosis in patients on hemodialysis is crucial and this area certainly warrants further investigation.

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Key Words: Chronic renal failure, Thrombosis.

1. Introduction

Although renal failure has classically been associated with a bleeding tendency, thrombotic events are common among patients with end-stage renal disease (ESRD) (Jalal et al., 2010). It is widely believed that not only chronic renal disease but also the haemodialysis process by itself activates platelets, coagulation and fibrinolysis (Sloand and Sloand , 1997, Kawabata et al., 1998). The haemodialysis procedure could influence haemostasis by two distinct pathways: first, by the effect of the dialysis membrane, the composition of the dialysis circuit, and changed rheology, and second, by the effect of added anticoagulants (Aggarwal et al., 2004). Noticeably, these factors have been significantly changed in the last decade (Rios et al., 2010).

Cardiovascular mortality and morbidity are higher in patients with chronic renal disease than in the general population (Aggarwal et al., 2002). Patients with chronic renal disease are in the highest risk group for thromboembolic disease and many clinical trials have demonstrated the greater safety and efficacy of low-molecular-weight heparin

(LMWH) versus unfractionated heparin (UFH) (Malhotra et al., 2001; Lai et al., 2010).

A variety of thrombosis-favoring hematologic alterations have been demonstrated in these patients. In addition, "non-traditional" risk factors for thrombosis, such as hyperhomocysteinemia, endothelial dysfunction, inflammation, and malnutrition, are present in a significant proportion of chronic dialysis patients (Naumnik et al., 2003).

Since activated platelets and coagulation could contribute to the occurrence of atherothrombotic events in haemodialysis patients, one could speculate that the haemodialysis procedure itself facilitates the development of atherothrombotic events (Sirolli et al., 2002). On the other hand, activation of fibrinolysis may lead to bleeding complications (Galbusera et al., 2009).

Hemodialysis vascular access thrombosis, ischemic heart disease, and renal allograft thrombosis are well-recognized complications in these patients. While deep venous thrombosis and pulmonary embolism are viewed as rare in chronic dialysis patients. Several ESRD treatment factors such as recombinant erythropoietin (EPO) administration (Wirtz et al., 1992), dialyzer bioincompatibility (Windus et al., 1996), and calcineurin inhibitor administration may have prothrombotic effects (Casserly and Dember, 2003).

This unanswered issue was the rationale for performing the present study in which we examine the abnormalities in the haemostatic pathway in patients with chronic renal failure on regular haemodialysis sessions to identify factors associated with thrombotic events.

2. Patients and Methods

Blood samples were obtained from 42 patients with CRF (19 females, 23 males; mean age 58 years, range 48–70) treated by chronic haemodialysis (at least 6 months); 20 of them had history of thrombotic events "group I" and the remaining 22 patients showed no history of thrombosis" group II". All patients had native vessels as a vascular access. All patients were treated by haemodialysis three times per week and received regular doses of low-molecular heparin before haemodialysis and no patient used aspirin or warfarin. Plasma levels of some blood coagulation-fibrinolysis parameters were measured including platelet count, prothrombin time/concentration (PT/PC), activated partial thromboplastin time (aPTT), thrombin time (TT), fibrinogen and D-Dimer, platelet aggregation (induced by adenosine diphosphate, collagen, Ristocetin, and Arachedonic acid), and the levels of natural anticoagulant protein C, protein S and antithrombin III. The results were compared with those obtained in a group of normal volunteers [twenty healthy subjects (9 females, 11 males; mean age 42 years, range 30–54) as controls].

Blood Sampling

Whole blood was obtained from venous needles without venous stasis was collected on:

- Tri potassium- EDTA containing tubes for standard hematology parameters (platelet count) were immediately prepared for testing on an automated blood cell counter (Beckman Coulter).
- Trisodium citrate 3.2% containing tube was centrifuged for 15 to 20 min at 3,000 *rpm* for measurements of coagulation and fibrinolytic parameters including PT/PC , aPTT, TT and AT-III [using Sysmex CA-1500- Siemens], fibrinogen was determined using STArt4- Diagnostica Stago, D-dimer, Protein C and protein S were measured by enzyme linked immunosorbent assay (ELISA) technique (Diagnostica Stago – France).
- Trisodium citrate 3.2% containing tube for platelet aggregation studies using adenosine diphosphate (ADP), collagen, ristocetin, and arachedonic acid (AA) (Crono-log company, USA) immediately prepared for testing using platelet aggregation profiler ® (PAP-4CD Bio/Data corporation) aggregometer. Platelet-rich plasma (PRP) was prepared by centrifugation of citrated blood at 1,000 *rpm* for 15 min at 20 °C.

Results were expressed as percentages of the maximal aggregation obtained after 5 min of stimulation.

Statistical Methods

Measured variables were expressed as mean±SD. Statistical analysis was done to compare the data between studied groups using t-test. P value < 0.05 was considered statistically significant and highly significant if $p < 0.001$. Statistical analyses were performed by the SPSS version 11).

3. Results

Markers of both coagulation and fibrinolysis activation (Table 1 & Figure1):

As regard Platelet count the mean platelet count were normal in patients groups, although mild thrombocytopenia occurred in several patients in both groups and thrombocytosis was observed in few patients in group I.

Higher mean value of platelet count was observed among patients in group I than group II with no statistically significant difference, while there was statistically significant differences when compared with controls, (216.7±122.5 and 169.4±63.8 *vs.* 284.1±37.8; range 91-535 and 79-291 *vs.* 225-336 $X10^3$/l; $p < 0.01, < 0.001$) in both groups respectively.

Longer PT/sec. and lower PC (%) in patients groups were observed, those differences were statistically significant between patients groups (15.4±1.4 *vs.* 14.4±1.0 and 68.1±12.8 *vs.* 76.6±9.7; $p < 0.05$) and were significantly increased in comparison with healthy controls (15.4±1.4 and 14.4±1.0 *vs.* 12.8±0.2; $p < 0.001$)

No statistically significant difference was observed between both patients groups as regard

aPTT/sec (37.6±11.6 *vs.* 36.3±7.6; $p > 0.05$); aPTT/sec was longer among patients groups compared with controls and the differences were statistically highly significant (37.6±11.6 and 36.3±7.6 *vs.* 26.6±2.2; $p < 0.001$).

There were statistically significant difference between both patients groups as regard TT/sec (21.4±2.7 *vs.* 19.1±3.0; $p < 0.05$); TT/sec was longer among patients groups compared with controls and the differences were statistically significant (21.4±2.7 and 19.1±3.0*vs.* 17.6±1.2; $p < 0.001$ & $p < 0.05$ respectively).

The mean plasma fibrinogen concentration was normal although levels above normal limits were noted in a few group I patients. Fibrinogen was significantly higher in group I than group II and normal controls fibrinogen levels (3.4±0.8 *vs.* 2.94±0.6 and 3.4±0.8 *vs.* 2.89±0.6; $p < 0.05$). The mean value of D-dimer (ng/ml) was significantly higher in group I than group II (391.9±144.5 *vs.* 231.7±132.7; $p < 0.001$) and in comparison with control group (391.9±144.5 and 231.7±132.7 *vs.* 97.3±13.6; $p < 0.001$).

In patients, platelet aggregation (induced by adenosine diphosphate, collagen and arachedonic acid and ristocetin) did not differ significantly in both groups and when comparing group I with the control, while there were statistically significant difference were observed between group II and control except for collagen (Table 2 & Figure 2).

Table 1. Screening test for coagulation and fibrinolytic system in different study groups

	CRF with thrombosis Group I N=20 *Mean±SD*	CRF without thrombosis Group II N=22 *Mean±SD*	Control N=20 *Mean±SD*	*P-value*
Platelet count	216.7±122.5	169.4±63.8	-	*NS*
	216.7±122.5	-	284.1±37.8	*
	-	169.4±63.8	284.1±37.8	**
PT (sec.)/ PC (%)	15.4±1.4/68.1±12.8	14.4±1.0/76.6±9.7	-	*
	15.4±1.4/68.1±12.8	-	12.8±0.2/96.0±3.5	**
	-	14.4±1.0/76.6±9.7	12.8±0.2/96.0±3.5	**
aPTT (sec.)	37.6±11.6	36.3±7.6	-	*NS*
	37.6±11.6	-	26.6±2.2	**
	-	36.3±7.6	26.6±2.2	**
TT (sec.)	21.4±2.7	19.1±3.0	-	*
	21.4±2.7	-	17.6±1.2	**
	-	19.1±3.0	17.6±1.2	*
Fibrinogen (g/l)	3.4±0.8	2.94±0.6	-	*
	3.4±0.8	-	2.89±0.6	*
	-	2.94±0.6	2.89±0.6	*NS*
D-Dimer (ng/ml)	391.9±144.5	231.7±132.7	-	**
	391.9±144.5	-	97.3±13.6	**
	-	231.7±132.7	97.3±13.6	**

PT: prothrombin time, PC: prothrombin concentration, aPTT : activated partial thromboplastin time, TT: thrombin time, NS: not significant, *: significant ($p < 0.05$), **: highly significant ($p < 0.001$).

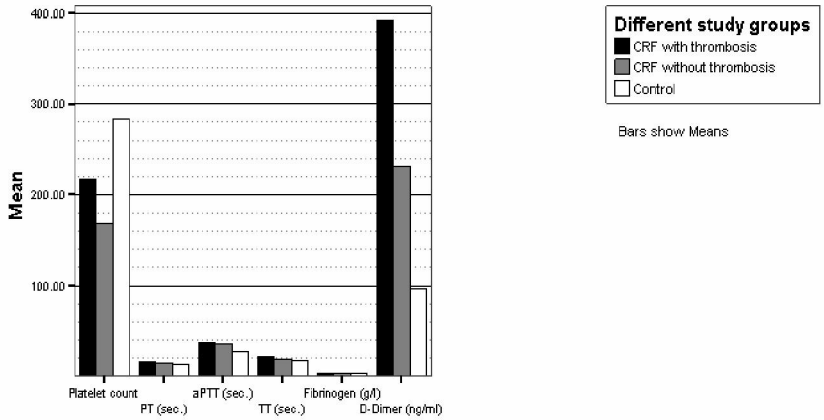

Figure 1.Plasma levels of some blood coagulation-fibrinolysis parameters

Table 2. Platelet aggregation test in different study groups.

	CRF with thrombosis Group I N=20 *Mean±SD*	CRF without thrombosis Group II N=22 *Mean±SD*	Control N=20 *Mean±SD*	*P-value*
ADP	68.6±23.4	45.9±29.8	-	*
	68.6±23.4	-	72.3±14.1	*NS*
	-	45.9±29.8	72.3±14.1	**
Collagen	64.9±21.6	61.8±26.4	-	*NS*
	64.9±21.6	-	72.5±6.9	*NS*
	-	61.8±26.4	72.5±6.9	*NS*
AA	64.4±24.5	43.2±28.3	-	*
	64.4±24.5	-	69.1±5.2	*NS*
	-	43.2±28.3	69.1±5.2	*
Ristocetin	72.3±16.4	58.0±26.9	-	*
	72.3±16.4	-	78.7±12.9	*NS*
	-	58.0±26.9	78.7±12.9	*

ADP: adenosine diphosphate, AA: arachedonic acid, NS: not significant, *: significant ($p < 0.05$), **: highly significant ($p < 0.001$).

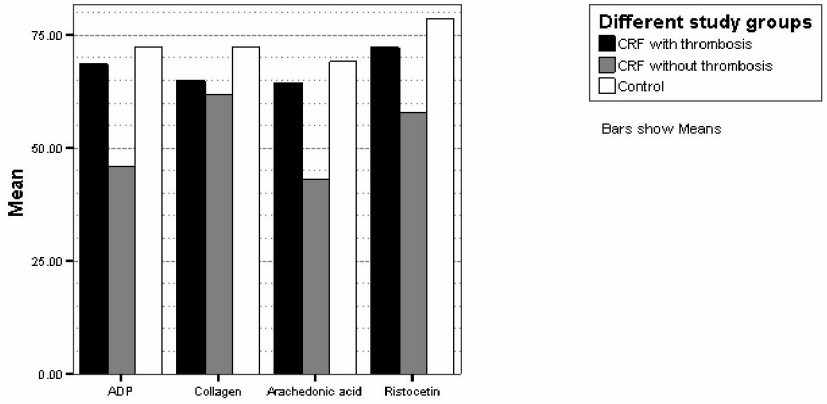

Figure 2. Platelet aggregation test in different study groups.

- In contrast, lower level of natural anticoagulants (protein C, protein S and AT-III) were observed in patients groups than control and they were statistically significant. The levels were lower in group I than group II and the differences between patients group were statistically highly significant for protein C (65.6±19.5vs. 93.2±18.3; $p < 0.001$), significant for protein S (68.6±10.6 vs. 76.3±10.9; $p < 0.05$), and not significant for AT-III (Table 3& Figure 3).

Table 3. Natural anticoagulant levels in different study groups.

	CRF with thrombosis Group I N=20 *Mean±SD*	CRF without thrombosis Group II N=22 *Mean±SD*	Control N=20 *Mean±SD*	*P-value*
Protein C	65.6±19.5	93.2±18.3	-	**
	65.6±19.5	-	106.7±15.6	**
	-	93.2±18.3	106.7±15.6	*
Protein S	68.6±10.6	76.3±10.9	-	*
	68.6±10.6	-	105.5±14.8	**
	-	76.3±10.9	105.5±14.8	**
AT-III	67.5±11.4	70.9±11.6	-	*NS*
	67.5±11.4	-	102.1±15.5	**
	-	70.9±11.6	102.1±15.5	**

AT: antithrombin, , NS: not significant, *: significant ($p < 0.05$), **: highly significant ($p < 0.001$)

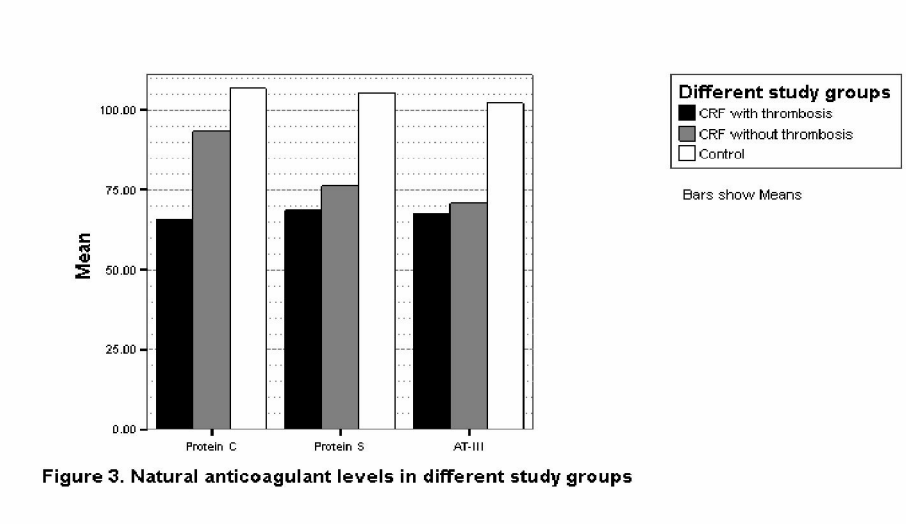

Figure 3. Natural anticoagulant levels in different study groups

4. Discussion

Chronic kidney disease (CKD) is a growing global health problem. CKD is typically associated with a prothrombotic tendency in the early stages of the disease, whereas in its more advanced stage, that is, end-stage renal disease, patients suffer from a prothrombotic tendency leading not only to possibly fatal conditions like ischemic heart disease or stroke (Irish, 1998), but also to thrombosis of the vascular access and, in many cases, a bleeding diathesis most frequently manifested by epistaxis and ecchymosis (Rios et al., 2010). The exact etiology behind the coexistence of these conflicting haemostatic disorders is poorly understood (Jalal et al., 2010).

Vascular complications represent 20-25% of all hospitalized patients on hemodialysis. Changes in the haemostatic system may play a major role in the pathogenesis of cardiovascular complications and vascular thrombosis; during haemodialysis, platelets, coagulation and fibrinolytic systems could be importantly affected due to several known (e.g. alterations in vessel wall integrity and platelet function, reduced blood flow in the native arteriovenous fistula, velocity of procedure, type of membrane, artificial vascular access, circuit composition, and the type of anticoagulation) and unknown factors (Naumnik et al., 2003; Aggarwal et al., 2004) .

Thus, it is essential to investigate haemostatic alterations in patients on hemodialysis so that adequate regimes for anticoagulant therapy could be implemented. The aim of the present study was to explore the effect of long term haemodialysis on the haemostatic system including some of coagulation,

fibrinolytic system, natural anticoagulant and platelet aggregation activation (induced by several agents).

Our results were in agreement with the majority of studies, which mostly showed activation of coagulation after haemodialysis procedure in some points, but are in line with other studies that showed lack or only low activation of coagulation during haemodialysis procedure (Irish, 1998; Naumnik et al., 2003) . Very likely, this seems to be due to the differences between previously used and improved, currently used modern procedures of haemodialysis by biocompatible membranes and better anticoagulation with low-molecular weight heparins (Vaziri et al., 1994).

Platelet count first increased then significantly decreased with increased serum creatinine in conservatively treated chronic uremias. The transient thrombocytopenia often seen with dialysis is thought to be due to the contact activation and deposition of platelet and fibrin on the dialysis membrane (Posadas et al., 2011). Blood and dialyzer membrane interaction can cause significant thrombocytopenia through the activation of complement system (Olafiranye et al., 2011)

Previous studies reported that neither platelet count nor platelet half life altered in uremia (Andrassy and Ritz, 1985). Our result revealed that the mean platelet number was normal, mild thrombocytopenia occurred in several patients in both groups and thrombocytosis observed in group I, the mean platelet number statistically lower in patients group than control group and it was higher among CRF patients presented with thrombotic complications.

This result found that the mean PT, aPTT, and TT were prolonged in patients groups than control and these differences were statistically significant. Increased levels of FDP (D-Dimer) can interfere with thrombin-fibrinogen reaction resulting in prolongation in the TT and aPTT.

Fibrinolysis is evidently activated during the modern haemodialysis procedure. The role of haemodialysis on fibrinolytic parameters has been investigated in several studies (Opatrny et al., 1991; Nakamura et al., 1992; Martin-Malo , 1993; Ishii et al., 1996). It seems that increased activation of fibrinolysis is the consequence of extra-corporeal circulation and that it is related to dialysis membrane biocompatibility (Opatrny et al., 1991; Martin-Malo , 1993). Synthetic membranes appear to be more biocompatible than others (Cianciolo et al., 2001; Sirolli et al., 2002). It might be that factors other than membranes are also important for fibrinolysis activation. The increase in fibrinolytic activity after haemodialysis may contribute to an increased bleeding tendency present in some susceptible haemodialysis patients (Kurz et al., 1985).

Fibrinogen level in our patients with thrombosis was significantly higher than control group. Elevated levels of fibrinogen was previously reported (Sagripanti et al., 1993) in some patients with CRF, the value increased almost with increased serum creatinine level to 10 mg/ 100ml due to new formation associated with a reactive process, which might also be extrarenal and afterward fell even with increased creatinine levels (Amiral et al., 1995); that might be due to a decreasing new formation in severe renal insufficiency (Boaz et al., 1999).

Also D- Dimer level was significantly higher in patients group than control this in agreement with Erdem and coworkers (1995), whom reported that D-Dimer were elevated in patients receiving dialysis implicated the dialysis procedures as a contributor to procoagulant activity.

Protein C deficiency has been described in ESRD patients with claciphylaxis, and a linked functional protein C deficiency with the development of deep venous thrombosis in dialysis patients was reported (Erdem et al., 1995).

The possible changes of natural anticoagulants (PC, PS, and AT III) were investigated, in accordance with Faioni *et al.* (1991) we found reduced protein C level in uremic patients, other studies reported that the effect of dialysis on protein C activity are conflicting partial normalization, reduction, and no change following dialysis treatment have been demonstrated (Kant et al., 1992).

The same finding were observed as regard Protein S and AT III, where lower significant levels of protein S was detected among patients group which might be attributed to peritoneal ultrafiltration of this factor (Kant et al., 1992).

The artificial circuit, changed rheology, and heparin might activate platelets (Kawabata et al., 1998; Aggarwal et al., 2004). These effects could be counterbalanced by defective aggregation of platelets due to ESRD (Aggarwal et al., 2002). Contradictory results have been reported in regard to the effect of haemodialysis on platelet aggregation (Sloand and Sloand , 1997; Malhotra et al., 2001), probably related to the complex interaction of the vessel wall, and different compositions of the dialysis circuit and anticoagulants on platelet function (Cianciolo et al., 2001; de Sa et al., 2001). Diminished platelet degranulation, reduction in stored platelet ADP and serotonin, and decreased platelet thromboxane are among the abnormalities that have been demonstrated *in vitro* studies of platelets from CRF patients (Remuzzi et al., 1983).

In this study we found diminished platelet aggregation response in uremic patients than control group and these differences were significant to ADP, AA, and ristocetin.

The detection of an abnormality of platelet AA metabolism in uremic patients has given a new possible clue in understanding the pathogenesis of uremic

thrombocytopathy (Remuzzi Gand Pusineri, 1988). Šabovi and coworkers (2005) results did not show differences in platelet aggregation before and immediately after haemodialysis procedure, suggesting that the haemodialysis procedure probably does not significantly activate platelets.

In comparison with healthy controls, haemodialysis patients with thrombosis had

an evidently activated coagulation system and activated fibrinolysis than those without thrombotic complication.

In conclusion, these results suggest that coagulation and fibrinolysis are enhanced in long term haemodialysis patients. Haemostatic abnormalities existed in patients on maintenance hemodialysis might contribute to thrombotic complications.

Thus, it is essential to investigate hemostatic alterations in patients on hemodialysis so that adequate regimes for anticoagulant therapy could be implemented, To date pharmacological prevention of thrombosis in patients on hemodialysis has been a major therapeutic challenge, as direct data are lacking on the management of anticoagulation in dialysis patients, so careful weighing of risks and benefits is crucial and this area certainly warrants further investigation.

Corresponding author
Dr. Eman Nasr Eldin
Departments of Clinical Pathology; Faculty of Medicine, Assiut University, EGYPT.
emannasr2000@yahoo.com.

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The Perception of Care- Givers of Mental Retarded Person towards Mental Retardation.

Mohebbi Z[1], Nooryan KH[2], Hashemi Mahmmod Abad N.[3], Najafi doulatabad SH[4].

[1] Department of Medical surgical nursing,School of Nursing and Midwifery, Shiraz University of Medical Sciences, Shiraz, Iran.
[2]Department of Mental Health, School of Nursing and Midwifery, Yasouj University of Medical Sciences, Yasouj, Iran.
[3]Department of Pschiatry, School of Medicin, Yasouj University of Medical Sciences, Yasouj, Iran.
[4] Department of Medical surgical nursing, School of Nursing and Midwifery, Yasouj University of Medical Sciences, Yasouj, Iran.
Corresponding author: Najafi doulatabad Shahla, shahlanajafi@gmail.com, shahlaiss@yahoo.com

Introduction: The number of persons suffering from retardation has been increased during the recent years. Most of mental retarded individuals are under the care at home and use family supporting services. The negative influences resulted from mental retardation may affect their family and care-givers. Considering that, the perception of care-givers may influences the care that they present therefore, a research was carried out with the aim to determine the perception of care- givers of mental retarded person towards mental retardation. **Method:** In this descriptive study, one hundred care- givers of mental retarded persons participated randomly. Data were collected using self-made questionnaire of perception of care- givers through face to face interview. Collected data were analyzed using SPSS software and x^2 test. **Results:** Most of care-givers (56.0%) had neutral perception towards mental retardation. In addition, a significant relation was observed between the education level and occupation of care-givers with their perception connected to mental retardation (P=0.001), while , no significant relation was observed between the age and sex of mental retarded person and age and sex of care-givers with their perception. **Conclusion:** Care-givers with lower education level and house-keepers had a lower perception towards mental retardation. Therefore, it is necessary to consider supporting programs by health care-givers in order to increase adaptation and reduce tension for aforesaid groups.
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Key words: Care-givers, Mental retarded, Perception, Mental retardation.

Introduction:

The number of persons suffering from mental retardation has been increased during the recent years (1). Mental retardation is being applied for a person whose mean of mental function is lesser than normal position by two standard deviation or his/her Intelligence Quotient (IQ) is below 70.0 (2). Parents of such children experience large chronic tensions and will affect not only parents but also sisters and brothers and communications of family members negatively (3). The experienced tension of these families will encounter parents with unstable mental situation, feeling sin, disturbance in psychical health and non-satisfaction from social health (4). In addition, incompatibility and disputation between couples and social seclusion are prevalent among such families (3) in such a way that, parents and family members may be exposed to psychological difficult specially anxiety and depression and suffer from disturbance in well-living sentiment, familial functions and self-concerned (2). In spite of available problems for care-givers and mental retarded persons, more contact of parents with mental retarded person may increases the adaptability of family members (5) followed by positive perception of parents from mental retarded child. Results of studies show that, positive feeling regarding retardation reduces he destructive effects resulted from tension, improves psychological flexibility of person and immunes person against neural disorders, endocrines and safety responses resulted from chronic tension that may causes the disease incidence (6). In addition, positive perception towards retardation will be followed by improvement of quality of life and family stability which will cause parents to try to make the abilities of their child appeared (3). Researchers also point out that, positive attitude and perception of parents towards mental retardation play important role in the process of caring child (7). Paying attention to the limitations of carried out studies regarding the perception of care-givers in relation with mental retardation and considering that, their perception may affect the care they present, a study with the aim to determine the perception of care-givers of mental retarded person towards mental retardation was carried out.

Method: This is a descriptive study carried out with the aim to determine the perception of care-givers of mental retarded person towards mental retardation. Research population was consisting of all care-givers of retarded persons under the support of Welfare Office (Behzisti) of Yasuj city. It is necessary to mention that, Kohgiloyeh and Boirahmad province is having 1,800 mental retarded out of which 500bolong to Ysuj city. Care-givers are being applied to those who have responsibility of protecting and caring of mental retarded person directly. Inclusion criteria were consisting of having Iranian nationality and ability of care-givers to complete questionnaire or doing interview. It was also necessary that, 1) Care-givers have the responsibility of protecting at least one mental retarded person, 2) Mental retarded person being care at home and 3) Care-giver and mental retarded person live at same place. The mental retarded person living in handicapped caring centers was eliminated from the study.

Considering the type of research and based on the produced information from previous studies, the required sample size to do research was estimated to be 100 persons. The data were collected randomly from available files in the Welfare office in such a way that, the name of all mental retardations was numbered successesively and then the table of random numbers was used to select samples randomly. After obtaining permission from moral committee, explaining regarding the object of research and being depositary in protection of information to care-givers and obtaining written letter of satisfaction to take part in the study, required information were collected.

Data collecting tool was consisting of questionnaire and demographic characteristic form. In demographic form, the age and sex of mental retarded person and the age, sex, education and occupation of care-givers were evaluated.

The main tool was a researcher made questionnaire of perception of care-givers that evaluated the perception of care-givers towards mental retardation. This questionnaire included 13 items (these items have come in table 2) and based on the 5 degree Likert scale, it has been divided into absolutely agreeable (5) to absolutely opposed (1). The obtained score was placed in the range of 13-65. More score showed the better perception of care-givers towards retardation. Thereafter, the obtained score was divided into 3 levels of positive perception (49-65), neutral perception (31-48) and negative perception (13-30). Data collection lasted for 6 months by attending at the house of persons. The validity of tool was specified via content validity in such a way that, 10 skilled personnel of Yasuj University of medical sciences evaluated and confirmed the tool. To determine the reliability of tool, internal consistency was used that the Cronbach′s coefficient alpha was obtained as α=0.84. Collected data were analyzed using SPSS software version 14 and applying descriptive statistics (mean) and inferential statistics (x^2 test).

Results: Results of the study showed that, most of care-givers participated in the research were men (56.0%). The mean and standard deviation of the age of care-givers was 40.63±10.89. The minimum and maximum age of care-givers was 22 and 70 years respectively. 31.0% of care-givers were illiterate and 37.0% of them had primary or high school education. The occupation of 30.0% of samples was employee. Most of care-givers were protecting boy mental retarded (62.0%). The mean and standard deviation of mental retarded persons was 12.45±4.80. In addition, the minimum and maximum age of mental retarded persons was 3 and 22 years respectively (table 1).

Results in relation with the object of research means "determining the level of perception of mental retarded person towards mental retardation" showed that, 44.0% of care-givers had positive perception and 56.0% of them had neutral perception towards retardation. The mean and standard deviation of score of perception of under studied samples was 45.31±7.82. The items of perception of care-givers of mental retarded person towards mental retardation have come in table 2.

The results of the study showed that, no significant relation was observed between the sex of care-givers and their age with their perception towards mental retardation (P=0.4 and P=0.80 respectively). Also, no significant relation was observed between the perception of care-givers with the sex and age of mental retarded person (P=0.9 and P=0.66 respectively). While, perception of care-givers towards retardation with the level of education had significant relation (P=0.00) and care-givers having higher level of education had a better perception towards mental retardation. Results of the study, using x^2 indicated that, the perception of care-givers had significant relation with their occupation (P=0.00), in such a way that, employee care-givers had a better perception towards mental retarded person and housekeepers had a lower perception towards mental retarded person (table 1).

Table 1- Personal characteristic of under studied samples and their relation with the perception of care-givers towards mental retardation.

Variable / The perception of care-givers towards mental retardation		Number (percentage)	x^2 test and P-value
Sex of care-givers	Man Woman	56 (56.0) 44 (44.0)	x^2=0.91 P=0.33
Age of care-givers (year)	≤35 36-45 ≥46 Mean±standard deviation	36 (36.0) 37 (37.0) 27 (27.0) 40.63±10.89	x^2=0.44 P=0.80
Education level of Care-givers	Illiterate High-school and lower Diploma University	31 (31.0) 37 (37.0) 15 (15.0) 17 (17.0)	x^2=25.66 P=0.0001
Occupation of care-givers	Unemployed Free Employee Worker Farmer	11 (11.0) 23 (23.0) 30 (30.0) 23 (23.0) 8 (8.0)	x^2=30.54 P<0.0001
The age of mental Retarded person (year)	≤ 7 8-12 13-17 ≥18 Mean±standard deviation	17 (17.0) 32 (32.0) 36 (36.0) 15 (15.0) 12.45±4.80	x^2=0.58 P=0.66
Sex of mental retarded person	Girl Boy	38 (38.0) 62 (62.0)	x^2=0.01 P=0.90

Table 2- Frequency distribution of perception of care-givers of mental retarded persons towards mental retardation.

Items of perception of care-givers of mental retarded person towards mental retardation.	Absolutely agreeable	Agreeable	No opinion	Oppose	Absolutely oppose
	Number (Pc.)	Number (Pc.)	Number (Pc.)	Number (Pc.)	Number (Pc.)
1-Family is the best shelter for mental retarded person.	8 (8.0)	13 (13.0)	4 (4.0)	33 (33.0)	42 (42.0)
2-The existence of retarded person causes the incidence of abnormal behaviors among healthy children.	24 (24.0)	17 (17.0)	11 (11.0)	30 (30.0)	18 (18.0)
3-The existence of retarded person at home causes the familial visits to be reduced.	12 (12.0)	14 (14.0)	10 (10.0)	40 (40.0)	24 (24.0)
4-The existence of retarded person causes the creation of depression among family.	8 (8.0)	20 (20.0)	3 (3.0)	22 (22.0)	46 (46.0)
5-Facing with a mental retarded person creates hopelessness.	21 (21.0)	12 (12.0)	13 (13.0)	34 (34.0)	20 (20.0)
6-A retarded person feels peace only when he/she is living with the same persons.	21 (21.0)	36 (36.0)	9 (9.0)	9 (9.0)	25 (25.0)
7-Retarded person should be guided towards self-sufficiency and independency.	9 (9.0)	10 (10.0)	3 (3.0)	40 (40.0)	38 (38.0)
8-It is better to consider some facilities for the family by Government for keeping mental retarded person.	40 (40.0)	31 (31.0)	7 (7.0)	14 (14.0)	8 (8.0)
9-Whole society should feel responsibility against mental retardation.	10 (10.0)	16 (16.0)	10 (10.0)	32 (32.0)	32 (32.0)
10-Allocating any type of expenditure to treat mental retarded person is useless.	23 (23.0)	19 (19.0)	8 (8.0)	26 (26.0)	24 (24.0)
11-Mental retarded persons have the right of possessing proper occupation with their condition.	11 (11.0)	17 (17.0)	8 (8.0)	31 (31.0)	33 (33.0)
12-The future of mental retarded persons is ambiguous and anxiousness.	14 (14.0)	12 (12.0)	10 (10.0)	29 (29.0)	35 (35.0)
13-Fortune plays role in creation of mental retardation.	37 (37.0)	23 (23.0)	11 (11.0)	18 (18.0)	11 (11.0)

Discussion and Conclusion: The perception of parents towards retardation is effective on relation with the child and social contacts of parents and family (3). Results of this research showed that, most of care-givers had neutral perception towards retardation. In addition, there was relation between the perception of care-givers towards retardation with their occupation and level of educations.

Asians specially Chinese also had relatively a low perception towards mental retardation and were the partisan of indiscrimination of persons suffering from mental retardation with other people of

the society. This is the case that, Mobbaraki and Zadbagheri have mentioned their attitude to be high (8). The beliefs of people of our country and believing on fate may have influenced on their perception. It seems that, this problem may also have caused the neutral perception among the samples of present research.

Most of care-givers were absolutely opposed or opposed with the fact that, family is the best shelter for mental retarded person. Family can play the most important role in caring of retarded person. But, several challenges and problems which are being created in these families can affect negatively on different dimension of the life of family members and influence their physical and psychical health (9). This problem may causes that, parents do not have propensity of keeping their retarded child at home and say that, a retarded person feels peace only when he/she is living with his/her similar persons.

On the other hand, 46.5% of samples believed that, the existence of retarded person in the family causes the incidence of abnormal behaviors in healthy children. Family members face several emotional problems like denial (10), shock, nervousness, feeling sin (2 and10), shame, depression, daydreaming and fear due to the retardation of their brother or sister(10).

Most of care-givers believe that, the existence of retarded person at home does not cause the reduction of familial visits, depression and creation of despair and hopelessness. Although the presence of retarded child at home can affect care-givers (11) but, increasing contact by parents, the adaptability of family members may be increased and they do not have depression, despair and hopelessness feeling. Also, increase of contact by parents with retarded person will be resulted in the attitude of parents towards retardation to become positive and improvement of their feeling towards the retarded person (5).

Most of care-givers (65.5%) mentioned that, retarded person must receive the necessary guidance for self-sufficiency and independency. Researchers state that, retarded person have the right to enjoy the appropriate educations so as to reach self-sufficiency via these educations (12). A large percentage of care-givers were absolutely agree or agree with the fact that, it is better to consider some facilities for the families by Government to keep the mental retarded person at home. The presence of mental retarded person at home can produce economical difficult for the family (3) because mother of family should takes care of retarded person all of her times. This problem will prevent her to have an occupation. On the other hand, retarded persons require several medical treatments due to the existence of physical and underline diseases that could impose large expenses on family. Therefore, these persons believe that, it s better to consider some helps for such families by Government. Of course, it should be attended that, other people are also responsible against such persons and parents expect to receive the supports of relatives other than Government (13).

A half of under studied samples believed that, allocating any type of expenditure to treat retarded person is not useless and considering expenditure for them is valuable. There is a large propensity for the presence of mental retarded children in normal schools, making social relations and employment of such persons during recent years (3). Considering that, some of these children require training programs and therapeutic interventions, it is better to spend some expenditure for such persons so as to observe their attendance in the society. 61.0% of parents stated that, these children do not have an occupation suitable to their condition. In spite of the facts that, Government programmers have decided to train such persons but still they do not possess a desirable occupation. Occupation plays an important role in producing well-being and healthy feeling and a better eco-social situation. Negative attitude and perception towards possessing occupation by retarded persons can leave negative effect on the health of such persons (14 and 15). Relatively a large percentage of care-givers believed that, the future of mental retarded persons is not ambiguous anxiousness. In such condition, the results of the qualitative study of Kermanshahi regarding the perception of care-givers towards retardation showed that, mothers are anxious about the future of their child. One of anxieties of mothers was that, whether they can take care of their children in the future or not? In addition, parents faced with such questions that, whether their children can do their own activities alone, get married and have an occupation in the future (2). The non-anxiety of parents regarding the future of their children could be attributed to the carried out planning in connection with the entrance of such children to the usual schools or the adaptability of parents.

Care-givers stated that, fortune plays role in creation mental retardation. In Iran′s culture, having a child is a present from God and God influences on physical, emotional and economical aspects of life of individuals. Families having mental retarded child feel that, they have been more attended by God and accept diseases and problems as God′s examination. This belief is effective in acceptance of retarded person in the family (2).

The results of the study indicated that, care-givers with higher level of education had a better attitude towards mental retardation. Wolff et-al. believe that, persons with a low level of education are having a negative attitude towards retardation (16). Other researchers also pointed out that, negative attitude towards retardation is observed rarely among persons with higher level of education (17).

There was a relation between the perceptions of care-givers with their occupation in this study. It means that, employee care-givers had a better perception towards mental retarded persons and house-keepers had a lower perception in this regard. Researchers believe that, the occupation of parents and their ability to provide the required expense of family and retarded person is effective in increase of their obligation for taking care of such person (18 and 19). Finally, doing this research sectionally is among it′s limitations. In order to obtain more information about challenges facing care-givers having a retarded child, it is suggested to carry out a long study and evaluate the effect of time-lapse on the perception of parents. Since various factors could influence on the perception and attitude of care-givers towards retardation, it is suggested to carry out qualitative researches regarding the personal and social perception and experiences of care-givers having a retarded child. In addition, carrying out more studies in relation with the quality of life of care-givers having a retarded child and doing more interventions to improve their quality of life is suggested.

Final Conclusion: In this study, care-givers having a lower level of education and house-keepers had a weaker perception towards the retarded persons. Therefore, health care-givers could identify these risky exposed groups and take a step to improve their perception by presenting more supports. In this study, care-givers also believed that, family is not the most important shelter for mental retarded persons. Therefore, care-givers are recommended to send retarded person to welfare and children caring centers occasionally so as to so as to reduce some of their responsibilities as well as probable tensions of family specially mother. Researchers hope that, more attention be made to mental retardations and their families and also consider huge plans to promote the perception of care-givers of retarded persons by responsible throughout the society.

Corresponding author:
Najafi doulatabad Shahla
Department of Medical surgical nursing,
School of Nursing and Midwifery,
Yasouj University of Medical Sciences, Yasouj, Iran.
Email: shahlanajafi@gmail.com, shahlaiss@yahoo.com

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Overcoming Actuators Saturation Problem in Structural Active Control

A. E. Bakeri

Department of Structural Eng., Faculty of Eng., Zagazig University, Zagazig, Egypt
aebakeri@zu.edu.eg

Abstract: Since the actuator capacity is limited, in the real application of active control systems under severe earthquakes, it is conceivable that the actuators saturate, hence the actuator saturation should be considered as a constraint in the design of optimal controllers. In this paper, a new procedure for structural active control is proposed to overcome the actuators saturation. This approach is based on elimination of structural response as early as possible to save the high control force required later due to the response generated from the small recent response taking into consideration the actuator capacity. The proposed approach is formulated and applied to single and multi-story buildings subjected to ground motion. Two types of ground excitations are considered. The first is sinusoidal and in resonance with building. The second type of excitation represented by several real earthquakes. The proposed approach is compared with the traditional optimal control in two manners, when the maximum control force in the two approaches is maintained the same and when the response reduction level is the same. It is found that the proposed approach does not only overcome the actuator saturation, but it also reduces the response for all cases considered, namely, single or multi-story building, light or heavy damped structures, and when buildings are subjected to sinusoidal or real ground motion.
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Keywords: Structural dynamic, Active control, Tendon, Earthquake, Actuator saturation

Nomenclature
[M]: mass matrix of the structure [C]: damping matrix of the structure [K] : stiffness matrix of the structure
{y} : displacement response vector of the structure $\{\dot{\mathbf{y}}\}$: velocity vector of the structure
$\{\ddot{\mathbf{y}}\}$: acceleration vector of the structure {m}: mass vector of the structure $\ddot{\mathbf{y}}_{\mathbf{g}}$: ground motion acceleration,
{b}: control force location vector u: control force

1. Introduction

During the last three decades, various methods have been developed and applied to suppress structural vibration in numerous areas. Vibration control of lightly damped and flexible structures such as high-rise buildings, long-span bridges, and so on has been widely researched. To improve their inherent low damping ratios, various kinds of passive, active, and semi-active devices have been developed and applied to many real-world structures. The idea of applying active control as a means of hazard reduction has become increasingly popular [1, 2]. In the design of an active control system, the objective is to reduce the structural response (accelerations, velocities, and displacements) under the limitation of both the control force level (limited by the actuator capability and the required amount of energy) and the number of measured signals. In active control of real-world building structures, active tendons, active mass damper, active tuned mass damper, and hybrid mass damper have been widely used and verified as promising devices [3- 5].

One matter that is encountered in controlling structures is the actuator saturation problem, which has been a topic of interest over the past several years [6–11].

Actuator saturation may lead to instability, and may also lead to serious deterioration in the performance of the closed-loop system. Numerous proposed solutions to the actuator saturation problem include Riccati and Lyapunov-type local and semi-global stabilization methods, the anti-windup technique, and absolute stability theory. In recent research [6,12–14], methods that can employ large gains of control input and provide guaranteed performance levels were developed.

Most controllers have been designed based primarily on linear control theories like optimal linear quadratic regulator formulation, H_2 control technique, direct velocity feedback method [15- 17]. However, linear control theories may not be effective in producing significant peak response reduction which is practically important for buildings safety. In addition, in some approaches the response of the controlled system exceeds the uncontrolled system. In order to overcome these problems of linear controllers and saturation, a new approach is developed. This approach is summarized in that it is preferred to eliminate the structure response as early as possible by

exerting control force within its limit because any small response may cause later higher response which require more control force. The new approach is applied to single story structures controlled actively by tendons and the results are compared with that of the traditional optimal linear approach. The approach is extended to apply on the multi-story structures.

2. THEORY:

2.1. Optimal active control approach:

The equations of motion of an active controlled multi-story structure shown in Figure 1, when subjected to ground motion are given by:

$$[M]\{\ddot{y}\}+[C]\{\dot{y}\}+[K]\{y\}=-\{m\}\ddot{y}_g+\{b\}u \quad (1)$$

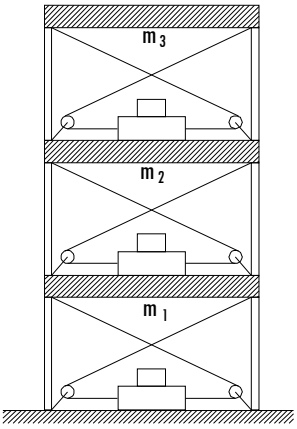

Figure 1. Controlled Structure

The response vector {y} is given by;

$$\{\mathbf{y}\}=[\mathbf{y_1} \quad \mathbf{y_2} \quad \cdots \quad \mathbf{y_n}]^{\mathbf{T}} \quad (2)$$

where y_i is the displacement response of the ith level. The equations of motion can be recasted in the *State Space Formulation* as follow;

$$\dot{\mathbf{X}}=\mathbf{A}\,\mathbf{X}+\mathbf{B_u}\,\mathbf{u}+\mathbf{B_f}\mathbf{F} \quad (3)$$

where {X} is the state vector which defined as

$$\mathbf{X}=\begin{Bmatrix}\mathbf{y}\\ \dot{\mathbf{y}}\end{Bmatrix}$$

and the matrices A, B_u, B_f and F are given by:

$$\mathbf{A}=\begin{bmatrix}\mathbf{0} & \mathbf{I}\\ -[\mathbf{M}]^{-1}[\mathbf{K}] & -[\mathbf{M}]^{-1}[\mathbf{C}]\end{bmatrix},\ \mathbf{B_u}=\begin{Bmatrix}\mathbf{0}\\ \mathbf{b}\end{Bmatrix},$$

$$\mathbf{B_f}=\begin{Bmatrix}0\\ \{-1\}_{n\times l}\end{Bmatrix} \text{ and } \mathbf{F}=\{\ddot{\mathbf{y}}_g\}_{l\times l}$$

The output response can be expressed using the state vector as follows;

$$\mathbf{Y}=\mathbf{H}\,\mathbf{X}+\mathbf{D}\,\mathbf{F} \quad (4)$$

where {Y} is the output response vector containing y, $\dot{\mathbf{y}}$ and $\ddot{\mathbf{y}}$ as follows;

$$\mathbf{Y}=\begin{Bmatrix}\mathbf{y}\\ \dot{\mathbf{y}}\\ \ddot{\mathbf{y}}\end{Bmatrix} \quad (5)$$

and the matrices H and D are given by;

$$\mathbf{H}=\begin{bmatrix}\mathbf{I} & \mathbf{0}\\ \mathbf{0} & \mathbf{I}\\ -[\mathbf{M}]^{-1}[\mathbf{K}] & [\mathbf{M}]^{-1}[\mathbf{C}]\end{bmatrix}, \text{ and } \mathbf{D}=\begin{Bmatrix}\mathbf{0}\\ \mathbf{0}\\ -\mathbf{1}_{n\times 1}\end{Bmatrix}$$

In linear optimal control theory, the control force (u) is represented as a linear function of the state vector X, i.e.

$$\mathbf{u}=-\mathbf{G}\,\mathbf{X} \quad (6)$$

and the constant time invariant gain vector (G) is obtained by minimizing the quadratic objective function (J), where J is given by;

$$\mathbf{J}=\int_0^\infty\left(\mathbf{x}^*\mathbf{Q}\,\mathbf{x}+\mathbf{u}^*\mathbf{R}\,\mathbf{u}\right)\mathbf{dt} \quad (7)$$

where Q is a positive-definite (or positive-semi-definite) Hermitian or real symmetric matrix and R is a positive-definite Hermitian or real symmetric matrix and they consider the relative importance factors for controlling the response and the control force.

The gain vector is given by [18] :

$$\mathbf{G}=\mathbf{R}^{-1}\mathbf{B}^*\,\mathbf{P} \quad (8)$$

where the matrix P is obtained using the Riccati equation ;

$$\mathbf{A}^*\,\mathbf{P}+\mathbf{P}\,\mathbf{A}-\mathbf{P}\,\mathbf{B}\,\mathbf{R}^{-1}\,\mathbf{B}^*\,\mathbf{P}+\mathbf{Q}=\mathbf{0} \quad (9)$$

2.2. The proposed approach:

The state space form of the equation of motion (Eq. 3) can be converted to discrete system and solved numerically as follow;

$$\mathbf{X_{k+1}}=\mathbf{e}^{\mathbf{A\,dt}}\mathbf{X_k}+\left(-\mathbf{B_u}\,\mathbf{u_k}+\mathbf{B_f}\mathbf{F_k}\right)\mathbf{dt} \quad (10)$$

The negative sign before the control force is due to the fact that its direction is opposite to the motion direction.

To eliminate the response in the next step, the left hand side of equation (10) should be zero, then the control force (u_k) can be calculated from the following equation;

$$\mathbf{u_k}=\mathbf{B_u^*}\left[\frac{\mathbf{1}}{\mathbf{dt}}\mathbf{e}^{\mathbf{A\,dt}}\mathbf{X_k}+\mathbf{B_f}\mathbf{F_k}\right] \quad (11)$$

where $\mathbf{B_u^*}$ is the Moore-Penrose pseudoinverse of the matrix (Bu).

The calculated control force may be within its limit, then applying this force will eliminate the response as possible as in the next step. If the

calculated control force exceeds its limit, the control force is applied as its upper limit and the response is calculated according to Equation (10).

Two Matlab programs are developed. The first follows the traditional optimal active control approach when variable weighting matrices are available. The second program apply the proposed approach to the same structures in two cases, the first case when the control force limit is adjusted as the maximum force obtained from the traditional approach to show that the same control force is reduced drastically in the structure response for the proposed approach. Then many indices are constructed as follow:

First Response Index (RI1): the ratio between the peak response of the traditional controlled and the uncontrolled.

Second Response Index (RI2): the ratio between the peak response of the proposed controlled and the uncontrolled.

Third Response Index (RI3): the ratio between the peak response of the proposed controlled and the traditional controlled.

Control Force Index (CFI): the ratio between the peak control force and the structure weight.

The second case when the same response reduction is adjusted to compare the control force and compare also the quadratic objective function (J). Then a control force index (CFI2) is constructed as a ratio between the control force of the proposed controlled and that of the traditional controlled. Also quadratic objective function index (JI) is created as ratio between the quadratic objective value of the proposed controlled and that of the traditional controlled. These indices are obtained for a constant level of response reduction (RI).

3. IMPLEMENTATION AND RESULTS

3.1. Single story structure:

A single story structures have a varied dynamic properties as shown in Table 1. These properties are selected to cover wide range of structures. The structures are controlled by the two approaches when subjected to many sinusoidal ground motion records with resonance frequencies of amplitude 0.3 g. The weighting matrices in the traditional control approach is adjusted as Q_{ij}=0; Q_{ii}=100; and R=0.1. The response indices are shown in Table 2. It is shown that the proposed approach is better than the traditional approach especially in case of flexible structures than the rigid ones, and in case of light damped than the heavy ones. This conclusion is shown in Figure 2, where the third response index (RI3) is plotted for all structures.

To emphasize the benefit of the proposed approach in reducing the control force, the eight selected structures are controlled by the two approaches to maintain the same level of response reduction. The control force is calculated by the two approaches. Table 3 shows the control force index and the quadratic objective function index when the response reduction level is adjusted to 0.1. It is clear that the proposed approach leads to lower control force (about 77 %) and in most cases lower quadratic index. The value of the quadratic objective function index (JI) less than unit means that the proposed approach is better than the traditional approach from the view point of traditional approach. This also means that the procedure that follow in optimal control approach does not lead to the optimal solution because there is another approach get better solution.

Table 1. Dynamic Properties of the Studied Structures

Structure No	Mass (kN/m.sec2)	Natural Frequency	Damping ratio
1	10	5	2
2	10	5	5
3	10	10	2
4	10	10	5
5	10	15	2
6	10	15	5
7	10	20	2
8	10	20	5

Table 2. Response indices for single story structure

Structure No	RI1	RI2	RI3	CFI (%)
1	0.101	0.001	0.007	28
2	0.172	0.010	0.057	25
3	0.147	0.003	0.018	26
4	0.303	0.109	0.358	21
5	0.197	0.008	0.039	24
6	0.428	0.271	0.633	17
7	0.250	0.039	0.155	23
8	0.534	0.406	0.761	14

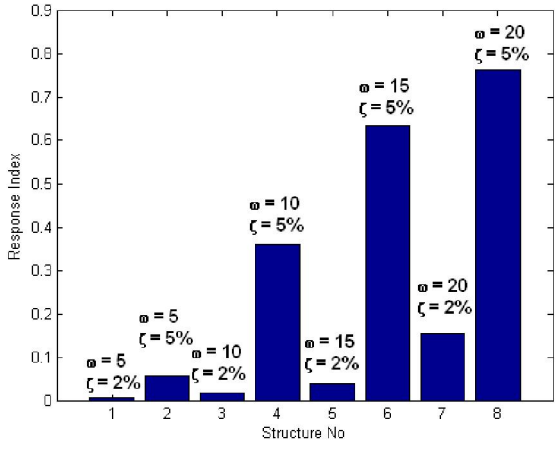

Figure 2. Third Response index (RI3) for single story structures

Table 3. Indices of single story structure for the same response reduction

Structure No	RI1=RI2	FI	JI
1	0.1	0.75	0.88
2	0.1	0.78	0.92
3	0.1	0.77	0.91
4	0.1	0.78	1.02
5	0.1	0.78	0.93
6	0.1	0.78	1.05
7	0.1	0.79	0.94
8	0.1	0.79	1.06

One of the problems that face the active control techniques is that the good results obtained in the case of ideal ground motion is diminished when applied to real ground motions. The structures number three and four are subjected to six earthquakes where their information are shown in Table 4. These earthquakes are scaled to 0.3 g. The two selected structures are subjected to these earthquakes when uncontrolled and when controlled by the two approaches when the same control force is adjusted. Table 5 shows the maximum displacement of the uncontrolled and the response indices for each earthquake. It is shown from the results of the third response index (RI3) that the proposed approach is more useful than the traditional approach in all cases and this benefit is clear in case of light damped structures than the heavy damped structures. It must be noticed that in some cases the proposed approach eliminates the structure vibration (zero values) with the same control force of the traditional approach, because the proposed approach apply the control force in early time. This early application of the force eliminates the small response which in the case of traditional approach causes additional response which require more control force later.

Table 4. Information of the applied six earthquakes

Earthquake name	Date	Magnitude	Location
El-centro	May 18, 1940	7.1	El centro, California
Hachinohe	May 16, 1968	7.9	Hachinohe city
Kobe	Jan. 17, 1995	7.2	Kobe japanese
Northridge	Jan. 17, 1994	6.8	Sylmar, California
Pacoima	Feb. 9, 1971	6.2	San fernando
Parkfield	Jun. 27, 1966	7.3	Parkfield, California

Table 5. Response Indices for Control approaches

Earthquake	Zeta = 2%				Zeta = 5%			
	Max. Disp.	RI1	RI2	RI3	Max. Disp.	RI1	RI2	RI3
El-centro	0.07	0.22	0.00	0.00	0.06	0.27	0.00	0.00
Hachinohe	0.06	0.29	0.00	0.00	0.04	0.42	0.02	0.04
Kobe	0.11	0.32	0.11	0.34	0.09	0.42	0.17	0.40
Northridge	0.03	0.49	0.00	0.00	0.02	0.63	0.00	0.00
Pacoima	0.03	0.37	0.22	0.59	0.02	0.51	0.32	0.63
Parkfield	0.13	0.21	0.02	0.09	0.10	0.27	0.04	0.14

3.2. Multi-story structure:

Two multi-story buildings are considered where they have the dynamic properties that shown in Table 1 for structures number three and four but for all stories and they have ten stories. The structures are controlled by the two approaches when subjected to sinusoidal ground motion with the lowest fundamental resonance frequency. Figure 3 shows the maximum drift of the uncontrolled, traditional controlled and proposed approach when the control force is adjusted as 2 % of the individual slab weight when the damping ratio for all modes is 2%. It is found that the new approach has a good reduction than the traditional approach in all stories by the same control force that calculated with the traditional approach.

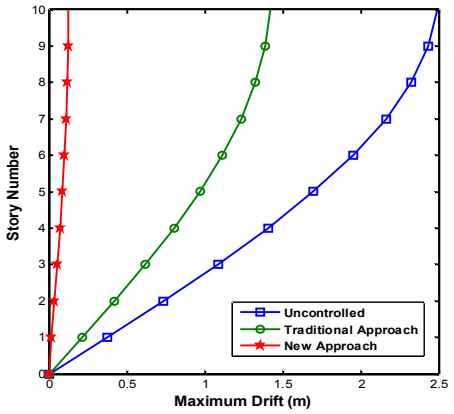

Figure 3. Maximum Drift for Ten story building

The ten story building with damping ratio 2% is subjected to Kobe earthquake when its record is adjusted to 0.3 g. Figure 4 shows the top story displacement of the uncontrolled, traditional controlled and new controlled structures. It is found that the new approach approximately eliminate the response.

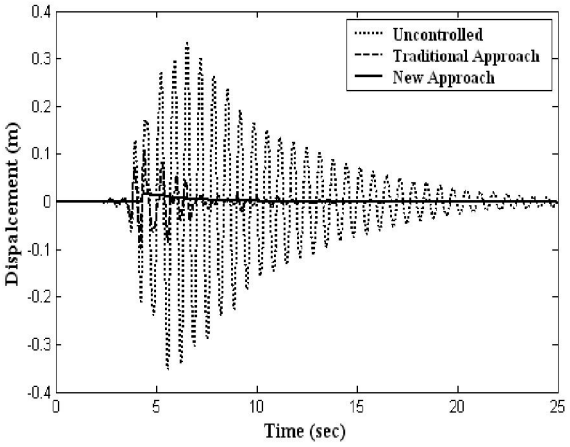

Figure 4. Top story drift of ten-stories building (zeta=2%) subjected to Kobe earthquake.

The multi-story structures are subjected to the records of the six earthquakes where the weighting factors in the traditional approach is adjusted as Q_{ii} = 10, R_i=0.01 when the control force in the new approach is adjusted within the limit of that obtained from the traditional approach. Table 6 shows the maximum drift ratio of the two buildings when controlled with the two approaches. It is shown that the new approach make more reduction in the response than the traditional approach with the same control force. It is found that the reduction of light damped structure is more than that of the heavy damped structure. The benefit of the new approach is not limited to the reduction of the structural displacement but it also reduces the velocity of the building as shown in Table 7.

Table 6. Displacement peak ratio of traditional and proposed approach

Earthquake	Zeta = 2%		Zeta = 5%	
	Trad.	New	Trad.	New
El-centro	0.50	0.17	0.55	0.24
Hachinohe	0.28	0.21	0.37	0.31
Kobe	0.32	0.05	0.48	0.11
Northridge	0.35	0.08	0.42	0.15
Pacoima	0.29	0.07	0.34	0.07
Parkfield	0.33	0.15	0.47	0.24

Table 7. Velocity peak ratio of traditional and proposed approach

Earthquake	Zeta = 2%		Zeta = 5%	
	Trad.	New	Trad.	New
El-centro	0.57	0.18	0.65	0.25
Hachinohe	0.36	0.21	0.40	0.28
Kobe	0.44	0.06	0.60	0.11
Northridge	0.36	0.10	0.41	0.16
Pacoima	0.31	0.08	0.36	0.09
Parkfield	0.36	0.13	0.52	0.21

To make a fair comparison between the traditional and proposed approach, a study was conducted to investigate the relation between the response ratio of the multi-story building and the required control force for the two approaches. Figure 5 shows the relation between Response ratio (which indicated the ratio between the maximum drift of the controlled and the uncontrolled structures) and the control force ratio (which indicated the ratio between the maximum control force and the individual slab weight) for ten story building with 2 % damping ratio. It is shown that the proposed technique is better than the traditional where the same control force makes more reduction and the same level of response reduction require smaller control force. The same conclusion is noticed for building with 5% damping ratio as shown in Figure 6.

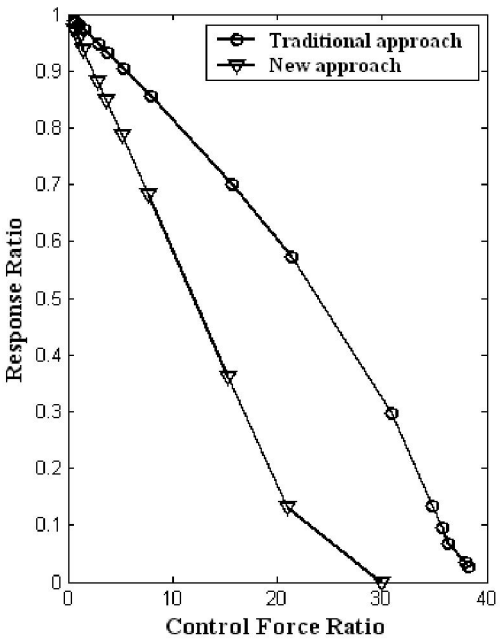

Figure 5. Relation between Response ratio and control force ratio for ten story building with 2 % damping ratio

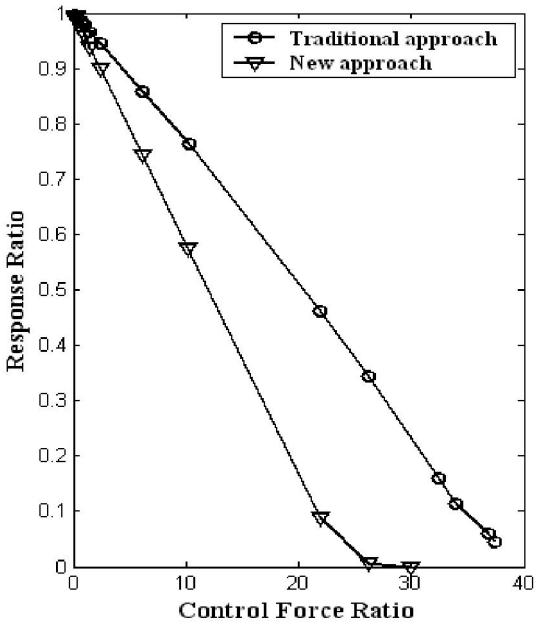

Figure 6. Relation between Response ratio and control force ratio for ten story building with 5 % damping ratio

4. Conclusions:

A new approach for structural active control is proposed to overcome actuators saturation problem. This approach is based on eliminating the structural response as early as possible to save the high control force required later due to the response generated from the small recent response. This approach is formulated and applied to single story and multi-story buildings. Firstly when the ground motion is ideal and in resonance with building and secondly when the ground motion is real earthquakes. The proposed approach is compared with the traditional optimal control in two manners; when the control force in the two approaches is maintained constant and when the response reduction level is the same. The main findings from this research are:

1. The proposed approach overcomes efficiently actuators saturation problem in structural active control.
2. The proposed approach decreases the structure response more than the optimal active control approach especially in case of flexible structures than the rigid ones, and in case of light damped than the heavy ones when the maximum control force in each approach is remained constant.
3. The proposed approach requires smaller control force than that of the traditional approach to make the same level of response reduction.
4. The benefit of the proposed approach is cleared when real ground motion is applied to the structure when highly response reduction is obtained.
5. The proposed approach reduces the velocity of the structure as that of the displacement.

Corresponding author
A. E. Bakeri
Department of Structural Eng., Faculty of Eng., Zagazig University, Zagazig, Egypt
aebakeri@zu.edu.eg

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Designing, construction and evaluation of tractor-back sprayer with Variable Rate Technology (VRT) by using aerial maps information

Mehrdad Fouj Lali[1], Parviz Ahmadi Moghadam[2]

[1] MSc in Mechanics of Agricultural Machinery, Urmia University, Iran
[2] Assistant Professor in Mechanics of Agricultural Machinery, Urmia University, Iran
Mehrdad.lali@gmail.com

Abstract: In recent years, concerns about utilizing various chemical toxicants in different sections of agriculture and also environmental pollutions increased and also convinced researchers and scientists in agricultural sector to discuss and offer new methods related to improve the toxicant management. One of the main disadvantages of increase the use of chemical toxicants in agriculture is transfer chemical materials to food through the soil and cause liver or respiratory cancer. Nowadays, in most countries, spraying fertilizer in farm fields is done without regard to changes in soil toxicant and also need of plant to toxicant and carried out uniformly to all parts of the farm and finally toxicant use and environmental pollution would increase. In developed countries, development of precision farming and using Variable Rate Technology (VRT) is growing rapidly. One of the vital nutrients for plant growth is nitrogen (N). Due to the lack of nitrogen in most agricultural soils, utilizing of nitrogen toxicant in agriculture is more than all other toxicants. On the other hand, excessive consumption of nitrogen would have negative effects on water quality and ecosystem of living organisms. In this paper, designing, construction and evaluation of chemical liquid toxicant spray with Variable Rate Technology (VRT) were carried out and it's used two types of electric valves in Variable Rate Technology (VRT). This technology has an intelligent processor system that spread the toxicant based on soil needs. System performance has been evaluated at different temperature and humidity. Finally, it has been concluded that valve opener have a stepper motor and busted water which have higher performance speed rather than analog gas valve cutter. In addition, humidity and ambient temperature had little effect on the system and toxicant spraying by the system saving $15 per acre and also is useful than traditional methods. By accurate available toxicant at the farm level, it's possible to plenty of products would die and has maximum environmental negative effects.

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Keywords: Toxicant spray, Variable rate, Electric valve.

1. Introduction:

Economic and environmental issues in addition to technical issues have been forced many agricultural producers to employ new methods in the management of agricultural production. Due to increasing agricultural production with the supply of mineral and organic resources for soils used by plant, therefore, the toxicant was sprayed on the crops in harvesting phase is very important. So, modern and accurate toxicant sprayed would have many benefits. All of its benefits including increase of crops quality, crops performance and also economic benefits for farmers. One of the main disadvantages of increasing the use of agricultural pesticides and weed removal is that pesticide transfer to food from the soil and cause liver and respiratory cancer. Nowadays, in most countries, fertilizing in farm fields is done without regard to changes in soil fertility and need of plants to the toxins and would carried out for all parts of farm uniformly. Non-normative broadcast of toxicants at the farm level would have negative economic effects and would increase social and environmental concerns. Many of chemical toxicants without being intake by plant would enter into surface water and groundwater and causing toxicity and environmental pollution (6). Philosophy of precise agriculture is that all agricultural inputs such as pesticides and toxicant and etc. that needs to be applied to any part of farm. In this type of agriculture, it is possible to calculate and evaluate differences among smallest possible levels and then different inputs are used based on various types. Variable Rate Technology (VRT) is a management strategy for addressing the spatial variability within the plantation. In other words, VRT is the optimal allocation of production inputs. In conventional methods using toxicants, farm and its products can be considered uniform and for use of toxicants for soil fertility in the farm and the medium for an additional one percent as a safety factor, amount of toxicant per hectare are determined and will be distributed equally in the field. With this method, it's possible to achieve the lowest cost and least environmentally damaging. Mainly, nitrates that are washed from the soil leads into groundwater.

Resources that provide exceed of 80% of drinking water for citizens. High levels of nitrogen in lakes would threaten aquaculture. Limit of toxicant spraying to areas with a shortage of farm organic materials could significantly increase the saving amount of toxicant. This approach is in order to optimize the amount of chemical toxicant consumption which led to decrease of cost for farmers and then would lead to increase in product in unit-level, maintain soil structure and increase fertility. Welsh et al. (2002) had used variable rate nitrogen application in the field of wheat and winter barley which their performance is 0.36 and 0.46 per hectare respectively rather than uniform spread of toxicant. Hong et al. (2011) prepared spatial changes of soil fertility factors (K.P.N) function crested wheat using satellite digital images in blocks of 5 for use in variable-rate machines. These maps show that in total spreading of urea, for maximum performance, only 13 percent of farm receives appropriate toxicant and other farms receive more or less amount of needed nitrogen. While in VRT methods, at least 52 kg per hectare of urea were saved. Paz et al. (2008) showed that level of nitrogen could be reduced, while have more production than uniform performance method. Scientists used this method in corn fields of Iowa State which its average rate was decreased 11 kg/ha than toxicant uniform performance and 15.66 USD per hectare was obtained as benefits. Ulson et al. (2011) constructed an intelligent system of toxicant performance with two neural networks which output of the first consider performance rate equal to satellite maps and also outline of second neural network as a flow rate. The flow rate had been controlled by a Korean valve with electrical sensor. Korean sensor with electrical motor for complete openness (from 10 to 90 degrees) would need to 0.4-1 second. This case was effective in reducing the load of hydraulic system. Besides, the main dimension of this research is the fast response of system. Roger et al. (2011) had applied variable rate in Australian farms and had concluded that would have 15 USD economical savings per hectare. Hong et al. (2011) had used ultrasonic sensor in variable rate system to evaluate latitude and longitude. In fact, the purpose of research was toxicant and toxin spreading on trees and so the had resulted that sensor has a low performance in open area, because by lowering the temperature, the error rate of system would increase, but other factors such as dust, had not significant effect on the system. Zaman et al. (2011) had used variable rate for blueberries and based on various experiments on the plant, they had concluded that plant had a better growth in comparison with traditional method and reduction of toxicant consumption in fertilizing process. However, based on the importance of issue, it would be necessary to savings, environment conservation and also protection of soils and waters against pollutions. The purpose of this study is designation, construction and evaluation of variable rate toxicant spraying which should be simple and low-cost. Finally, it should be possible to spreading toxicants by this machine.

2. Materials and Methods:

For variable rate in system, to type of valves had been designated and constructed. For the first valve, it had been used a combination of stepper motor and busted water. In regards to this issue that stepper motors are under control in any round and angular, they were appropriate for this research rather than direct current motors. For this valve, we had used a 24 (V), 1.5 (A) stepper motor which could endure 12 Kg of forces in 200 (rpm). Connection between circle pin and engine was done by an interface copper metal. Since there is fluctuation probability in Circle-based workflow engine, so by connection of this coupling, this probability takes aside (Figure 1). By receiving necessary voltage and frequency to determine round amount in engine from central control system, stepper started to work and would open and close the valve. This kind of situation changes from open state to close state, would take 3 seconds. In the second valve of a direct current motor with a transmission that is in the range of 90 degrees, one gas valve was built (Figure 2). By reaching the positive 12 voltage signal to the engine, lever connected to the gas valve would positioned clockwise and otherwise would positioned anti-clockwise which lead to the openness or closeness of valve.

Figure 1. First electric valve

Figure 2. Second electronic valve

The used valve in this experiment was selected based on 0.25 inches pump pressure. The designated transmission would enable to changes the valve position just in 4 seconds from openness state to the completely close state. Inlets of two electrical valves were connected through 3 branches connected to the pump outlet (Figure 3). Inlet of pump suction was connected by plastic siphon filter to the tankers and 0.75 inches busted water is put between tankers and siphon to toxicant do not thrown out from tanker when testing and troubleshooting apparatus.

Figure 3. Water pomp and main valves

30 Designation and construction of electronic circuit and its installation on spraying liquid toxicant:

To evaluate flow rate amount and time of passing tractors in the region and total number of openness and closeness of electrical valves, an electrical circuit was designated and written by C program by using a microprocessor. This circuit composed of input, output and central processing unit. First, analyzed data of aerial maps include the needed amount of flow rate in the region and also needed time for passing of tractor from that region was entered to the apparatus by a keyboard. In the system menu, 20 routes were fitted as an arable land zoning. This was done to both tested valves. Processor sector was composed of a programmable microprocessor from RAM family which its task was receiving, storage, analysis and display of data. Since this microprocessor has the latest technology of programmable processor, its processing speed is high. In order to movement of tractors in farms, it would need to the high speed order to the electrical valve, so this processor had been used. In order to show the route information e.g. passing time of tractor and position of electrical valves, a LCD display was embedded in the circuit (Figure 4).

Figure 4. The central controller of apparatus

The input power in circuit would fed from tractor's battery and 12 input voltage from battery of tractor convert to 10 voltage by regulator and also current and voltage of circuit was set up by regulator in the process (Figure 7). In order to prevent noise interference on the tractor and the machine performance, the frame of circuit was selected from a metal around it was covered by aluminum tape. To communicate with the apparatus and receive the information about time and flow rate amount through computer instead of keyboard, input and output of serial was designated for circuit.

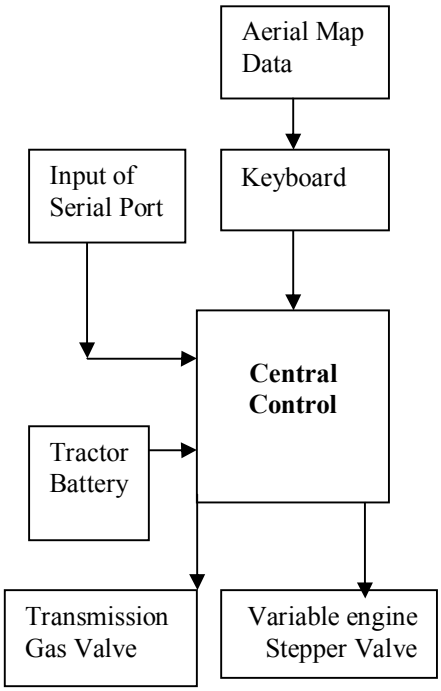

Figure 5. Systematic diagram of apparatus

Before entering the apparatus to the farm, it should be calibrate the precise of apparatus, its delay time and its speed. In this regard, evaluation of apparatus was carried out by virtual network on the asphalt in the laboratory and also by real networks in the farm. In this research, the parameters of consumption rate of toxicant location were studied spatially and uniformly. The accuracy and error rates of the machine in any particular area n both laboratory and field tests were evaluated. After testing and data recording, data were analyzed.

Results and discussions:

Amount of toxicant consumption: comparison of average liquid toxicant consumption in different plots based on spatial performance and uniform application had been showed in Figure 6.

As it's shown in the chart, the amount of consumption toxicant in spatial performance is 1949.52 ml and in uniform performance is 4680 ml (based on 4 nozzles and 50 meters movement). This matter means that in spatial performance method, we could have 34.58 percent of saving in toxicant consumption.

Diagram of Figure 7 shows comparison of toxicant applied in both ways in every 6 plots.

Increase or decrease of applied amount of toxicant in spatial performance method is depending on needed amount of toxicant in farm from theoretical data. While the amount of toxicant applied in the particular location is always fixed and not related to changes in soil nitrogen.

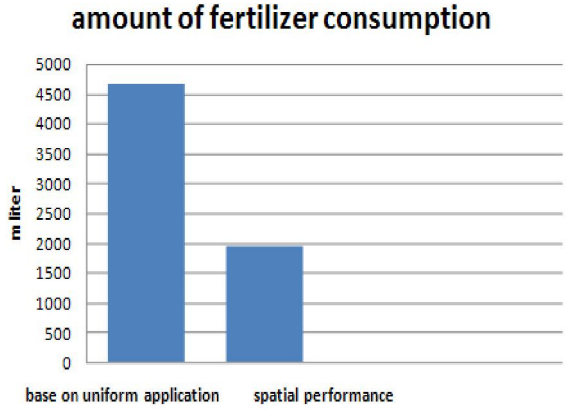

Figure 6: Chart of comparison of average liquid toxicant consumption in different plots based on spatial performance and uniform application

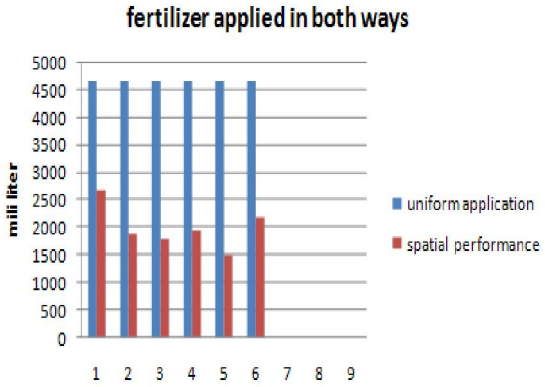

Figure 7. Comparison of fertilizer applied in both ways in every 6 plots.

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Comparison between Flexible Pavement Damage Due to Conventional and Wide-Base Tires of Heavy Multiple Axles

Hassan Salama[1], Ahmed Shehata[1]; Mahmoud Solyman[2]and Mohamed El Refaey[1]

[1]Civil & Environmental Engineering Department, Al Azhar University, Egypt
[2] Construction Engineering and Utilities Department, Zagazig University, Egypt
elsaied2000@yahoo.com

Abstract: Trucks are considered one of the most important means in transporting. Recently, the tire designers introduced new wide-base tires to replace the conventional dual tires system. The objective of this study is to investigate flexible pavement damage due to different heavy multiple axle configurations with wide-base tires. Several axle configurations including single, tandem, tridem and quad with conventional and wide-base tires were considered in this study. Two flexible pavement sections were analyzed, thick and thin pavement sections with thicknesses and material properties representing majority of the pavement cross-sections. To quantify and compare the damage for thick and thin pavement sections due to heavy axle load configurations, the forward analyses were conducted using KENLAYER program to calculate the pavement response. The horizontal tensile strain at the bottom of the hot mix asphalt and the vertical compressive strain on top of the subgrade and at the middle of each pavement layers as well as the six consecutive sub-layers of the subgrade soils were calculated from the structural model. These pavement responses were utilized in the performance models to calculate the two main pavement distress, fatigue cracking and pavement surface rutting. The strain area model for fatigue and VESYS rutting model for rutting were utilized to calculate the pavement damage. The Axle Factors were calculated for each axle configurations to compare the pavement damage due to axles with conventional and wide-base tires. The results indicate that axle loads with wide-base tires impose more fatigue and rutting damage than axles with conventional tires.
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Key words: Wide-base tire, dual tire, pavement rutting, pavement fatigue, pavement damage, multiple axles

1. Background

The 21st century witness enormous trading activities due to the economic alliances between the countries. This is in return increased the transportation activities on the road networks. Heavy trucks have major share in transporting freights. Recently, the truck industry introduces the wide-base tires which replacing the conventional tires to reduce fuel consumption, tire cost and repair, emission and noise, and recycling impact of scrap tires. Also, using the wide-base tire increase hauling capacity, ride and comfort, and improve handling, braking, and safety.

Since the introduction of wide-base tires, researchers started to compare the contact area, contact stress, the pavement response and damage effect on the pavement of both conventional and wide-base tire. For two types of wide-bases (385/65R22.5 and 425/65R22.5) at a constant load, the tire inflation pressure variation primarily affected the contact stresses in the central region of the contact area; the higher the inflation pressure, the greater the contact pressures in this central region. The contact pressures in outer portions of the tires were essentially not affected. In contrast, at a constant inflation pressure, the tire load variation explicitly influenced the contact stresses in the outer regions of the contact area; the higher the load, the higher those stresses. The maximum contact stress was still located at the center of the contact area, as **Yap, 1988** reported in a similar study. He compared the tire load increase due first to an inflation pressure increase and then to a tire load increase for a 11-24.5 radial tire, a 11R24.5 radial tire and a 385/65R22.5 wide-base tire (all manufactured by Goodyear). The wide-base tires exhibited higher increase in the contact stresses in the case of the increase of the inflation pressure, but they had the lowest increase as the tire load increased. Despite this fact, in both cases wide-base tires had higher vertical contact stresses. **Myers *et al.*, 1999** measured the three components of the contact stresses under various truck tires. Results were presented for the vertical and transverse contact stresses for a bias ply tire and R299 radial tire (for both load 25 KN and inflation pressure 115 psi), and M844 wide base radial tire (load 41.7 KN and inflation pressure 115 psi). The results indicate that, the vertical and transverse contact stresses are higher for wide-base tires because wide-base tires have a higher load per tire ratio than any other type of tire. The distribution of the vertical contact stresses was also not uniform. The maximum value was found to occur at the center of the contact area and equal to approximately 2.3 times the inflation pressure. Also, it is observed that the maximum vertical stresses of the

wide-base tire are about 1.5 times greater than those of the bias ply and radial tires. With respect to the transverse stresses, again the wide-base tires exhibit higher values in the central region of the contact area. Maximum transverse stress (of the wide-base tire) is about one third of the maximum vertical contact stress. It should be noted that the relationship between pavement response (stress, strain, and deflection) are not linear relationship with the pavement performance (Fatigue, rutting, etc.) which urge for quantifying the pavement damage due to these axles with wide-base tires.

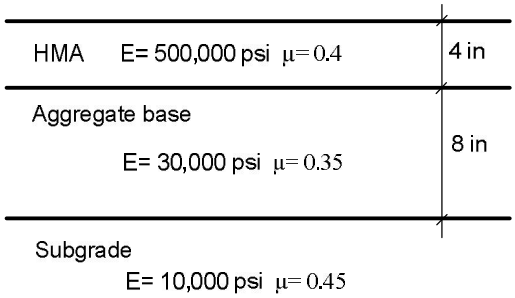
Al-Qadi *et al.*, 2002, measured the pavement response for dual tire and new wide-base tire with the same tire pressure at Virginia Smart Road Test Facility. The results showed that the newly developed wide-base tire induce approximately the same horizontal strain under the hot mix asphalt layer as do equivalent dual tires. Therefore, they expect the same fatigue damage for both newly developed tires and dual tires. In contrary, the vertical compressive stresses induced by wide-base tire are greater on the upper hot mix asphalt layers of the tested pavement. The difference diminishes with depth and become negligible at the bottom of the subbase layer.

Kim *et al.*, 2005, used plane-strain two-dimensional and three-dimensional static and dynamic finite element analyses to assess the larger stresses generated by wide-base tires and their effect on the subgrade. They compared between the response of conventional and wide-base tires under elastic-plastic conditions, wide-base tires induce approximately four times larger permanent strains in the pavement layers than conventional tires. Therefore, design of a pavement using Load Equivalency Factor (LEF) values for dual tires leads to overestimation of the pavement design life.

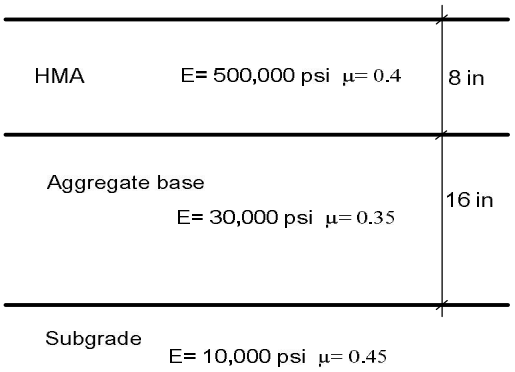
Since the relation between the pavement response and pavement damage is not linear, researchers have investigated the pavement response and predicted the pavement damage to determine the effect of wide-base tires on pavement damage. **Sebaaly and Tabatabaee, 1992** investigated the effects of tire pressure, tire type, axle load, and axle configuration under actual truck loading and highway speed on instrumented test sections. The various tire types are tested against the 11R22.5 wide base tire to evaluate their relative damage to pavements. The results showed that the wide-base single tires consistently have significantly higher strains and deflection than dual tires. The fatigue and rutting damage factors for the wide-base single tires range from 1.5 to 1.7 and from 1.2 to 2.0 for the single and tandem axles, respectively.

1.1 Damage Calculation Due to Multiple axle loads

Several laboratory fatigue tests such as simple fracture, support fracture, direct axial, diametral, triaxial, fracture tests, and wheel tracking tests were performed to determine the fatigue damage due to traffic loads, (**Matthews *et al.*, 1993)**. Researchers stated the basic concept of each test where some of these tests were stresses-controlled while others were strain-controlled. However, all of these tests have been performed using either a single pulse with rest period or a continuous sinusoidal load. Similar to pavement fatigue, several trials have been made to predict pavement rutting based on laboratory experiments (**Ayres, 2002**); however all of these trials were based on single load pulse. In reality, the pavement is subjected to multiple load pulses due to the passage of large axle group trucks.



a) Thin section



b) Thick section

Figure 1: Thicknesses and material properties of thick and thin pavement

Due to the fact that the damage resulting from multiple axle load were not correctly characterized since there were no laboratory tests based on multiple pulses. Recently, a massive laboratory tests simulating the multiple axle loads for both flexible and rigid pavement are conducted at Michigan State University.

Salama and Chatti, 2011 got advantages of these tests and evaluated fatigue and rut damage prediction methods for asphalt concrete pavements subjected to multiple axle loads. Different summation methods of calculating pavement damage caused by multiple axles were evaluated using laboratory data, with the evaluation criterion being the degree of agreement with the measured laboratory performance. They concluded that for fatigue damage, dissipated energy and strain area methods have an excellent agreement with the laboratory determined axle factors. For rutting damage, the peak strain method has good agreement with the laboratory determined axle factors. In this study, strain area and peak strain methods will be used to calculate the fatigue and rutting damage of pavement, respectively. The damage of pavement were calculated for thick and thin pavement with thicknesses and material properties as shown in Figure 1 a and b. The axle factor of fatigue and rutting damage can be calculated from strain area and peak strain equations as illustrated in the following sections.

1.1.1Fatigue

Fatigue is one of the main distress types in flexible pavements. The main pavement response that causes fatigue cracking in pavement is the tensile strain at the bottom of the hot mix asphalt. KENLAYER computer program will be used to calculate the horizontal tensile strain at the bottom of the hot mix asphalt layer under the stander axle and all axles considered in the study (single, dual, tridom and quad) with conventional dual tires and wide-base tires, **Huang, 1993**. Hence, strain area proven that it is the most candidate method to quantify the fatigue damage. Equation 3.1 shows the number of fatigue cycles until failure using strain area method. To compare the damage due to multiple axle relative to the stander axle, fatigue strain area model will be used to calculate the Axle Factors (AF).

$$N_f = 18.865 * A_o^{-0.478} \quad (1)$$

Where:

N_f = is the number of cycles to failure, and

A_o = is the initial area under the strain curve for stander axle or any axle or truck.

and

AF = Damage of axle / Damage of the stander axle

$$= N_{f\,\text{std axle}} / N_{f\,\text{axle or truck}} = (A_{o\,\text{std axle}} / A_{o\,\text{axle or truck}})^{-0.478} \quad (2)$$

1.1.2 Rutting

Similar to fatigue, rutting is one of the main distress types in flexible pavements. The main pavement response that causes pavement rutting is the vertical compressive strain. KENLAYER computer program will be used to calculate the vertical compressive strain on top of the subgrade layer, at the middle of the hot mix asphalt layer, at the middle of the base layer and at the middle of the subsequent six subgrade layers each with thicknesses of 40 inches until the vertical compressive strain becomes negligible and no resultant permanent deformation due to truck load.

To calculate the total rutting at the pavement surface (rutting in HMA plus rutting in base plus rutting in subgrade), VESYS rutting model is the most appropriate model which has this capability, Moavenzadeh, 1974. Equation 3.7 shows the form of the model.

$$\rho_p = h_{AC}\frac{\mu_{AC}}{1-\alpha_{AC}}\left(\sum_{i=1}^{K}(n_i)^{1-\alpha_{AC}}\left(\varepsilon_{ei,AC}\right)\right) + h_{base}\frac{\mu_{base}}{1-\alpha_{base}}\left(\sum_{i=1}^{K}(n_i)^{1-\alpha_{base}}\left(\varepsilon_{ei,base}\right)\right)$$
$$+h_{SG}\frac{\mu_{SG}}{1-\alpha_{SG}}\left(\sum_{i=1}^{K}(n_i)^{1-\alpha_{SG}}\left(\varepsilon_{ei,SG}\right)\right) \quad (3)$$

Where:

ρ_p = total cumulative rut depth (in the same units as the layer thickness),

I = subscript denoting axle group,

K = number of axle group,

H = layer thickness for HMA layer, combined base layer, and subgrade layer,

n = number of load applications, assume $n = 1*10^6$ (one million repetitions)

ε_e = compression vertical elastic strain at the middle of the layers,

μ = permanent deformation parameter representing the constant of proportionality between plastic and elastic strain, and

α = permanent deformation parameter indicating the rate of change in rutting as the number of load applications increases.

Since the rutting calculation using VESYS model will be used for relative comparison for different axles with conventional and wide-base tires, Table 1 shows an average values for permanent deformation parameters which was presented in previous research, Salama, 2005.

Table 1: Average values of permanent deformation parameters

Pavement layer	α	μ
HMA	0.65	0.8
Base	0.7	0.4
Subgrade	0.75	0.025

The rutting damage factors for axles can be calculated from equation (3.8). However, the truck factor will be calculated by summing the axles factor of the truck axle.

Damage factor = Rutting $_{(\text{any axle})}$ / Rutting $_{(\text{stander axle})}$ (4)

1. Research Procedure

The following table summarizes the research methodology in term of axle and truck configuration, the forward analysis software, the performance model, and axle load values that will be used to calculate the pavement damage due to conventional tire and wide-base tire. Figure 2 shows the flow chart of research plan which satisfy the research objectives.

Table 2: Summary of the research methodology

Item	Availability	Considered in the research
Axle configuration	Single to eight axle group	Single to quad axle
Axle load values	Different axle load values	Single =10 ton, Tandem = 20 ton, Tridem =30 ton, and Quad = 40 ton
Forward analysis software	Several MLET and FEM software	KENLAYER (MLET)
Fatigue model	Several fatigue models	Strain area model
Rutting model	Several Rutting models	Subgrade rutting using AI model
		Hot mix asphalt using Peak strain model
		Total rutting at the pavement surface using VESYS model

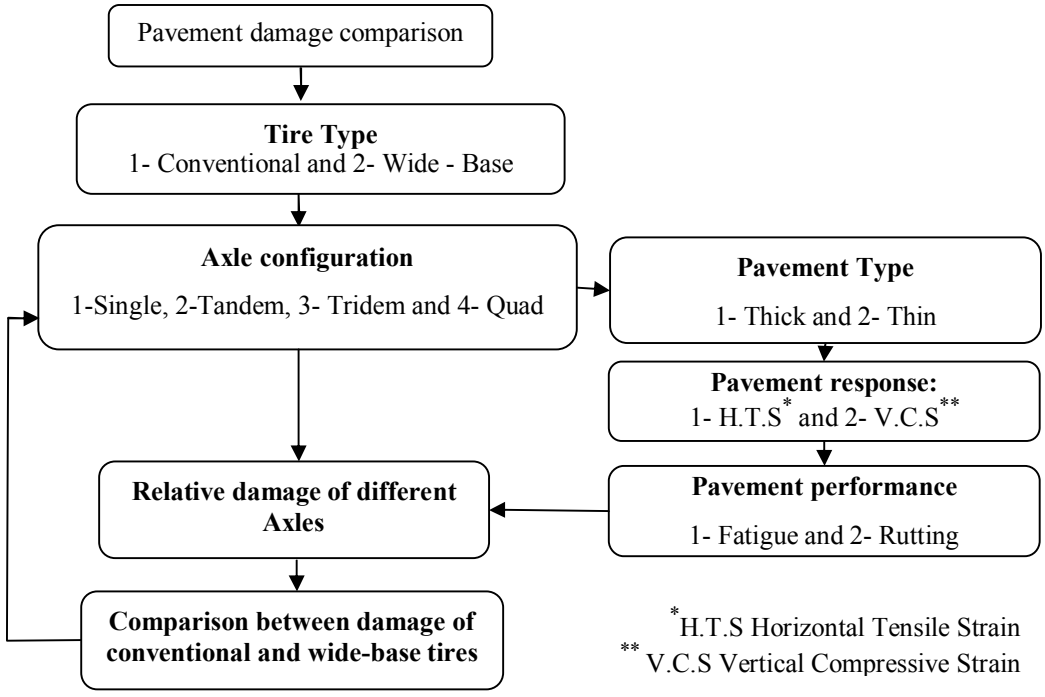


Figure 2: Flowchart of the analysis

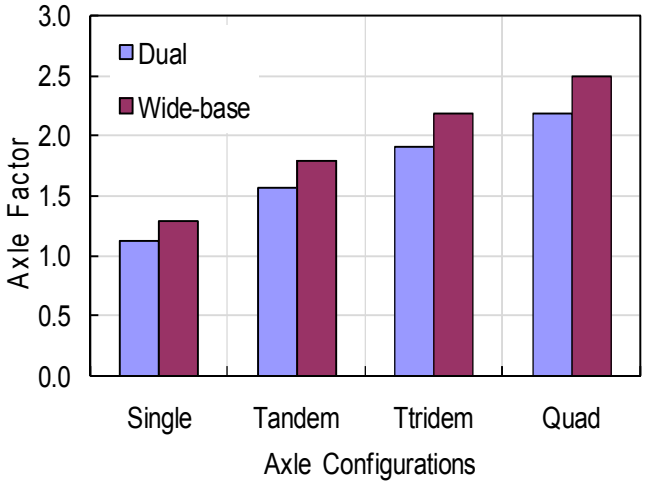
3. Analysis and Discussions

Figures 3 a and b shows the Axle Factor calculated from the strain area method for single, tandem, tridem and quad axle with dual and wide-base tires for both thin and thick pavements. The results show that the wide-base tires impose more fatigue

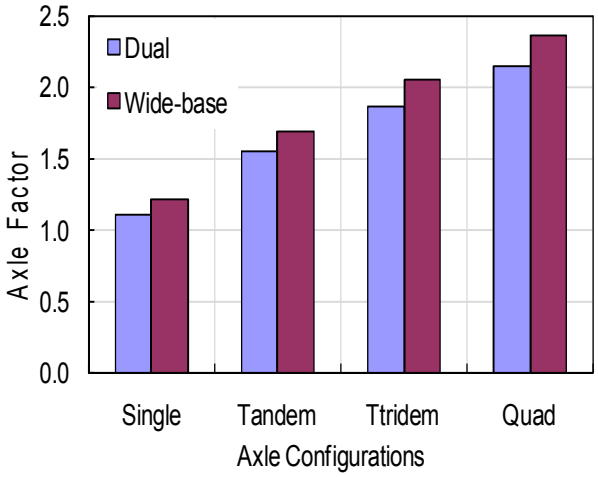
damage for both thin and thick pavements. Almost similar trend of increasing in magnitude observed for the four axle types for thin and thick pavements. The increase in the fatigue damage under wide-base tire due to the larger area under the strain pulse which resulting from smaller surface contact area of the wide-base tire than the dual tire, see Figures 3 a and b. The increase in fatigue damage due to axles with wide-base tires are 9.9%, 9.0%, 10.2%, and 9.8% for single, tandem, tridem and quad axles, respectively. Whereas for thin pavement, these percentages of increase in fatigue damage are 15.2 %for single and 14.7% for tandem, tridem and quad axles.

Figures 4 a and b shows the calculated total surface rutting Axle Factors due to different axles with dual and wide-base tires for thick and thin pavements. The results show that the Axle Factors for thick pavements due to axles with conventional and wide-base are very close and there is no significant difference between the rutting damage. On the other hand, these differences are relatively higher in the thin pavement which indicates that axles with wide-base tires cause more rutting damage than axles with conventional tires. The wide-base tire cause more rutting damage in the thin pavement since the thin hot mix asphalt do not provide enough protection for the sub-layers especially the aggregate base to sustain the heavy axle loads. The percentages of rutting damage increase for thick pavements due to axles with wide-base tires are 16.7, 4.7, 4.2, and 4 for single, tandem, tridem and quad axles, respectively. These results indicate that increasing the number of axles within an axle group decreasing the rutting damage. For Thin pavement, these percentages become 31.3, 21.6, 21.1, and 20.9 for single, tandem, tridem and quad axles, respectively.

Comparing the overall increase in the fatigue damage resulting from axles for thin and thick pavements due to the wide-base tires indicate that the wide-base tires impose more fatigue damage ranges between 9 % and 10.2 % with average 9.7 % in compare to fatigue damage with dual tires for thick pavements whereas this percentage ranges between 14.7 % and 15.2 % with average 14.8 % for the thin pavements, see Figure 5. Whereas comparing the overall increase in the total surface rutting damage resulting from axles for thin and thick pavements due to the wide-base tires indicate that the wide-base tires impose difference in the overall rutting damage ranges between 4 % and 16.7 % with average 7.4 % in compare to dual tires for thick pavements whereas this percentage ranges between 20.9 % and 31.3 % with average 23.7 % for the thin pavements, see Figure 5.

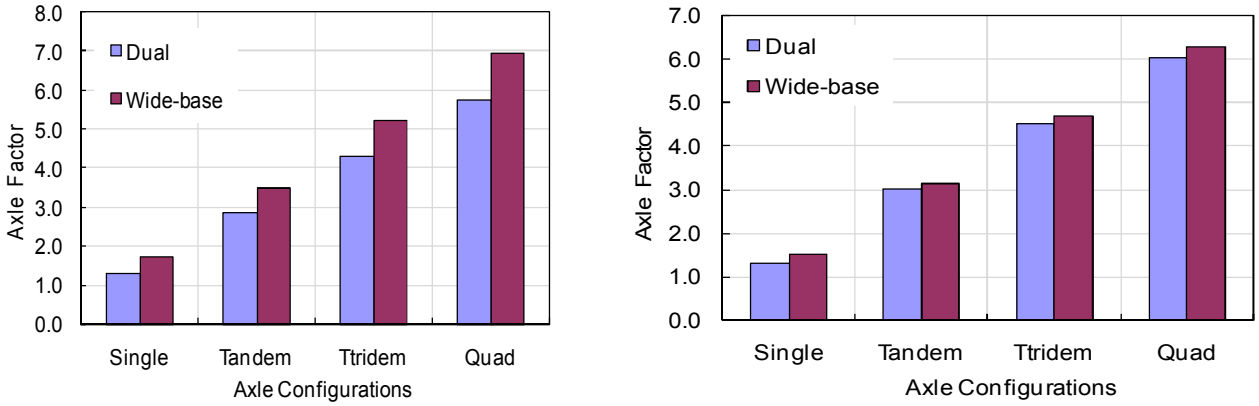


a) Thin pavement



b) Thick pavement

Figure 3: Fatigue axle factors



a) Thin pavement b) Thick pavement

Figure 4: Rutting axle factors

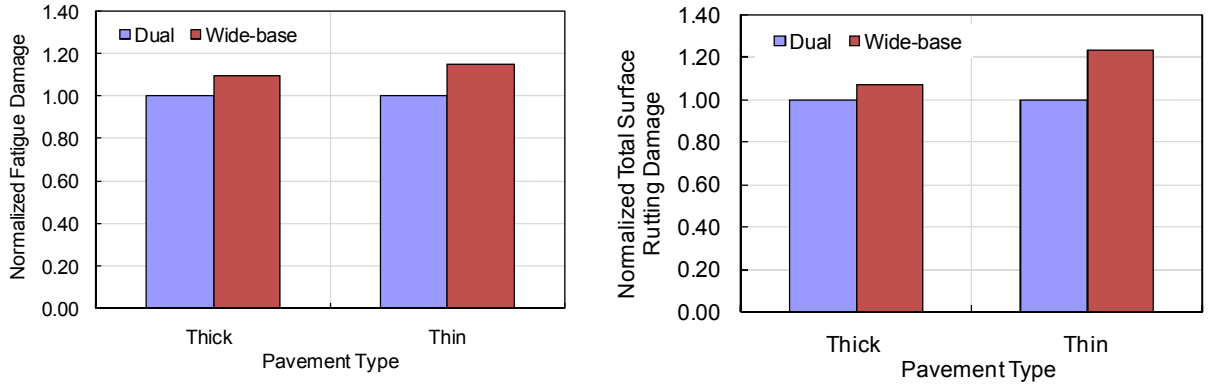
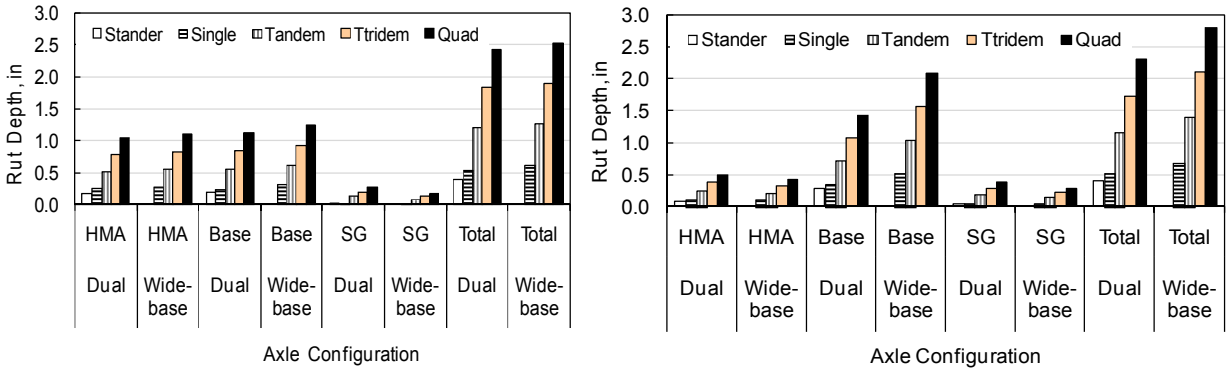


Figure 5: Normalized average fatigue and rutting damage for thick and thin pavement

Figure 6 a and b shows the total surface rutting and the layer rutting due to different axle configuration with dual and wide-base tires for both thin and thick pavements. Figure 6 a shows that the resulting layers rutting in thick pavement due to axles with dual or wide-base are almost the same. This indicates that the rutting is more affected by the total weight of the axle loads rather than the distribution of the load underneath the tires, as long as the weak layers are protected by the hot mix asphalt. Unlike thick pavement, thin pavement has no enough hot mix asphalt to protect the base layer to carry heavy axle loads. Hence axles with wide-base tires create more rutting damage in the base layer than axles with dual tires which resulting in more total rutting damage due to axles with wide-base tires, see Figure 6 b.



a) Thick pavement b) Thin pavement

Figure 6: Total and layer rut depth due to different axle configurations

4. Conclusion

This study involves mechanistic evaluation of flexible pavement damage due to axle loads with wide-base tires. The analysis includes comparisons between pavement damage due to axle loads with wide-base tires and axle loads with conventional tires. The pavement damage includes fatigue and total surface rutting damages. Based on the analysis of fatigue and rutting damage due axle loads with conventional and wide-base tires for thin and thick pavement, the following conclusions are drawn:

- In general, axle loads with wide-base tires impose more fatigue and rutting damage than axles with conventional tires.
- Axles with wide-base tires impose an average 9.7 % (from 9 % to 10.2%) fatigue damage more than the axles with conventional tires for thick pavements whereas this percentage become on average 14.8 % (from 14.7 to 15.2 %) for the thin pavements.
- Axles with wide-base tires impose on average 7.4 % (4% to 16.7%) rutting damage more than the axles with conventional tires for thick pavements whereas this percentage become on average 23.7 % (20.9 % to 31.3 %) for the thin pavements.
- An overall agreement between the layer rutting damage resulting from total surface rutting approach (VESYS rutting model) with subgrade and hot mix asphalt rutting.

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Simultaneous determination of Human $CD4^+CD25^+$ regulatory T cells suppressing anti-TB immune responses of $CD4^+$, $CD8^+$ and $V\gamma2V\delta2^+$ T cells in vitro

Jie Qin[1], Guangming Gong[2*], Shilei Sun[1], Bo Song[1], Ying Du[2], Xuan Yang[2], Na Wang[2], Sha Zhu[2], Xiaoyan Xuan[2], Pingping Liu[2], Yuming Xu[1]

[1.] Department of Neurology, First Affiliated Hospital of Zhengzhou University, Zhengzhou, Henan 450052, China
[2.] Department of Microbiology and Immunology, College of Basic Medicine, Zhengzhou University, Zhengzhou, Henan 450001, China.
gmgong@zzu.edu.cn

Abstract: To evaluate the negative regulatory effect of human $CD4^+CD25^+$ regulatory T cells (Treg) on anti-Tuberculosis (TB) immune responses of T cells including $CD4^+$, $CD8^+$ and $V\gamma2V\delta2^+$ T cells simultaneously in vitro, a versatile carboxyfluorescein succinimidyl ester (CFSE)-based proliferation assay was exploited to determine the suppressive effect of Tregs on the purified protein derivative (PPD)-specific proliferations of such T-subsets in peripheral blood mononuclear cells (PBMC) of BCG-infected donors. We demonstrated that PPD-stimulation droved synchronously the proliferations of both $CD4^+$ and $CD8^+$ T cells and $V\gamma2V\delta2^+$ T cells in PBMCs. Such PPD-specific proliferations of $CD4^+$, $CD8^+$ and $V\gamma2V\delta2^+$ T cells can be prohibited by human Treg concurrently. Similarly, Treg has the ability of suppressing the CD3/CD28 antibody-induced proliferations of such T-subsets. Our results indicated that the versatile CFSE-based proliferation assay can be applied to determine the complicated anti-TB immune response of multi-T-subsets simultaneously, and may facilitate human Tregs as potential tool to down-regulate overdue anti-TB immune responses involved in multiple effective T-subsets to enhance protection against *Mycobacterium tb* (*M. tb)* infection.

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Key words: $CD4^+CD25^+$ regulatory T cells (Treg), anti-TB immune response, T cells, carboxyfluorescein succinimidyl ester (CFSE)

1. Introduction

As one of the leading causes of death worldwide, tuberculosis (TB) is threatening human health seriously. There are more than 9 million new cases of TB and an estimated 2 million cases of deaths annually in the world. The most severe burden of TB disease are happening in developing countries(Lawn and Zumla, 2011). Notoriously, the shortage of the fundamental understanding of the pathogenesis and protective immunity against *Mycobacterium tb (M. tb)* and increasing rates of drug-resistant *M. tb* are the huge persisting challenges to public health worldwide, which greatly impede the promising progress in TB prevention and control (Lawn and Zumla, 2011). Therefore, it is urgently needed to seek and identify new reliable approaches to the prevention and treatment of TB based on exactly understanding the mechanisms of anti-TB immune responses.

The effectiveness of live Bacillus Calmette-Guerin (BCG) vaccination has been proved in against *M. tb* infection in children but not adult (Lawn and Zumla, 2011). As an attenuated strain of *M. bovis*, BCG encodes the same antigens of *M. tb* that can drive protective anti-*M. tb* immunity of human newborns via inducing potent specific and functional $CD4^+$ and $CD8^+$ T cell responses (Li et al., 2011). Therefore, elucidating BCG-elicited anti-TB immunity may facilitate us to find new effective measures to prevent and control *M. tb* infection(Gong et al., 2009; Qin et al., 2011).

T cells-mediated immunity plays a central role in host resistance to *M. tb* infection. It has been well documented over the past decades that the important role of $CD4^+$ T cells secreting cytokines such as IFN-γ, TNF-α and IL-2 to recruit monocytes, granulocytes and effective T cells migrating to disease sites and activate or amplify the effective functions of such immune cells to contributing collectively to antimicrobial immunity in *mycobacterial* infection (Li et al., 2011). Interestingly, the contributions of $CD8^+$ T cells(Chen et al., 2009) and Vγ2Vδ2 T cells(Chen, 2011; Shen et al., 2002) in *mycobacterial* infection have also been indicated recently. Likewise, growing studies revealed that these T cells contributed collaboratively to potent anti-*M. tb* immunity, for each of them may function as regulator to reciprocally enhance Ag-specific immune response of such T-subsets in infections (Chen, 2011).

Despite being a $CD4^+$ T cells, naturally occurring $CD4^+CD25^+$ regulatory T cells (Treg) play a key role in maintaining immune homeostasis in infection by suppressing the undue activation, proliferation and/or effective function of different types of T cells (Chen, 2011; Gong et al., 2009; Qin et al., 2011). Recently,

growing studies (Chen et al., 2012; Chen, 2011; Gong et al., 2009; Scott-Browne et al., 2007) had demonstrated that human Tregs have the negative regulatory effects on antigen-specific immune responses of independent $CD4^+$, $CD8^+$ T cells(Li et al., 2011) and $V\gamma2V\delta2^+$ T cells(Li and Wu, 2008) in *mycobacterial* infection. However, it is little known that Treg has the same effects on the anti-TB immune responses of a mixed pool of effective T cells including $CD4^+$, $CD8^+$ and $V\gamma2V\delta2^+$ T cells in peripheral blood mononuclear cells (PBMC) of *mycobacterial*-infected human, which reflects the delicate balance between such human T-subsets in clinical course and disease outcome in *M. tb* infections and the reality of human immune system in resisting pathogenic microorganism *in vivo*.

Accumulating evidence have indicated that Ag-specific immune response is very important in clearing various kinds of pathogens(Chen, 2011; Shen et al., 2002). To determine Ag-specific immune response during *M. tb* infection, numerous studies continued to rely on the ability of *M. tb*-specific T cells to produce cytokines, which may not accurately, completely and systematically reflect the functions of TB-specific T cells that including the activation, proliferation, division, autocrine and paracrine, etc. (Li et al., 2011). Attributable to further development in the application of flow cytometry, increased studies have showed that the multiple functions of Ag-specific poly-T-subsets can be assessed simultaneously by a versatile CFSE-based proliferation assay(Gong et al., 2009; Li et al., 2011; Qin et al., 2011). In the current study, we successfully conducted a CFSE-based proliferation assay to simultaneously determine the regulatory function of human Treg on anti-*M. tb* immune responses of $CD4^+$, $CD8^+$ and $V\gamma2V\delta2^+$ T cells in BCG-infected donors' PBMCs *in vitro*.

2. Material and Methods

2.1. Subjects

8 male healthy volunteers aged from 21 to 40 years old were enrolled from Zhengzhou University. 5~15 mL of EDTA-anticoagulant blood samples were collected from each volunteer. All protocols of this study were approved by the Biomedical Ethics Review Committee at Zhengzhou University, China.

2.2. BCG-infection

All volunteers were intradermally infected with BCG (Shanghai Institute of Biological Products, China) at a dose of 0.05mg in 0.1 ml volume within 3 months.

2.3. Cell isolation

PBMCs were separated from EDTA-anticoagulant peripheral blood using density gradient method. $CD4^+CD25^+$ T Cells were purified by using $CD4^+CD25^+$ Regulatory T Cell Isolation Kit (Miltenyi Biotec). Briefly, $CD4^+$ T cells were purified from PBMCs by depletion of non-$CD4^+$ cells with negative selection. From purified $CD4^+$ T Cells, $CD25^+$ T Cells were isolated by positive selection of $CD4^+CD25^+$ regulatory T cells using $CD25^+$ magnetic microbeads. $V\delta2^+$ T cells were purified by using purified mouse-anti-human Vδ2 (Clone: 15D, Endogen, Rockford, IL) Abs and goat-anti-mouse IgG microbeads (Miltenyi Biotec) with positive selection.

2.4. Labeling cells with CFSE and PKH26 red

Total 6×10^6 of $CD4^+CD25^+$ T cells-depleted PBMCs were labeled with CFSE using the CFSE Cell Proliferation Kit (Invitrogen-Molecular Probes) following the manufacturer's protocol. Briefly, the cells were suspended gently in 1 ml of prewarmed 0.1 % BSA -PBS containing CFSE at a 2.0 μM concentration and then incubated for 15 min at 37°C in dark. 5 volumes of ice-cold culture media were added to the cells and incubated 5 min on ice to quench the staining. Then the cells were washed by resuspending the pellet in fresh media for there times.

The purified 1×10^6 of $CD4^+CD25^+$ T cells were also labeled with PKH26 red using the PKH26 red fluorescent cell linker kit (Sigma) following the instructions. Briefly, the cells were suspended in a 2 ml total volume at final concentrations of 2×10^{-6} M PKH26 dye at room temperature for 5 minutes. Stop the staining reaction by adding an equal volume of complete medium. Then the cells were washed for total 4 times before being used.

2.5. CFSE-based proliferation assay

It was done following the standard protocols as previously described(Gong et al., 2009; Qin et al., 2011). The CFSE-labeled, $CD4^+CD25^+$ T cells-depleted PBMCs were added at 2×10^5 cells per well to individual wells of Costar round-bottom 96-well plates supplied with 0.2 ml of prewarmed R1640 containing with 10% FBS and 50 U/ml penicillin and 50 μg/ml streptomycin. Then these PBMCs were respectively stimulated by 15 μg/ml PPD (Shanghai Institute of Biological Products, China), or 5 ug/ml purified mouse anti-human CD28 (BD Pharmingen) and CD3 (BD Pharmingen). Meanwhile, PKH26 red labeled $CD4^+CD25^+$ T cells were then added to the CFSE-labeled, $CD4^+CD25^+$ T cells-depleted PBMCs at 2×10^5 per well in the absence or presence of PPD, CD28 and CD3.

After cultured for 7 days in the CO_2 incubator at 37℃, 95 % humidity and 5 % CO_2, the cells were collected and stained with surface Abs specific for Vγ2 (Endogen) in each tube at 4℃ for 20 min. After washed 3 times with 5% FBS-PBS, cells in each tube were added 5 uL Goat anti-mouse IgG (Biolegend) at 4℃ for 20 min in dark. Then wash 3 times again and add surface Abs specific for CD3 (Dako Cytomation), CD4 (BD Pharmingen), CD8 (Dako Cytomation) in each tube at 4℃ for 20 min in dark. Proliferation response was analyzed by flow cytometry to determine CFSE signal intensity and to exclude $PKH26^+$ cells, and the percentage of proliferation was calculated on the number of $CFSE^{dim}$

cells divided by the number of $CFSE^+$ cells. Flow cytometry was performed with CyAn ADP flow cytometer (DakoCytomation, Carpinteria, CA) and analyzed using Summit Data Acquisition and Analysis Software (DakoCytomation).

2.6. Statistical analysis

Unpaired student *t* test and one-way ANOVA were exploited to determine the differences between groups in vitro. p <0.05 was considered significant (GraphPad, San Diego, CA, USA).

3. Results

3.1. PPD induced antigen-specific proliferations of human $CD4^+$, $CD8^+$ and $V\gamma2V\delta2^+$ T cells

To determine whether human Treg has the function of suppressing the anti-*M. tb* immunity induced by effective T cells such as $CD4^+$, $CD8^+$ and $V\gamma2V\delta2^+$ T cells simultaneously, we used PPD as antigen-specific stimulation to induce the proliferations of $CD4^+$, $CD8^+$ and $V\gamma2V\delta2^+$ T cells in the PBMCs of volunteers that recently infected with BCG, since protein antigen of *M. tb* can be recognized directly by memory $CD4^+$ T cells, $CD8^+$ T cells and Vγ2Vδ2 T cells (Gong et al, 2009; Li et al, 2008; Qin et al., 2011).

We found that PPD induced the proliferations of not only $CD4^+$ T cells (p <0.01) and $CD8^+$ T cells (p <0.01), but also $V\gamma2V\delta2$ T cells (p <0.01) of BCG-infected volunteers (Figure 1, 2). Our results verify that human memory $V\gamma2V\delta2$ T cells directly recognized protein antigen of BCG, which is in accordance with the results in human study (Li et al, 2008).

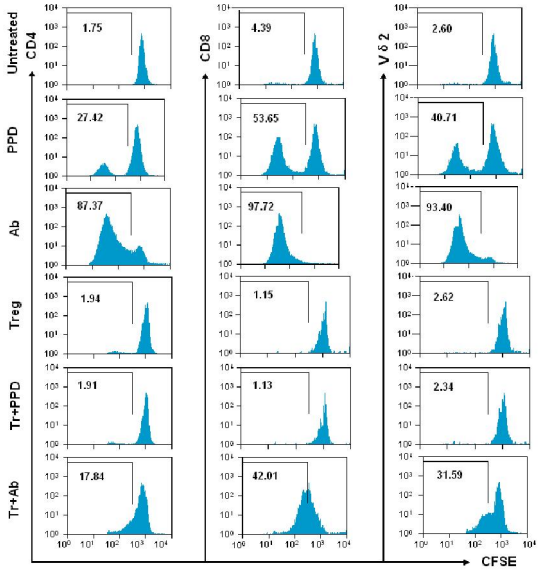

Figure 1. Hisgram figures of one representative volunteer of eight male volunteers showing PPD Ag-specific suppression of human $CD4^+CD25^+$ T cells on $CD4^+$, $CD8^+$ and $V\gamma2V\delta2^+$ T cells in PBMCs of BCG-infected donors in vitro. Tr: $CD4^+CD25^+$ T cells; Treg: $CD4^+CD25^+$ T cells.

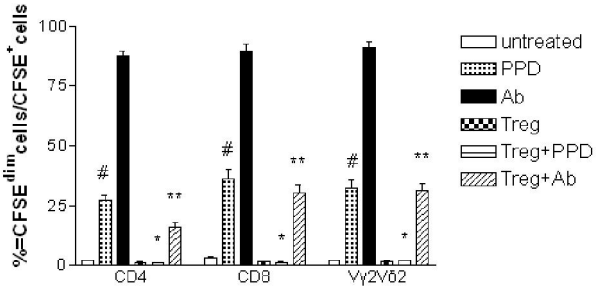

Figure 2. The suppressing percentage of $CD4^+CD25^+$ Treg on $CD4^+$, $CD8^+$ and $V\gamma2V\delta2^+$ T cells in vitro were shown as the mean values of triplicate measurements ± SEM (n=8): # Untreated *vs* PPD: $CD4^+$, $CD8^+$ and $V\gamma2V\delta2^+$ T cells, p <0.01 individually; * PPD *vs* Treg+PPD: $CD4^+$, $CD8^+$ and $V\gamma2V\delta2^+$ T cells, p <0.01 individually; ** Ab *vs* Treg+Ab: $CD4^+$, $CD8^+$ and $V\gamma2V\delta2^+$ T cells, p <0.001 individually.

3.2. Human Treg suppressed the PPD-specific proliferations of $CD4^+$, $CD8^+$ and $V\gamma2V\delta2^+$ T cells simultaneously

To further determine the suppression of human Treg on anti-*M. tb* immunity induced by effective T cells such as $CD4^+$, $CD8^+$ and $V\gamma2V\delta2^+$ T cells simultaneously, CFSE-based proliferation assay was exploited under the situation that Ag-specific immune responses of effective T cells can be driven by PPD-stimulation that results have been shown as above.

We found that Treg had the function of suppressing the PPD-specific induced activation/proliferation $CD4^+$ (p <0.01), $CD8^+$ (p <0.01) and $V\gamma2V\delta2^+$ T cells（p <0.01）, as well as the CD3/CD28 antibody-induced antigen non-specific proliferations of those T cells *in vitro* (p <0.001 individually) (Figure 1, 2).

4. Discussions

We conducted a sensitive, versatile CFSE-based proliferation assay for detecting the suppressing function of human Treg on anti-TB immunity *in vitro* (Gong et al, 2009; Li et al, 2008; Qin et al., 2011). A traditional assay for measuring such function of Treg is a coculture system by which a quantitive $CD4^+CD25^-$ responder T cells are incubated with different amounts of Treg under polyclonal stimulation. The proliferations of responder cells can be evaluated by measuring the incorporation of radioactive ^{3}H-thymidine into the DNA of cells in S phase during the last pulse 6~24h of culture. The advantages of CFSE-based method are obvious compared with ^{3}H-thymidine-based assay. Firstly, CFSE-based assay can be used directly to determine several phenotypes, surface or inner molecules such as cell cycle and cytokine profile of proliferated responder cells

simultaneously. At the same time, the proliferation of Treg droved by IL-2 in the coculture is excluded and then the suppressive mechanisms of that can be further analyzed. Secondly, CFSE-based assay is more sensitive than ^{3}H-thymidine-based assay which have high levels of background for the detection of rare antigen-specific T cell responses(Venken et al., 2007). The proliferation detected by later method is often underestimated. Thirdly, the proliferation of CFSE-based assay can be analyzed directly by flow cytometry to avoid using radioactive materials and machines. Fourthly, ^{3}H-thymidine-based assays can not distinguish proliferations of different cell populations from mixed pool, but CFSE-based method can.

For the past decades, the evaluations on the suppressing function of Treg focused on its inhibiting role in the proliferation of a single cell types, for example, $CD4^{+}CD25^{-}$ effective T cells. Thanks to the recent rapid progress in the regulated targets of Treg, effective $CD8^{+}$ and $V\gamma2V\delta2^{+}$ T cells were added into the pool of its regulatory objects that covered almost all the current types of immune cells. And that the cooperative and competitive roles of these T-subsets in immunity against *M. tb* were also indicated in the recent studies(Chen et al., 2009; Gong et al., 2009; Li and Wu, 2008). Therefore, we decided to determine the *M. tb* Ag-specific and non-specific proliferations of $CD4^{+}$, $CD8^{+}$ and $V\gamma2V\delta2^{+}$ T cells in the PBMCs of BCG-infected donors with CFSE-based proliferation assay. Indeed, our results were in agreement with references that $CD4^{+}$, $CD8^{+}$ and $V\gamma2V\delta2^{+}$ T cells contributed simultaneously to anti- *M. tb* immunity because the PPD-specific proliferations of such T-subsets can be observed *in vitro*. In addition, our results supported that CFSE-based proliferation assay can be applied to detect concurrently the Ag-specific immune responses of multiple effective T-subsets, which will make it easy to probe deeply into the mechanisms of extensive anti-mycobacterium immunity mediated by poly-T-subsets *in vitro*.

Furthermore, by flow cytometric analysis of CFSE-based proliferation assay, we found the powerful suppressing functions of Treg on $V\gamma2V\delta2$ T cells, as well as $CD4^{+}$, $CD8^{+}$ T cells in BCG-infected human. Treg did suppress not only the PPD-specific proliferation of $CD4^{+}$ T cells which play most important roles in anti-*M. tb* immunity, but also that of $CD8^{+}$ and $V\gamma2V\delta2^{+}$ T cells. Likewise, Tregs suppressed not only PPD Ag-specific, but also CD3/CD28 antibody-induced non-specific proliferations of conventional $CD4^{+}$, $CD8^{+}$ T cells, and nonclassical $V\gamma2V\delta2$ T cells, which suggested Treg may control anti-mycobacterium immunity that mediated not only by conventional $CD4^{+}$, $CD8^{+}$ T cells, but also by nonclassical $V\gamma2V\delta2$ T cells during anti- *M. tb* immunity. These evidences demonstrated that human Treg has versatile regulatory functions on a mixed pool of effective cells including $CD4^{+}$, $CD8^{+}$ T cells (Chen et al., 2009; Chen et al., 2012) and $V\gamma2V\delta2$ T cells (Chen et al., 2012; Gong et al., 2009; Li and Wu, 2008) that contribute collectively to the extensive ongoing anti-mycobacterium immunity.

The results of this study may open up the possibilities to exploit Treg as potential tool for immunotherapy during infectious or other obstinate diseases. For example, pre-activated Treg may be considered to be used for treating acute TB with brain and pulmonary lesion caused by overactive effective memory T cells which contribute to excessive immune response to result in cerebrocortical necrosis and pulmonary cavity(Chen et al., 2012).

In summary, our results demonstrated that human Treg had the function of suppressing the anti-*M. tb* immunity of multiple effective T cells such as $CD4^{+}$, $CD8^{+}$ and $V\gamma2V\delta2^{+}$ T cells that detected simultaneously by the versatile CFSE-based proliferation assay (Chen, 2011; Li and Wu, 2008; Scott-Browne et al., 2007; Shen et al., 2002; Qin et al., 2011). The results may facilitate the clinical application of Treg in controlling and treating intractable *M. tb* infections, and the further application of CFSE-based proliferation assay to detect Ag-specific immune responses of multiple cells during infectious diseases.

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Corresponding Author:
Dr. Guangming Gong
Dept. of Microbiology and Immunology
College of Basic Medicine, Zhengzhou University
Zhengzhou, Henan province 450001, China
E-mail: gmgong@zzu.edu.cn

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Hasimoto Surfaces

Nassar H. Abdel-All, R. A. Hussien and Taha Youssef

Department of Mathematics Faculty of Science, Assiut University, Assiut, Egypt
nhabdeal2002@yahoo.com, tahaussef@yahoo.com

Abstract: The purpose of the present work is to construct a Hasimoto surface from its fundamental form coefficients via numerical integration of Gauss-Weingarten equations and fundamental theorem of surfaces.
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Keywords: Evolution of curves, Hasimoto surface, Gauss-Weingarten equations.

1. Introduction

There are a catalogue of surfaces that can be described by integrable equations such as surfaces of constant negative Gaussian curvature, surfaces of constant mean curvature, minimal surfaces, affine spheres. This paper continues the program by adding Hasimoto surfaces to the catalouge. These surfaces are obtained by evolving a regular space curve x in R^3 as it evolves over time according to this evolution equation:

$$x_t(s,t) = x_s \wedge x_{ss} = \kappa(s,t)b \qquad (1)$$

this is an evolution of the curve in its binormal direction with velocity equal to its curvature. Eq.1 Known as the vortex filament flow or Localized Induction Equation (LIE). Here, $\mathbf{x}(s,t)$ is a position vector for a point on the curve, t is the time, s is the arc--length parameter, κ is the curvature of $\mathbf{x}$, $\mathbf{b}$ is the unit binormal and the subscripts indicate the differentiation with respect to the indicated variables. The subject of how space curves evolve in time is of great interest and has been investigated by many authors. Hasimoto [1] showed that the evolution of a thin vortex filament regarded as a moving space curve can be mapped to the nonlinear Schrodinger equation. Rick Mukherjee and Radha Balakrishnan [2] are studied moving curves of the sine-Gordon equation. Nassar, et al [3, 4, 5, 6] studied evolution of plane curves, motion of hyper surfaces and evolution of space curves in R^n. The authors in [7] constructed Hasimoto surface via integration for Frenet--Serret equations using fundamental existence and uniqueness theorem for space curves. Here we take another method different from them, the outline of this method is to construct six fundamental quantities $\{g_{11}, g_{12}, g_{22}, L_{11}, L_{12}, L_{22}\}$ for Hasimoto surface after then we integrate Gauss-Weingarten equations numerically. Since the surface generated form the evolution of the curve so in the next section we introduce geometry of space curve evolution.

Our Goal**.** The goal of this paper is to construct Hasimoto surfaces and display it via numerical integration of Gauss-Weingarten equations.

The article is organized as follows**.** In section 2 we introduce geometry of space curve evolution, derive (CNPDEs) which formulates the problem directly in terms of the curvatures and get exact solution for them. In section 3, we reconstruct the curve from its curvatures. In section 4, we introduce differential geometry of surfaces. In section 5, we study the geometric properties of Hasimoto surfaces. In section 6, we reconstruct the surface from its fundamental forms via numerical integration of Gauss-Weingarten equations and display it.

2. General Curve Evolution

If $\mathbf{x} = \mathbf{x}(s,t)$ is the position vector of a curve C moving in space, then the unit tangent, principal normal and binormal vectors ,which are denoted by $\{t, n, b\}$ respectively vary along C according to the well−known Serret−Frenet relations [8]

$$\begin{aligned} t_s &= \kappa n, \\ n_s &= -\kappa t + \tau b, \\ b_s &= -\tau n, \end{aligned} \qquad (2)$$

where s measures arc length along C, κ is its curvature and τ its torsion. The general temporal evolution in which the triad $\{t, n, b\}$ remains orthonormal adopts the form [9],

$$\begin{aligned} t_t &= \alpha n + \beta b, \\ n_t &= -\alpha t + \gamma b, \\ b_t &= -\beta t - \gamma n. \end{aligned} \qquad (3)$$

Here α, β and γ are geometric parameters which are generally functions of s and t. These

describe the evolution in t of the Frenet frame $\{t,n,b\}$ on the curve. For non−stretching curves, the triad must satisfy the compatibility conditions

$$t_{ts} = t_{st}, \quad n_{ts} = n_{st}, \quad b_{ts} = b_{st}. \tag{4}$$

Using Eqs.(2) and (3) , the compatibility conditions become

$$\begin{aligned} \kappa_t &= \alpha_s - \tau\beta, \\ \tau_t &= \gamma_s + \kappa\beta, \\ \beta_s &= \kappa\gamma - \tau\alpha. \end{aligned} \tag{5}$$

If the velocity vector $v = \mathbf{x}_t$ of a moving curve C has the decomposition

$$\frac{\partial \mathbf{x}}{\partial t} = \lambda\mathbf{t} + \mu\mathbf{n} + \nu\mathbf{b}, \tag{6}$$

then imposition of the condition $\mathbf{x}_{ts} = \mathbf{x}_{st}$ yields

$$\begin{aligned} 0 &= \lambda_s - \mu\kappa, \\ \alpha &= \lambda\kappa + \mu_s - \nu\tau, \\ \beta &= \mu\tau + \nu_s. \end{aligned} \tag{7}$$

where λ, μ, ν as functions of s and t, correspond to the normal, binormal and tangent projections of the velocity. Below we restrict our attention to a purely local form for these velocities as in the form,

$$\begin{aligned} \lambda &= \lambda(\kappa, \kappa_s, ..., \tau, \tau_s, ...), \\ \mu &= \mu(\kappa, \kappa_s, ..., \tau, \tau_s, ...), \\ \nu &= \nu(\kappa, \kappa_s, ..., \tau, \tau_s, ...). \end{aligned}$$

The dynamical equations for the curvature κ and the torsion τ of the evolving curve C may now be expressed in terms of the components of velocity λ, μ, ν by substitution of Eq.(7) in to Eq.(5) to obtain

$$\begin{cases} \kappa_t = (\lambda\kappa + \mu_s - \nu\tau)_s - (\mu\tau + \nu_s)\tau \\ \tau_t = \dfrac{1}{\kappa}((\mu\tau + \nu_s)_s + \tau(\lambda\kappa + \mu_s - \nu\tau)) + (\mu\tau + \nu_s)\kappa \end{cases} \tag{8}$$

where

$$\gamma = \frac{1}{\kappa}[(\mu\tau + \nu_s)_s + \tau(\lambda\kappa + \mu_s - \nu\tau)] \tag{9}$$

Eqs.(8) link the curves with non linear partial differential equations. For a given λ, μ, ν the above coupled nonlinear partial differential equations (CNPDES) determined the motion of the curve . Now let a curve C moving in the space according to

$$\frac{\partial \mathbf{x}}{\partial t} = \kappa\mathbf{b}. \tag{10}$$

Known as the vortex filament flow or localized induction equation (LIE).

For this motion we have

$$\{\lambda, \mu, \nu\} = \{0, 0, \kappa\} \tag{11}$$

From Eqs.(5), (7) the velocities of the moving frame are

$$\begin{aligned} \alpha &= -\kappa\tau, \\ \beta &= \kappa_s, \\ \gamma &= \frac{\kappa_{ss} - \kappa\tau^2}{\kappa}. \end{aligned} \tag{12, 5}$$

The evolution of the moving frame w.r.to t is given from Eq.(3)

$$\begin{aligned} t_t &= -\kappa\tau n + \kappa_s b, \\ n_t &= \kappa\tau t + \frac{\kappa_{ss} - \kappa\tau^2}{\kappa} b, \\ b_t &= -\kappa_s t - \frac{\kappa_{ss} - \kappa\tau^2}{\kappa} n. \end{aligned} \tag{13}$$

and from Eq.(8) The evolution equations for curvature and torsion are

$$\begin{aligned} \kappa_t &= -2\kappa_s\tau - \kappa\tau_s, \\ \tau_t &= \kappa\kappa_s - 2\tau\tau_s + (\frac{\kappa_{ss}}{\kappa})_s. \end{aligned} \tag{14}$$

A (CNPDES) (14) was formulated by Da Rios in [10]. We used MATHEMATICA package software (computational software program used in scientific, engineering, mathematical fields and other areas of technical computing) for solving the system Eq.(14) which apply the tanh−and sech−methods [11]. Thus the above system has a solution in the form,

$$\kappa = 2c_2 \operatorname{sech}(c_1 t + c_2 s + c_3), \quad \tau = -c_1/2c_2. \tag{15}$$

where c_1, c_2, c_3 are arbitrary real constant.

3. Constructing a Curve from the Curvature and Torsion

One of the basic problems in geometry is to determine exactly the geometric quantities which distinguish one figure from another. For example, line segments are uniquely determined by their lengths, circles by their radii, triangles by side-angle-side, etc. It turns out that this problem can be solved in general for sufficiently smooth regular curves. We will see that a regular curve is uniquely determined by two scalar quantities, called curvature and torsion, as functions of the natural parameter.

Theorem 3.1 (Fundamental existence and uniqueness theorem for space curves) *[12] Let $\kappa(s)$ and $\tau(s)$ be arbitrary continuous functions on $a \le s \le b$. then there exists, except for position in space , one and only one space curve C for which*

$\kappa(s)$ *is the curvature,* $\tau(s)$ *is the torsion and s is a natural parameter along C.*

The figure 1 in the section 6 represent snapshot of the evolving space curve obtained by solving the Frenet – Serret Eqs. (2) for a specified curvature and torsion using Mathematica [13]. Any moving space curve can be studied from two perspectives,namely, the shape of the curve and the evolution of the curve .At every fixed t, we clearly have a representation of the corresponding static space curve at that instant.

4. Differential Geometry of Surfaces

Let $x = x(s,t)$ denote the position vector of a generic point P on a surface S in R^3. Then, the vectors x_s and x_t are tangential to S at P, at such points at which they are linearly independent,

$$N = \frac{x_s \wedge x_t}{| x_s \wedge x_t |} \qquad (16)$$

determines the unit normal vector to S. The first and second fundamental forms (abbreviated FFF, SFF) on S are defined respectively by

$$\begin{aligned} I &= \langle dx.dx \rangle = g_{11}ds^2 + 2g_{12}dsdt + g_{22}dt^2 \\ II &= \langle -dx.dN \rangle = L_{11}ds^2 + 2L_{12}dsdt + L_{22}dt^2 \end{aligned} \qquad (17)$$

where g_{ij} and L_{ij} are given by

$$\begin{aligned} g_{11} &= \langle x_1.x_1 \rangle, \quad g_{12} = \langle x_2.x_1 \rangle, \quad g_{22} = \langle x_2.x_2 \rangle \\ L_{11} &= \langle x_{11}.N \rangle, \quad L_{12} = \langle x_{12}.N \rangle, \quad L_{22} = \langle x_{22}.N \rangle. \end{aligned} \qquad (18)$$

where $\langle , \rangle$ is the Euclidean scaler product. The Gauss and Weingerten equations give us the rate of change of (X_1, X_2, n) associated with the surface S, which take the form [8]

$$\begin{aligned} x_{ss} &= \Gamma_{11}^1 x_s + \Gamma_{11}^2 x_t + L_{11}\mathbf{N}. \\ x_{st} &= \Gamma_{12}^1 x_s + \Gamma_{12}^2 x_t + L_{12}\mathbf{N}. \\ x_{tt} &= \Gamma_{22}^1 x_s + \Gamma_{22}^2 x_t + L_{22}\mathbf{N}. \end{aligned} \qquad (19)$$

$$\begin{aligned} N_s &= \frac{g_{12}L_{12} - g_{22}L_{11}}{g} x_s + \frac{g_{12}L_{11} - g_{11}L_{12}}{g} x_t. \\ N_t &= \frac{g_{12}L_{22} - g_{22}L_{12}}{g} x_s + \frac{g_{12}L_{12} - g_{11}L_{22}}{g} x_t \end{aligned} \qquad (20)$$

where

$$g = g_{11}g_{22} - g_{12}^2 \qquad (21)$$

The quantities Γ_{ij}^k are called the Christoffel symbols of the second kind and given by

$$\Gamma_{ij}^k = \frac{1}{2} g^{kl} \left(\frac{\partial}{\partial u^i} g_{lj} + \frac{\partial}{\partial u^j} g_{il} + \frac{\partial}{\partial u^l} g_{ij} \right), i,j,k,l = 1,2 \qquad (22)$$

where (g^{ij}) is the inverse of (g_{ij}). In the above, the Einstein convention of summation over repeated indices has been adopted. The Gaussian curvature κ_g and the mean curvature κ_m are

$$\kappa_g = \frac{L}{g} = \frac{L_{11}L_{22} - L_{12}^2}{g_{11}g_{22} - g_{12}^2}, \qquad (23)$$

$$\kappa_m = \frac{L_{11}g_{22} - 2L_{12}g_{12} + L_{22}g_{11}}{2g}. \qquad (24)$$

where $g = det(g_{ij}), L = det(L_{ij})$. The compatibility conditions $(\mathbf{x}_{ss})_t = (\mathbf{x}_{st})_s$ and $(\mathbf{x}_{st})_t = (\mathbf{x}_{tt})_s$ applied to the linear Gauss system (19) produce the Gauss and Mainardi-Codazzi system

$$\begin{aligned} L &= g_{11}((\Gamma_{22}^1)_s - (\Gamma_{12}^1)_t + \Gamma_{22}^1\Gamma_{11}^1 + \Gamma_{22}^2\Gamma_{12}^1 - \Gamma_{12}^1\Gamma_{12}^1 \\ &- \Gamma_{12}^2\Gamma_{22}^1) + g_{12}((\Gamma_{22}^2)_s - (\Gamma_{12}^2)_t + \Gamma_{22}^1\Gamma_{11}^2 - \Gamma_{12}^1\Gamma_{12}^2) \end{aligned} \qquad (25)$$

$$\begin{aligned} \frac{\partial L_{11}}{\partial t} - \frac{\partial L_{12}}{\partial s} &= L_{11}\Gamma_{12}^1 + L_{12}(\Gamma_{12}^2 - \Gamma_{11}^1) - L_{22}\Gamma_{11}^2 \\ \frac{\partial L_{12}}{\partial t} - \frac{\partial L_{22}}{\partial s} &= L_{11}\Gamma_{22}^1 + L_{12}(\Gamma_{22}^2 - \Gamma_{12}^1) - L_{22}\Gamma_{12}^2 \end{aligned} \qquad (26)$$

Theorem 4.1 (Fundamental existence and uniqueness theorem Of Surfaces) *[12] Let* g_{11}, g_{12} *and* g_{22} *be functions of s and t of class* C^2 *and let* L_{11}, L_{12} *and* L_{22} *be functions of s and t of class* C^1 *all defined on an open set containing* (s_0, t_0) *such that for all (s, t),*

(i)

$$g_{11}g_{22} - g_{12^2} > 0, \qquad g_{11} > 0, \qquad g_{22} > 0$$

(ii) $g_{11}, g_{12}, g_{22}, L_{11}, L_{12}, L_{22}$ satisfy the compatibility equations (25),(26) Then there exists a patch $X = X(s,t)$ of class C^3 defined in a neighborhood of (s_0, t_0) for which $g_{11}, g_{12}, g_{22}, L_{11}, L_{12}, L_{22}$ are the first and second fundamental coefficients. The surface represented by $\mathbf{X} = \mathbf{X}(s,t)$ is unique except for position in space.

5. Geometric Properties of Hasimoto Surfaces

For Hashimoto surfaces $\mathbf{x} = \mathbf{x}(s,t)$ the tangent vectors are

$$\mathbf{x}_s = t, \qquad \mathbf{x}_t = \kappa b$$

The coefficients of the FFF are

$$\begin{aligned} I &= \langle d\mathbf{x}.d\mathbf{x}\rangle \\ &= \langle (\mathbf{x}_s ds + \mathbf{x}_t dt).(\mathbf{x}_s ds + \mathbf{x}_t dt)\rangle \\ &= ds^2 + \kappa^2 dt^2 \end{aligned}$$

so that

$$\boxed{g_{11} = 1, \quad g_{12} = 0, \quad g_{22} = \kappa^2.} \qquad (28)$$

The unit normal to $\mathbf{x}$:

$$\mathbf{N} = \frac{x_s \wedge x_t}{|x_s \wedge x_t|} = -\mathbf{n} \qquad (29)$$

The coefficients of the SFF are

$$\begin{aligned} II &= \langle -d\mathbf{X}.d\mathbf{N}\rangle \\ &= \langle (\mathbf{t}ds + \kappa\mathbf{b}dt).(\mathbf{n}_s ds + \mathbf{n}_t dt)\rangle \end{aligned}$$

$$= -\kappa ds^2 + 2\kappa\tau ds dt + (\kappa_{ss} - \kappa\tau^2)dt^2$$

where we use the time evolution equations of the triad $\{\mathbf{t}, \mathbf{n}, \mathbf{b}\}$ with respect to s and t respectively Eqs.(2), (13) to obtain

$$\boxed{L_{11} = -\kappa, \quad L_{12} = \kappa\tau, \quad L_{22} = \kappa_{ss} - \kappa\tau^2.} \qquad (31)$$

It is easily verified that the Eqs. (28), (31) with (15) satisfies the compatibility conditions (25),(26) which are determined the surface up to its position in space as we shall show in the next section. The Gaussian curvature κ_g and the mean curvature κ_m for Hasimoto surface are

$$\kappa_g = \frac{-\kappa_{ss}}{\kappa} \qquad (32)$$

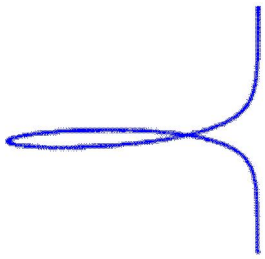
$$\kappa_m = \frac{1}{2\kappa}\left(\frac{\kappa_{ss}}{\kappa} - \kappa^2 - \tau^2\right) \qquad (33)$$

6. Geometric Visualization of the Hasimoto Surfaces and its Generator

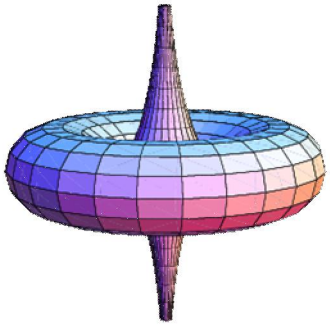
We recall that a curve in E^3 is uniquely determined by two local invariant quantities, curvature and torsion, as functions of arc length. Similarly, a surface in E^3 is uniquely determined by certain local invariant quantities called the first and second fundamental forms. Now by using Fundamental Theorem Of Surfaces 4.1 which states that the sextuplet $\{g_{11}, g_{12}, g_{22}, L_{11}, L_{12}, L_{22}\}$ determines the surface S up to its position in space. The surfaces below generated by evolution of space curve obtained via solving the Gauss-Weingarten equations (19,20) for a specified the coefficients of FFF and SFF using Mathematica [14].

(27)

Figure 1: Hasimoto surface corresponding to (30)
$(\kappa = 2sech(s+2t), \tau = -1)$



(a) $(\kappa = 2sech(s), \tau = 0)$



(b) surface generated by evolution of the above curve

Figure 2: Hasimoto surface and its generator

7. Conclusions

we constructed the Hasimoto surface from its fundamental form coefficients via numerical integration of Gauss-Weingarten equations and fundamental theorem of surfaces.

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A Comparative Study between Virtual Colonoscopy (CT Colonoscopy) and Conventional Colonoscopy in Different Presentations of Suspected Colonic Disorders

Wael M. Aref[1]; Ahmed El-Mazny[1] and Farid G. Amin[2]

[1]Department of Internal Medicine, Faculty of Medicine, Cairo University
[2]Department of Radiodiagnosis, Faculty of Medicine, Cairo University
waelaref@yahoo.com

Abstract: Background: Virtual colonoscopy is a promising new modality for investigating suspected colonic disorders, it is relatively safe, can be done without sedation and in less time compared to conventional colonoscopy. **Aim of this work:** to evaluate the application of virtual colonoscopy in different indications of conventional colonoscopy and compare between both procedures as regards sensitivity and specificity of both methods, putting the hypothesis that virtual colonoscopy can replace the conventional colonoscopy. **Subjects and Methods**: a group of eighty two patients having different indications for colonoscopy were included; all patients underwent full medical history, examination and any needed investigations. Patients were scheduled to undergo both conventional and virtual colonoscopy on the same week, both endoscopist and radiologist were unaware of the other report. **Results:** Both conventional and virtual colonoscopy detected colonic masses in 18 patients, colonic diverticulae in 5 patients and colonic strictures in 2 patients with no missed or false positive results with 100% sensitivity and specificity; and 100% positive and negative predictive values. Meaning that virtual colonoscopy was accurate in detection of masses, diverticulae and strictures. However detection of polyps by virtual colonoscopy was 88% sensitive and 77% specific with 3 missed polyps (small polyps) and 13 false positive polyps detected by virtual colonoscopy. Virtual Colonoscopy Could not detect any of the following lesions**:** angiodysplasia (2 patients), ulcerative colitis (without pseudo polyps) (3 patients), flat ulcers and non-specific colitis (11 patients), with a Sensitivity 0%. **Conclusion:** Virtual Colonoscopy can be used in evaluation of patients presenting with constipation, weight loss or abdominal pain in whom colonoscopic examination was indicated (in these patients colonic lesions were masses, strictures and diverticulae, so virtual colonoscopy is sensitive in detecting these lesions). But the use of virtual colonoscopy is limited in patients presenting with anemia and positive occult blood in stools, bleeding per-rectum and chronic diarrhea (in these patients the colonic lesions were angiodysplasia, flat ulcers and non specific colitis, so virtual colonoscopy is not sensitive in detecting these lesions). Also, virtual colonoscopy is a good diagnostic tool for screening for colorectal carcinoma**,** however using the recent technology in virtual colonoscopy as new faster CT multi-slice machines with the least possible slice thickness in order not to miss a small lesion is recommended.

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Key words: Virtual Colonoscopy - Conventional colonoscopy – Lower GIT symptoms - Colorectal carcinoma

1. Introduction

Three-dimensional Computed tomographic (CT) Colonography or Virtual Colonoscopy, is a promising new imaging method. The technique combines the use of rapid helical CT with computer software capable of rendering images of the whole colon. This method is being promoted by some as a noninvasive screening test for colorectal neoplasia **[1].** Current Computed tomographic (CT) Colonography protocols use submillimeter detector collimation, resulting in more than a thousand images for a single examination. Various image display techniques are used to interpret these large data sets. Image interpretation may include a two-dimensional (2D) axial reviewed by **[2],** 2D multiplanar reformation reviewed by **[3],** Primary 2D reviewed with three-dimensional (3D) comparison for problem solving by **[4],** primary 3D reviewed with 2D comparison for problem solving by **[5],** also computer-aided diagnosis**,** use of non radiologists as second readers, and use of various 3D display options including virtual dissection reviewed by **[6-9].**

Virtual colonoscopy has a number of advantages over conventional colonoscopy. With virtual colonoscopy, the examination is performed without sedation, in less time than and involves little risk of complications observed with conventional colonoscopy such as perforation and distension. It can help to examine the colon in case of failed colonoscopy due to either stricture or a large mass partially obstructing the colon, also it has a better patient compliance in patients who refused the conventional colonoscopy. The radiologist, using a number of static and dynamic display options, can examine and reexamine segments of the colon after the procedure has been performed. The localization of abnormalities is precise, and both sides of the bowel folds can be visualized **[10]**. The disadvantages of virtual colonoscopy include the need for bowel cleansing and infusion of gas to expand the

colon. Scanning hardware is expensive, and the interpretation of the images is relatively difficult and time consuming. Retained stool or fluid or contracted segments of the bowel interfere with the detection of lesions **[11].**

The current cost of virtual colonoscopy probably prohibits its use as a screening test. A major component of the cost is the time now required for a radiologist to perform the procedure. To be economically feasible for use as a screening method, the cost probably would need to drop below the cost of conventional colonoscopy, since virtual colonoscopy is only a diagnostic test. An appreciable number of patients undergoing screening would need a subsequent colonoscopy to evaluate abnormalities and resects polyps. This additional procedure must be included in any analysis of cost. The relatively low specificity of virtual colonoscopy in most series (i.e., the many false positive results) magnifies its cost, because it leads to unnecessary conventional colonoscopies. **[12]**

The use of CT imaging for the detection and staging of CRC (Colorectal carcinoma) was proposed as early as 1980 **[13]**. In 1983, **Coin et al., [14],** found that CT had potential mass screening method for colorectal polyps**.** Over a decade later, in 1994, the term "virtual colonoscopy" was formally introduced by **[15].** Since then, great advances in software and hardware have occurred**.** Since 1996, studies have been conducted using multiple scanning parameters, different risk populations, multiple stool and fluid tagging techniques, multiple colon preparation techniques, different image processing techniques, and differing radiologist experience with CTC to determine the best technique for screening.

So the aim of this study is to evaluate the applications of virtual colonoscopy in different indication of conventional colonoscopy and compare between both procedures in patients having different lower GIT complaints, putting the hypothesis that virtual colonoscopy can replace the conventional colonoscopy.

2. Subjects and Methods

Our study was a cross section study that included eighty two patients (42 male and 40 female). They were referred to Kasr El Aliny Hospital, El-Ebrashy Endoscopy unit, Internal Medicine Hospital, Cairo university between May 2008 and January 2010 for evaluation of different indications for colonoscopy including: iron deficiency anemia with positive occult blood in stool, bleeding per-rectum, repeated abdominal pain, chronic diarrhea, constipation or weight loss (as a screening tool for colorectal carcinoma). Those patients were invited to enroll in the study. Upper GI endoscopy was done for all patients with anemia with positive occult blood in stools, weight loss and abdominal pain before colonoscopy and all patients have irrelevant upper GIT findings.

All patients underwent full medical history, examination and investigations to exclude other causes of their complaint. Patients were scheduled to undergo both conventional and virtual colonoscopy on the same week; both endoscopist and radiologist were unaware of the other's report. There were no post-procedure complications after both conventional and virtual colonoscopy.

All patients were examined by using a four channel Multi-slice CT scanner (light-speed plus; GE Medical System, Milwaukee), The data was recorded in DICOM format and re-evaluated using a "GE medical system advantage window 4.0 sun works" for post-processing.

Preparation for Conventional and Virtual colonoscopy:

Patients were instructed to: maintain a clear liquid low fiber diet 48 hours prior to examination, followed by oral intake 500 ml of Mannitol 20 % which gives the best results in emptying the colon from its natural contents**.** Rectal cleaning enema was done few hours before the study.

Patient position during CT colonography:

Thirty patients underwent scanning in both prone and supine positions, while the remaining fifty eight patients, were examined in the supine position only. The choice of whether additional prone position scanning was needed, was taken based on adequate preparation of the patient judged at the scanning time.

Bowel distension during CT colonography:

A rectal tube was placed, and air was insufflated to maximum patient tolerance, with an average of 30-40 blub compressions, the scout CT image allowed rapid assessment of colonic distension. When necessary, further insufflations were performed to maximum patient tolerance before data acquisition. The rectal tube was removed for improved patient comfort and to prevent possible rectal lesions from being obscured.

Image processing:

Performed with computer workstation with commercially available software (NAVIGATOR; GE medical system) that provided image reconstruction that was performed by using an interval of 1.5 mm. The processed images included sagittal and coronal two-dimensional (2D) reformatted, endoluminal and virtual dissection "Colon Splitting" images. The 2D CT reformatted images and endoluminal images were represented in a multiple-image display format. The endoluminal images viewed continuously in the interactive mode provided an endoscopic- like examination.

All images were interpretated on the computer workstation by radiologists blinded to the patient's history and to results of standard conventional colonoscopy. The evaluation consisted of initial review

of the magnified 2D transverse CT images followed by review of the endoluminal images in the interactive (Fly-through) mode. Endoluminal viewing was performed in both antegrade and retrograde directions and with the patients in both supine and prone positions by using a step interval of 3-5 mm. the transverse and reformatted coronal and sagittal 2D CT images were displayed alongside the endoluminal images in a four-quadrant display format.

Segmentation:

The colon was classified into 6 segments: Rectum-Sigmoid Colon- Descending Colon- Transverse Colon (including both hepatic and splenic flexures)-Ascending Colon- Caecum.

Statistical analysis

Data were checked, coded, entered and analyzed using computer based statistical package for social sciences (SPSS) for windows 16 program. Comparison between data of the study group was done using sensitivity, specificity, positive predictive value, negative predictive Value, total Accuracy, and measure of agreement. The "*p"* value of 0.05 was considered the limit below which the difference of the values would be statistically significant.

3. Results

The studied group consisted of eighty two patients [42(51%) male and 40 (49%) female]. The age of studied patients ranged from 14-70 years their mean age was 45.90 ± 15.76 years.

The prevalence of the main indications for colonoscopic examination among the studied group showed 22 (27%) had anemia, 15(18%) had bleeding per rectum, 14 (17%) had constipation, 12 (15%) chronic diarrhea, 10 (12%) had weight loss and 9 (11%) had abdominal pain.

The lesions found among studied group at conventional colonoscopy were as follows: polyps {small in 6 patients (7.3%), medium in 3 patients (3.6%), large in 16 patients (19.5%)}, mass in 18 patients (22%), stricture in 2 patients (2.4%), diverticulae in 5 patients (6%), angiodysplasia in 2 patients (2.4%), ulcerative colitis in 3 patients (3.6%), flat ulcers & non-specific colitis in 11 patients (13.4%), internal piles in 2 patients (2.4%) and no lesions in 14 patients

The lesions found among studied group at virtual colonoscopy included the following : polyps { small in 2 patients (2.4%). medium in 2 patients (2.4%), large in 20 patients (24.4%)}, mass in 17 patients (20.7%), stricture and large polyp in 2 patients (2.4%), medium and small polyp in 1 patient (1.2 %), large and small polyp in 3 patient (3.6%), mass and medium sized polyp in 1 patient (1.2%), diverticulae in 5 patients (6%) and no lesions in 29 patients (35.3%) (more than one lesion may be present in the same patient).

By comparing the findings in both conventional and virtual colonoscopy in each indication for colonoscopy we found **(22)** patients with anaemia that were indicated to undergo colonic examination, **(15)** of them have lesions on conventional colonoscopy and **(7)** had no lesions,. Only **(9)** had lesions on virtual colonoscopy with **(7)** missed lesions and (1) false positive lesion, with 53.33% Sensitivity and 85.7% Specificity of virtual colonoscopy in detection of lesions in patients with anemia. Also **(15)** patients with bleeding per-rectum were indicated to undergo colonic examination all of them had lesions on conventional colonoscopy while in the **(15)** patients, **(18)** lesions were detected on virtual colonoscopy (more than one lesion may be present in same patient), with **(3)** missed lesion and (6) false positive lesions with a Sensitivity 80% and Specificity 60% in finding lesions in patients with bleeding per-rectum.

As regards patients with constipation, **(12)** out of **(14)** had lesions on conventional colonoscopy, while **(14)** lesions were detected in the **(12)** patients on virtual colonoscopy (more than one lesion may be present in same patient), with **no** missed lesion and (2) false positive results, with Sensitivity 100% and Specificity 85.7% in finding lesions in patients with constipation. We found that **(10)** patients with chronic diarrhea out of **(12)** had lesions on conventional colonoscopy while in virtual colonoscopy, only **(3)** out of the **(12)** patients had lesions, with **(10)** missed lesions and **(3)** false positive lesions, with **0** % Sensitivity and Specificity of virtual colonoscopy in finding lesions in patients with chronic diarrhea

As regards patients presenting with unexplained weight loss**, (9)** out of **(10)** had lesions on conventional colonoscopy while in virtual colonoscopy, **(10)** lesions were detected in the **(9)** patients with no missed lesions and (1) false positive lesion, with 100 % Sensitivity and 90% Specificity of virtual colonoscopy in finding lesions in patients with unexplained weight loss. Also **(7)** patients with repeated abdominal pain out of **(9)** had lesions on conventional colonoscopy, while in virtual colonoscopy there were also **(7)** lesions on virtual colonoscopy detected in the **(7)** patients with **(1)** missed lesion and (1) false positive lesion, with 85.7% Sensitivity and 88.8% Specificity of virtual colonoscopy in finding lesions in patients with abdominal pain.

By comparing the finding in both conventional and virtual colonoscopy regarding each colonic lesion. Regarding polyp detection, the total number of polyps detected by conventional colonoscopy were **(25)**, (**6** small, **3** medium, **16** large), while by virtual colonoscopy were **(35)**, (**6** small, **4** medium, **25** large). There were **(13)** false positive results and **(3)** missed results in virtual colonoscopy with overall Sensitivity for detection of polyp lesions 88% and Specificity 77%. Summary of the results and statistical analysis in polyp

detection by both conventional and virtual colonoscopy were shown in table (1).

Table (1): Comparison between both conventional and virtual colonoscopy as regards polyp detection

Polyp size	**Small < 5 mm**		**Medium 5-.9mm**		**Large > 10 mm**	
The procedure	VC	CC	VC	CC	VC	CC
Detected No.	6	6	4	3	25	16
Missed polyps	3	0	0	0	0	0
False positive polyps	3	0	1	0	9	0
Sensitivity%	50%	100%	100%	100%	100%	100%
Specificity%	96%	100%	98.5%	100%	86.5%	100%
PPV	50%	100%	75%	100%	64%	100%
NPV	96%	100%	100%	100%	100%	100%
Total Accuracy	88.2%	100%	100%	100%	89.7%	100%
Measure of agreement(Ķappa)	0.363	1.00	1.00	1.00	0.752	1.00
P value	0.02		<0.001		<0.001	

PPV= positive predictive value, NPV= negative predictive value, CC=conventional colonoscopy, VC=virtual colonoscopy.

The results of both conventional and virtual colonoscopy in polyp lesions detections at different colonic segments were shown in table (2).

Table (2): The results of both conventional and virtual colonoscopy in polyp's lesions detections at different colonic segments

	Small < 5 mm		Medium 5-9.9mm		Large > 10 mm	
	CC	VC	CC	VC	CC	VC
Rectum	1	2	1	1	6	8
Sigmoid C.	2	3	1	2	6	7
Descending	2	1	0	0	2	2
Transverse	0	0	0	0	0	0
Ascending	1	0	1	1	2	3
Caecum	0	0	0	0	0	5

CC= Conventional colonoscopy, VC=Virtual colonoscopy**.**

The total number of masses detected by both conventional and virtual colonoscopy were **(18)** with no missed or false positive results with a **Sensitivity 100%**, **Specificity 100%, Positive Predictive Value 100%, Negative Predictive Value 100%** and **Total Accuracy 100%** with **measure of agreement (Ķappa) = 1.00**, **(P < 0.001).** (table 3)

Both conventional and virtual colonoscopy could detect strictures in 2 patients, one with a sigmoid colon stricture and the other with a descending colon stricture. In both patients, the scope of the conventional colonoscopy failed to pass through the stricture, while in virtual colonoscopy, examination was completed. In patient with sigmoid stricture, there was a 2 cm ascending colon polyp while the patient with descending colon stricture, virtual colonoscopy detect a 1 cm caecal polyp. So, virtual colonoscopy had a Sensitivity 100%, Specificity 100%, Positive Predictive Value 100%, Negative Predictive Value 100% and Total Accuracy 100%, (P < 0.001). (table 3)

The total number of diverticular lesions detected by both conventional and virtual colonoscopy were **(5)** with no missed or false positive results and with a Sensitivity 100%, Specificity 100%, Positive Predictive Value 100%, Negative Predictive Value 100% and Total Accuracy 100%, with measure of agreement (Ķappa) = 1.00, (P < 0.001) (table 3).

Table (3): comparison between conventional and virtual colonoscopy as regards mass, stricture and diverticulosis

Lesion	**Mass**		**Stricture**		**diverticulosis**	
The procedure	VC	CC	VC	CC	VC	CC
Detected No.	18	18	2	2	5	5
Missed lesions	0	0	0	0	0	0
False positive lesions	0	0	0	0	0	0
Sensitivity%	100%	100%	100%	100%	100%	100%
Specificity%	100%	100%	100%	100%	100%	100%
PPV	100%	100%	100%	100%	100%	100%
NPV	100%	100%	100%	100%	100%	100%
Total Accuracy	100%	100%	100%	100%	100%	100%
Measure of agreement(Ķappa)	1.00	1.00	1.00	1.00	1.00	1.00
P value	<0.001		<0.001		<0.001	

PPV= positive predictive value, NPV= negative predictive value.

In two patients with internal hemorrhoids, virtual colonoscopy detected the piles as false positive rectal polyps. In the rest of the lesions detected by conventional colonoscopy, virtual colonoscopy could not detect any of the following lesions: angiodysplasia: (2 patients), ulcerative colitis (without pseudopolyps) (3 patients), flat ulcers and non-specific colitis (11 patients).

CT colonography detected several extra-colonic lesions as enlarged lymph nodes (8 patients), abdominal aortic atherosclerosis (6 patients), abdominal aortic aneurysm (3 patients), focal hepatic lesions (5 patients), liver hemangiomas (4 patients), hepatic steatosis (15 patients), solid renal mass (2 patients), renal cysts (4 patients), pleural effusion (7 patients), solid pancreatic mass (2 patients), ovarian teratoma (1 patient), bilateral renal hydronephrosis (1 patient), ileal wall thickening (1 patient), vertebral body lytic lesion (1 patient). All these lesions could not be detected by conventional colonoscopy.

4. Discussion

Virtual colonoscopy is a non-invasive relatively novel health technology used to examine the large bowel mainly in screening of colorectal cancer **[15]**. The accuracy of virtual colonoscopy in assessing different pathologies in different indications for colonoscopy was the aim of this study.

Comparison between virtual and conventional colonoscopy as regards their results and sensitivity for colonic lesions detection has been the goal for several studies in order to assess the accuracy of virtual colonoscopy. In our study, the overall sensitivity of Virtual colonoscopy in polyp detection was **88%**; for the detection of large polyps (10 mm or more) the sensitivity was **100%** and specificity was **86.5%**; for detection of medium sized polyps (5-10 mm) the sensitivity and specificity was **100%** and for detection of polyps smaller than 5 mm the sensitivity was **50%** and specificity **was 96%.** These results matched the results of **Gluecker et al. [19],** who reported an overall sensitivity of virtual colonoscopy in polyp detection **85.3%,** with sensitivity for small polyp detection **65%** and medium sized polyp detection **97%** and large polyp detection **100%.** Also **Kalra et al. [20]**, who reported Sensitivity **65 %** and specificity **92%** in small polyps detection, Sensitivity **97%** and specificity **93%** in medium sized polyp detection and sensitivity **100%** and specificity **88%** in large polyp detection.

In this study the colon was divided into six segments with highest prevalence of polyps at the rectum and sigmoid colon **"34.6%"** for each. Our results agreed with **Zalis et al. [18]**, who has divided the colon into the same six segments and showed predominant variable sized polyps detected at the rectum and sigmoid colon each constitutes "**33.3%"** of the number of detected polyps**.**

In our study, the false positive results in detecting large polyps by virtual colonoscopy was largely (five patients) due to misinterpretation of the ilieo-caecal valve as a large caecal polyp which is one of the disadvantages of virtual colonoscopy. This agreed with **[21], w**ho concluded that the ileocecal valve (ICV) can have a polypoid shape and is a common cause of false-positive findings during CT Colonography.

In our study, The main missed lesions in virtual Colonoscopy were 3 polyps smaller than 5 mm (from total 6 small polyps detected by conventional colonoscopy) with 50% sensitivity. Other missed lesions included internal hemorrhoids in 2 patients, hyperemic mucosa and flat ulcers in 3 patients with ulcerative colitis, colonic angiodysplasia in 2 patients and 11 patients with flat ulcers and non-specific colitis. These results are matching with the conclusions of **Park et al. [22],** who reported that flat lesions, ulcers, vascular lesions, non-complicated flat inflammatory lesions and small polyps are the main causes for missed lesions at CT colonoscopy. In this study, when all flat, sessile or pedunculated lesions are included, sensitivity was **75%** for lesions 10 mm or larger, and **79%** for those 6 mm or larger. When only sessile and pedunculated lesions were included, corresponding sensitivities were **100%** and **98%** respectively which matches our results. All missed lesions larger than 10 mm were flat. Sessile or pedunculated polyps 5 mm or smaller were more likely to be missed more than those 6 mm or larger**.** On the other hand, our study did not agree with **Bond [23],** who reported a **95%** sensitivity of virtual colonoscopy in small polyp detection and this may be due to the use of a multislices CT used was with more advanced technology and higher speed with very thin slice thickness.

In our study, there were two patients with colonic strictures in whom conventional colonoscopy failed to pass and to complete the study, but Virtual colonoscopy was able to detect both strictures and was able to complete the study in both patients, in both of them we discovered a polyp in each one proximal to the stricture in Virtual Colonoscopic examination, which agreed with **Iannaccone et al. [24],** who has reported a Sensitivity of **100%** for Virtual colonoscopy in the detection of both strictures and colonic mass lesions and discovered lesions proximal to the occlusive growth (mass or stricture) in 45 out of 100 patients.

In patients presented with anemia with positive occult blood in stool, virtual colonoscopy overall sensitivity in lesions detection was 53.33% and has high sensitivity and specificity in patients presenting with anemia due to large or medium-sized polyps, but in small polyps, it has sensitivity 60% only. It detected diverticulae in one patient presenting with anemia, but it couldn't detect angiodysplasia, flat ulcers, ulcerative colitis in other 4 patients. So, we concluded that the

use of Virtual Colonoscopy can be helpful in some but not all patients presenting with this type of anemia.

Many authors **[17-18-21-26-29],** stressed the role of virtual colonoscopy in colorectal cancer (CTC) screening, with excellent sensitivity for polyps (the precursor of colorectal cancer) masses and malignant strictures with safety and acceptability. Our results meets the results of **[24],** who reported a sensitivity of 100% in masses, strictures and large polyps detection by the virtual colonoscopy, also our study agreed with **Lieberman et al.[30],** who reported a 100 % sensitivity of virtual colonoscopy in large polyps and masses detection.

This high sensitivity in virtual colonoscopy in patients with constipation is due to the presence of colonic masses or large polyps in this group of patients, which were all detected by the virtual colonoscopy (sensitivity of virtual colonoscopy in mass and large polyps detection was **100%** for both).

So, the application of virtual colonoscopy in patients presenting with weight loss and constipation can be tried as a screening tool due to its high sensitivity in detecting the lesions causing these symptoms which proved to be malignant lesions in our study. Also it has advantages over the conventional colonoscopy due to its ability to diagnose extra-colonic spread and detect the mural tumor invasion with high efficacy in colorectal carcinoma staging with detection of lymph node or liver metastasis. But on the other hand, Virtual Colonoscopy has a very unaccepted pifall in such patients as we cannot perform a diagnostic biopsy from the causative lesion which is essential to diagnose the nature of such lesions.

A limitation of the present study is the small sample size in some patient groups regarding each indication. Hence, our results need to be verified in a larger prospective study.

In Conclusion, multi-detector CT Colonography (Virtual Colonoscopy) is a reliable tool for detecting colonic mass lesions larger than 5 mm, polyps larger than 5 mm, strictures and colonic diverticulae. CTC is of value in evaluating the colonic segment lying proximal to colonic cancers including those with occlusive growths or strictures. Contrast-enhanced CTC is also useful in identifying extra-colonic findings.

Virtual Colonoscopy is a good screening tools for malignant or premalignant lesions in patients presenting with constipation or weight loss. Also it helps in staging of colorectal carcinoma regarding the detection of tumor mural growth, lymph node or liver metastasis and in diagnosis of associated extra-colonic lesions as ascites which could not be done by conventional colonoscopy. But its use as a good diagnostic tool is limited due to inability for a diagnostic biopsy from such lesions.

From this study we recommended further evaluation of some patient groups as those with bleeding per-rectum, diarrhea and anemia as our study has some limitations due to small sample size.

It is recommended to apply the use of virtual colonoscopy as a follow up tool in patients with previous known precancerous lesions and familial polyposis or suspicious lesions who need at least annual colonoscopic follow up using the new faster CT multi-slices machines with the least possible slice thickness in order not to miss a small lesion that cannot be seen between two slices of the CT.

Corresponding author
Wael M. Aref
Department of Internal Medicine, Faculty of Medicine, Cairo University
waelaref@yahoo.com

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Studies on Coccidia of Egyptian Balady Breed Chickens

Ahmed A. Al-Gawad[1]; Olfat A. Mahdy[1]; Aida A. N. El-Massry[1] and Mohamed S. A. Al-Aziz [2]

[1]Faculty of Veterinary Medicine Cairo University. Department of Parasitology
[2]Middle East Business Unit Manager Pfizer Animal Health
dr.olfat.mahdy@gmail.com

Abstract: A total of 711 Balady breed chickens of different ages and sex were collected from houses and farms of 4 localities: Cairo & Giza, governorates Western delta governorates; El-Gharbiyah, El-Behiera, Kafer El- Sheikh, Eastern governorates; El- Sharqiyah, Ismailia & Upper Egypt governorates; Qina and Aswan, during the period between September 1999 - August 2003 were sacrificed and their intestine were examined for the presence of *Eimeria* species. Microscopical identification of Eimeria oocysts species revealed that 21.24% of these chickens were found infected with five species of Eimeria; which were *E.necatrix* (58.27%), *E.tenella* (25.82%), *E.acervulina* (19.20%), *E.mitis* (10.59%) and *E.maxima* (4.66%), respectively. It was found that chickens of 1-21 day old were found free from infection (0%), while chicken of 64 – 84 day old showed high infection rate (62.37%).The high rate of infection was noticed in winter season (45.13%), while the lowest rate was recorded during summer season (1.86%). The highest incidence of *Eimeria* species (37.16%) was found in (Cairo & Giza).While, the lowest incidence (7.32%) was found in Delta areas. The prepatent period, age resistance beside histological examination of the five previously identified *Eimeria* species, which were experimentally isolated and propagated, was also studied.
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Keywords: Five *Eimeria* species - Egyptian Balady Breed Chickens – Incidence – Histopathology.

1. Introduction

Avian Coccidiosis is the major problem in poultry worldwide; it causes serious problem and causing huge economic loss to poultry industry **Jadhav et al. (2011).** The occurrence of different *Eimeria* species combinations and the intensity of infection vary considerably, both globally and locally **Oikawa et al. (1979), Williams (1996)** and **Amer et al. (2010)** and with time **Braunius, (1986b)** and **Haug et al. (2008).**

Coccidiosis also, causes weight loss, lower feed conversion rate, delayed sexual maturity and decrease of egg production. **Lobago et al. (2005).** Lesions of the intestinal mucosa and loss of pigmentation may also become apparent during the latter stages of infection **Conway & McKenzie (1997), Mc Dougald & Reid (1997)** and **Amer et al. (2010).**

In Egypt, numerous research papers were carried on coccidiosis of commercial white broilers, but few of them carried on Egyptian Balady breed chicken, which seem to be more resistant to infectious diseases **Abu El-ezz (1994).** Therefore, this study was designed to determine the incidence of coccidiosis in local strain (balady breed chickens) and to identify the prevalent *Eimerian* species in 4 different localities in Egypt. Experiment was planned to study the isolation and identification of the most predominant *Eimeria* species by morphology and detection of microscopic lesions as well as studying the pathogencity of each isolates species.

2. Materials & Methods

A total of 711 sacrificed Egyptian Balady breed chickens of different sex and ages (1-21days), (22– 42 days), (43 – 63 days), (64 - 84 days) and (< 84 days) were collected from 4 localities in Egypt; Cairo & Giza, Western delta governorates; El-Gharbiyah, El-Behiera, Kafer El-Sheikh, Eastern governorates; El- Sharqiyah, Ismailia and Upper Egypt governorates; Qina & Aswan. The study was conducted from September 1999 to August 2003.

Concentration floatation technique was applied for the collection of *Eimeria* oocysts from intestinal content of chickens **Davies et al. (1963).** Isolation of *Eimeria* oocysts was depended on the measurements by using a calibrated ocular micrometer at 400x magnification **Long** and **Reid (1982),** 30 random oocysts from each sample were identified by a combination of the following criteria according to **Conway** and **Mckenzie (1997);** (1) Location & characteristics of intestinal lesions (2) oocyst morphology (3) Sporulation time of *Eimeria* species. *Eimeria* oocysts measured and categorized into three groups (Table 1): a small oocysts group, 17.8-14.1µm; *Eimeria mitis* in the middle part of intestine (ileum) and 18.2-14.1µm *Eimeria acervulina* (duodenum), a medium group sized oocysts, 20.1-16.9µm; *Eimeria necatrix* (ileum) and 21.3-17.9µm *Eimeria tenella* (caecum); a large oocysts group, 29.9-23.8µm; *Eimeria maxima* (ileum).

Gross lesion examination and lesion scores: Investigated sacrificed chickens in the laboratory by cervical dislocation using the technique described by **Zander (1978).** The gastrointestinal tract was grossly examined carefully. The intestinal portions were divided into 4 sections, the upper part (duodenum and

jejunum), the middle part (ileum), lower part (distal ileum and rectum) and cecal pouches. Intestinal gross lesions in any part of the sections were graded from 0 to 4 based on lesion score key **Conway** and **McKenzie (1997).** The lesion score zero represents absence of lesion and lesion score four is for very severe intestinal /cecal mucosa lesion and fatal cases. The location of the lesion was recorded; intestinal contents from the respective sections were taken and duplicate mucosal scrapping smears made from each section of the intestine.

From each part of infected intestine of *Eimeria* oocysts species was collected the content and prepared according to **Conway** and **McKenzie (1997)**. Then identification of each species of *Eimeria* depending on the three criteria previously recorded. Each species of *Eimeria* was spread out in shallow Petri dish 2.5% potassium dichromate solution for sporulation. **Ryley et al. (1976).** The isolated oocysts were counted by Mc Master Technique **Long et al. (1976).**

Selected number (10^3) of identified species of sporulated oocysts; (*E.necatrix, E.acervulina*, *E.maxima & E.mitis*) and (10^4) for *E .tenella,* were inoculated orally to experimentally chickens for propagation and histopathological studies of investigated *Eimeria* species.

Experimental infection: In this experiment 5 isolated oocysts which are; *E.mitis*, *E.acervulina, E.necatric, E.tenella & E.maxima,* were inoculated in 28 old age Balady breed chickens. In this experiment 80 Balady free chicks reared from one day old in disinfected wire cages. The ration used for the chicks was completely free from antibiotics and anticoccidials drugs. The eighty balady chicks were divided into 6 groups; the first 5 groups (10 chicks/ group) were inoculated by 10^3 sporulated oocyst of each pre-isolated species of *Eimeria* through its crop. The last group (30 chicks) was kept as non infected control group (Table2). For examination of intestinal lesions and endogenous stages of the parasites, one of the infected chickens from each group was slaughtered at 3 to 8 day's post-inoculation (dpi) depending on the *Eimeria* species. Faeces were collected once daily and examined for the presence of oocysts and detection prepatent time, Oocysts count and morphology were also determined **Thebo et al. (1998).** The rest of infected chickens were slaughtered on 9^{th} of infection to detect the P.M lesions and histopathological changes for each *Eimeria* species. The intestinal tissues of infected chickens showing gross lesions were fixed by formal saline 10% to apply histopathological staining according to **Carleton et al. (1967).**

3. Results

From table (3): it was found that 151(21.24%) chickens from total 711 were infected by five *Eimeria* species (in four different localities from Egypt). The rates of infection by *Eimeria* species were 58.27%, 25.82%, 19.20%, 10.59% and 4.66% for *E.necatrix E.tenella, E.acervulina E.mitis* and *E.maxima*, respectively. (Fig.1); showed unpopulated oocysts of *Eimeria* spp.; (A).*E. maxima*, (B) *E.acervulina*, (C) *E. mitis* (D) *E.tenella and* (E). *E.necatrix*. Mixed infection was recorded in 33% of positive samples.

Table (4); revealed that the Cairo & Giza regions showed highest incidence of infection (37.16%) followed by Eastern delta regions (36.30%).While, the lowest incidence was recorded in Western delta regions (7.32%).

Concerning the age susceptibility, it was found that chickens of 1-21 days age showed low rate of infection (0%), while chicken of 64 – 84 day old showed high infection rate (62.37%).

Regarding the season's variation, table (5) showed that a high rate of infection has been noticed during the winter season (45.13%), while lower rate of infection was recorded during summer season (1.86%). Autumn and spring seasons showed 18.30% & 18.21 % rate of infection.

From experimental results in table (6), determined the prepatent period from each infected groups they recorded 100 hrs. for *E. mitis* & *E.acervulina*, 120 hrs. for *E. maxima* & *E.tenella* and 168 hrs. for *E. necatrix*. Also, described the predilection sites and P.M lesions for different *Eimeria* species which beginning from 4^{th} dpi. for *E. mitis, E.maxima, E. necatrix and E. tenella,* While, started on the 5^{th} dpi. for *E.acervulina.* The clinical signs concentrated mainly in loss of weight, severe anemia and bloody diarrhea in *E.tenella.*

Tissue specificity and gross lesions were preliminary diagnostic of samples especially for *E.acervulina* (Fig.2), *E.necatrix* (Fig.3) and *E.tenella* (Fig.4), during the experimental infection gross lesions.

Histological finding of duodenum of experimentally infected chicken with *E. acervulina* showed presence of hyperplastic changes in the epithelial mucosa with activation of goblet cells, sometimes there was epithelial desquamation. The lamina propria was infiltrated with inflammatory cells Plate (1A-B), accompanied with hemorrhagic areas (Plate 2A). Gametocyte was observed (Plate2B) and the muscular layers suffer from edema. In addition, plate (2 A-H) explains the histological finding of the middle part of small intestine of naturally infected balady chicks with *E. necatrix* which showed its characteristic coagulative necrosis and focal hemorrhagic areas and deeply embedded gametocyte in tunica musculosa and serosa.

Moreover, the caecum of naturally infected balady chicks with *E.tenella* Plate (3A-C): showed considerable numbers of oocysts in lamina propria beside sever hemorrhage and complete desquamation of epithelium and edema of muscular tissue.

Table (1): Identification of five *Eimeria* species in Balady chickens.

Site of lesion	Postmortem lesions	Shape	Size of oocyst (µm)	Shape index	Sporulation time	Species of *Eimeria* identified
Ileum	Mucoid, enteritis	Ovoid	17.8-14.1	1.26	18 hrs.	*E.mitis*
Duodenum	Transverse Whitish band on duodenal loop.	Ovoid	18.2-14.1	1.29	17 hrs.	*E.acervulina*
Ileum	Balloning of intestine Mucoid blood filled exudates	Oblong Ovoid	20.1-16.9	1.19	18 hrs.	*E.necatrix*
Caecum	Haemorrhages & clotted blood in caecal pouches	Ovoid	21.3 -17.9	1.19	18 hrs.	*E.tenella*
Ileum	Thickened intestine wall. Patechiae.	Ovoid	29.9-23.8	1.25	30 hrs.	*E.maxima*

Table (2): Experimental infection of 50 Balady free chicks (28 days) old with five *Eimeria* species.

Main Group	1	2	3	4	5	Control
No. of chicks	10	10	10	10	10	30
Eimeria spp.	*E.necatrix*	*E.tenella*	*E.acervulina*	*E mitis.*	*E. maxima*	0
Inoculation dose	10^3	10^4	10^3	10^3	10^3	0

Table (3): Incidence of *Eimeria* species in examined chickens.

Species	Number of examined chickens (711)	Number of infected chickens (151) 21.24%	No. inf. chickens	%	Mixed infection
E.necatrix			88	58.27	9
E.tenella			39	25.82	28
E.acervulina			29	19.20	18
E.mitis			16	10.59	16
E.maxima			7	4.66	7

Table (4): Incidence of *Eimeria* spp. in four different localities in Egypt.

localities group	No. of ex.	No. of inf.	Incidence (%)	Isolated species
Cairo & Giza group	148	55	37.16	*E.acervulina, E.maxima, E.mitis, E.necatrix and E.tenella*
Western Delta area (El-Gharbiyah, El-Bihiera, Kafer El-Sheikh)	287	21	7.32	*E.acervulina, E.maxima, E.mitis and E.tenella*
Eastern Delta governorate (El Shrquiyah &Ismailia)	157	57	36.30	*E.acervulina, E.maxima, E.necatrix and E.tenella*
Upper Egypt (Qina & Aswan)	119	18	15.13	*E.acervulina, E.maxima, E.mitis, E.necatrix and E.tenella*
Total	711	151	21.24	*E.acervulina, E.maxima, E.mitis, E.necatrix & E.tenella*

Table (5): Seasonal incidences of *Eimeria* spp. in Balady breed chickens:

Season	Examined	Infected	%	Isolated species
Summer	107	2	1.86	*E.necatrix*
Autumn	213	39	18.30	*E.acervulina, E.necatrix and E.tenella*
Winter	144	65	45.13	*E.acervulina, E.mitis, E.necatrix & E.tenella*
Spring	247	45	18.21	*E.acervulina, E.maxima, E.mitis &E.tenella*
Total	711	151	21.24	*E.acervulina, E.maxima, E.mitis, E.necatrix and E.tenella*

Table (6): Illustrations of Prepatent period and gross lesions of experimentally infected Balady chickens.

gp. no.	Species	Prepatent period (hrs)	Clinical signs Day post Infection (dpi)	PM lesions	Gross lesions
1	*E.mitis*	100	Decrease in weight gain from 5th dpi.	4th day	Slight enteritis in middle part of intestine (+1)
2	*E.acervulina*	100	Decrease in weight gain from 5th dpi.	5th day	Duodenum had lesions from pinpoint white necrotic focci to sever ladder like white batches (+1 to +3)
3	*E.maxima*	120	Decrease in weight gain from 5th dpi.	4th day	Slight enteritis in middle part of intestine (+1)
4	*E.tenella*	120	From 3rd dpi. Severe depression, wing drops, strains, white diarrhea to bloody, no mortality.	4th day	Lesions began from 4th day as typhlitis ranging from slight to bloody. Lesion score from +2 to +4 in 100% of chicks
5	*E.necatrix*	168	From 4th dpi. Sever anemia, wing drops, strains, bloody diarrhea.	4th day	Bloody enteritis in middle part of intestine, lesion score (+2 to +3)

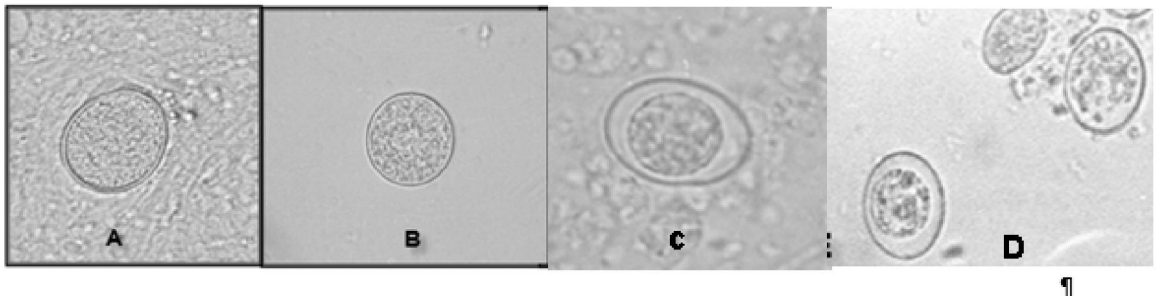

Fig.1; unpopulated oocyst of *Eimeria* spp.; (A). *E.acervulina*,, (B) *E. mitis* (C) *E.tenella* (D). *E.necatrix* (X400).

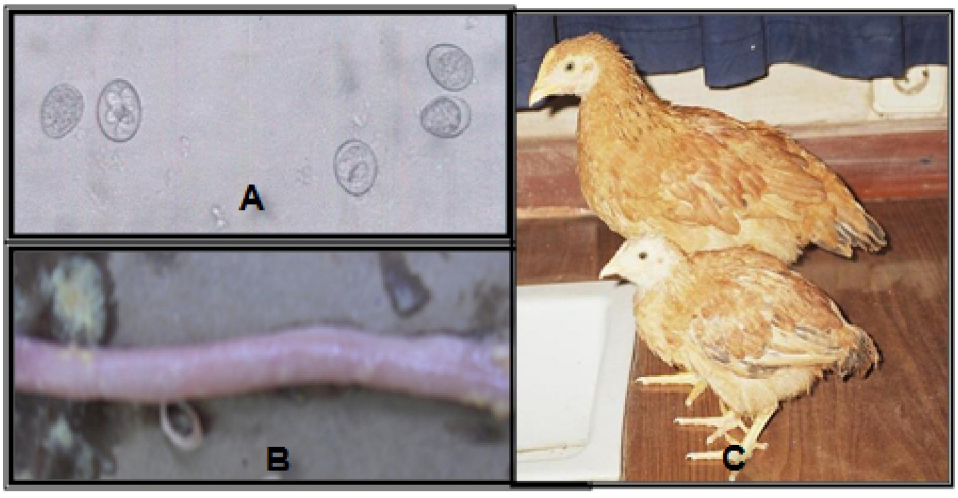

Fig.2: *Eimeria acervulina* (A) sporuolated oocysts direct smear X100 (B) White necrotic focci appear from the serosal surface of duodenum 6th dpi. (C) Infected chicken against control chicken showing loss of weight

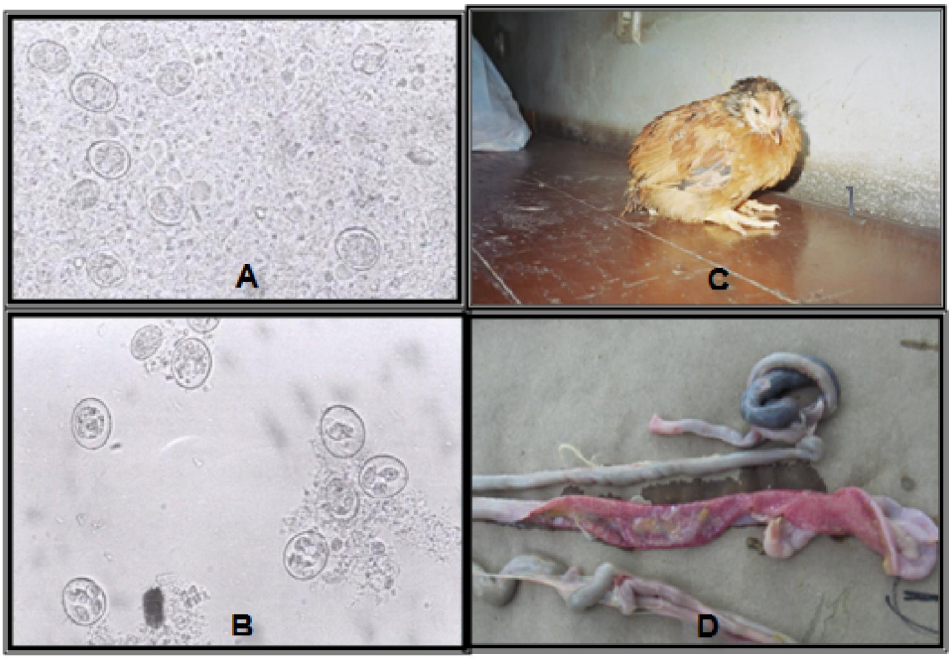

Fig.3: *E.necatrix* (A) unsporulated oocyst (B) sporulated oocysts from direct smear X100. (C) Infected chicken showing depression, ruffling and off food. (D) Ileum showing hemorrhagic enteritis 7thdpi.

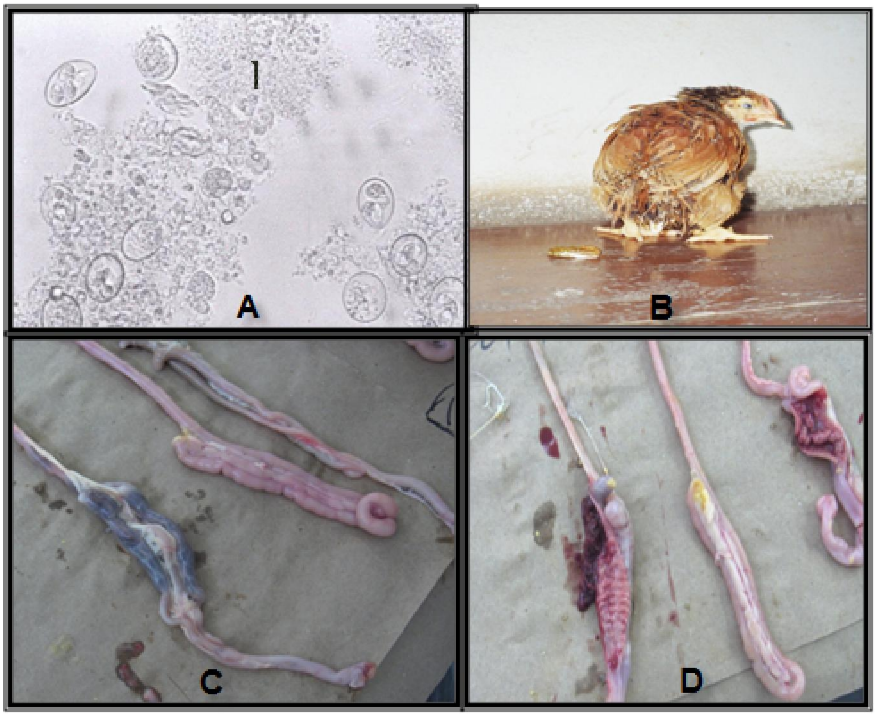

Fig.4*: E. tenella* (A) sporulated oocysts X100. (B) Infected chicken showing diarrhea (C&D) Two coeci of infected chicken showing bloody content 6th dpi.

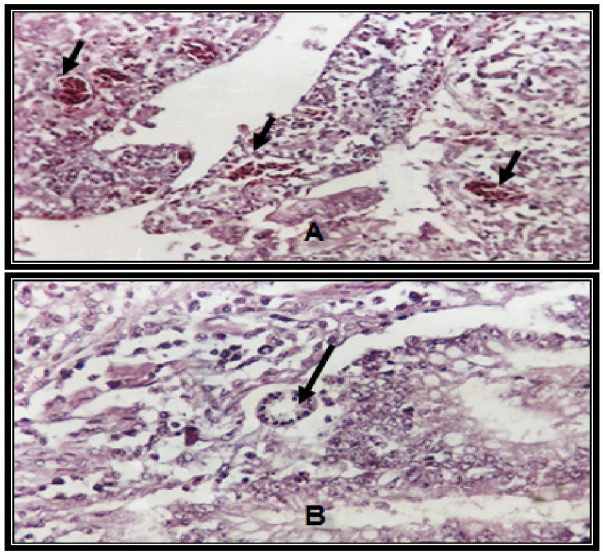

Plate 1 (A&B): showing histological finding of duodenum experimentally infected balady chicks with *E. acervulina*. A: showing focal hemorrhagic area (H. & E. X250).B: showing gametocyte (H. & E. X400).

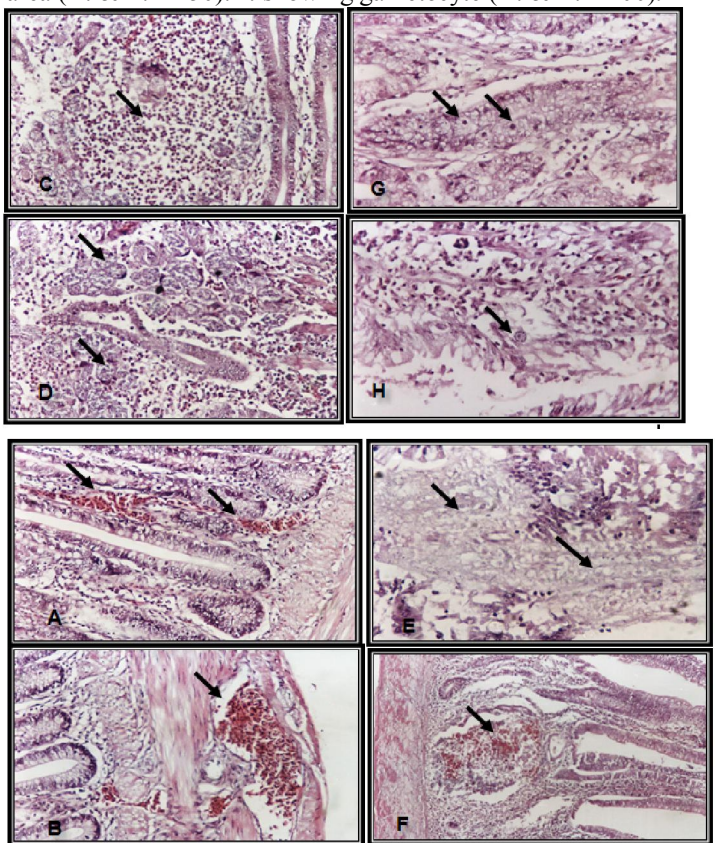

Plate 2 (A-H): showing the middle part of intestine of experimentally infected balady chicks with *E. necatrix*

A. showing mild hemorrhagic lamina propia (H&E X250). B. showing congestion in the muscularis (H&E X250)
C. showing inflammatory cells aggregation (H&E X250) D. showing great number of schizonts (H&E X250)
E. showing coagulative necrosis (H&E X 40) F. showing focal hemorrhagic area (H&E X100).
G. showing intracellular oocysts (H& E X400) H. showing gametocyte (H&E X250)

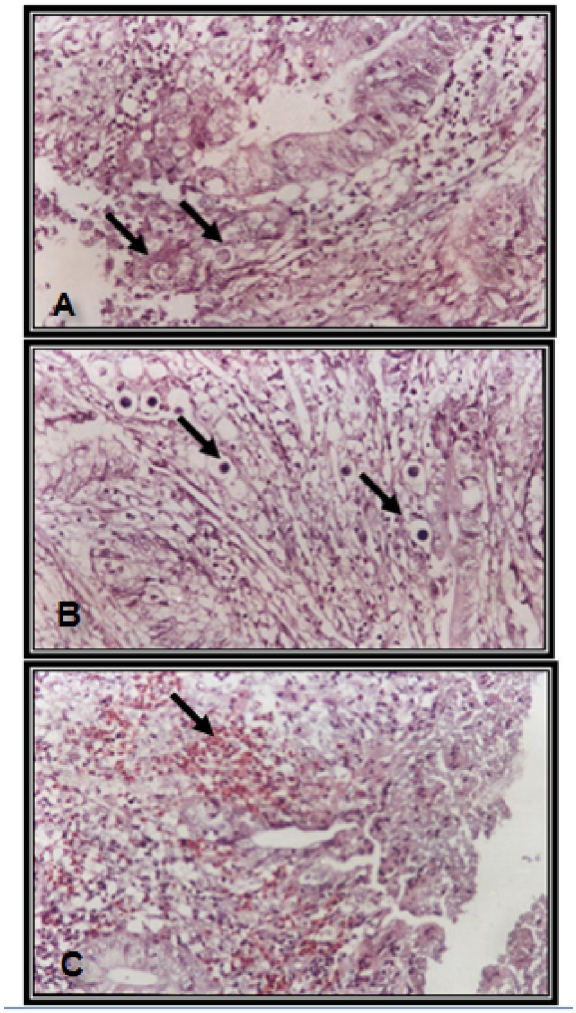

Plate 3(A-C) showing caceum of experimentally infected chickens with *E.tenella*
A. Showing different stages of coccidian (H&E X 250)
B. Showing numbers of intracellular oocysts (H&E X250)
C. Showing sever hemorrhage (H&E X250)

4. Discussion

In the present study, a total of 711 sacrificed Balady chickens of different ages and sex were collected from 4 different geographical localites in Egypt. The incidence of coccidiosis in native breeds was 21.24 %. This result nearly agreed with **Lunden & Thebo (2000) and Ashenafi et al. (2004)** who recorded 19.3 % incidence in layer farms in Sweden at the age ranged between 19-32 weeks and 25.8 % incidence in 190 chicken samples examined in Ethiopia. This result disagree with **Ahmed et al. (2003), Khelfa (1982) and Amer et. al. (2010),** who recorded 43.9 %, 82.24% & 90% rate of infection respectivly in chickens in Egypt. **Norcross &Washko (1970)** and **Allen & Fetterer (2002)** who mentioned that differences in incidence according to age are due to different age susceptibility to different *Eimeria* species**.**

Pinard-van Der Laan (1997) found that Fayomi Line breed was the most resistant agansit coccidiosis which showed no mortality, less sever lesion than the other lines, the white Leghorn lines were the most susceptabile.

In the present study, it was found that the most prevalent *Eimeria* species among the examined chickens were *E. necatrix* & *E. tenella* (58.27% & 25.82%). This result agrees with that reported by **Shakshouk (1984)** who stated that incidence with the same Eimeria species were 32.2% & 67.8% in broilers in Beheira and Alexandria governorates. In addition, this result agree partially with that result recorded by **Abu Elezz (1994)** who stated that, the ceccal coccidiosis *E.tenella* is the most prevalent species in Balady chicks in Egypt. However, **Haug et al. (2008)** who recoded *E. tenella* and *E. maxima* were the most

prevalent species associated with medium-sized and large oocysts, respectively in broiler chickens in Norway.

The present study, revealed that the incidence of *E. acervulina*, *E. mitis* and *E. maxima* incidences were 19.20%, 10.59% and 4.66% respectively. These results agree with **Khelfa (1982)** who stated that the mentioned species incidences were 10-80%, 10-40% and 4-10%, respectively, in the Upper Egypt. **Ahmed et al. (2003)** reported that the presence of *E.acervulina, E.maxima and E.mitis* species was 43.9% in Egypt. This previous result disagrees with the finding of **Kucera (1990), Mc Dougald et al. (1997)** and **Lobago et al., (2005)** who stated that the prevalence differences were normal due to the differences in the epidemiological situation among different countries. Morover, **Haug et al. (2008)** who found the incidence of *E. acervulina and E. maxima* was 100% and 27.5% in broiler chickens in Norway.

The absence of *E. brunetti* & *E. praecox* among the examined balady chicks agree with the finding recorded by **Shakshouk (1984), Ahmed et al. (2003)** and **El Behairy (2005)** and disagree with the finding recorded by **Khelfa (1982)** and **Haug et al. (2008)** who recorded the presence of *E. preacox* in 5-10% and 9.8 % broiler chickens.

Moreover, mixed infection was found in the rate of 33% among native breed Balady chicks under investigation. . This result agree with the finding by **Oikawa et al. (1974), Kucera (1990), William (1996)** and **Lobago et al. (2005),** who noticed the mixed infection with different species of Eimeria in the chickens. The present finding showed a difference in incidence of coccidial infection between different geographical localities, this result agreed with **Shirley (1992)** who stated that the effect of the environment (temperature & moisture) on the course & severity of coccidial infection has a great impact **Ashenafi et al. (2004) and Haug et al. (2008)** who confirmed the incidence of coccidiosis is varied in related to different selected climatic zones; there were a significant difference in coccidiosis prevalence from 42.2% to 13.1% chickens in central Ethiopia and 36.25% to 70.9% in broiler chickens in Norway.

The present study showed different age susceptibility among Egyptian native breed of different *Eimeria* species, *E. acervulina* and *E.tenella* which occur in 4th week and in older ages. In the contrary, *E.necatrix*, *E.maxima* and *E.mitis* infections weren't begin before 42 days of age. All examined samples of age less than 21 days was completely free. This result disagree with normal broiler age susceptibility, finding by **William (1996)** and **Mc Dougald et al. (1997)** who found several species of *Eimeria* oocysts from 15th and before 21th days old in the flocks. The differences of age susceptibility between native breed (Balady) and normal broiler might be explained in relation to genetic factores.

The present study showed clear difference in incidence of coccidiosis, among different seasons of the year, they were 45.13% in winter; 18.30% in autumn & spring and 1.86% in summer. These results agree with **Shirley (1992)** and **Ashenafi et al. (2004)** who explained the effect of humidity percent which increase in winter on the coccidiosis incidence. Moreover, **Lunnden &Thebo (2000)** and **Badawy et al. (2000)** also explained that the stocking density which increase in winter by 30% has a direct effect on the increasing incidence in winter. On the other, hand, **Haug et al. (2008)** found that high incidence (90.7%) of *Eimeria* species was recorded during summer of the year 2003-2004 in Norway.

The experimental infection of Balady breed chickens by Eimeria species isolates is aimed to study the biological characters of each isolate ,and confirming the diagnosis of each species of Eimeria .The protocol which used also by **Kucera (1990), William (1996)** and **Mc Dougald et al. (1997).** The study is aimed also for further immunological investigations of Eimeria species in Balady breed chickens.

The histological finding in this study confirmed the diagnosis of each species as *E. acervulina* showed presence of gametocyte with the characteristic inflammatory cells in duodenal part of intestine. The fact which agreed with **Hein (1971)** *E. necatrix* showed its characteristic coagulative necrosis and focal hemorrhagic areas and deeply embedded gametocyte in tunica musculosa and serosa as had been shown by **Hein (1971).** *E.tenella* showed considerable numbers of oocyst in lamina propria of coecum beside sever hemorrhage and complete desquamation of epithelium and edema of muscular tissue which agreed with the finding by **levine (1985).**

Corresponding author
Ahmed A. Al-Gawad
Faculty of Veterinary Medicine Cairo University.
a_abdelgwad @hotmail.com

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Reliability Equivalence of Independent Non-identical Parallel and Series Systems

Yousry H. Abdelkader[1,3]; A. I. Shawky[2] and M. I. Al-Ohally[1]

[1]Dept. of Math., Faculty of Science, University of Dammam, KSA
[2]Dept. of Statistics, Faculty of Science, King Abdulaziz University, P.O. Box 80203, Jeddah 21589, Saudi Arabia
[3]Math. Dept., Faculty of Science, Alexandria University, Egypt
yabdelkader@yahoo.com

Abstract: The reliability equivalence factors of parallel and series systems with *n* independent non-identical components are obtained. Three different methods are used to improve such systems: (i) improving the quality of several components by decreasing their failure rates, (ii) adding a hot component to the system, and (iii) adding a cold redundant component to the system. The survival function is used as a performance measure of the system reliability to compare different system designs. The *rth* moment time to failures will be derived in parallel and series systems in Weibull Distribution.

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Key words: Reliability; Series and Parallel Systems; Reduction method; Hot duplication; Cold duplication; Weibull Distribution.

1. Introduction

Reliability evaluation is an important and integral feature of the planning, design and operation of all engineering systems. The failure of mechanical devices such as ships, trains, and cars, is similar in many ways to the life or death of biological organisms. A reliability equivalence factor is a factor by which a characteristic of components of a system design has to be multiplied in order to reach equality of a characteristic of this design and different designs regarded as a standard. Equivalent of different system designs with respect to a reliability characteristic such as mean time to failure (MTTF) or survival function in case of no repairs is needed. Råde (1989) has introduced the concept of reliability equivalence. Råde (1990, 1991, 1993a, 1993b) has applied such concept to some simple series and parallel systems of two independent and identical components. Råde (1993a and 1993b) has used three different methods to improve the reliability of a system. In these methods it is assumed that the reliability of a system can be improved by: (i) improving the quality of one or several components by decreasing their failure rates; (ii) adding a hot component to the system; (iii) adding a cold redundant component to the system. Sarhan (2000) has introduced more generals improving methods of a system. In such methods, the reliability of a system can be improved by: (i) improving the quality of some components by reducing their failure rates by a factor ρ, $0< \rho <1$; (ii) assuming hot duplications of some components; (iii) assuming cold duplications of some components; (iv) assuming cold redundant standby components connected with some components (one for each) by random switches. Råde (1993a, 1993b) and Sarhan (2000) have used the survival function as the performance measure of the reliability system. Råde (1993a, 1993b) has calculated the reliability equivalence factors of series and parallel systems which consist of independent and identical components with constant failure rate. Sarhan (2000) has applied the concept of reliability equivalence on a series system consists of *n* independent but non-identical components. He assumed that the lifetime of each component follows the exponential distribution. Sarhan *et al.* (2004) have studied the equivalence of different designs of a four independent and identical components series-parallel system. They have tried to deduce the reliability equivalence factors of a series-parallel system. In obtaining these factors, the reliability function and mean time to failure (MTTF) of the system are used as performance measures to compare different system designs of original system and others improved systems. Sarhan (2005) obtained the reliability equivalence factors of a parallel system with *n* independent and identical components using the general method which is established in (2000). However, Sarhan (2000) obtained the MTTF in the case of independent and non-identical components series systems and some special cases studied in parallel system when the failure rates of the system's components are constants. Sarhan and Mustafa (2006) introduced different vectors of reliability equivalence factors of a series system consisting of *n* independent and non-identical components. They assumed that the failure rates of the system components are follow the exponential distribution and used the reliability function and the MTTF as performances to derive the reliability equivalence of the system. Their work generalized the results presented in Sarhan (2000). Sarhan *et al.* (2008) discussed the equivalence factors of *m* modules (subsystems) connected in series where each module *i* consisting of n_i components in parallel.

They assumed that the system components are independent and identical exponentially distributed. El-Damcese and Khlifa (2008) have considered the equivalence factors of *m* modules connected in parallel with subsystems *i* consisting of n_i components in series. They assumed that the system components are independent and identical Weibull distribution. Sarhan (2009) has studied the equivalence of different designs of a general series-parallel system based on the system reliability function and the MTTF. He assumed that the system components lives are independent and follow the exponential distributions. Generally speaking, to obtain the MTTF the first moment of order statistics is used, see, e.g., Arnold *et al.* (1992) and Asadi (2006). That is, compute $\mu_{n:n}=E(X_{n:n})$ for parallel system and $\mu_{1:n}=E(X_{1:n})$ for the series system. The *rth* moment of order statistics arising from independent non-identically distributed random variables have been studied by many authors see, e.g., Barakat and Abdelkader (2000, 2004), Abdelkader (2004 a and 2004 b), Abdelkader and Abotahoun (2006) and Abdelkader (2010, 2011).

Since the time to failure of an item is a random variable, Rausand and Høyland (2004) are used several measures of the center of a life distribution. The mean, the median and the mode are used to measure the time to failure of an item. Therefore, a higher moment such as the variance can also be used, the variance is one of the most commonly used measure for analyzing error. In this paper we shall use the survival function and the *rth* moment time to failure (MOTTF) to calculate the reliability equivalence factors for series and parallel systems, consisting of n independent and non-identical components. That is, we compute the *rth* moment time to failure, $\mu_{n:n}^{(r)} = E(X_{n:n}^{(r)})$, for the parallel system and, $\mu_{1:n}^{(r)} = E(X_{1:n}^{(r)})$, for the series system. These components are assumed to be follow the Weibull distribution in the case of independent non-identically distributed. The results presented here generalize those given in the literature.

The rest of the paper is organized as follows. The reliability functions and MOTTF for the parallel and series systems are introduced in Section 2. In Section 3, we derived the reliability functions and MOTTF for the parallel and series systems when the system components lives follow the Weibull distribution. Two different methods for improving the system designs are introduced in Section 4. In Section 5, the reliability equivalence factors and γ -fractiles of the original and improved designs are presented. Concluding remarks are drawn in Section 6.

2. The reliability functions and MOTTF

Assume a system composed of *n* independent but not identical components. Each component has a life time T_i , (*i*=1, 2,…, *n*). The reliability function of the system which consists of *n* components connected in parallel and series are defined, respectively, as

$$R(t) = 1 - \prod_{i=1}^{n} \left(1 - R_i(t)\right), \qquad (1)$$

and

$$R(t) = \prod_{i=1}^{n} R_i(t), \qquad (2)$$

where R_i (*t*) is the reliability function of each component *i,(i=1,2,…,n).* The *MOTTF* is defined by

$$MOTTF = r\int_o^\infty t^{r-1} R(t)dt. \quad (3)$$

Let $MOTTF_p$ and $MOTTF_s$ be the moment time to failure for the parallel and series systems. They are, respectively, defined by

$$MOTTF_p = r\int_o^\infty t^{r-1}\left(1 - \prod_{i=1}^{n}\left[1 - R_i(t)\right]\right)dt, \quad (4)$$

and

$$MOTTF_s = r\int_o^\infty t^{r-1}\prod_{i=1}^{n} R_i(t)\,dt. \qquad (5)$$

3. The Weibull distribution

Let $T_i, i = 1,2,...,n$ be random variables having Weibull distribution with reliability functions

$$R_i(t) = e^{-a_i t^\beta}, t \geq 0,\ a_i, \beta > 0. \quad (6)$$

The Weibull distribution generates a family of distributions as β changes its values. For instance, when $\beta = 1$ and $\beta = 2$, the Weibull distribution reduces to exponential and Rayleigh distribution with parameters, α_i, i = 1,2,…, respectively.

The Weibull distribution has a broad variety of monotone increasing failure rates when $\beta \geq 1$. That is, the failure rates, $r_i(t)$, , are functions of time

$$r_i(t) = \beta\,\alpha_i\, t^{\beta-1} \qquad (7).$$

The moment time to failure for the parallel and series systems will be derived in the following Theorem.

Theorem 1. The $MOTTF_p$ and $MOTTF_s$ for the Weibull distribution are given, respectively, by

$$MOTTF_p = \Gamma\left(\frac{r}{\beta}+1\right)\sum_{j=1}^{n}(-1)^{j+1} D_j, \quad (8)$$

and

$$MOTTF_s = \Gamma\left(\frac{r}{\beta}+1\right) D_n, \quad (9)$$

Where,

$$D_j = \sum_{1\le i_1<i_2\ldots<i_j\le n}\ldots\sum \frac{1}{\left(\alpha_{i_1}+\alpha_{i_2}+\ldots+\alpha_{i_j}\right)^{\frac{r}{\beta}}},$$

$(i_1, i_2, \ldots, i_n)$ are the permutations of $(1,2,\ldots,n)$ for which $1 \le i_1 < i_2 < \ldots < i_j \le n$ and $\Gamma(.)$ is the gamma function.

Proof. It is easy to see that

$$MOTTF_p = r\int_o^\infty t^{r-1}\left(1-\prod_{i=1}^{n}(1-e^{-\alpha_i t^\beta})\right)dt$$

$$= r\int_o^\infty t^{r-1}\Big(\sum_{i=1}^{n}e^{-\alpha_i t^\beta} - \sum_{1\le i_1\le i_2\le n}\sum e^{-(\alpha_{i_1}+\alpha_{i_2})t^\beta} +$$

$$\sum_{1\le i_1<i_2<i_3\le n}\sum\sum e^{-(\alpha_{i_1}+\alpha_{i_2}+\alpha_{i_3})t^\beta} + \ldots + (-1)^{n+1}e^{-\sum_{i=1}^{n}\alpha_i t^\beta}\Big)dt.$$

Upon using

$$\int_o^\infty t^{r-1}e^{-\theta t^\beta}dt = \frac{\Gamma(\frac{r}{\beta})}{\beta\theta^{\frac{r}{\beta}}}, \quad \forall\theta>0, \quad (10)$$

we get Eq.(8). The proof of Eq.(9) follows from the definition

$$MOTTF_s = r\int_o^\infty t^{r-1}\prod_{i=1}^{n}e^{-\alpha_i t^\beta}dt = r\int_o^\infty t^{r-1}e^{-\sum_{i=1}^{n}\alpha_i t^\beta}dt,$$

and using Eq.(10).

Corollary 1. The $MOTTF_p$ and $MOTTF_s$ for the exponential distribution are given, respectively, by

$$MOTTF_p = \Gamma(r+1)\sum_{j=1}^{n}(-1)^{j+1}D_j, \qquad (11)$$

and

$$MOTTF_s = \Gamma(r+1)D_n, \qquad (12)$$

Where,

$$D_j = \sum_{1\le i_1<i_2\ldots<i_j\le n}\ldots\sum \frac{1}{\left(\alpha_{i_1}+\alpha_{i_2}+\ldots+\alpha_{i_j}\right)^{r}}.$$

Proof. Setting β=1 in Theorem 1, we get the results.

4. Designs of improved the series and Parallel systems

In this section, we shall introduce two different methods to improve the system designs. The first is the reduction method and the second is called redundancy method which is composed of hot and cold duplication methods. In reduction method, it is assumed that the failure rates of some of the system components are reduced by a factor ρ, 0<ρ<1. In the hot duplication method, it is assumed that some of the system components are duplicated in parallel while some of the system components are duplicated in parallel by a perfect switch in the cold duplication method. Therefore, one can make equivalence between the reduction method and the duplication method based on some reliability measures. In other words, the design of the system that is improved according to the reduction method should be equivalent to the design of the system according to one of the redundancy method. The comparison of the designs produce the so-called reliability equivalence factors.

4.1 The reduction method for series and Parallel systems

In this method, it is assumed that the system can be improved by improving l, $1\le l\le n$ of its components. That is, the failure rates of l components are reduced from α_i to $\rho\,\alpha_i$, $0<\rho<1$. Let $R_{s,\rho}^{(l)}(t)$ and $R_{p,\rho}^{(l)}(t)$ denote the reliability function of the series and parallel systems improved by reducing the failure rates of l of its components by the factor ρ.

Theorem 2. The moments of the series and parallel systems under the reduction method, denoted by $MOTTF_{s,\rho}^{(l)}$ and $MOTTF_{p,\rho}^{(l)}$, are given, respectively, by

$$MOTTF_{s,\rho}^{(l)} = \frac{\Gamma(\frac{r}{\beta}+1)}{\left(\rho\sum_{i=1}^{l}\alpha_i+\sum_{i=l+1}^{n}\alpha_i\right)^{\frac{r}{\beta}}}, \qquad (13)$$

and

$$MOTTF_{p,\rho}^{(l)} = \Gamma(\frac{r}{\beta}+1)\Big(\sum_{j=1}^{l}(-1)^{j+1}Z_j(\rho) +$$

$$\sum_{k=l+1}^{n}(-1)^{k+1}Z_k - \sum_{j=1}^{l}\sum_{k=l+1}^{n}(-1)^{j+k+2}Y_{jk}(\rho)\Big), \quad (14)$$

Where,

$$Z_j(\rho) = \sum_{1\le i_1<i_2\ldots<i_j\le l}\ldots\sum \frac{1}{\left(\rho\sum_{j=1}^{l}\alpha_{i_j}\right)^{\frac{r}{\beta}}},$$

$$Z_k = \sum_{l+1\le i_1<i_2\ldots<i_k\le n}\ldots\sum \frac{1}{\left(\sum_{k=l+1}^{n}\alpha_{i_k}\right)^{\frac{r}{\beta}}},$$

$$Y_{jk}(\rho) = \sum_{\substack{1\le i_1<i_2<\ldots<i_j\le l\\ l+1\le i_1<i_2\ldots<i_k\le n}}\ldots\sum \frac{1}{\left(\rho\sum_{j=1}^{l}\alpha_{i_j}+\sum_{k=l+1}^{n}\alpha_{i_k}\right)^{\frac{r}{\beta}}},$$

The subscript of the last summation means we have two summations. One runs for j to l and the other runs for k to n.

Proof. The reliability function of the series system, $R_{s,\rho}^{(l)}(t)$ is given by

$$R_{s,\rho}^{(l)}(t) = \prod_{i=1}^{l} e^{-\rho\alpha_i t^\beta} \times \prod_{i=l+1}^{n} e^{-\alpha_i t^\beta}$$

$$= e^{-(\sum_{i=1}^{l} \rho\alpha_i + \sum_{i=l+1}^{n} \alpha_i)t^\beta} \qquad (15)$$

The reliability function of the parallel system, $R_{p,\rho}^{(l)}(t)$, is given by

$$R_{p,\rho}^{(l)}(t) = 1 - \prod_{i=1}^{l}(1 - e^{-\rho\alpha_i t^\beta}) \times \prod_{i=l+1}^{n}(1 - e^{-\alpha_i t^\beta})$$

$$= \sum_{j=1}^{l}(-1)^{j+1} Z_{i_j}(\rho t) + \sum_{k=l+1}^{n}(-1)^{k+1} Z_{i_k}(t) - \sum_{j=1}^{l}\sum_{k=l+1}^{n}(-1)^{j+k+2} Z_{i_j}(\rho t) Z_{jk}(t), \quad (16)$$

where, $Z_{i_j}(\rho t) = \sum_{1\le i_1<i_2\ldots} \ldots \sum_{<i_j\le l} e^{-\rho\sum_{j=1}^{l} \alpha_{i_j} t^\beta}$,

$$Z_{i_k}(t) = \sum_{l+1\le i_1<i_2\ldots<i_k\le n} \ldots \sum e^{-\sum_{j=1}^{l} \alpha_{i_j} t^\beta}.$$

From the definitions of the moments which are given by Eqs. (4) and (5) and using the integral in Eq. (10), we get the results.

4.2 The hot duplication method

In this case, it is assumed that the system can be improved by improving m of its components, where $1 \le m \le n$.

Lemma 1. The reliability function of the parallel and series systems, denoted by $R_{p,H}^{(m)}(t)$ and $R_{s,H}^{(m)}(t)$, under the hot duplication method are given by

$$R_{p,H}^{(m)}(t) = \sum_{j=1}^{q}(-1)^{j+1} I_j, \qquad (17)$$

$$R_{s,H}^{(m)}(t) = I_q, \qquad (18)$$

where, $I_j = \sum_{1\le i_1<i_2\ldots} \ldots \sum_{<i_j\le q} e^{-\sum_{v=1}^{l} \alpha_{i_v} t^\beta}$,

and q=n+m.

Proof. The reliability function of the system, $R_{p,H}^{(m)}(t)$, is given by

$$R_{p,H}^{(m)}(t) = 1 - \prod_{i=1}^{q}(1 - e^{-\alpha_i t^\beta})$$

$$= \sum_{i=1}^{q} e^{-\alpha_i t^\beta} - \sum_{1\le i_1\le i_2\le q}\sum e^{-(\alpha_{i_1}+\alpha_{i_2})t^\beta} +$$

$$\sum_{1\le i_1< i_2< i_3\le q}\sum\sum e^{-(\alpha_{i_1}+\alpha_{i_2}+\alpha_{i_3})t^\beta} + \ldots + (-1)^{q+1} e^{-\sum_{i=1}^{q} \alpha_i t^\beta}$$

We can rewrite the last equation in the form of Eq.(17). The proof of Eq.(18) follows from the definition

$$R_{s,H}^{(m)}(t) = \prod_{i=1}^{q} e^{-\alpha_i t^\beta} = e^{-\sum_{i=1}^{q} \alpha_i t^\beta} = I_q,$$

and hence the proof.

Theorem 3. The moment time to failure for the parallel and series systems, $MOTTF_{p,H}^{(m)}$ and $MOTTF_{s,H}^{(m)}$, are given by

$$MOTTF_{p,H}^{(m)} = \Gamma(\frac{r}{\beta}+1)\sum_{j=1}^{q}(-1)^{j+1} D_j, \quad (19)$$

$$MOTTF_{s,H}^{(m)} = \Gamma(\frac{r}{\beta}+1) D_q, \quad (20)$$

where,

$$D_j = \sum_{1\le i_1<i_2\ldots<i_j\le q} \ldots \sum \frac{1}{\left(\alpha_{i_1} + \alpha_{i_2} + \ldots + \alpha_{i_j}\right)^{\frac{r}{\beta}}}.$$

Proof. By replacing n by q in Theorem 1, we get the results.

4.3 The cold duplication method

Under the cold duplication method, the system will be improved by improving m of its components, $1 \le m \le n$. Let $R_{s,C}^{(m)}(t)$ and $R_{p,C}^{(m)}(t)$ be the reliability functions of the series and parallel systems. Then

$$R_{s,C}^{(m)}(t) = \prod_{i=1}^{m} R_{1,C}(t) \prod_{i=m+1}^{n} R_1(t), \qquad (21)$$

and

$$R_{p,C}^{(m)}(t) = 1 - \prod_{i=1}^{m}(1 - R_{1,C}(t)) \prod_{i=m+1}^{n}(1 - R_1(t)), \qquad (22)$$

where $R_{1,C}(t)$ denotes the reliability of a system's components after it was improved according to cold duplication method. So, we can write

$$R_{1,C}(t) = (1+\alpha_i t^\beta)e^{-\alpha_i t^\beta}, \qquad 1 \le i \le m, \quad (23)$$

see Billinton and Allan (1983).

Theorem 4. The moments of the series and parallel systems, written as $MOTTF_{s,C}^{(m)}$ and $MOTTF_{p,C}^{(m)}$, in cold duplication method are given, respectively, by

$$MOTTF_{s,C}^{(m)} = \sum_{a_1=0}^{1} \cdots \sum_{a_m=0}^{1} \frac{r\prod_{i=1}^{m} \alpha_i^{a_i} \Gamma(\frac{r+\sum_{i=1}^{m} a_i}{\beta})}{\beta(\sum_{i=1}^{n} \alpha_i)^{(\frac{r+\sum_{i=1}^{m} a_i}{\beta})}}, \quad (24)$$

and

$$MOTTF_{p,C}^{(m)} = \frac{r}{\beta}(\sum_{j=1}^{m} (-1)^{j+1} C_1(i_j) T_j +$$

$$\sum_{k=m+1}^{n} (-1)^{k+1} C_2(i_k) T_k -$$

$$\sum_{j=1}^{m} \sum_{k=m+1}^{n} (-1)^{j+k+2} C_1(i_j) C_2(i_k) T_{jk}), \quad (25)$$

Where,

$$C_1(i_j) = \sum_{1 \le i_1 < i_2 \ldots < i_j \le m} \cdots \sum_{a_1=0}^{1} \cdots \sum_{a_m=0}^{1} \prod_{i=1}^{m} \alpha_{i_j}^{a_j},$$

$$C_2(i_k) = \sum_{m+1 \le i_1 < i_2 \ldots < i_k \le n} \cdots \sum,$$

$$T_j = \frac{\Gamma(r+\sum_{i=1}^{m} a_i)}{(\sum_{j=1}^{m} \alpha_{i_j})^{(r+\sum_{i=1}^{m} a_i)}},$$

$$T_k = \frac{\Gamma(r)}{(\sum_{k=m+1}^{n} \alpha_{i_k})^{r}},$$

$$T_{jk} = \frac{\Gamma(r+\sum_{i=1}^{m} a_i)}{(\sum_{j=1}^{m} \alpha_{i_j} + \sum_{k=m+1}^{n} \alpha_{i_k})^{(r+\sum_{i=1}^{m} a_i)}}.$$

Proof. From Eq.(21), we can write

$$R_{s,C}^{(m)}(t) = \prod_{i=1}^{m} (1+\alpha_i t^{\beta}) e^{-\alpha_i t^{\beta}} \prod_{i=m+1}^{n} e^{-\alpha_i t^{\beta}},$$

$$= e^{-\sum_{i=m+1}^{n} \alpha_i t^{\beta}} \prod_{i=1}^{m} \sum_{a=0}^{1} (\alpha_i t^{\beta})^{a} e^{-\alpha_i t^{\beta}}$$

$$= e^{-\sum_{i=m+1}^{n} \alpha_i t^{\beta}} T_m(t),$$

where,

$$T_m(t) = \sum_{a_1=0}^{1} \cdots \sum_{a_m=0}^{1} \prod_{i=1}^{m} \alpha_i^{a_i} t^{\beta \sum_{i=1}^{m} a_i} e^{-\sum_{i=1}^{m} \alpha_i t^{\beta}}.$$

Therefore,

$$MOTTF_{s,C}^{(m)} = r\int_o^{\infty} t^{r-1} R_{s,C}^{(m)}(t) dt.$$

Upon using the integral of Eq. (10), we get Eq. (24).

The reliability function of the parallel system can be written in the form

$$R_{p,C}^{(m)}(t) = \sum_{j=1}^{m} (-1)^{j+1} T_{i_j}(t) + \sum_{k=m+1}^{n} (-1)^{k+1} T_{i_k}(t) -$$

$$\sum_{j=1}^{m} \sum_{k=m+1}^{n} (-1)^{j+k+2} T_{i_j}(t) T_{jk}(t). \quad (26)$$

Since we can write

$$\prod_{i=m+1}^{n} (1-e^{-\alpha_i t^{\beta}}) = 1 - \sum_{k=m+1}^{n} (-1)^{k+1} T_{i_k}(t),$$

$$T_{i_k}(t) = \sum_{m+1 \le i_1 < i_2 \ldots} \cdots \sum_{< i_k \le n} e^{-\sum_{k=m+1}^{n} \alpha_{i_k} t^{\beta}},$$

$$\prod_{i=1}^{m} \left(1-(1+\alpha_i t^{\beta}) e^{-\alpha_i t^{\beta}}\right) = 1 - \sum_{j=1}^{m} (-1)^{j+1} T_{i_j}(t),$$

$$T_{i_j}(t) = \sum_{1 \le i_1 < i_2 \ldots} \cdots \sum_{< i_j \le m} \sum_{a_1=0}^{1} \cdots \sum_{a_m=0}^{1} \prod_{i=1}^{m} \alpha_{i_j}^{a_j} t^{\beta \sum_{j=1}^{m} a_j} e^{-\sum_{j=1}^{m} \alpha_{i_j} t^{\beta}}.$$

Therefore, Eq.(26) can be obtained. Hence,

$$MOTTF_{p,C}^{(m)} = r\int_o^{\infty} t^{r-1} R_{p,C}^{(m)}(t) dt,$$

Upon using the integral of Eq. (10), we get Eq. (25) and hence the proof.

5. The reliability equivalence factors and γ - Fractiles

In this section, the survival and the moments reliability equivalence factors for the series and parallel system will be introduced. The γ -Fractiles of the original and improved systems are also presented.

5.1 Hot-Cold series and parallel reliability equivalence factors

The survival reliability equivalence factors, written $\rho_F = \rho_{G,F}^{K}(\gamma)$ where G=s or p (for series and parallel systems); F=H(C) for hot(cold); $K = l$ or m components and $0 < \gamma < 1$, is defined as that factor ρ by which the failure rates of l of the system's components should be reduced in order to reach the reliability of the system which improved by improving m of the original system components according to hot(cold) duplication methods see, Sarhan (2009). The following set of non-linear equations give the solutions of ρ_F for the series and parallel systems:

$$R_{G,\rho}^{(l)}(t) = R_{G,F}^{(m)}(t) = \gamma.$$

Therefore, from Eqs. (15) - (18) we can write for the hot reliability equivalence factors (series and parallel systems) that

$$R_{s,\rho}^{(l)}(t) = e^{-(\rho\sum_{i=1}^{l} \alpha_i + \sum_{i=l+1}^{n} \alpha_i) t^{\beta}}$$

$$= R_{s,H}^{(m)}(t) = e^{-\sum_{i=1}^{n} \alpha_i t^{\beta}} = \gamma, \quad (27)$$

$$R^{(l)}_{p,\rho}(t) = 1 - \prod_{i=1}^{l} \left(1 - e^{-\rho\alpha_i t^{\beta}}\right) \times \left(1 - e^{-\alpha_i t^{\beta}}\right)$$

$$= R^{(m)}_{s,H}(t) = \sum_{j=1}^{q} (-1)^{j+1} I_j = \gamma, \qquad (28)$$

where I_j is defined in Lemma 1.

Similar equations for the cold reliability equivalence factors (series and parallel systems) can be written using the Eqs. (15), (21) and (16), (22). That is,

$$R^{(l)}_{s,\rho}(t) = R^{(m)}_{s,C}(t) = \gamma, \qquad (29)$$

and

$$R^{(l)}_{p,\rho}(t) = R^{(m)}_{p,C}(t) = \gamma, \qquad (30)$$

The solutions of the system of Eqs. (27) - (30) have no closed form. So, a numerical technique can be used to get the solutions with respect to ρ_F.

On the other hand, the moment reliability equivalence factors can be used to reach the equality (or nearness) of the moment time to failure in the reduction method and the moment time to failure of the designs obtained from the hot(cold) duplication methods for the series and parallel systems, see Sarhan (2009). That is,

$$MOTTF^{(l)}_{s,\rho} = MOTTF^{(m)}_{s,H} = MOTTF^{(m)}_{s,C}, \qquad (31)$$

$$MOTTF^{(l)}_{p,\rho} = MOTTF^{(m)}_{p,H} = MOTTF^{(m)}_{p,C}. \qquad (32)$$

Therefore, using Eqs. (13), (20), (24) and (14), (19), (25) we get non-linear system of equations which can be solves numerically.

5.2 γ-Fractiles

Let $L(\beta,\gamma)$ be the γ-Fractiles of the original systems and $L^{(m)}_{G,F}(\beta,\gamma)$ be the γ-Fractiles of the improving systems obtained by improving *m* of the system's components according to G=s(p) for series and parallel systems and F=H(C) duplication methods.

The γ-Fractiles of the systems having reliability functions $R_G(t)$ and $L_G(\beta,\gamma)$ can be obtained by solving the equations

$$R\left(\frac{L_G(\beta,\gamma)}{\alpha}\right) = \gamma, \qquad (33)$$

and for the hot and cold duplication methods, we have to solve the following equations

$$R^{(m)}_{G,F}\left(\frac{L^{(m)}_{G,F}(\beta,\gamma)}{\alpha}\right) = \gamma, \qquad (34)$$

with respect to L. The above equations have no closed form solutions in L. So, the numerical techniques are the best solutions in these cases.

6. Concluding Remarks

The case of independent non-identical arises in many situations, we enumerate two. The first, we are not guarantee that all components of the system are work with the same efficiency even they are produced from the same factory. The second, we may assume that the components are came from different factories with different efficiency.

The results of this paper extend those available in the literature see, e.g., Sarhan (2000, 2002, 2005, 2009), Montaser and Sarhan (2008) and Sarhan *et al.* (2004, 2008). The system reliability function and the system moment time to failure (MOTTF) in the case of independent non-identical components are used to study the equivalence of different designs for the series and parallel systems. Moreover, the mentioned papers in the references assumed that the failure rates of the system components are follow the exponential distribution (constant failure rates) and computed the mean time to failure. While this paper presents the MOTTF and assumes the failure rates of the system components are functions of time and follow the Weibull distribution.

It is obvious from the foregoing Theorems to see that the following remarks: (i) from Eqs. (9) and (13), the $MOTTF^{(l)}_{s,\rho}$ is greater than or equal $MOTTF_s$ after reducing the failure rates of l of the components by the factor ρ Since the denominator of Eq. (9) is greater than the denominator of Eq. (13); (ii) similarly, the $MOTTF^{(l)}_{p,\rho}$ is greater than $MOTTF_p$, which are given by Eqs. (8) and (14), for the same reason; (iii) also, it is easy to deduce that $MOTTF^{(m)}_{s,C} > MOTTF^{(m)}_{s,H}$ and $MOTTF^{(m)}_{p,c} > MOTTF^{(m)}_{p,H}$. That is, the moment time to failure in the cold duplication method is greater than the moment time to failure in the hot duplication method. In reliability equivalence factors including the survival and the moments, most of the results established in the literature showed that

$$R_s(t) < R^{(m)}_{s,H}(t) < R^{(m)}_{s,C}(t).$$

and

$$R_p(t) < R^{(m)}_{p,H}(t) < R^{(m)}_{p,C}(t).$$

$$MOTTF^{(l)}_{s,\rho} < MOTTF^{(m)}_{s,H} < MOTTF^{(m)}_{s,C},$$

$$MOTTF^{(l)}_{p,\rho} < MOTTF^{(m)}_{p,H} < MOTTF^{(m)}_{p,C},$$

apart from the components are independent identical or non-identical see, e.g., Sarhan (2000, 2002, 2005, 2009), Sarhan and Mustafa (2006), Sarhan *et al.* (2004, 2008), Xia and Zhang (2007), El-Damcese and Khalifa (2008) and Montaser and Sarhan (2008).

Moreover, the results in the mentioned references showed that the amount of increasing of the γ-fractile in cold duplication method is greater than the γ-fractile obtained by using the hot duplication method.

In the future, we may study the reliability equivalence factors for other family of distributions in the case of independent non-identical components along with high quality software programs to perform the associated calculations.

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Corresponding author
Yousry H. Abdelkader
Dept. of Math., Faculty of Science, University of Dammam, KSA
Math. Dept., Faculty of Science, Alexandria University, Egypt.
yabdelkader@yahoo.com

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Cytogenetic and molecular variation on *Vicia faba* treated with creatine monohydrate

Nora H. Al-zahrani, Kadija H. Alamoudi and Salha M. Al-shamrani*

Faculty of Science for Girls, King Abdulaziz University, Jeddah, Saudi Arabia Kingdom
*Corresponding author**: nlalzhrani@kau.edu.sa

Abstract: The cytogenotoxicity of creatine monohydrate conc. (1.5, 2, 2.5 and 3g/100 ml) were examined on *Vicia faba* plant, i:e: meiotic division behavior, leaf protein by using SDS-PAGE protein electrophoresis and changes in DNA of M_2 faba bean plant by using RAPD analysis. All creatine monohydrate treatments showed abnormal pollen mother cells (PMCs) which increased as the concentration and treatment period increased. The most common abnormalities were stickines, disturbed, laggard, bridges and micro-multi nuclei. The electrophoretic study of leaf proteins showed alteration of some minor protein bands after creatine treatments. The highest concentration of creatine showed a polymorphic number of genetic bands by using RAPD-PCR product comparing with control. Results strongly suggested that creatine monohydrate is clastogenic.
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Key words: *Vicia faba* plant, RAPD analysis, SDS-PAGE protein electrophoresis

1. Introduction

Creatine monohydrate has become one of the most popular sports supplements among professional and a mateur atheletes. In human body, creatine is converted to creatine phosphate which helps to fuel skeletal muscles, provides support for immediate energy production, high intensity workout. Over the past several years, numerous studies have shown that creatine supplementation may help improvement atheletic performance (Henein, 2004). On the other hand, many plant products were widely used by human for increasing activity and human capacity to physical work; improve the general body function; enhance the natural body resistance; help to restore the immune system mechanism, help to maintain activity and fitness from these plant products such as ginseng, nigella, pollen grains, garlic oil, beta carotene, selenium, and others (Henein, 2004).

However, the use of these compounds may lead to many complications and side effects. Many investigators have suggested that the study of chromosomal aberration in mitotic and meiotic division, total DNA or RNA contents, changes in storage protein banding patterns and RAPD-PCR profile changes are suitable systems to detect the potential cytological and molecular effects caused by these compounds. Chromosomal aberrations, alterations in protein banding pattern and DNA alteration by using RAPD analysis were observed in several reports such as Chen *et al., (*2000) caffeinein in *Vicia faba*, Mohamed (2000) some biofertilizers on many plants, Polit *et al.,(*2000) BAP and lAA on *Vicia faba*), Abd EL-Hamied (2001) extracts of some umbelliferrous plants on *Vicia faba*, Maslam (2004) some medicinal extracts on *Allium cepa*, Mohamed–(2004)some medicinal plant extracts *on Allium cepa*, Usciati, *et al.,* (2004) 6- benzl aminopurine on *Cicer arietinum,*Baeshin *et al.,* (2009), leaf extract of Rhaza stricta (Decne) on *Allium cepa*,Priti *et al.,* (2009)antibiotics in *Pisum sativum*, Gabriele *et al.,* (2010) paraquat in *Hordeum vulgare* and human Lymphocytes, Ganguly *et al.,(*2010) organophate and zadirctinbase, insecticides on *Lathyrus sativus L*., Guzin *et al, (*2010) boron on wheat *(Triticum aestivum L.)* and bean (*Phaseolus vulgaris L*.), Haroun (2010) *kockia indica* extract on *Vicia faba L.)*, Korpe (2010) copper induced stress on egg plant *(Solanum melongena L*), Min et al., (2010) (Aluminum on *Vicai faba)*, Pinho *et al., (*2010)(*Baccharis trimera* (Less) DC using the *Allium cepa)*, (energy drink:bison and ovulation iducer drug :clomid on *Vicai faba*)

The aims of the present study were to detect the mutagenic effects of creatine monohydrate on *Vicia faba* by estimating meiotic aberrations, changes in leaf protein banding patterns and determine the genetic alteration using RAPD analysis.

2. Material and Methods

2.1. Materials:

2.1.1. Samples:

Plants of *Vicia faba* (Giza 40 variety) were used in this study, fresh leaves and the plants in flower stage.

2.1.2..Reaction Mixture:

Amplification of DNA was performed in 10 µl reaction mix containing 20 ng genomic DNA, 0.5 U *Taq* polymerase (Promega, USA), 200 µM of each of

dNTPs, 10 pmole random primer (Operon,Tech., Inc., USA), 10 mM Tris-HCl and 1.5 mM $MgCl_2$, pH 9.0).

2.1.3.RAPD primers :

10-mer random primers used are illustrated in table (1).

Table 1. RAPD primers and their sequences

Primers	Name	Sequence 5- 3-
Produce variations	A 20	GTTGCGATCC
	B 12	CCTTGACGCA
	C 11	AAAGCTGCGG
	C 16	CACACTCCAG
	G 17	ACGACCGACA
Unable to produce variation	A 3	AGTGAGCCAC
	A 18	AGGTGACCGT
	B16	TTTGCCCGCA
	E18	GGACTGCAGA
	G18	GGCTCATGTG

2.2. Methods:

2.2.1. Cytogenetic analysis

At the flowering stage, plants of *Vicia faba* (Giza 40 variety) were sprayed with four concentrations of creatine monohydrate 1.50, 2, 2.50 and 3g/100 ml (dietar supplement produced by General Nutrition Corporation, pittsburgh, PA15222, USA). Control plants were sprayed with distilled water. Ten flower buds from ten different plants were gathered through duration of 24, 48h and 15 days. For meiotic studies the appropriate flower buds were collected and fixed in carnoy's solution (ethyl alcohol absolute and glacial acetic acid in the ratio 3:1) for 24 h and then transferred to 70% ethyl alcohol and kept in refrigerator. The cytological analysis was carried out by using 2% acetocarmine stain as described by Darlington and La Cour (1979). The data recorded for different treatments were statistically analyzed using t-test to determine significant differences between the treatments.

2.2.2. SDS-PAGE protein analysis

Fresh leaves were taken from *Vicia faba* plants after sprayed with four creatine concentrations and distilled water after 15 days and then decoated and milled to fine powder. Soluble water proteins were extracted over night using Tris-HCl buffer (pH 6.8) according to Laemmli (1970). Centrifugation was performed at 10,000 rpm for 10 min and 40 µl supernatant of soluble proteins were loaded in SDS-slab gel of 15% acrylamide containing 10% SDS. Gel was run at a current of 15 m A for 1 h followed by 25mA for 4-5 h. Molecular weights of different bands were calibrated using the wide range protein marker ranged from 25-230 kDa according to Matta *et al.,* (1981).

2.2.3. RAPD-PCR analysis

DNA extraction and RAPD amplification conditions

DNA was extracted from *Vicia faba* leaves sprayed with the highest creatine concentration (3g/100 ml) using CTAB method (Doyle and Doyle 1990). DNA concentration was determined by comparing with serial dilutions of Lambda DNA, electrophorsed in 0.8% agarose gel, stained in 0.2 µg/ml ethidium bromide and photographed under UV illumination. RAPD analysis was performed using UNO thermal cycler (Perkin Elmer, Germany) 10-mer random primers Table (1).

Amplification was performed in 10 µl reaction mix [containing 20 ng genomic DNA, 0.5 U *Taq* polymerase (Promega, USA), 200 µM of each of dNTPs, 10 pmole random primer (Operon,Tech., Inc., USA), 10 mM Tris-HCl and 1.5 mM $MgCl_2$, pH 9.0)]. Amplification was performed for 45 cycles, using UNO thermal cycler (Biometra, Germany) as follows: One cycle at 92^{o}C for 3 min., 45 cycles at 92^{o}C for 30 sec., 35^{o}C for 60 sec. and a final extension of 10 min at 72°C. PCR products were analyzed using 2% agarose gel electrophoresis and visualized with 0.2 µg/ml ethidium bromide staining. The fragments were photographed with Gel Doc 2000 (Bio RAD). The sizes of the fragments were estimated based on a DNA ladder of Perkin Elmer (Germany).

3. Results and Discussion

3.1.Meiotic abnormalities of *Vicia faba* treated with creatine monohydrate

A wide spectrum of meiotic abnormalities was recorded in ten flower buds from different plant after different treatments with creatine monohydrate. Data in Table (2) shows that the total abnormal PMCs% was increased by the increasing of creatine concentration in the most treatments. Also, this trait was increased by increasing the period duration from 24 to 28 h in all treatments; however this trait was decreased in 15 days period duration in the most treatments as a result of recovery in this period. On the other hand, the abnormal PMCs% in the second division was lower than those recorded in the first division in the most creatine treatments as a result of recovery in this cell age. The most frequent types of abnormalities were: stickiness, disturbed, (micro-multi) nuclei, laggards, bridge and multipolar after creatine treatments.

Table 2. Numbers and percentages of abnormal PMCs in 1st & 2nd meiotic divisions and total mean of meiotic abnormalities after spraying of *Vicia faba* plants with creatine monohydrate for (24, 48 h & 15 days)

Time	Con.g/100ml	Abnormality % in 1st division			Abnormality % in 2nd division			PMCs meiotic divisions		
		dividing cell No.	Abnormal cell.	%	dividing cell No.	Abnormal cell	%	Total dividing cell No.	Abnormal cell	Mean of % abnormal PMCs±se.
Control		276	6	2.17	234	12	5.13	510	18	3.44 ± 1.07
24h.	1.5	45	8	17.78	96	28	29.17	141	36	25.26 ± 2.18**
	2	238	98	41.18	332	114	34.34	570	212	37.10 ± 2.96**
	2.5	414	222	53.62	356	176	49.44	770	398	51.5 ± 2.32**
	3	306	154	50.33	294	124	42.18	600	278	45.95 ± 2.99**
48h.	1.5	90	40	44.44	98	54	55.10	188	94	49.86 ± 0.95**
	2	290	158	54.48	328	112	34.10	618	270	43.66 ± 1.05**
	2.5	440	266	60.45	312	154	49.36	752	420	55.92 ± 3.18**
	3	286	168	58.74	344	168	48.84	630	336	53.39 ± 0.61**
15days	1.5	154	48	31.17	68	8	11.76	222	56	20.09 ± 2.18**
	2	274	118	43.07	318	118	37.11	592	236	39.82 ± 2.19**
	2.5	380	200	52.63	308	138	44.81	688	338	48.82 ± 1.76**
	3	356	188	52.81	326	190	58.28	682	378	55.58 ± 0.78**

The results in Table (3) and Figure (1) revealed that the abnormalities were present in metaphase, anaphase and telophase stages of the meiosis with all treatments. The induction of meiotic abnormalities appears to be a common effect of most chemicals (Shehata *et al.,* 2008; Fisun and Goc Rasgele 2009).

The stickiness and disturbed stages were the most common abnormalities. The first type of abnormalities is the stickiness found in most phases of meiosis after different creatine treatments. (Fig. 1a, b, c, d, e, f, g and k). The number of sticky cells increased in all stages of meiotic division as the creatine concentration increased in the most treatments. Also, this trait was increased by the increasing of duration period from 24 to 48h then decreased in the 15 days period duration in most treatments, Table (3). The obtained results are in agreement with many reports, for instance (Laemmli 1970; Matta *et al.,* 1981; Shehata *et al.,* 2008; Fisun and Goc Rasgele 2009). They suggested that the chromosome stickiness may results from breakage and exchange between chromatin fibers on the surface of adjoining chromosomes.

The second type of abnormalities is the disturbed which observed in metaphase and anaphase in all treatments and the percentage of this trait was not depended of the creatine concentration or period duration (Fig. 1g, h, I and j). This abnormality was observed by other reports such as: Polit *et al.,* 2000 (BAP and lAA on *Vicia faba*), Maslam 2004 (some medicinal extracts on *Allium cepa*), Usciati, *et al.,* 2004 (6- benzlaminopurine on *Cicer arietinum)* after many chemicals treatments they suggested that the chromosomes disturbed may results from the effect of the chemical treatment on proteins constituting the spindle apparatus.

Laggard chromosomes were observed in some creatine treatments in metaphase anaphase and telophase (Fig. 1f, k, l and n). Laggard at metaphase could by attributed to the failure of the spindle apparatus to organize and function in a normal way (Atef *et al.* 2011).

Figures 2 and 3 show the SDS-PAGE banding patterns of water soluble proteins for *V .faba* plants after sprayed with four concentrations of creatine monohydrate and genomic DNA analysis.

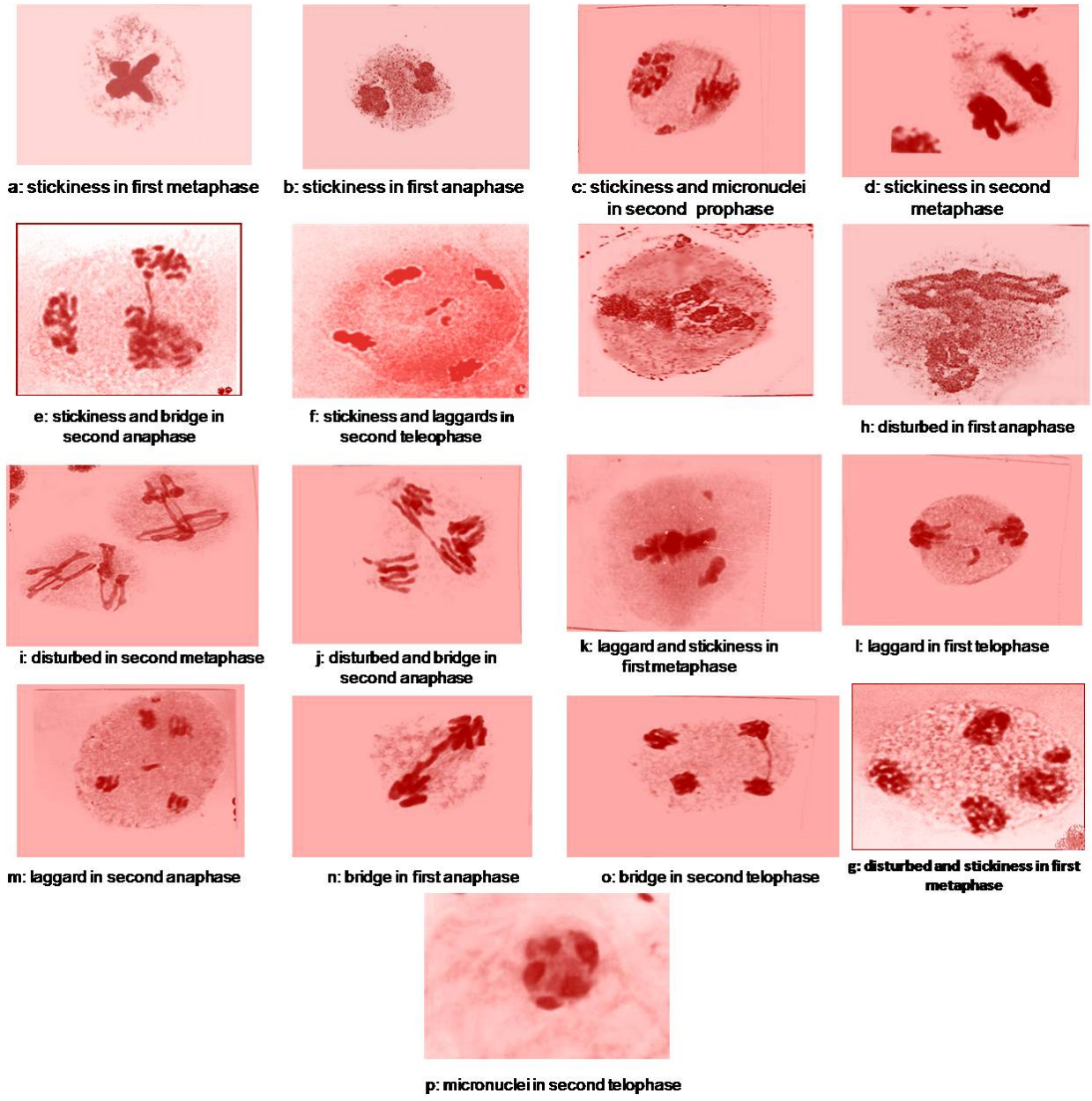

Fig 1. Meiotic abnormalities produced after different treatments with creatine monohydrate in *Vicia faba* plant.

Fig. 2. SDS-PAGE banding patterns of water soluble proteins for *V .faba* plants after sprayed with four concentrations of creatine monohydrate.
(omit)

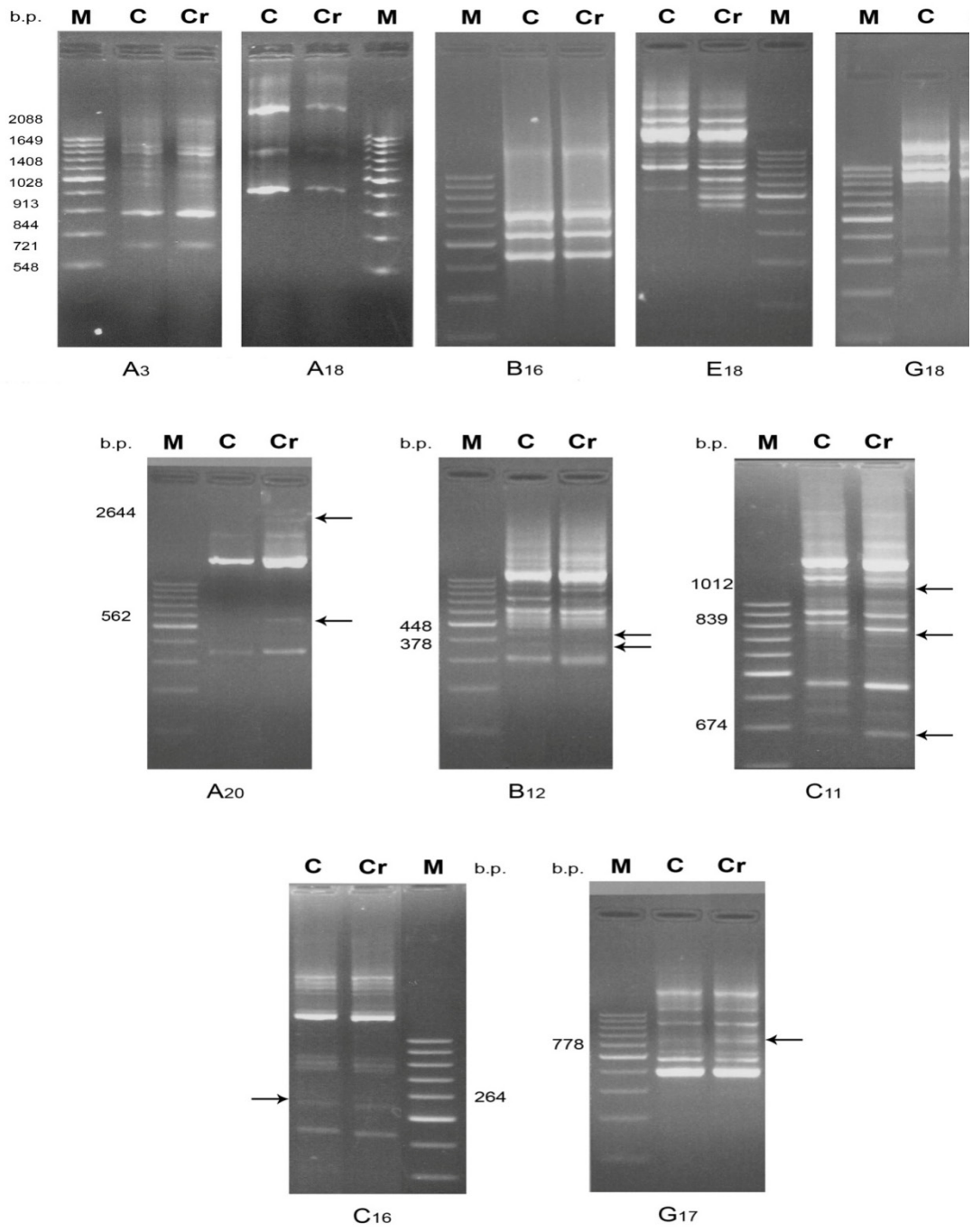

Fig.(3): RAPD profiles of genomic DNA from M_2 *Vicia faba* plants treated with 3g/100 ml creatine monohydrate by using 10 primer.

(M: DNA maker, C: control, Cr: creatine)

Table 3.Types and Percentages of abnormalities in 1st & 2nd meiotic divisions after sparaying of *Vicia faba* plants with creatine monohydrate for (24, 48 ,hours & 15 days).

Time	Cont.	%abnormal in 1st division				%abnormal in 2nd division					
		stickiness	Disturbed	Laggards	Bridge	stickiness	Disturbed	Laggards	Bridge	Multipolar	(Mico-Multi) Nuclei
Control		__	2.17	__	__	.85	4.27	__	__	__	__
24h.	1.5	__	17.78	__	__	12.50	16.67	__	__	__	__
	2	26.05	10.13	__	__	14.46	19.88	__	__	__	__
	2.5	29.95	23.67	__	__	32.02	15.17	1.12	__	__	1.12
	3	42.48	7.84	__	__	27.21	11.56	.68	1.36	.68	.68
48h.	1.5	20.00	24.44	__	__	24.49	20.41	__	__	__	10.20
	2	28.97	20.69	3.45	1.38	17.68	15.24	__	__	__	1.21
	2.5	39.09	17.73	2.27	1.36	29.49	14.74	__	1.28	__	3.85
	3	44.06	11.19	2.10	1.40	28.49	8.72	2.33	2.33	__	6.98
15days	1.5	11.69	19.48	__	__	__	11.76	__	__	__	__
	2	25.55	16.79	__	.73	19.50	13.84	1.26	__	__	2.50
	2.5	33.16	14.74	2.11	2.63	22.08	13.64	1.95	1.30	.65	5.19
	3	30.90	16.85	3.37	1.69	33.74	13.50	2.45	1.23	__	7.36

These laggerd may be distributed randomly to either poles at both anaphase and telophase I and II which result ultimately in aneupliody, Amer and Ali (1988). Or may they give micronuclei at telophase, Ozturk (2008). The induction of laggard chromosomes could be attributed to irregular orientation of chromosomes, Patil and Bhat (1992). In addition to previous common abnormalities, it was observed more on meiotic division including bridges, micronuclei and multinucleate. Bridges were induced in some treatments (Fig. 1e, j, m and o) and they could be due to the breakage and reunion, Asita and Makhalemele (2009) or due to the general stickness of chromosomes, Ozturk (2008).

Table 4 shows the RAPD profile using 5 primers in M^2 treated *Vicia faba* plants treated with 3 g /100 ml creatine.

Table 4. RAPD profile using 5 primers in M^2 treated *Vicia faba* plants treated with 3 g /100 ml creatine.

	Ms (bp)	Control	Creatine
A 20	2644	-	+
	562	-	+
B 12	448	+	-
	378	+	-
C 11	1012	+	-
	839	+	-
	674	-	+
C 16	264	+	-
G 17	778	-	+

+ and - = appearance and disappearance of fragments

While, micronuclei and multinucleate were also recorded in some treatments in the second meiotic division (Fig. 1c, p and q) and our results are in agreement with the results of Srivastsva and Singh (2009). Finally, the inductions of these chromosomal abnormalities were pointed to the mutagenic potential of creatine monohydrate

4.References

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Cardiovascular Diseases in Mena Hospital during Hajj (1429H) at Makkah, Saudi Arabia

Abdulhalim Salim Serafi and Abdulmonim Ahmad Alqasim

Faculty of Medicine, Umm al-Qura University, Makkah, Saudi Arabia
ASSerafi@uqu.edu.sa

Abstract: Background: The numbers of Hajj pilgrims are increasing yearly. During Hajj period of 1428H (2007G), cardiac problems have been reported as one of the commonest causes of admissions in hospitals. However, the data regarding the commonest presentations and types of cardiac diseases were not reported in the literature. **Objectives:** To identify the cardiovascular diseases in Hajj pilgrims for the year 1429H (2008G) In Mena Hospitals. To determine the proportion of heart diseases most common among the pilgrims, with a focus on patients with heart failure (HF) and ischemic heart disease (IHD). It is known that patients who suffer from HF have elevated some of cardiac enzymes, that originated the idea of this research is trying to reach an early signs in patients with HF and try to diagnose their condition and refer them to a cardiologist early to treat and prevent complications of the disease. **Methods:** Analysis of the data was carried out for all patients, who were admitted at Mena Emergency Hospital over a period of 15 days in Hajj season 1429H (2008G). A questionnaire has been filled out and blood samples were taken from all patients admitted to Coronary care unit (CCU) and cardiology wards in the hospital. **Results:** A total number of 507 patients were admitted to the hospital including 120 patients with heart diseases, 85 of them were admitted in CCU and 35 in cardiology ward. The patients suffering from various cardiac diseases were in the following order: heart failure cases 70%; ischemic heart disease 20%; and valvular heart disease 10%. Brain Natriuretic Peptide (BNP) was significantly increased in all patients with heart failure. By analyzing the questionnaire in patients who have HF with high BNP we found that 80% of them admitted to the hospital after they perform physical effort and more than 50% of them had started the symptoms they have after they throwing pebbles. **Conclusions:** Most common cardiac diseases were found in hospitalized patients for Hajj 1429H, belonging to different countries over the globe. Circulating levels of the BNP can help in the diagnosis of cardiovascular disease and provide prognostic information not only in patients with HF but also the general population and other patient groups. The BNP test is used as an aid in the diagnosis and assessment of severity of heart failure (HF). BNP testing on clinical outcomes of patients presenting to the emergency department with acute dyspnea could be helpful and may lead to a decrease in admission rates and decrease in mean length of stay. The BNP test is also useful for the risk stratification of patients with acute coronary syndrome.

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Key words: Cardiovascular disease, Hajj, BNP, pilgrim, Makkah.

1. Introduction

Once in a lifetime every Muslim is expected to undergo a holy pilgrimage, known as Hajj, which takes place in the 12th month of the Islamic lunar calendar. The Kingdom of Saudi Arabia has been privileged to host the event of Hajj being one of the five pillars of Islam. It brings millions of pilgrims of several Nationalities from different countries of the world every year. Although, this important pilgrimage need mental, physical and financial fitness, for its performance, which is ignored, especially for the physical abilities, by the visiting Hajji themselves and health authorities of their home countries. Therefore, yet a lot of pilgrims come to Makkah with major cardiovascular diseases. In addition, overcrowding and the hot climate subject the pilgrims further towards environmental and health hazards. The rituals performed during hajj include a walk around Kaaba, a cube-shaped building in Makkah considered the most sacred site in Islam, followed by the Sa'i consisting of walking between two hills (Safa and Marwa) seven times, each with a distance of about 450 m to a total of 3.15 km. Other rites include a 14.5 km journey to the desert Arafat, a night spent at Muzdalifah where pebbles to be thrown the following day at Mena (about 5 km from Makkah).

In a previous study, heart diseases (**Serafi, 2008**), whether exacerbations of pre-existing disease or the occurrence of new ones have been reported to account for (20%) of all diseases seen during the 2-week periods of the Hajj. A person could be ill at any time in his life and to seek medical aid, he might be admitted to a hospital for as long as needed. But during the Hajj days, too many Hajj pilgrims get medical problems ranging from minor flu to major illnesses and some may need surgical intervention. It is worth to mention that excellent medical services

are provided in every hospital of Makkah, Muzdalifa, Arafat and Mena (**Gazzaz *et al*., 2004**). It results in only 2 to 3% casualties among huge admissions in various hospitals. Yousef and colleagues (**Yousaf *et al*., 1995**) have recorded the health problems of pilgrims seen as outpatients. Of these, the commonest diseases were pneumonia, diabetes and ischemic heart disease. This resulted in a high admission rate to the medical departments, as in our current study.

The pattern of surgical problems alone was studied by Elhassan & Hameed (**1990**) and Al-Harthi & Al-Harbi (**2001**). The commonest problems listed were blunt abdominal trauma due to traffic accidents, obstructed inguinal hernia, and intestinal obstruction. In recent years, there has been a change in the pattern of diseases among pilgrims (from cholera and meningitis, to diabetes and ischemic heart disease), perhaps due to improved health education and hygiene (**Ahmed, 2002**) as highlighted in Hajj studies for specific diseases (**Ataur-Rahim, 1986; Yousaf, 2000; Ahmed, 2002**).

Recently In a 2012 review article (**Al Shimemeri, 2012**) examining the pattern of cardiovascular disease in Hajj Pilgrims found that over the past few years cardiovascular diseases have emerged as an important cause both of intensive care unit (ICU) admission and of mortality during Hajj. For instance, in a study analyzing Hajj hospital admissions in 2004 as a function of pilgrims' geographical origin, myocardial infarction was identified as the major cause of admission into the intensive care units (ICU) of seven hospitals (four in Mena, three in Arafat), ahead of pneumonia, asthma, chronic obstructive pulmonary disorder (COPD) and pulmonary edema (**Madani *et al*., 200**4). More than 60% of the ICU admissions came from cardiovascular causes of which myocardial infarction and left ventricular failure occurred with the highest frequencies.

In 2005 the Ministry of Health of the Kingdom of Saudi Arabia identified deaths resulting from cardiovascular diseases as the highest recorded during Hajj (**Health Statistics: Saudi Ministry of Health, 2005**).

Serafi (**2010**) studied the commonest cardiac diseases at AL Noor specialist Hospital for Hajj 1429 and he concluded that hospitalized patients in Al-Noor Hospital for Hajj 1429H, were already suffering from most common cardiac diseases before coming for Hajj, and belongs to different countries over the globe. It clearly indicates loop holes in the health services of their home countries that did not verify the physical fitness of their pilgrims and allowed them to proceed for Hajj. This study will also serve as a helping tool for the Ministry of Hajj in Saudi Arabia to take appropriate measures for demanding strictness for the physical fitness of Hajj pilgrims and anticipated health services for them.

It is recommended that Health authorities of other countries should undertake counseling & medical testing of all persons planning for Hajj beforehand (at least six months earlier to the start of their journey for Hajj) and arrange an awareness program to advise and educate Hajj pilgrims regarding health care. And do not allow cardiac risk patients to proceed for Hajj unless they obtain acceptable vital values with medicine and care. Such patients should also possess a medical card with them; stating brief history regarding their main cardiac problem and medicines (generic/ chemical names) prescribed to them. This will reduce hospitalization rate and the burden on health services in Makkah and Medina during Hajj season (**Serafi, 2010**).

Cardiovascular disease (CVD) remains the leading cause of death in both men and women in the United States, and CHF remains the most common cause of hospitalization in patients older than 65. Recent statistics from the American Heart Association (AHA) indicate that CHF-related mortality rose 35% from 1992 to 2002. The high disease prevalence and high mortality associated with CHF mandate aggressive diagnostic and management strategies. The revised American College of Cardiology/AHA heart failure guidelines incorporate a new classification system to more readily identify high-risk patients and to direct primary and secondary prevention efforts (**Dickstein *et al*., 2008**).

Approximately 5 million adults have congestive heart failure (CHF) in the USA, and approximately 550,000 new cases of heart failure are diagnosed in USA every year. The incidence of CHF increases dramatically in the elderly population: Approximately 10% of men and women older than 75 years have the disease (**Redfield *et al*., 2002**).

Circulating levels of the BNP system can help in the diagnosis of cardiovascular disease and provide prognostic information not only in patients with HF but also the general population and other patient groups. Changes over time also carry prognostic information and studies are assessing BNP-guided treatment strategies. New insights regarding the biology of the BNP system are emerging with identification of circulating molecular forms of BNP, which may improve the diagnostic and prognostic value of BNP (**Costello-Boerrigter *et al*., 2006**)

The BNP test is used as an aid in the diagnosis and assessment of severity of congestive heart failure (also referred to as heart failure). A recent meta-analysis concerning effects of BNP testing on clinical outcomes of patients presenting to the emergency department with acute dyspnea revealed that BNP

testing led to a decrease in admission rates and decrease in mean length of stay (**Redfield *et al*., 2006**). The BNP test is also used for the risk stratification of patients with acute coronary syndromes (**Dickstein *et al*., 2008; Maisel *et al*., 2008**).

The above mentioned background shows a clear urgency for carrying out a study on patients with heart disease of pilgrims in Makkah and the holy sites. This study aimed to determine the proportion of heart disease most common among the pilgrims, with a focus on patients with heart failure (HF) and Ischemic heart disease (IHD). Since the mortality rate has risen significantly as a result of heart disease, especially in patients with coronary artery disease and heart failure we must search for ways and means to diagnose the disease early to try to control it and treat its complications.

It is known that patients who suffer from heart failure have higher levels of some cardiac enzymes that originated the idea of this research. This emphasize to observe the early signs in patients with heart failure (HF) and to try to diagnose their condition and refer them to the cardiologist early for treatment and to avoid, God willing, the incidence of complications as a result of heart diseases. The importance of the present research is for an attempt to find out the initial indicators early and try to treat and avoid them if possible. It is well known and is common to treat any disease or its complications, the results are far better off when detected early to try to control the patient.

Prevention is better than cure is the saying completely honest and this research may help patients with heart predicting the consequences that may affect them and try to prevent them, including through the organization of early treatment and referring to cardiac clinics.

There are great economic importance as well as early diagnosis may reduce the complications of heart attack, which will help States, individuals and health care institutions and organizations of the health insurance for early diagnosis and treatments, and to prevent complications of heart disease, such as treatments of cases of heart failure and coronary artery disease and other heart diseases. This reduces the cost as a result of early diagnosis of this deadly disease.

2. Methods:

The study was performed in Mena emergency hospital in Mena, Makkah, Kingdom of Saudi Arabia. The researchers were divided into two groups the first group was at the emergency department (ER), and the second group in coronary care unit (CCU). All researchers had to complete a questionnaire and to take blood samples from all patients attending the two departments in the hospital. The blood samples were saved and then transferred for future analysis and all results were saved in an Excel sheet for future statistical analysis.

This study was conducted for 507 (297 males, 196 females and 14 children) pilgrims of Hajj, admitted in the Mena emergency hospital, 120 cardiac patients of them were admitted in Coronary care unit (CCU) (85) and cardiology wards (35), Makkah, Saudi Arabia. It was done from the first 15 days of Hajj season (Month Zul-Hajjah) of the Islamic year 1429H, corresponding to 29th November to 13th December 2008G. The patient's data collection includes, statistic of all patients admitted to the hospital, questionnaire and blood samples for patients with suspected heart disease and those with shortness of breath. Brain Natriuretic Peptide (BNP) was estimated for all patients with heart diseases and those with shortness of breath.

3. Results:

Table 1 shows that total numbers of admission were 507 patients, 82% of them were discharged, only 17% of patients were still in hospital and 1% of patients were dead at the end of Hajj season. Majority of admitted patients were non-Saudi 76% with 78% of them were discharged and 20% inpatients with 2% mortality, while the admitted Saudi patients were 24% with 92% of them discharged and 7.5% patients stayed in hospitals with only 1 patient died less than 0.5%.

Patients with heart diseases were 120 patients, 100 of them were Non-Saudi patients, and 20 Saudi patients. They presented with different types of heart diseases. Table 2 showed that majority of non-Saudi patients 37 (37%) presented with heart failure while the majority of Saudi patients 8 (40%) were post coronary arteries bypass graft surgery (CABG).

As shown in table 3 that the number of male patients admitted with heart diseases were more than female patients. Patients with heart failure (40%) were the commonest presentations for both genders.

Table 4 shows that the number of males admitted to CCU were 68 (80%) and female patients were (20%) with majority of them with heart failure for both genders.

BNP were measured from Blood samples of all patients admitted to CCU and who are admitted to hospital suffering from shortness of breath or suspicion of HF with a total number of 150 patients. BNP ranged from 759 to13740 pg/ml with the highest levels in female patients with congestive heart failure.

Table 1. Anthropometric characteristics of all Saudi and Non-Saudi admitted patients.

	Saudi				Non Saudi				Total
	Male	Female	Children	Total	Male	Female	Children	Total	
Admission	71	48	1	120	226	148	13	387	507
Discharged	65	44	1	110	168	123	13	304	414
Inpatients	5	4	0	9	51	23	0	74	83
Dead	1	0	0	1	8	1	0	9	10

Table 2. Anthropometric characteristics of the Saudi (n=20) and Non-Saudi patients (n=100) with heart diseases.

	Rheumatic Heart Disease	Post CABG	Congestive Heart Failure (CHF)	Hypertension	Heart Arrhythmias	Heart Failure (HF)
Saudi	1	8	3	3	3	2
Non-Saudi	6	15	17	17	8	37
Total	7	23	20	20	11	39

Table 3. Anthropometric characteristics of Male (n=98) and Female (n=22) patients with heart diseases.

	Rheumatic Heart Disease	Post CABG	Congestive Heart Failure (CHF)	Hypertension	Heart Arrhythmias	Heart Failure (HF)
Male	5	20	17	17	9	30
Female	2	3	3	3	2	9
Total	7	23	20	20	11	39

Table 4. Anthropometric characteristics of Male and Female patients admitted to CCU.

	Heart Failure (HF)	Congestive Heart Failure (CHF)	Heart Diseases	Total
Male	30	17	21	68
Female	9	3	5	17
Total	39	20	26	85

Table 5. BNP Values in Male and Female patients with heart diseases, data (means±standard deviations).

	Heart Failure (HF)	Congestive Heart Failure (CHF)	Heart Diseases
Male	358 ± 1370	1310 ± 12490	67 ± 759
Female	512 ± 2421	1718 ± 13740	87 ± 870

4. Discussion:

This study was done in continuation of previous studies (**Serafi, 2008; 2010**) to further evaluate the Hajj Pilgrims admitted in hospital with respect to their classification as cardiac patients. It may be tempting to ask the question: should the Saudi Arabian authorities institute an age based exclusion criterion for granting admittance onto the pilgrimage? Perhaps, it would be of greater help; however, to have the different national and regional authorities work together with the Saudi Arabian authorities in ensuring intended pilgrims are adequately screened for likely risk factors such as cardiovascular diseases with a special attention to how well such chronic diseases have been managed by the individual.

Expectedly, this will parametrically consider age as a risk factor; however, such screening should primarily focus on disease risk factors. This is already in force in certain cases where intended pilgrims from Turkey and Malaysia with severe heart conditions are prevented from performing the Hajj. It is important that the main objectives of Hajj could not be achieved either individually or collectively, if health authorities are not care full. It is worth to note that the health ministry of Saudi Arabia is spending millions of Riyals on best care of these pilgrims in Makkah and Madinah, coming from all around the world. However, during main Hajj Ritual period, emergency admissions in hospitals offer great difficulty to pilgrims themselves and challenge to the local health authorities. This situation clearly reflects carelessness of health authorities or Hajj missions belonging to the home countries of these Pilgrims. The Pilgrims from Malaysia and Turkey with heart diseases are prohibited from coming to perform Hajj. However, only one Hajji from Malaysia was admitted to al-Noor hospital with acute coronary syndrome with no previous history of heart disease.

This is a pilot study with limitations. It highlights only those cases admitted in Mena

emergency hospital for the year 1429H and refers to a previous studies for the year 1427H, 1429H (**Serafi, 2008; 2010**)1. It is only a reflection of status of Hajj pilgrims and their heart diseases from our perspective. Pilgrims are somewhat careless about their health matters, as they want to avail every single minute to perform rituals. However, they are forced to seek medical care when they fall ill. Approximately 507 patients were admitted during 1429H in this emergency medical care center (Mena emergency hospital).

Treatment of HF can be challenging, especially since common symptoms and signs have only limited specificity. For that reason, a sensitive, objective, and cost-effective measure of patient status is highly desirable. The cardiac-derived natriuretic peptide BNP and its related peptides may be such markers. Given that myocardial stretch stimulates BNP production and release, that the heart is the major source of BNP, and that BNP can easily be measured in plasma, there is a straightforward rationale for evaluating circulating BNP as a biomarker for cardiac overload (**Dickstein *et al*., 2008; Maisel *et al*., 2008**).

We found that majority of admissions were males. Maximum numbers of admission were observed in CCU suggesting that these patients were in acute stage whether this was a new acute episode or a de compensation of old heart diseases. A total number of 507 patients were admitted to the hospital including 120 patients with heart diseases, 85 of them were admitted in CCU and 35 in cardiology ward. The patients suffering from various cardiac diseases were in the following order: heart failure cases 70%; ischemic heart disease 20%; and valvular heart disease 10%. Brain Natriuretic Peptide (BNP) was significantly increased in all patients with heart failure. By analyzing the questionnaire in patients who have HF with high BNP we found that 80% of them admitted to the hospital after they perform physical effort and more than 50% of them had started the symptoms they have after they throwing pebbles. It is known that patients with controlled hypertension can go easily into decompensation and presented with heart failure after an exertion and that was the case in our study as majority of patients developed their symptoms after performing physical efforts.

Gazzaz *et al*. (**2004**) have recorded 20% of admissions to Al-Noor specialist hospital is due to heart diseases during the Hajj 1422. Al-Ghamdi *et al.* (**2003**) conducted a study during the Hajj 1422 session but for the hospitals of Al-Mashaer areas (four in Mena and three in Arafat) and gave similar results. These are considered as primary and secondary care facilities that cater to the urgent medical needs of pilgrims. Although this is a small study and needs further research in different aspects of Hajj and in other hospitals, it provides a brief overview of heart diseases in pilgrims. Screening of patients with shortness of breath by measuring BNP in their blood could help in diagnosing more patients and send them early to cardiac clinics so my lead to reducing the length of stay and prevent complications.

Conclusions:

It is concluded that hospitalized patients in Mena emergency hospital for Hajj 1429H, were already suffering from most common cardiac diseases before coming for Hajj, and belongs to different countries over the globe. It clearly indicates loop holes in the health services of their home countries that did not verify the physical fitness of their pilgrims and allowed them to proceed for Hajj.

This study will also serve as a helping tool for the Ministry of Hajj in Saudi Arabia to take appropriate measures for demanding strictness for the physical fitness of Hajj pilgrims and anticipated health services for them. It is recommend that Health authorities of other countries should undertake counseling & medical testing of all persons planning for Hajj beforehand (at least six months earlier to the start of their journey for Hajj) and arrange an awareness program to advise and educate Hajj pilgrims regarding health care. And do not allow cardiac risk patients to proceed for Hajj unless they obtain acceptable vital values with medicine and care. Such patients should also possess a medical card with them; stating brief history regarding their main cardiac problem and medicines (generic/chemical names) prescribed to them. This will reduce hospitalization rate and the burden on health services in Makkah and Medina during Hajj season. This study will also serve as a helping tool for the Ministry of Hajj in Saudi Arabia to take appropriate measures for demanding strictness for the physical fitness of Hajj pilgrims and anticipated health services for them.

Prevention is better than cure 'theorem is completely honest and this research may help heart patients that may predict complications and try to acquire them and protect them by regulating the treatment and lack of exposure to physical stress and refer them to clinics early to prevent complications. There are great economic importance as well as early diagnosis may reduce the complications of the heart diseases and that will help states and individuals and health care institutions and institutions of health insurance for early treatments, prevent complications of heart disease, such treatments are invasive and costly especially for cases of heart failure and

coronary artery disease which reduces the cost as a result for early diagnosis of this deadly disease.

Recommendations:

1. Young and healthy pilgrims to substitute the elderly patients to throw pebbles.
2. Transfer the elderly and the sick pilgrims by cars to throw pebbles.
3. Use of an electrical course to and from the Jamarat to transport pilgrims

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Correspondence author
Abdulhalim Salim Serafi
Faculty of Medicine, Umm al-Qura University, Makkah, Saudi Arabia
ASSerafi@uqu.edu.sa

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Developing skills in managing Objective Structured Clinical Examinations (OSCE)

Samira Alsenany[1] and Amer Al Saif[2]

[1]Assistant Professor in Gerontology, Nursing Department, Faculty of Applied Medical science, King Abdulaziz University, Jeddah, Saudi Arabia. Salsenany@kau.edu.sa.
[2]Assistant Professor in Physical Therapy, Physical Therapy Department, Faculty of Applied Medical sciences, King Abdulaziz University, Jeddah, Saudi Arabia. Aalsaif @ kau.edu.sa

Abstract: The objective structured clinical examination (OSCE) was originally developed in 1975 by Harden to avoid the many disadvantages of the traditional clinical examination and to improve feedback between staff and students; in making the examination more objective, a marking strategy was decided in advance. Furthermore, clinical competence assessment is an important issue in clinical health education: assessing clinical practice is long-standing and receives substantial attention in health care education. The OSCE mode is very useful to monitor the abilities students, and stations can be designed to address different skills and knowledge. OSCEs are valuable way of assessing proficiency in range of clinically- focused skills and knowledge, so they are widely used as fundamental assessment strategy in across the world. The greatest advantage of using OSCE is that it can be set up to integrate theory and practice in forms of small scenarios, simulations, case studies, standardized patient (SP) and the students can improve their own learning and reflection in a safe environment. In the OSCE evaluation of clinical skills is essential feedback and it plays an important motivating role between students and teachers to ensure the quality and appropriateness of a learning process. It may be used for exploration of the relationship between competence and knowledge as an assessment method through meeting specific objectives of the teaching process and integrating technical and theory "stations" to advanced clinical practice. However, OSCE can also be used in a formative way, as problem-based exercises to enhance skill acquisition and integrate other key skills (e.g. critical thinking, communication, and reflective practice). There are a number of methods to evaluate the knowledge, skill and attitudes of students in academic program such as written examinations, projects / papers / presentations, and clinical examinations. The Objective Structured Clinical Evaluation (OSCE) is a clinical examination, utilizing a standardized patient (SP) setting in order to test the student's understanding and performance knowledge, skills and attitudes. Additionally, OSCEs involve the Year Coordinators, Instructors, Examiners, Standardized Patients Students. Each of these stakeholders has a particular role and set of responsibilities towards an OSCE. During an OSCE, the students are evaluated on their skill sets of communication, assessment and treatment, safety, and patient feedback and education. Examiners and Standardized patients receive additional training to ensure continual quality of the OSCE. However, the potential of OSCE as a flexible teaching and evaluation method to avoid examiner variation has been recognized in health education. The greatest advantages of using OSCE are that it can be set up to integrate theory and practice in the form of small scenarios, simulations, case studies and standardized patient (SP), and the students can improve their own learning and reflection in a safe environment, as has been identified. Although there are a few drawbacks in using OSCE, such as time, cost, number of clinical instructors requested with the high number of students, we should not neglect it. Several studies were found in the literature review that are on assessment of clinical competence and objective structured clinical examination (OSCE). Studies were reviewed from the Cumulative Index of Nursing and Allied Health Literature (CINAHL); MEDLINE and ASSIA were searched using Ovid and CSA.

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Keywords: OSCE, assessment clinical competence, change, evaluation and performance.

1. Introduction

Clinical competence assessment is an important issue in clinical health education: assessing clinical practice is long-standing and receives substantial attention in health care education, as shown in Watson et al's (2002) study of clinical competence assessment . This reports that the assessment of clinical competence is centre stage in health education and it is problematic due to difficulties in deciding what to assess, whether competence should be assessed globally or through multiple competencies, and issues behind the lack of objectivity of assessment methods with little evidence to support the use of clinical competence and a wide variety of methods for its use in health care team. Alinier (2003) proposes that OSCE can be a useful method of teaching because it is a safe practice to help students gain more confidence when confronted by technical instruments present in the hospital environment. Latif (1992) pointed out that OSCE and

clinical examination both scored high for their ability to assess clinical competence. Bondy (1983) comments that evaluation of clinical competence of students is usually considered subjective and inconsistent. He proposed the criteria for a five-point rating scale for evaluation of student clinical performance to be a fair assessment of performance.

Another study in clinical competence assessment tools for reliability and validity (Norman et al, 2002) found that no single method is appropriate for assessing clinical competence among health care students and points out that health care education needs a multi-method strategy for clinical competence assessment. Mahara (1998) comments that the evaluation of student learning in the clinical area has been concentrating on how much educators are familiar with issues arising from the subjective nature of clinical evaluation and the role of clinical instructors as both teachers and evaluators.

The clinical competence evaluation it considers as a teaching assessment method is not the only evaluation method. Bradley and Postlethwaite (2003) analyzed that it is essential for evaluation of clinical skills to give feedback and play an important role in motivation between students and teachers to ensure the quality and appropriateness of a learning programme. They suggest use of the objective structured clinical examination (OSCE) at both undergraduate and postgraduate level. The objective structured clinical examination (OSCE) was originally developed in 1975 by Harden to avoid the many disadvantages of the traditional clinical examination and to improve feedback between staff and students; in making the examination more objective, a marking strategy was decided in advance (Harden et al, 1975). The OSCE stations can be designed in the form of small scenarios where students have to set up or interact with technical instruments or communicate with 'patients': a layperson is called a standardized patient (SP).

Whether OSCEs assess clinical safety or role competence is an area of debate. If we strictly adhere to inflexible parameters for safe practice, a student could potentially, in the name of safety, refer every patient they see to either a medical practitioner or a specialist nurse for a second opinion. While this may be a safe practice, it does not conform to the everyday level of independent practice realistically required from an advanced nurse practitioner to assess, plan, deliver and evaluate patient care. Therefore, there is a baseline at which we expect an advanced nurse to operate. Students need to demonstrate their ability to work bounded by the limits of their advanced role competence within an overall safe approach. Accordingly, students need to be able to identify potentially serious clinical signs and symptoms in their OSCE stations, and conversely OSCE stations need to be designed to give students an opportunity to identify serious clinical signs and symptoms.

2. Literature review

Several studies were found in the literature review that are on assessment of clinical competence and objective structured clinical examination (OSCE). Studies were reviewed from the Cumulative Index of Nursing and Allied Health Literature (CINAHL); MEDLINE and ASSIA were searched using Ovid and CSA. The database Keywords for searching included: OSCE, assessment clinical competence, change, evaluation and performance. The problem of assessing clinical practice is long-standing and receives substantial attention in the literature research. Norman et al (2000) suggest a need for a multi-method strategy for clinical competence assessment for health care students. Their study collected assessment data from a sample of 257 nursing and 43 midwifery students in four educational institutions and administered additional assessment measures. They assert that the different methods address different abilities. A clear finding from this study is that no single method is appropriate for assessing clinical competence. Similarly, Mahara (1998) claims, in a perspective on clinical evaluation in health care education, that clinical learning is the heart of the educational experience for students. He reviewed and discussed the objectivity-subjectivity debate and the limits of evaluation practices based solely in positivism and teacher-evaluator and formative-summative distinction. He reported that clinical evaluation processes are more than one aspect of clinical learning and he suggested that curricula judging of a student's clinical practice as a teaching-learning strategy must be based on the concepts of meaning-making, reflection and teacher-student feedback, providing a basis for evaluation approaches.

In similar vein, Waston et al (2002) propose that the assessment of clinical competence remains almost universally accepted in health education. Their study was designed to investigate the evidence for the use of clinical competence assessment through a review using systematic methods of literature of different assessments of clinical competence, like use of objective structured clinical examination (OSCE) as a clinical competence tool for assessment. This points out that some students may perform less well than they would in clinical practice due to the examination nature of the OSCE.

There is still considerable confusion about the definition of clinical competence and most of the methods in use to define or measure competence have not been developed systematically. There has been a change in theoretical frameworks of assessment, as a

lack of consistency in the training of student assessors in the clinical areas was identified. In Calman's (2002) study, data were collected by postal questionnaire.

The directors of the 13 programmes (seven nursing and six midwifery programmes) were surveyed and also 12 group interviews were conducted with students (six nursing and six midwifery student groups) from seven institutions. Students from all four branches were represented and 72 students (36 nurses and 36 midwives). Students' views suggested that they had little confidence in methods of clinical competence assessment and there was no formal validity and reliability of testing. Some of these issues may be resolved with the development of an instrument for competence assessment. In another study, Bukinghams (2000) asserts that effective assessment of competency of student in clinical practice is a vital issue. Another study by Howley (2004) criticizes the traditional method of assessment in clinical competence, saying that the assessment tool is becoming increasingly complex. Reviewing the performance assessment with standardized patients based on various literatures, Howley proposed several areas for the future direction of performance assessment, including (a) toward evidence-based locally-developed assessments, (b) toward an understanding of educational outcomes and non-cognitive assessment factors, and (c) toward more student-driven assessments.

When we review the framework for assessing clinical competence for health care students to help me understand the process of psychomotor acquisition in my Advanced Practice in order to gain some confidence in how to assess students' competence, I turn to Miller (1990) Psychologist George Miller 1990 proposed a framework for assessing clinical competence. At the lowest level of the pyramid is knowledge (knows), followed by competence (knows how), performance (shows how), and action (does). In this framework, Miller distinguished between "action" and the lower levels.

"Action" focuses on what occurs in practice rather than what happens in an artificial testing situation. Other common methods of assessment of clinical competence in health care students, such as multiple choice questions, simulation tests, and objective structured clinical examinations (OSCEs) target the lower levels of the pyramid (Norcini, 2003). A review by Miller (1990), on the assessment of clinical skill, competence and performance, raises an interesting point concerning the performance and action component of future graduates.

Examinations should be designed to test students in performance closely related to their future professional function, such as objective structured clinical examination (OSCE). Ananthakrishnan (1993) defines OSCE as an "assessment tool in which the components of clinical competence such as history taking, physical examination, simple procedures, interpretation of lab results, patient management problems, communication, attitude etc. are tested using agreed check lists and rotating the student round a number of stations some of which have observers with check lists." OSCE is considered a powerful tool in evaluation and an effective facilitator in learning in health education.

Ross et al (1988) point this out in their study of objective structured clinical examination (OSCE) to measure the psychomotor learning outcome and the programme designed to assist students to learn to conduct a nursing neurological examination. They report that OSCE has a tradition in medicine, having been developed by Ronald Harden in Scotland and first reported in the British Medical Journal in 1975, and educators have a challenge in the measurement of clinical skills performance. An examination of the literature on OSCE clarifies the advantages and limitations of the method as follows.

A. Advantages of objective structured clinical examination (OSCE)

In Objective Structured Clinical Examination (OSCE) the students practise the clinical skill in a safe area, such as with standardized patients (SP): simulated, artificial models or manikins are utilized with an examiner present. This is one of the advantages of OSCE: according to Alinier (2003) study, when assessing students and lecturers in use of this hybrid formative OSCE, two questionnaires have been designed.

The first questionnaire was aimed at collecting information from students (n=86); the second questionnaire was distributed to lecturers (n=39) who have assessed students during OSCE. The study received positive feedback regardless of teaching method and shows that OSCE is favourably perceived because the aim of OSCE is to teach safely to help students gain more confidence when confronted by technical instruments present in the hospital environment.

Following this line, Langford et al (2004) report that OSCE can help the students to gain some confidence; practising in a safe environment will reduce stressful feelings and fear from high numbers of errors if real patients were to be present in the exam, which may lead to a lack of competence in the required skill among the students. In a similar vein, Lee et al (2003) propose that OSCE competency assessment may reduce the incidence of errors in information reported and an OSCE is a reliable, valid, and practical method for assessing continued skill

competency. Following along these lines, skills and competences need to be acquired because they are used in a formative way to enhance skill acquisition through simulation.

Preparation and implementation of the OSCE is explored in students and tutors, and the strengths and problems are examined in the study by Anderson (2002) on the implementation of an objective structured clinical examination (OSCE) in the assessment of mental health nursing students with discussion of the development of OSCE. The study concludes advocating the use of the OSCE assessment tool as a formative exercise. Similarly, Coovadia and Moosa (1985) suggested that OSCE can measure both clinical competence and theoretical knowledge. Advanced nursing practice is concerned about decision-making based on a theoretical background, as in objective structured clinical examination (OSCE) that will be considered as an import issue in nursing education. As Bartfay (2004) suggested, objective structured clinical examinations (OSCE) promote the mastery of clinical skills and decision-making for nursing students in controlled and safe learning environments, which lead to advanced nursing education and practice.

B. Disadvantages of objective structured clinical examination (OSCE)

Objective structured clinical examination (OSCE) consists of different stations. All stations should be capable of being completed in the limited time. The students are rotated through all stations and have to move to the next station at the call from the examiner. Since the stations are generally independent, students can start at any of the procedure stations and complete the cycle. These stations are independent of each other, broken down into components and tested separately. This condition of OSCE's different stations is considered to be a limitation: Chabeli (2001) criticizes the objective structured clinical examination (OSCE) and suggests use of varied alternative methods for clinical assessment and evaluation for nursing students because OSCE does not measure the learners' clinical competence holistically; the data were collected from perceptions of 20 nurse educators, regarding the use of OSCE as a clinical evaluation method within a qualitative and descriptive research strategy. Three focus group interviews were conducted in different sessions.

A descriptive content analysis was formulated and he found positive and negative aspects toward OSCE from nurse educators. This suggestion is also supported by Senanayake (2001), who found that OSCE tests skills, attitudes and knowledge in separate compartments, and ability to look at patients as a whole is not assessed; however, clinical decision making can be incorporated in an OSCE. Another reason for limitation of OSCE in clinical teaching is that it is time consuming: OSCE exams need extra time than traditional assessment tools in clinical teaching to cover all stations in clinical assessment, preparation, displaying and time management needed in the exam. As shown in Anisur (2005), objective structured clinical examination (OSCE) can lead to increase in teaching time.

On the other hand, Cusimano et al (1994) found that OSCE is more expensive and time-consuming than traditional exams because of the need for more human resources and materials in, for example, the need for enough examiners, standardized patients (SPs), support staff and equipment for the procedure. Brazeau et al (2002) found OSCE did not meet higher standards of reliability and would need more time of testing per student to meet those standards. When comparing OSCE with other methods, Alnasir (2004) created a similar method to OSCE called Watched Structured Clinical Examination (WSCE), which can test student competence in clinical skill and knowledge communication skill in a short time. The method of study is illustrated by a total of 62 students for the WSCE, seated in two halls, which were equipped with video projectors. Five stations were presented in the session, which lasted for 60 minutes. Complete instructions on how to interpret each station and how to answer the questions related to each station were clearly written in the WSCE booklet. Alnasir discovered WSCE to be more useful than OSCE because it is possible to examine a large number of students in certain clinical skills in a short period, with an advantage over the OSCE in that it is less time-consuming, more cost-effective, requires less supervising staff to conduct the examination and it is less stressful to the students.

The image of health care has improved over the past 130 years, since Nightingale initiated a transition to professional status by introduction of advanced clinical practices. It is evident that health care staff have been involved in change across the decades (Joellen & Janice, 1996). Other writers also suggested that clinical education needs to change and introduce students to Objective Structured Clinical Evaluation (OSCE) as an effective method (McCourt & Thomas, 2001; O'neill, 1996).

Similarly Nicol (1998) proposed that 'Bart's OSCE' is an innovative approach to the assessment of clinical skills, through the medium of simulated professional practice. This has prompted changes in teaching-learning and assessment of clinical skills because of the change in nature of clinical placements due to shortage of staff in clinical areas or in-patient episodes to increasing workloads.

Objective structured clinical examination (OSCE) needs to be implemented in a proper way by creating some changes. Using the theory of Lewin, K (1958) as a source of classical change theory, this viewed changes as occurring in three steps: unfreezing, moving, and refreezing. He also emphasized the need to identify those forces that support change (driving forces) and those that mediate against it (restraining forces). The first step of a change process is unfreezing, meaning getting people to think differently about a problem or way of doing something. For example, for assessment of clinical competence by OSCE, we would need to meet with my colleagues at. This would be to discuss: students problems in evaluation and help students to gain more confidence in practising in a safe environment; and how the nursing curriculum lacks a professional assessment tool, with emphasis on the need to change from traditional evaluation and use OSCE as a teaching and evaluation method. The provision of evidence in previous research, and a compilation of literature to support this change should satisfy the practice outcome for educators and students.

The second step in the change process is moving: meaning trying something new, given the idea of a trial. In our college the objective structured clinical examination is considered to be a new idea, needing assessment with an arranged check list: the practical objectives and feedback from students and staff is a requirement to measure reliability and validity of the exam. As Cohen et al (1990) defined, "OSCE reliability refers to precision of the examination and construct validity to the degree to which the examination can discriminate between different levels of training". This reliability and validity of the exam is an important issue before implementation as a clinical assessment tool because the examination is more objective, and a marking strategy can be decided in advance.

Then we should recognize the stakeholders in change and power of organization to acceptance intervention and teambuilding sessions can helpful too. The third step of the change process is refreezing, which means solidifying the change so that it becomes universal practice. By adapting the objective structured clinical competence (OSCE) as an effective assessment tool, this may be used for evaluation of students as a routine part of care.

Data on students' perception about OSCE examination yield important information that was helpful for the driving forces supporting a change because nursing students possibly may become qualified in practice due to feeling more confident, having reduced stress, and obtaining satisfaction from the OSCE evaluation tool. Students are able to reflect on their performance and solve any problems they might have with some of the stations in a safe and comfortable environment. Support and positive attitude of organization members as feedback information also acknowledges the high education value of the OSCE, which may also have potential as a driving force.

There are a few restrictions, such as the number of students involved, the rigidity of the time so that the session runs in a coordinated way, the large number of qualified people required to assess the students, and adequate funding maintenance. Confrontation can be useful in effecting change when an advanced practice role is introduced and, in appropriate situations, such as when faced with previous resistance, the power of the organization has a positive effect in facilitating and supporting the change in advanced practice.

In conclusion, the OSCE mode is very useful to monitor the abilities students, and stations can be designed to address different skills and knowledge. The greatest advantage of using OSCE is that it can be set up to integrate theory and practice in forms of small scenarios, simulations, case studies, standardized patient (SP) and the students can improve their own learning and reflection in a safe environment. In the OSCE evaluation of clinical skills is essential feedback and it plays an important motivating role between students and teachers to ensure the quality and appropriateness of a learning process. Although there are a few drawbacks in using OSCE, such as time, cost, number of clinical instructors requested with a high number of students, it should not be neglected.

Corresponding Authors:

1-. Dr. Samira Alsenany, BSc (Hons), RN, MSc, PGCert ANP, PhD
Assistant Professor in Gerontology
Nursing Department,
Faculty of Applied Medical Sciences
King Abdulaziz University
Salsenany@kau.edu.sa

2. Dr. Amer A. Al-Saif, PT, MPT, DPT, PhD
Assistant Professor, Physical Therapy Department
Neuro Rehab Track; Balance and Dizziness Therapy
Faculty of Applied Medical Sciences
King Abdulaziz University
Aalsaif@kau.edu.s

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Effects of Vitamin A Supplementation on Reducing Toxicity of Aflatoxin B1 on the Ovary of Young Female Rats

Ismail, N. H.

Zoology Dept., Faculty of Girls for Art Sciences and Education, Ain Shams University
nadia-hussein@hotmail.com

Abstract: Aflatoxins are highly toxic, mutagenic, teratogenic and carcinogenic compounds produced by some species of Aspergillus, especially *A. flavus* and A. parasiticus. This study was designed to investigate the possible therapeutic dose of Vit. A on ovary of young female rats treated with aflatoxin B1 (AFB1). Animals were divided into 5 equal groups each group contains 6 rats. Group I animals of this group had been kept as normal without any treatment and considered as controls. **Group 2:** Animals of this group were orally administered vehicle 50% DMSO (dimethylsulfoxide) alone .**Group3**: Animals of this group were orally administered vehicle with Vitamin A (132 IU double the human therapeutic dose).**Group4:** Animals of this group were orally administered .0.05 µg AFB1 per kg dissolved in 50% DMSO (dimethylsulfoxide) .Group 5: Animals of this group were orally administered 0.05 µg AFB1 per kg with Vitamin A (132IU double the human therapeutic dose).The experiment lasted for 14 weeks, animals were dissected 24 hours after last doss. Ovarin sections of treated female rats showed pathological changes represented by reduction number and deformed follicles, with Absence of mature follicles. In addition, semithin ovarian sections exhibited, follicles without oocytes, residual in zona granulosa cells with reduction in theca layer. Also serum follicle stimulating hormone(FSH), luteinizing hormone(LH) levels were decreased and estradiol level was increased. Vitamin A showed a partial improvement of histopathological as regards ovary sections observed with numerous follicles in various stages of development (primary, secondary and Graafian follicle ,corpora lutea), with presence of some deformed follicles. Also serum follicle stimulating hormone(FSH), luteinizing hormone(LH)and estradiol levels were improved with supplementation of vitamin A. In conclusion, this study provides evidence that AFB1 adversely indirectly damages ovarian tissue through increasing estradiol , while vitamin A treatment effectively attenuates the toxic effect of AFB1 in the ovary
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Keywords: Aflatoxin B1- Vitamin A- Ovary - young female rats

1. Introduction

Aflatoxins are produced by fungi of the genus Aspergillus which grow on corn grain, soybeans, dry beans, cottonseed, wheat and peanuts. The most common AF are AFB1, AFB2, AFG1 AFG2, AFM1 and AFM2. Aflatoxin B1 (AFB1) is the most toxic and is usually predominant (**FAO and WHO, 1997**).). Aflatoxins are not only contaminate our food stuffs, but also are found in edible tissues, milk and eggs after consumption of contaminants for feed by farm animals (**Fink-Gremmels, 1999; Bennett and Klich**, **2003 ,Aycicek *et al.*,2005 and Giray *et al.*, 2007).** The toxico-pathological spectrum of AFB1 (in a broad spectrum of vertebrates) is very wide encompassing acute toxicological effects, carcinogenicity, teratogenicity, genotoxicity, immunotoxicity and sometimes death **(Wild and Turner, 2002).**

The fungal metabolites namely mycotoxins represent the most significant contaminants of food and feed **(Aly,1993).** Various members of myoctoxins were detected in animal sera, feed and food and produced severe dangerous changes in active organs (**Hassan *et al.*, 2004 , 2007and 2008).** AFB1 was reported to exert deleterious effects on the reproductive capacity of lab and domestic female animals **(Ibeh, *et al.*, 2000; and Abdelhamid *et al.*, 2004)**. Histopathological examinations of the ovaries in aflatoxin-treated mature domestic fowls showed follicular atresia, accompanied by cessation of egg production during the whole feeding period **(Hafez *et al.*, 1982)**. Aflatoxin is known to be a human carcinogens based on sufficient evidence of carcinogenicity in humans **(Yaling *et al.*, 2008)**.

The mycotoxins in feed consumed by animal and their serum cause disturbances in the hormonal profile related to fertility including follicle stimulating hormone (FSH), luteinizing hormone (LH), testosterone (TES.), and can cause abnormal fetal development in farm animals which affect the normal function of reproductive organs and elsewhere the productivity of animals **(Tiemann and Vanselow, 2003).**

On the other hand vitamin A is ,significantly prevents aflatoxin induced alterations in the tissue such as liver, kidney and gizzard of chicks. Considerable interactions exist between vitamin A and aflatoxin. In essence vitamin A is anti-mutagenic, both *in vivo* and *in vitro* to prevent aflatoxin induced liver damage. **Gradelet *et al.* (1998)** reported that carotenoids exert their protective effect through the deviation of AFB1 metabolism towards detoxication pathways. Carotenoids are also effective in reducing DNA damage but less effective than vitamin A. Various studies have demonstrated toxin binding compounds such as vitamins E and C **(Hoehler and Marquardt, 1996**). However, no data are currently

available on the ability of these to prevent aflatoxin toxicity in rat ovary. Young animals are more susceptible, with the sex and mode of administration of the toxin affecting the response. Therefore, this study was conducted to evaluate the ability of vitamin A to reduce toxicosis of aflatoxins in ovary of young female rat.

2. Material and Methods

Animals and treatments

The present investigation was carried out on young female albino Wistar rats weighing 80-100 g. Animals were acclimatized for two weeks and the commercial food and tap water were supplemented *ad labium* during acclimatization period. The animals were subsequently divided into five groups of 5 rats each.

Group1: Animals of this group had been kept as normal without any treatment and considered as controls.

Group2: Animals of this group were orally administered vehicle 50% DMSO (dimethylsulfoxide) alone.

Group3: Animals of this group were orally administered vehicle with Vitamin A (132IU double the human therapeutic dose)

Group4: Animals of this group were orally administered 0.05µg AFB1 per kg dissolved in 50% DMSO (dimethylsulfoxide).

Group 5: Animals of this group were orally administered 0.05µg AFB1 per kg with Vitamin A (132IU double the human therapeutic dose).

The experiment lasted for 14 weeks, .animals were dissected 24 hours post treatment then animals were sacrificed by ether overdose.

Chemicals

Aflatoxin B1 used in this study were obtained from Sigma Chemical Company (St. Louis, USA). It was dissolved in sun oil and orally given at dose 0.05 µg/ kg body weight/day.Vitamin A is avaibale in market as capsule contain 50000 1U(132IU double the human therapeutic dose), as described by **Sinha and Dharmshila (1992)**.The therapeutic dose for rat was calculated according to the table given by **Paget and Barnes(1964)**.The dose was given orally and estimated according to the weight of the rat.

Histological preparation

Immediately after sacrificed, ovaries were quickly removed and fixed in Bouin's fluid then dehydrated in an ascending series of alcohol, cleared in two changes of xylene and embedded in paraffin wax. Sections of 5 micrometers thickness were cut using rotary microtome and mounted on clean slides, for histological examination sections were stained with Ehrlich's haematoxylin and eosin.

Semithin sections Studies:

The following steps in preparing sections for semithin were carried out. Fresh small pieces of ovarian tissue up to 1 mm^3 in size were fixed in 4% glutaraldehyde-formaldehyde for 5 hr. then in (0.2 M) Na cacodylate for 2hrs. at 4°C, then washed in phosphate buffer pH. 7.2 for 30 min. and post fixed in 1% osmic acid (2% OsO_4+ 0.3 M of Na cacodylate) for 2 hrs. at 4°C, then washed in phosphate buffer (pH 7.2) for 30 min. at 4°C. Samples were dehydrated through ascending grades of ethanol and embedded in epoxy resin in an oven at 60°C for 14 hrs. to produce a firm block, then processed for preparation of semithin sections which were stained by 1% toluidine blue **(Hunter, 1984)**. Sections were prepared and examined at the Central Lab., Faculty of Science, Ain Shams University.

Biochemical analyses

For hormone determination, blood samples were withdrawn through a heart puncture and then centrifuged. Serum were stored at -20 ºC until assayed for the biochemical parameters. FSH, Determination of FSH by enzyme-linked immunosorbent assay (ELISA) kits according to **Rose (1998)**. Determination of LH by ELISA kits according to **Rebar *et al.* (1982)** and estradiol determination of total E2 by radioimmunoassay (RIA) kits according to **Xing *et al.* (1983).**

Statistical analysis

The results were expressed as mean ± SD of different groups. The differences between the mean values were evaluated by Student's t-test **(Fowler *et al.*, 1998)** .

3. Results

Histological Observations

Sections of ovary of control rat revealed that it consists of spindle shaped cells, fine collagen fibres and ground substance which together constitute the ovarian stroma. The peripheral zone of the stroma, the cortex, contains numerous follicles in various stages of development (primary, secondary and Graafian follicle). In addition, corpora lutea and atretic follicles are present (Figs.1a,b&c).Ovarian sections of rats treated daily with vehicle 50% DMSO (dimethylsulfoxide) for 14weeks showed normal structure of germinal epithelium as well as healthy follicles and stromal cells(Fig.1d),Sections in ovaries of rats daily treated with vehicle and Vitamin A (132IU double the human therapeutic dose)for 14 weeks revealed the stroma containing numerous developing follicles (Figs.1e&f). On the other hand section in ovary of rats orally receiving 0.05 µg AFB1 / kg dissolved in 50% DMSO (dimethylsulfoxide), showed decrease in the number of developed follicles where only primary follicles appeared with necrotic cells of their columnar border (Fig.2a). Many

deleterious histological changes were induced on the tertiary follicle such as, destruction and separation of basement membrane of oocyte and the theca follicle from the zona granulosa appeared with vacuoles (Fig.2b).In addition a large number of developing follicles were severely affected including some abnormal Graafian follicles that appeared elongated with accentric nucleus, and without corona rediata and cumulus oophorus and abnormal feature zona pellucida and zona granulose (Fig.2c) . Other follicles appeared containing wide zona pellucida in one and vacuolation another, and dilated congested blood vessel (Fig.2d).Also noted that the histological sections was not show any mature follicles. Ovarian sections of rats treated daily with AFB1 with Vitamin for 14 weeks exhibited marked improvement in the histological state compared with those of animals treated with AFB1 alone . Normal like stages of oogenesis, primary, secondary, tertiary and Graafian follicles were observed .Yet some deformed follicle appeared where the ovarian medulla contained large number of vacuoles and hemorrhagic lesions.(Figs.3a&b).On the other hand some sections showed dilated and congested blood vessels ,and many deformed follicles including elongated Graafian follicles and antrum follicular. Also oocytes appeared without corona rediata and cumulus oophorus . Zona granulose cell layers and their theca became thiner (Fig.3c). Secondary follicles appeared with degenerated zona granulosa and theca, where zona pellucida was not appearent (Fig.3d).

Semithin sections:

Histopathological examination of the semithin ovarian sections in G1,G2 and G3 showed normal histological pattern including numerous follicles in various stages of development (Fig.4a) . Secondary and tertiary follicles, appeared with normal oocytes and zona granulosa and antrum follicular (Figs.4b &c). Sections in ovaries of rats daily treated with AFB1 for 14weeks G4 revealed many histological changes such as reduced number of developing follicles and hemorrhages (Figs.4d&f). Many follicles appeared without oocytes (Figs.5b,c,d&e). Antrum follicular and zona granulosa were only seen in tertiary follicles (Fig.5c).Some follicles appeared containing residual cells of zona granulosa and thecal layer became thiner (Fig.5c). Examination of ovary sections of rats treated with AFB1 followed by Vit.A showed marked improvement in development follicles including those that contain oocytes, antrum follicular compared with animals treated with AFB1(Fig.5f) .

Biochemical Results

Changes in FSH, LH and estradiol.

Animals treated with AFB1 (G4) revealed very highly significant(P<0.001) decrease in serum follicle stimulating hormone (FSH) and serum luteinizing hormone (LH) levels reached 5.80±0.43 and (16.24±0.44) compared with control group (8.98±0.45) and(25.56±0.64) as shown in table(1). Animals treated with AFB1 and vitamin A (G5) the results indicate partial improvement in serum follicle stimulating hormone (FSH) and serum luteinizing hormone (LH) levels significant decrease (P<0.05) reach (7.44±1.73d and 22.28±2.37), while animals in (G2) and(G3) showed non-significant changes in(FSH) and (LH) levels when compared with control group (Table1). The data shown in table(1) indicate that AFB1 administration(G4) induced very highly significant (P<0.001) increase in estrdiol level reached(16.0 ±0.83) compared with control (2.56±1.03) while animals treated with vitamin (G5) showed significant decrease (4.61±1.36) when compared with AFB1 (G4) treated rats showed highly significant increase in estradiol level in serum of animals(G5) compared with control. Treating animals (G2 and G3) showed non- significant difference in levels of FSH, LH and esradiol when compared with control group.

Table(1): Showing the effect of administration of aflatoxin B1 and vitamin A on serum follicle stimulating hormone(FSH) and serum luteinizing hormone (LH) concentration and estradiol level in female rats for 14 weak.

Groups	(FSH) m1u/ m1 (M±SD)	(LH) m1u/ m1 (M±SD)	(E2) ng/ m1 (M±SD)
Control	8.98±0.45	25.56±0.64	2.56±1.03
Control +Vehicle	8.50±0.67	26.42±2.59	2.68±1.04
Control+ Vit. A	8.84±1.51	26.08±2.21	3.04±0.83
Aflatoxin	5.80±0.43***	16.24±0.44***	16.0 ±0.83***
Aflatoxin + Vit A	7.44±1.73*	22.28±2.37*	4.61±1.36**

P < 0.05 *Significant P < 0.01** Highly significant

P <0.001*** Very highly significant

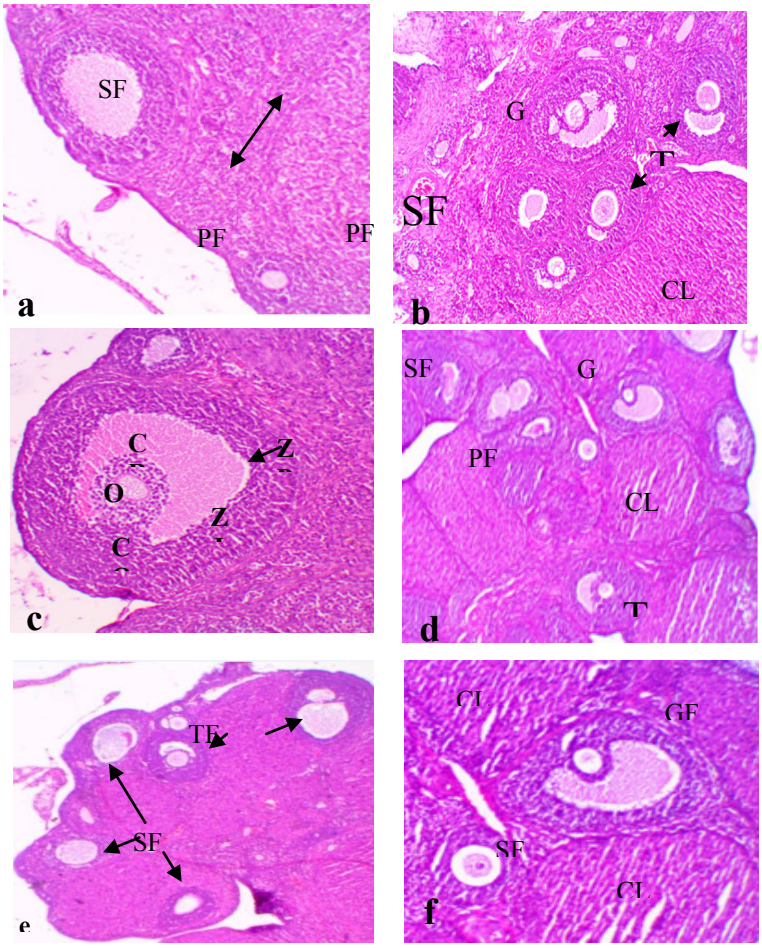

Fig. 1: Photomicrograph of ovarin sections of rat (a, b &c) control showing primary follicle (PF), secondary follicle (SF), tertiary follicle (TF), Graaffian follicle (GF) corpus luteum (CL) ,(d): control with vehicle and (e & f) control with vitamin A showing all types of follicles. (H&E a, b &,dX100 - eX40 - cX400).

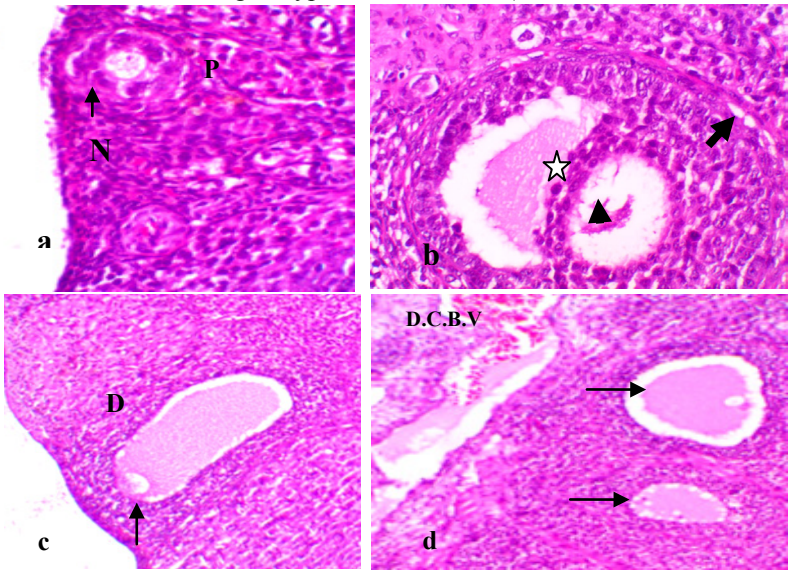

Fig.(2):Photomicrographs of ovarian sections of rat treated with aflatoxin showing large number of severely affected, developing follicles,(a)showing only primary follicle with nicrosis in columnar cells(N),(b) showing tertiary follicle with loss of nuclear membrane of oocyte(arrow head), vacuoles in theca extrena(arrow) and normal follicular antrum( ), (c)showing deformed follicle(DF),accentric ,nuclus (arrow) ,(d)showing dilated,congested blood vessel,and deformed follicle(arrow) Note that the histological sections do not show any mature follicle (H&E aX40 - bX400 –and c, dX100).

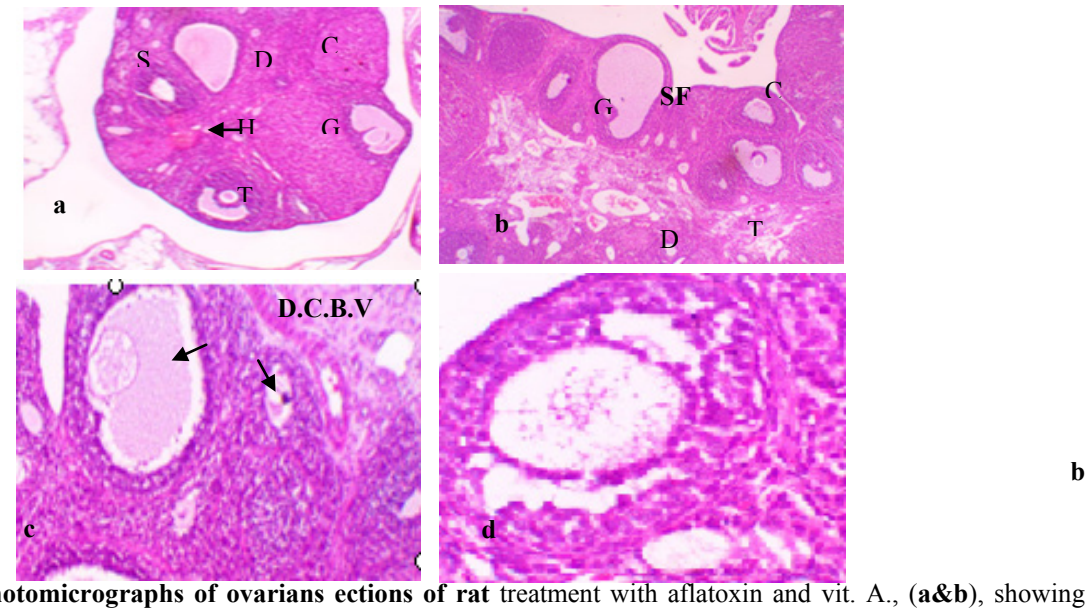

Fig. 3 : Photomicrographs of ovarians ections of rat treatment with aflatoxin and vit. A., (**a&b**), showing improvement of developing follicles secondary follicle (SF),tertiary follicle(TF), Graaffian follicle(GF),with deformed follicle (DF) hemorrhage(H) and vacuolation(arrow).(c),showing deformed of secondary follicle and, graaffian follicle(DF), and dilated congested blood vessel(D.C.B.V.).(d)showing severely affected secondary follicle(arrow) . (H&E aX40 - bX40 – c X100,and dX400).

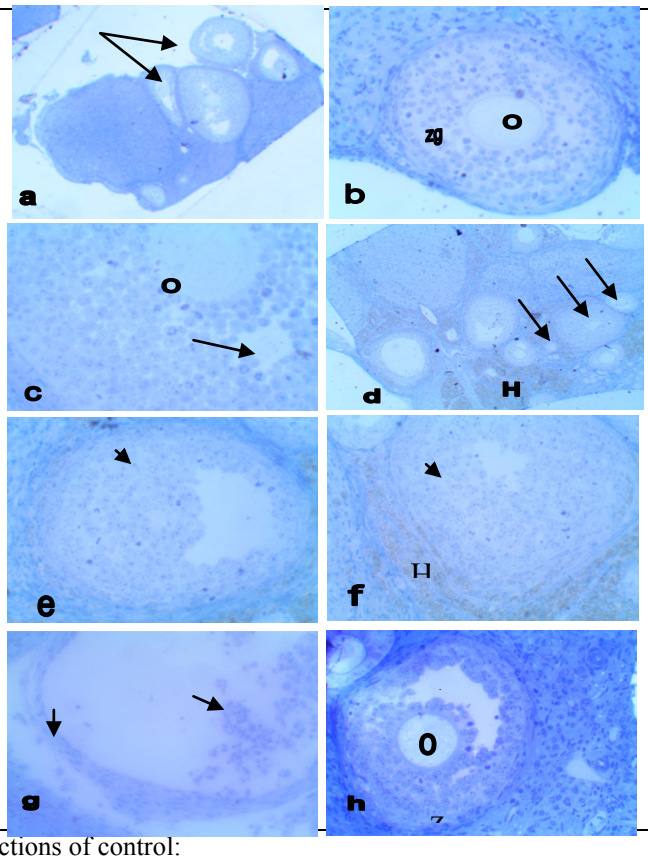

Fig.(4) Semithin ovarian sections of control:

(a) showing large number of developing follicles (arrows), (b&c) magnified of (a) showing normal oocytes (o) zona granulose (zg)and antrum follicular (arrow);

(d,e,f&g) ovarian sections of treated with aflatoxin:

(d) showing malformations in large number of developing follicles (arrows), hemorrhage (H) in (e&f)showing absence of oocytes (short arrow) hemorrhage (H)

(g) showing residual cells of zona granulosa and thin theca layer (arrows);

(h) ovarian sections of treated with afl. +Vit. A showing normal follicle, oocytes (0), zona granulose (zg) and antrum follicular (Af). Toluidine blue (a&d) x 40, b, c, e,f,g &h x 400

4. Discussion:

Aflatoxins (AFs) are highly toxic secondary metabolites produced by the species of Aspergillus, especially *A. flavus* and *A. parasiticus*, in most livestock as well as humans by their continuing intermittent occurrence in both feeds and foods **(Abdelhamid *et al.*, 1990; Robens and Richard, 1992; Abdelhamid, 2008).** Several factors may enhance the occurrence of mycotoxin in the human diet in developing countries. These include eating habits, existing marketing problems which encourage long storage periods; the pre- and post-harvest practices that encourage accumulation of moisture and thus mold growth, ignorance, and poverty. This is aggravated by the fact that there are no strict regulations that impose limits on the concentration of mycotoxins in crops that are marketed in these countries as well as lack of relevant technology required in monitoring fungi and mycotoxins in **grains (Wilkister and Nyaora, 2008)**. In the present study, many histopathological changes were seen in the ovary of albino rats after treatment with aflatoxin including decrease in number, and deformities in of developing follicles present with degenerated zona granulosa cells and absence of mature follicles, hemorrhage and dilated congested blood vessels. Semithin sections displayed the deformed follicles such as absence of oocytes, residual cells in zona granulosa , and reduction in theca layers. This may be attributed to the direct effects of AFB1 on reproductive cycle. Data showed disturbances in estrus cycle, significant reductions in the number of oocytes and large follicles, as well as inhibition and reduction in conception rates **(Ibeh and Saxena, 1997)**. Decrease of zona granulosa may caused immature follicles. Some studies explained the granulosa cells of the follicles to exhibited a great number of mitochondria, that might afford a greater developmental potential if the oocytes of such follicles were allowed to mature *in vitro***(Mobarak, 2009)**.Accordingly failure of *in vitro*- matured oocytes may be a partly attributed to a reduced number of mitochondria,resulting in insufficient production of adenosine triphosphate required for developmental events **(Pizzo and Pozzan, 2007)** .These results are similar to that obtained by some investigators, **Kourousekos and Lymberopoulos (2007)** reported deleterious effects of aflatoxin on the reproduction system, i.e., sexual maturation, growth and maturation of the follicles, levels of hormones, gestation, and growth of fetus. **Abdelhamid (2005)** found that aflatoxin lowered the fertility to 13% and increased the mortality of embryo. Mycotoxins (including aflatoxins) adversely affect the reproductive systems of various animal species **(Abdelhamid, 2008)**. The ovaries showed follicular atresia which has a detrimental effect on egg production **(Hafez *et al.*, 1982; Del Bianchi *et al.*,2005**; **Pandey and Chauhan, 2007).** These findings may be referred in part due to the adverse effects of aflatoxin. **Abd El–Wahhab (1996**), noted from microscopic examination of ovaries of female rabbits treated with 0.15 mgAFB1/kg BW that there were some pathological alterations in the form of (1) coagulative necrosis which appeared mainly in the growing and mature follicles and (2) decrease in number and size of Graffian and growing follicles with increased number of atretic follicles and small areas of degenerative changes. Moreover, chronic exposure to aflatoxin decreased reproductive efficiency of ruminants **(Diekman and Green, 1992).** Fertility of pregnant rats decreased after aflatoxin and embryonic resorptions,malformations, and developmental retardations occurred **(Cilievici, *et al.*, 1980).**

Effect of aflatoxin-contaminated diet on performance of laying hens showed a significant decrease in egg production and egg weights **(Rizzi *et al.*,2003; Zaghini *et al.*,2005; Pandey and Chauhan, 2007)** On another way, the results were disagree with **Oliveira *et al.* (2000) and Oliveira *et al.* (2003)** who found that in laying hens fed on AFB1, production and egg weight were not significantly affected .

So present data indicate that the carry over of aflatoxin B1 residues is relatively most probable to occur in laying hens when the birds are continuously exposed for long periods to low level of aflatoxin in the diet. This fact may be related to the lower capacity of laying hen in detoxifying aflatoxin B 1**(Hassan, 1995; Del Bianchi *et al.*,2005)**. Aflatoxins incorporated into the feed of laying hens may cause relevant lesions in liver and in kidneys, heart and ovaries. Results also, indicated that prolonged administration of aflatoxins, may cause economic losses to egg producers, besides aflatoxins in egg even in small amounts may cause public health problems due to its cumulative effects for egg consumers as concluded by **Chowdhury and Smith,(2004) and Ogido, *et al.* (2004).**

In the present study, Aflatoxins significantly decreased the levels of both LH and FSH. Aflatoxin treatment significantly increased serum estradiol level compared with control group. This decrease and increase may be due to enhanced synthesis or impaired metabolism. Similar to the obtained results of decrease of both LH and FSH, , it was reported that mycotoxins produced a variety of adverse health effects reduced progesterone synthesis by inhibition of the follicle stimulating hormone secretion (FSH) **(Tiemann and Vanselow, 2003)**. The main effect of these toxins is the inhibition of protein synthesis throughout binding with DNA and RNA perhaps as a result of interference with nitrogen metabolism produced immunosuppresion and reduced antibody formation **(Zaghloul and Shehata, 1991; Hassan *et al.*,1997 and 2004)**. Comparison between groups administrated

AflatoxinB1 resulted in detection of a significant difference in levels of LH and FSH of female rats compared to controls. The aflatoxin has a hypophysotoxic effect, especially on adenohypophysis **(Clarke *et al.*, 1987)**. Thus, decreases in levels of the LH could be related to the effect of the toxin on hypophysis. Some organophosphates inhibit G-protein activities and could lead to inactivation of LH receptors **(Zou *et al.*,2006)**; hence, it may reduce progesterone level.

Serum FSH levels tend to be elevated when the testes are damaged and circulating inhibin-B is reduced **(Jensen *et al.*,2004).** It is obvious that this degenerative effect of the toxin on germinal epithelium of the seminiferous tubules would breakout into sertoli cells, bringing about a decrease in inhibin B1 level and, consequently, due to reduction of the inhibitory effect of the inhibin B1 on the production and secretion of FSH, the level of this hormone increases. According to the results of a study in female rats, increase in levels of estradiol and inhibin B causes a decrease in the level of the FSH in the follicular phase **(Erickson and Shimasaki, 2001; Padhy *et al.*,2009)**. Aflatoxin contamination can reduce the birds' ability to withstand stress by inhibiting the immune system. This malfunction can reduce egg size and possibly lower egg production. In addition, one must pay special attention to the use of contaminated corn in layer rations because eggs are promptly used as human food and aflatoxin metabolites have been found in egg yolks **(Bray and Ryan, 2006).**The presence of aflatoxins in egg is a potential threat to the health of the consumer. Growing children are more sensitive than adults, as egg is one of their main sources of nutrients that indicated the above mentioned histopathlogical changes in ovary of young female rats.

Vitamin A (consisting of retinol and its active metabolites) is vital for vision; controlling the differentiation program of epithelial cells in the digestive tract and respiratory system, skin, bone, nervous system, and immune system; and for hematopoiesis **(Gursu *et al.*,2002)**.

The administration of vitamin A in the present investigation showed partial improvement of induced histopathological lesions as regards ovary sections observed with numerous follicles in various stages of development (primary, secondary and Graffian follicle, corpora lutes) , with presence of some deformed follicles. Also follicle stimulating hormone (FSH), luteinizing hormone (LH) and estradiol levels were improved with supplementation of vitamin A.

In poultry, immune responses and disease susceptibility have been linked to vitamin A deficiencies. In fact, there is a current interest in the relationship between vitamin A status or availability and overall health of poultry **(Aye *et al.*,2000 a, b; Dalloul *et al.*,2002).**

Some studies have shown that a vitamin A deficiency in the diets of coccidiosis-challenged broilers resulted in compromised immune defenses as reflected in lymphocyte profiles, oocyst shedding, and interferon- levels **(Dalloul *et al.*, 2002). Alpsoy *et al.* (2009)** showed that AFB1 significantly decreased the level of GSH and the activities of superoxide dismutase and GPx and increased level of malondialdehyde. Simultaneous supplementation with vitamin A, C, and E restored these parameters to that of normal range. **Webster *et al.* (1996)** reported that vitamin A thus may control carcinogenesis by manipulating molecular events at the initiation stage. As a result, more studies are needed to understand the mechanism of vitamin A antioxidant activity in mycotoxicosis.

5. Conclusion

Present results reported the significant influence of mycotoxins on some endocrine function of reproductive organs which were reflected on the low productivity .The present study concluded that both physiological and histopathological the main source of these changes is attributed to the environmental pollution of food and feeds by fungi and their toxins. Therefore, every hygienic care must be undertaken during all steps of feed and food production and other factors related to the environment of animal to prevent such pollution. Hence the productivity of animal and human health become under control. These results demonstrate that vitamin A plays a complex role in the process of chemical aflatoxicosis and when added at double therapeutic dose in the diet can provide protection against the harmful effects of AFB1 for experimental period.

Corresponding author
Ismail, N. H.
Zoology Dept., Faculty of Girls for Art Sciences and Education, Ain Shams University
nadia-hussein@hotmail.com

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Combined Effect of Systemic Bisphosphonates, Calcium and Vitamin D on Alveolar bone in Osteoporotic Postmenopausal Females having Chronic Periodontitis Following Surgical Periodontal Therapy

Basma Mostafa[1], Ebtehal Hamdy[2] and Nermine Nassif[1]

[1]Department of Surgery and Oral Medicine, National Research Centre, Cairo, Egypt
[2]Department of Oral Medicine and Periodontology, Cairo University, Cairo, Egypt
boshta@hotmail.com

Abstract: This study was conducted to evaluate the systemic use of Alendronate (ALN), an aminobisphosphonate in combination with calcium and vitamin D supplementations on the alveolar bone in osteoporotic postmenopausal females following surgical periodontal treatment of chronic periodontitis. **Subjects and Methods**: Forty postmenopausal osteoporotic females having chronic periodontitis were divided into two groups. Group (1) which is the control group (n=20) received systemic ALN for 6 months. To group (2) which is the study group (n=20) systemic ALN was given in combination with calcium and vitamin D for 6 months. Initial therapy including scaling, root planning and oral hygiene instructions followed by surgical periodontal therapy using the modified Widman flap procedures was performed. The pocket depth (PD) and clinical attachment level (CAL) in addition to radiographic linear, density and angular defect measurements were done at baseline before starting the initial therapy and at 6 months postoperatively. The results revealed a significant gain in clinical attachment level and reduction of pocket depth in both groups with presence of a significant improvement of all the radiographic measurements at the end of the study period. The study group always showed higher percentages of improvement than the control group in all the measurements. It can be concluded that systemic ALN in combination with calcium and vitamin D administration to postmenopausal osteoporotic females is a valuable treatment modality in adjunct to surgical therapy in the management of chronic periodontitis.
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Keywords: Alendronate, calcium, vitamin D, osteoporosis, periodontitis, alveolar bone

1 Introduction

Osteopenia and Osteoporosis are systemic skeletal disorders characterized by compromised bone strength and mass, with a consequent increase in bone fragility and susceptibility to fracture. Osteopenia is associated with reduction in the amount of bone in addition to the presence of microarchitectural changes without the occurrence of clinical fracture. Osteoporosis on the other hand is associated with high incidence of clinical fracture. It is characterized by reduction in bone mineral density to below the minimum level required to ensure sufficient mechanical support. There is also deterioration of bone tissues due to imbalance between bone resorption and formation, favoring resorption (Koduganti *et al.,* 2009 and Passos *et al.,* 2010).

According to the general diagnostic criteria proposed by the World Health Organization (WHO) and the modification of the International Osteoporosis Foundation, osteoporosis is considered to be present when the bone mineral density (BMD) is 2.5 Standard Deviation (SD) below the average peak bone density achieved in young adults, matched by gender and race. While osteopenia is defined as bone mineral density between one and 2.5 SD below normal BMD level (Wende, 2001 and Shum *et al.,* 2010).

Osteoporosis occurs mainly in postmenopausal females; although younger women and men can be also affected. It was estimated that one in four women during menopause and one in three women > 65 years of age are affected by osteoporosis (Passos *et al.,* 2010).

Postmenopausal osteoporosis in females is characterized by progressive loss of bone tissue that begins after natural or surgical menopause leading to fracture within 15–20 years from the cessation of the ovarian function. The loss of the ovarian function seen in postmenopausal women is accompanied by significant overall changes in skeletal homeostasis (Robet and Louis, 2000 and Kanis *et al.,* 2008).

With regard to periodontal disease, it has been shown that its worldwide prevalence is

10% to 15%, although it may reach 80% in certain regions. Periodontal disease is considered to be the greatest cause of tooth loss and edentulism among adults. The impact of periodontal disease on affected individuals is increasingly apparent and significant with the disease progression, from gingival recession at a relatively early stage with dentin hypersensitivity, toward tooth mobility and pathologic migration leading eventually to tooth loss, thereby affecting chewing and speech functions, esthetics, psychological aspects, and the overall quality of life (Shum *et al.,* 2010).

The evaluation of the relationship between osteoporosis and periodontitis is complicated by the fact that both diseases are multifactorial in the etiology. Multiple systemic factors influence the progression of osteoporosis, including age, race, diet, gender, hormone therapy, smoking, genetic factors, exercise, and body weight. Several of these factors are also risk factors for periodontal disease (Al Habashneh *et al.,* 2010).

It was proven that the periodontal destruction could be significantly influenced by the systemic loss of bone accompanying osteoporosis (Shum *et al.,* 2010). It was also reported that postmenopausal women with osteoporosis and periodontitis are extremely susceptible yielding an excessive response to dental plaque and calculus, as shown by greater bleeding on probing, dentoalveolar bone loss, and decreased alveolar bone mineral density (Passos *et al.,* 2010).

Although periodontal diseases are initiated mainly by bacteria that colonize the tooth surface and gingival sulcus, the host response is believed to play an essential role in the breakdown of connective tissue and bone which are key features of the disease process (Graves *et al.,* 2008).

Periodontal therapy generally consists of mechanical and surgical procedures including the treatment with antibiotics and non steroidal anti-inflammatory drugs (NSAIDS) in some forms of the disease (Rocha *et al.,* 2004). There are data from studies in animals and human trials indicating that pharmacologic agents that modulate the host response believed to be involved in the pathogenesis of periodontal destruction may be effective in slowing the disease progression (Pradeep and Sharma, 2012).

Among these agents, bisphosphonates which are chemical analogs of pyrophosphate; product of human metabolism were found capable of modulating bone mineralization by inhibition of osteoclastic bone resorption. They also have shown osteostimulative properties *in vivo* and *in vitro*, as evidenced by the increase in matrix formation. It was documented that the systemic use of these drugs in postmenopausal women having osteoporosis brings about significant improvement in bone density, resulting in reduced incidence of hip, vertebral, and forearm fractures (Rocha *et al.,* 2004). These agents have been tested in several animal studies and found also effective in treating periodontitis (Jeffcoat *et al.,* 2005).

Alendronate (ALN), an aminobisphosphonate is one of the second generation of bisphosphonates, which was found to be a potent inhibitor of bone resorption. Various studies proved that the systemic use of ALN in humans and some animal models decreased bone loss and increased bone density (Pradeep and Sharma, 2012).

Some anthropological records revealed that humans are exposed to considerably less ultraviolet radiation (required for the synthesis of vitamin D) and consume considerably less calcium than did the early ancestors (Hildebolt, 2005). If calcium intakes are not at or above threshold values, skeletal calcium is resorbed to maintain the body's calcium homeostasis. Dietry calcium intake is important for acieving peak bone mass and maintaining bone density. Dietry intake of vitamin D is essential for calcium absorption. Chronically low intake of calcium and vitamin D can lead to a negative calcium balance, thus causing a secondary increase in calcium removal from bone, including the alveolar bone. Such bone loss may contribute to weakening of the tooth-attachment apparatus (Nishida *et al.,* 2000 and Hildebolt, 2005).

The Food and Nutrition Board (FNB) of the Institute of Medicine has published the well-known recommended dietary allowances (RDA). The adequate intake (AI) that sustains normal health of 1,200 mg/day of calcium and 10.0 g (400 IU)/day of vitamin D for ages 50 to 70 was set (Hildebolt, 2005).

There is evidence that there are possibilities for the management of periodontitis by controlling the systemic risk factors. These observations hypothesize that the combined use of bisphosphonates to regulate skeletal and alveolar bone density with adequate levels of calcium and vitamin D to manage alveolar bone loss associated with periodontal infection is recommended (Nishida *et al.,* 2000, Hildebolt, 2005 and Miley *et al.,* 2009).

The aim of this study is to evaluate the effect of systemic bisphosphonates alone and in

combination with calcium and vitamin D on the alveolar bone in osteoporotic postmenopausal females having chronic periodontitis following surgical periodontal therapy.

2 Subjects and Methods

Study Design

The research was conducted on a selected group of postmenopausal women (≥ 50 years of age) who attended the outpatient clinic in the medical unit of the National Research Center (NRC), Cairo, Egypt for bone densitometry testing in the routine yearly check up. Only women who experienced natural menopause (no menstruation for at least one year) were chosen and invited to participate in this study. They received systemic bone mineral density (BMD) assessment as an initial screening using dual-energy x-ray absorptiometry (DXA) of the hip (Norland XR46 version 3.9.6).

Systemic BMD was classified according to the WHO criteria, where osteoporosis was defined as BMD ≥ 2.5 SDs below the optimal mean BMD of young healthy individuals of the same race and gender. Only postmenopausal females with BMD T-score less than -2.5 SD, where T-score is the expression of BMD values in terms of standard deviations from the normal value of a female young adult mean were included in this study. Women with a history of a systemic condition or medication intake that might influence the BMD or periodontal disease severity were excluded (i.e., women with a history of diabetes mellitus, thyroid diseases, chronic renal problems, and connective tissue diseases). Postmenopausal females on corticosteroids, chemotherapy, recent peptic or esophageal disorders were also excluded. Exclusion criteria also included postmenopausal females treated with drugs that inhibit gastric acid secretion for more than 2 weeks in the last 6 months; chronic treatment with NSAIDs, hormone replacement therapy or any other drug known to alter bone calcium metabolism were not included. Smoking females were not allowed to participate in the study. All patients were systemically reviewed. The participants were chosen to be of the same socio-economic level.

The postmenopausal osteoporotic females who accepted the participation in the study were then referred to the dental clinic at the NRC medical services unit and to the dental clinic at the Faculty of Oral and Dental Medicine, Department of Oral Medicine and Periodontology, Cairo University after making prior appointments for evaluation of their oral condition. All participants received further information about the study protocol and objectives at the dental clinics.

Participants diagnosed as having chronic periodontitis according to the criteria of the American Academy of Periodontology (2000) were chosen. Each patient presented with probing depth (PD) ≥ 5 mm in at least three teeth or periodontal attachment level (PAL) ≥ 4 to 6 mm and vertical bone loss ≥ 3 mm with no history of periodontal therapy or use of antibiotics in the preceding 6 months was selected to be part of the study.

The study included forty non-smoking females, 50-65 years old, who were at least one year postmenopausal, osteoporotic and have not undergone hysterectomy or ovariectomy. All patients were also diagnosed as having chronic periodontitis. Subjects were age-matched into 2 groups of 20 patients each;

Group (1) which is considered the control group included 20 osteoporotic postmenopausal females diagnosed as having chronic periodontitis. The patients in this group received systemic ALN 70 mg/week for 6 months for the treatment of osteoporosis as described in accordance with their physician. In conjunction conventional scaling and root planning followed by surgical periodontal therapy using the modified Widman flap (MWF) procedures as described by Ramfjord and Nissle, 1974 was done.

Group (2) which is considered the study group included 20 osteoporotic postmenopausal females also diagnosed as having chronic periodontitis. This group served as the study group which received ALN 70 mg/week in addition to 1000 mgms of calcium and 400 IU of vitamin D for 6 months. This group was also treated with conventional scaling and root planning followed by surgical periodontal therapy using the same modified Widman flap (MWF) procedures as in group (1).

For both groups detailed oral hygiene instructions were given and full mouth scaling and root planning using ultrasonic scalers and periodontal curettes under local anesthesia was completed before the surgical intervention. Scaling and root planning was performed for each patient in two sessions, one session for each half and completed over one week. Occlusal adjustment was done whenever indicated. Four weeks after the initial therapy the sites that presented pocket depth ≥ 5 mm were allocated to surgical treatment using MWF. Following the surgery sutures were removed after 7 days and

the teeth were polished. Post surgical care included patient's instruction to use 0.12% chlorhexidine gluconate twice daily for assisting plaque control for 7 days. Neither brushing nor manipulation of the surgical site is attempted for 10 days. Patients were seen every 3 weeks for 6 months at which time the teeth were polished and the oral hygiene instructions were reviewed. The participating women were informed about the nature, objectives, and possible risks of the study, and they signed informed consent statement that authorized their inclusion in the study.

Clinical Study

Women in both groups received ALN. They were given blister packs of pills containing 70 mg ALN. They were instructed to take one tablet of ALN in the morning, at least 30 minutes before breakfast or at least 2 hours after breakfast once weekly. Patients in group (2) were instructed to take 70 mg/week ALN in addition to 1000 mgms calcium and 400 IU vitamin D/ daily. Patients were followed up every 2 weeks for 6 months. Medication compliance was assessed at each visit by counting the tablets remaining in the blister packs. During each visit, bacterial plaque (BP) was assessed using plaque detection tablets and flossing technique was reviewed. During the study period, all patients within the same group received identical periodontal assessment and treatment.

Periodontal Assessment

Periodontal assessment was carried out at baseline before starting the initial therapy and the surgical procedures and at the end of the study (6 months postoperatively). At the baseline evaluation, all clinical parameters were measured and mechanical treatment including removal of all supra and subgingival calcified deposits to obtain a smooth, hard surface was done. Scaling and root planning was carried out by one of the investigators in two successive sessions. Patients were taught and encouraged to maintain their dental health and plaque control through brushing and flossing. All assessment measurements were taken by the same investigator. The condition of all teeth was assessed and recorded. The mean was taken for the following measurements: whole mouth probing depth and clinical attachment level.

Pocket depth (PD) was measured according to the standard procedure described by Glavind and Loe, 1967 using a periodontal probe with Williams' calibrations at the free gingival margin and recorded at six locations (mesiobuccal, distobuccal, midbuccal, mesiolingual, distolingual and midlingual) on each tooth parallel to the long axis of the examined tooth. The total of the mean probing depth at the six locations on each tooth for each patient was calculated in millimeters. The distance from the cemento-enamel junction (CEJ) to the free gingival margin and the distance from the free gingival margin to the bottom of the pocket/sulcus (PD) were measured at the mesio-buccal and mid-buccal surfaces using also a calibrated probe. From these two measurements, individual subject mean attachment level (the distance from the CEJ to the bottom of the pocket or sulcus) was calculated in millimeters. All the measurements were taken at baseline and 6 months.

Radiographic parameters:

Bone mineral density (BMD) was measured for each patient by Dual-energy X-ray absorptiometry (DXA) of the hip using Norland XR46 version 3.9.6 for diagnosis of osteoporosis. Standardized intraoral periapical radiographs using the paralleling technique were taken at baseline and 6 months post-operatively using Trophy x-ray machine with exposure parameters of 60 KVP, 10 mA and 0.14 sec (Trophy radiology, 94300 Vincennes, type 6510, made in France). The paralleling technique was used with Rinn XCP film holder (KKD Germany), which consists of interchangeable acrylic bite blocks, a plastic aiming ring and a metallic indicator arm.

For each patient an occlusal stent was constructed to confirm reproducibility and standardization of the technique. The alveolar bone changes were measured from the radiographs using the measurement system of the Digora software (Orion Corporation, Sordex Medical System, Finland).

1- Linear Measurements calculated in millimeters (mm):

a) A line was drawn from C.E.J to the alveolar crest in each defect parallel to the long axis of the studied tooth at the most radiographically accentuated point (A).

b) Another line was drawn from the alveolar crest to the apex to the long axis of each studied tooth (B).

The same investigator performed the measurements twice and the mean of both trials was calculated as an attempt to eliminate intra-observer errors.

2- Densitometric Measurements (gram / cubic centimeter):

For performing standardized densitometric analysis, 3 successive straight lines were drawn each 1 mm apart and parallel to each other and to the root surface reaching the most radiographically accentuated points.

a) The first line was drawn from C.E.J to the alveolar crest in each defect parallel to the long axis of the studied tooth (A).

b) The second line was drawn from the alveolar crest to the apex to the long axis of each studied tooth (B).

c) The third line was drawn from C.E.J to the apex parallel to the long axis of each studied tooth (C). The mean value of each line was measured and their means were calculated.

The same investigator performed all the measurements for each line twice and the mean of the trials was calculated as an attempt to eliminate intra-observer errors.

3- Radiographic defect angle measurements (degree):

The radiographic defect angle was calculated and defined by two lines that represented the root surface and the bone-defect surface. The defect angle is the angle between the two lines one drawn from CEJ to the bone defect and the other one is drawn from the bone defect to the alveolar bone crest at the most radiographically accentuated point (Fernanda *et al.*, 2011).

Statistical Analysis

Descriptive statistics including the mean and standard deviation were calculated. Also the *P* value was measured. The Wilcoxon matched pairs test was used to compare pre and post treatment values. The Mann-Whitney test was used to compare between the two groups. The significant level was set at $P \leq 0.05$ (Dawson and Trapp 2001). Statistical analysis was performed with SPSS 16.0 (Statistical Package for Scientific Studies) for Windows (SPSS, Inc., Chicago, IL, USA).

3. Results

A) Clinical assessment:

1- Probing depth (PD) measurements:

Comparison of the mean PD values of the control and study groups at baseline and six months postoperatively revealed that there was no statistically significant difference between the two groups before treatment. After surgical treatment with MWF, the study group showed more statistically significant reduction of the PD as compared to the control group as presented in table (1).

Table (1) shows the mean ± SD, percentages of change and *P* values of PD measurements of both control and study groups at baseline and six months postoperatively. Significance was set at $P \leq 0.05$.

Group	Control group	Study Group	
Treatment	Mean ± SD	Mean ± SD	*P*-value
Baseline	6.66 ± 1.4	6.69 ± 1.3	0.388
Six months	5.12 ± 1.4	4.48 ± 1.1	0.354
Mean Difference	1.54	2.21	0.001
% of PD reduction	23.12%	33.03%	

2- Clinical attachment level (CAL) measurements:

Comparison of the mean CAL values of the control and study groups at baseline and six months postoperatively revealed that there was no statistically significant difference between the two groups before treatment. After surgical treatment with MWF, the study group showed more statistically significant gain of the clinical attachment after six months as compared to the control group as shown in table (2).

Table (2) shows the mean ± SD, percentages of change and P values of CAL measurements of both control and study groups at baseline and six months postoperatively. Significance was set at $P \leq 0.05$.

Group	Control group	Study Group	
Treatment	Mean ± SD	Mean ± SD	*P*-value
Baseline	5.66 ± 1.2	5.66 ± 0.9	0.154
Six months	4.55 ± 1	4.35 ± 0.8	0.717
Mean Difference	1.11	1.31	0.001
% of CAL gain	19.61 %	23.14%	

B) Radiographic assessment:

1) Linear measurements of the control group:

Table (3) demonstrates the linear measurements (A) and (B) pre and post treatment for the control group. There was a significant difference in the Wilcoxon matched pairs test between pre and post treatment values with a *P*-value of (0.0001) for both measurements.

Table (3) is showing the mean ± SD, percentages of improvement and *P* values of linear measurements (A) and (B) pre and post treatment for the control group.
SD: standard deviation, *P*: probability, I: improvement, S: significance or significant, NS: non-significant.

Control group	Linear measurement (A)		Linear Measurement (B)	
	Baseline	Six months	Baseline	Six months
Mean±SD	6.49 ±1.41	5.6 ±1.42	12.37 ±2.28	13.08±2.33
P-value	0.0001		0.0001	
S	S		S	
% of improvement	13.55%		5.73%	

2) Linear measurements of the study group:

Table (4) shows the linear measurement (A) and (B) pre and post treatment for the study group. There was a significant difference in the Wilcoxon matched pairs test between pre and post treatment values with a *P*-value of (0.0001) for both measurements.

Table (4) Showing Mean ± SD, percentages of improvement and P values of linear measurements (A) and (B) pre and post treatment of the study group.

Study group	Linear measurement (A)		Linear measurement (B)	
	Baseline	Six months	Baseline	Six months
Mean ±SD	6.32 ±1.32	4.48 ±1.11	12.92 ±1.99	15.63 ± 1.83
P-value	0.0001		0.0001	
S	S		S	
% of I	23.41 %		20.97 %	

3) Comparison between control and study groups in the linear measurements:

Table (5) revealed the Mann-Whitney test results for the linear measurements (A) and (B) pre and post treatment between control and study groups. There was no significant difference in pre treatment values as the *p*-value was (0.54), while there was a significant difference in the post treatment values where *p*-value was (0.02) in measurement (A). While for measurement (B) there was also no significant difference in pre treatment values as the *p*-value was (0.39), while there was a significant difference in the post treatment values where *p*-value was (0.0001).

Table (5) Showing Mann-Whitney test results between control and study groups for linear measurements (A) and (B) pre and post treatment.

Mann Whitney test	Linear measurement (A)		Linear measurement (B)	
	Baseline	Six months	Baseline	Six months
Mann Whitney U value	281.0	195.5	269.0	113.0
P-value	0.54	0.02	0.39	0.0001
S	NS	S	NS	S

4) Density measurements of the control group:

Table (6) demonstrates the density measurements (A), (B) and (C) pre and post treatment of the control group. There was a significant difference in the Wilcoxon matched pairs test between pre and post treatment values as *P*-value was (0.001) for the (A) measurements. Also for the (B) measurements as *P*-value was (0.002). In addition there was a significant difference in the
Wilcoxon matched pairs test between pre and post treatment values as *P*-value was (0.0001) for the (C) measurements.

Table (6) Showing Mean ± SD, percentages of improvement and *P* values of density measurements (A), (B) and (C) pre and post treatment for the control group.

Control group	Density (A)		Density (B)		Density (C)	
	Baseline	Six months	Baseline	Six months	Baseline	Six months
Mean ±SD	40.5 ±18.05	44.98 ±16.43	128.31 ±13.48	131.28 ±14.37	110.4 ±24.28	115.2 ±21.39
P	0.001		0.002		0.0001	
S	S		S		S	
% of I	11.03%		2.31%		4.34%	

5- Density Measurements for the study group:

Table (7) demonstrates the density measurements (A), (B) and (C) pre and post treatment for the study group. There was a significant difference in the Wilcoxon matched pairs test between pre and post treatment values as *P*-value was (0.0001) for all the measurements (A), (B) and (C).

Table (7) Shows Mean ± SD, percentages of improvement and *P* values of density measurements (A), (B) and (C) pre and post treatment for the study group.

Study group	Density (A)		Density (B)		Density (C)	
	Baseline	Six months	Baseline	Six months	Baseline	Six months
Mean ±SD	45.09 ±32.66	65.31 ±36.49	132.0 ±15.01	143.28 ±14.82	111.92 ±15.35	129.88 ±16.55
P-value	0.0001		0.0001		0.0001	
S	S		S		S	
% of I	44.84%		8.53%		16.03%	

6- Comparison between the density measurements in the control and study groups:

Table (8) reveals the Mann-Whitney test results for the density measurements (A), (B) and (C) pre and post treatment between control and study groups. There was no significant difference in pre treatment values while there was a significant

difference in the post treatment values of (A), (B) and (C) measurements.

Table (8) Mann-Whitney tests between control and study groups for density measurements (A), (B) and (C) pre and post treatment.

Mann Whitney test	Density (A)		Density (B)		Density (C)	
	Baseline	Six months	Baseline	Six months	Baseline	Six months
Mann Whitney U value	300.0	202.0	282.0	206.0	284.0	206.5
P-value	0.8	0.03	0.55	0.03	0.55	0.04
S	NS	S	NS	S	NS	S

7- Angle defect measurements

Table (9) demonstrates the defect angles pre and post treatment for control and study groups.

There was a significant difference in the Wilcoxon matched pairs test between pre and post treatment values as *P*-value was (0.02) in the control group and *P*-value was (0.00001) in the study group.

Table (9) shows the Mean ± SD, percentages of improvement and P values of defect angles pre and post treatment for the control and study groups.

Defect angle measurements	Control Group		Study group	
	Baseline	Six months	Baseline	Six months
Mean ±SD	57.0 ±14.12	59.96 ±13.15	55.11 ±13.64	67.72 ±10.87
P-value	0.02		0.0001	
S	S		S	
Percentage of improvement	5.19%		22.88%	

8- Comparison between the defect angle measurements in the Control and Study groups:

Table (10) reveals the Mann-Whitney test results for the defect angles pre and post treatment between control and study groups. There was no significant difference in pre treatment values where the *p*-value was (0.77), while there was a significant difference in the post treatment values where *p*-value was (0.04).

Table (10): shows the Mann-Whitney test of defect angles between control and study groups pre and post treatment.

Mann-Whitney test	Angles	
	Baseline	Six months
Mann-Whitney U value	298.0	207.0
P-value	0.77	0.04
S	NS	S

Table (11) shows percentages of improvement of all the measurements in both groups revealing that in all the clinical and radiographic measurements the study group showed higher percentages of improvement than the control group.

Group	Control group	Study Group
Measurements		
PD	23.12	33.03
CAL	19.61	23.14
Linear (A)	13.55	23.41
Linear (B)	5.73	20.97
Density (A)	11.03	44.84
Density (B)	2.31	8.53
Density (C)	4.34	16.03
Defect angle	5.19	22.8

4. Discussion

Osteoorosis is often called the "silent disease" because bone loss occurs without symptoms. People are not aware that they have osteoporosis until their bones become so weak that a sudden strain, bump or fall causes a fracture or a vertebra to collapse (Ghozlani *et al.,* 2009). Osteoporosis is one of the risk factors that have been implicated in the progression of periodontitis (Graves & Cochran, 2003). A number of studies showed that there is a relationship between oral and systemic bone loss as well as an association of osteoporosis with periodontal diseases (Brennan *et al.,* 2007, Nackaerts *et al.,* 2008 and Shum *et al.,* 2010).

Understanding the association between periodontal disease and osteoporosis and the mechanisms underlying this association may aid health professionals in the prevention, early detection, and treatment of these common diseases (Shum *et al.,* 2010). Bisphosphonates are widely utilized pharmacological agents in the management of systemic metabolic bone diseases (including osteoporosis) due to their ability to inhibit bone resorption. During bone resorption they can be taken up by the osteoclast, resulting in osteoclast de-activation and apoptosis. Various studies have demonstrated that bisphosphonates not only induce the osteoblasts to secrete inhibitors of osteoclast-mediated resorption but also stimulate the formation of osteoblasts precursors and mineralized nodules, thereby promoting early osteoblastogenesis (Rocha *et al.,* 2004). There is also reduction of activity and prevention of the development of osteoclasts from hematopoietic precursors. Bone resorption is suppressed followed by a secondary mineralization resulting in increased bone mass, improving bone strength

and reduction in fractures (Menezes *et al.,* 2005). Bisphosphonates are often considered the first-line therapy for the treatment of post-menopausal osteoporosis. They are the most widely prescribed anti-resorptive agents.

Randomized clinical trials of ALN demonstrated increase in bone mineral density in post-menopausal women with osteopenia or osteoporosis. In women with osteoporosis a reduction in the incidence of hip, vertebral and non-vertebral fractures of nearly 50% were noted (Koduganti *et al.,* 2009). In addition ALN was found capable of preventing periodontal ligament destruction in several experimental periodontitis studies. It was also documented that the systemic administration as well as the local delivery of ALN reduced alveolar bone loss without interfering with bone formation in procedures involving mucoperiosteal flap surgery (Reddy *et al.,* 2005 and Pradeep & Sharma, 2012).

ALN was proven to preserve alveolar bone through its anti-inflammatory and antibacterial activities in experimental periodontitis. ALN is capable of inhibiting the neutrophil influx which has been linked to tissue destruction in a number of inflammatory diseases such as rheumatoid arthritis and periodontitis. ALN is also capable of reducing the mononuclear cell infiltration in gingival tissue. Circulating monocytes may differentiate locally into osteoclasts, thereby exerting bone resorbing activity; this may contribute to the bone sparing effect of ALN. Regarding the antibacterial activity ALN can inhibit the growth of the bacteria characteristic of periodontal disease. It is possible that the antibacterial activity of ALN might result, at least partially, from the prevention of bone destruction and reduction of the periodontal pocket which was noted in the present study as explained by Menezes *et al.,* 2005.

Additionally Rocha *et al.,* 2001 have used systemic ALN for 6 months in the treatment of periodontitis patients with type II diabetes and reported significant improvement in the healing response compared to the placebo group. Another study found significant decrease in clinical parameters such as plaque index; gingival index and PD with significantly gain in CAL. The percentage of bone fill in the ALN group was 40.4 ± 11.71% compared to 2.5 ± 1.02% in the placebo group after 6 months therapy as reported by Rocha *et al.,* 2004. This was in line with the present study in which there was a significant reduction of pocket depth and a significant gain in clinical attachment level in both control and study group. Also the radiographically measured parameters should a significant improvement at the end of the 6 months treatment period.

The improvement noted in the present study can be contributed to the combined mechanical treatment, and the oral hygiene instructions our patients received in addition to the surgical periodontal therapy and the administration of ALN. In this study postmenopausal women were selected because they are at increased risk for bone loss including alveolar bone and are more likely affected by osteoporosis as a result of estrogen deficiency (Haas *et al.,* 2009 and Pradeep & Sharma, 2012).

Females at the postmenopausal period experience diverse physical and emotional symptoms. Change in dietary habits, and oral changes are frequently found among these women. There is also higher prevalence of periodontal disease and osteoporotic jaws (Rocha *et al.,* 2004).

It appears that as people age, they take less dietary calcium and vitamin D possibly because of their decrease of total food intake. They are also less exposed to the sun due to the limitations of their movements. So it is reasonable that they take supplements to fulfill their requirements to prevent bone resorption or future disease as stated by Nishida *et al.,* 2000.

It was proven that elderly women who took calcium and vitamin D supplementation were less likely to be periodontitis cases. Various data suggests that calcium and vitamin D intake by adult periodontal maintenance patients is associated with better periodontal health. It was documented that lower dietary intake of calcium increased the risk of periodontal disease in a dose-response relationship (Al Habashneh *et al.,* 2010).

The National Osteoporosis Foundation as well as the National Academy of Sciences thus recommended a daily intake of 1200 mgms of calcium and 400-600 IU of vitamin D (Koduganti *et al.*,2009). This is why the postmenopausal females in our study group were given calcium and vitamin D supplementations.

To our knowledge the combined use of ALN, calcium and vitamin D and its effect on alveolar bone especially following surgical management of intrabony lesions in chronic periodontitis patients was not investigated. So we hypothesized that this combination will yield better results than using ALN alone. This hypothesis was proven to be correct because all the linear, density and angular defect measurements in addition to the clinical

parameters records (PD and CAL) of our study revealed that the results of the study group were always showing a higher percentage of improvement than the control group. This was in agreement with studies documenting that vitamin D and calcium oral supplementation were effective in improving the periodontal condition and were used as a useful adjunctive treatment. This adjunct treatment was found capable of decreasing the damage (clinical attachment and alveolar crest height loss) caused by periodontal disease .The investigators also suggested that vitamin D may reduce the susceptibility to gingival inflammation through its anti-inflammatory effect. It was also demonstrated in a 3-year study that increased intake levels of calcium and vitamin D had a beneficial effect on tooth retention (Miley *et al.,* 2009) which is in conformity with the present study. This is why clinicians always rely on parameters such as PD reduction, CAL gain and radiographs to evaluate a treatment modality as performed in the current study (Froum *et al.,* 2001).

Many studies where osseous surgery was performed, pocket reduction was greater than with scaling or curettage (Becker *et al.,* 2001). The main advantage of the modified Widman flap surgery done in the current study over any other periodontal surgical procedure is the intimate postoperative adaptation of healthy collagenous tissues to all tooth surfaces. It has been shown experimentally in animals and humans that with a close adaptation of gingival tissues to the tooth surface, a marginal new epithelial attachment forms which tends to seal off the deeper areas of separation between the tooth and the surrounding tissues. Thus the healing connective tissues may adapt as closely to the tooth surfaces as to an implant and reattachment with formation of new cementum may develop gradually from the apical aspects of the lesion. In such cases minimal or no inflammation is present indicating that the pathologic periodontal pocket has been eliminated as a source of irritation (Ramfjord and Nissle, 1974 and Becker *et al.,* 2001).

DXA performed at the start of the study in the diagnosis of osteoporosis is capable of detecting small changes in bone mineral content at multiple anatomic sites with excellent precision (0.5% to 2%) and accuracy (3% to 5%). Assessment of alveolar bone following surgical treatment of intrabony defects was evaluated by a digital imaging system using standardized periapical radiographs. Much diagnostic information is available from digital radiographs which allows for detection of mineral changes as little as 5% (Woolhiser *et al.,* 2005). They are valuable in the detection of early to moderate bone changes, approximating the amount of bone loss and its location, and helping in the prognosis of the affected teeth serving as baseline data and as means of evaluation of post treatment results (Tugnait and Hirschmann, 2003).

Conclusion

The combined use of ALN, calcium and vitamin D showed better improvement in treatment outcomes in both the clinically and radiographically measured parameters proving that this combination is a valuable treatment modality in management of chronic periodontitis in adjunct to the surgical periodontal therapy.

Corresponding author

Basma Mostafa, BDS, MDS, DDS.
Department of Surgery and Oral Medicine,
National Research Centre, Cairo, Egypt
boshta@hotmail.com

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