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CONTENTS

Environmental fluoride exposure and reproductive hormones in male living in endemic fluorosis villages in China

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Abstract: Objective To explore the influence of high fluoride exposure on reproductive hormones in male living in endemic fluorosis villages in China. Methods A cross sectional study was conducted in Tongxu county in Henan Province, China. Endemic fluorosis villages and control villages were selected by random sampling according to fluoride concentration in drinking water and the prevalence of endemic fluorosis. Local male residents aged from 18 to 50 years old who were born and grew up in the investigated villages were recruited as subjects by cluster sampling. Fasting blood and urine samples were collected. The serum level of GnRH was detected using ELISA. The serum level of luteinizing hormone (LH), follicle-stimulating hormone (FSH), estradiol (E_2), and testosterone (T) were determined by chemiluminesence immunoassay (CLIA). Results The serum levels of FSH were 7.82 mlU/ml, 10.20 mIU/ml and 9.57 mIU/ml in male from defluoridation villages (DFPG), high fluoride villages (HFG) and control villages (CG) respectively. FSH level in male from DFPG was significant lower than that from HFG and CG (P<0.05). The serum levels of E_2 were 33.67 ng/mL, 29.17 ng/mL and 28.99 ng/mL in DPFG, HFG and CG respectively. E_2 level in male from DFPG was significant higher than that from HFG and CG (P<0.05). Serum levels of E_2 in CG were associated with LH (r=0.343, P=0.000), age (r=0.195, P=0.015), and inversely associated with serum FSH (r=-0.237, P=0.003), whereas this correlation was not observed for serum E_2 level in DFPG and HFG. Conclusion Long-term fluoride exposure in drinking water may influence the reproductive hormones in males living in endemic fluorosis villages.
[Zhou T, Ding Z, Xi Y, Yang RP, Duan LJ, Li SH, Cheng XM, Hou JX, Wen SB, Liu J, Chen J, Cui LX, Ba Y. **Environmental fluoride exposure and reproductive hormones in male living in endemic fluorosis villages in China.** *Life Sci J* 2012;9(4):1-7] (ISSN:1097-8135). http://www.lifesciencesite.com. 1

Key words: fluoride, reproductive hormone, hypothalamus-pituitary-testis, male

1.Introduction

Fluorine is a necessary element to human body and plays a key role in the prevention and control of dental caries. However, fluoride has a tendency to accumulate in the organisms if the exposure persists over time, even at low concentration (Kebsch et al., 2007). Dental and skeletal lesions are the major clinical feature of endemic fluorosis, which is an important public health concern worldwide and caused by chronic persistent fluoride exposure through ingestion or inhalation and most commonly occurs as a result of high fluoride levels in drinking water or industrial exposure from fumes or dust. It is currently estimated that approximately 43 million children have dental fluorosis and over 2 million adults have skeletal fluorosis in China (Yang and Heng, 2009). Excessive fluoride intake can also affect hormone secreting and soft tissues such as liver, kidney, brain, reproductive, pancreas and so on (Tiainen et al., 2011; Susheela et al., 2005; Ma et al., 2008; Lu et al., 2010). Many researches have been conducted to explore the relationship between fluoride ingestion and reproductive structure or function since 1980. The reported reproductive toxic effects include increases in numbers of abnormal spermatozoa, loss of spermatogenesis in rats, decreased sperm quality and quantity in rats and mice, and decreased reproductive output in mice. Our previous studies also showed that exposed to fluoride in drinking water suppressed rats reproductive ability by affecting sperm quality and quantity, sperm structure and reducing activity of the enzymes (Jiang et al., 2005; Xu et al., 2005).

The neuroendocrine system of the hypothalamus-pituitary-gonad (HPG) axis regulates reproduction in vertebrates and can be influenced by chemicals, therefore affects the reproductive system. Ma et al. observed in the experiment in male rats that fluoride could affect hormone levels of each layer of the hypothalamus-hypophysis-testis axis, and then may disturb the reproductive endocrine function (Ma et al., 2008). However, most of the studies used

animal models, primarily rodents, and often gave high daily doses for short durations to evaluate structural or functional alterations in the male reproductive tract associated with fluoride (Rico et al., 1991; Raymakers et al., 1990). Fewer studies involved in human populations. We conducted, here, a cross sectional study to explore the relationship between sex hormone levels and fluoride exposure in male residents who lived in the high fluoride areas.

2.Materials and methods

2.1.Location and population

A corss sectional study was conducted in six villages of Tongxu county in Henan Province, China in 2011 by using simple cluster sampling method. It included three endemic fluorosis villages and three non-endemic fluorosis villages. Endemic fluorosis villages were defined as villages with fluoride levels exceeding 1.0 mg/L (Chinese water quality standard) in drinking water. Two of the three villages were conducted defluoridation project of drinking water at the end of 2008. Non-endemic fluorosis villages were defined as villages with fluoride levels of less than 1.0 mg/L in drinking water. There were no significant differences in the natural environment, socioeconomic status, life styles, dietary habit, and other demographic characteristics among the six villages. A total of 354 male residents who were aged between 18 and 50 years and who were born and grew in the six villages were considered eligible for the study. Among them, 55 male residents were excluded from the study because of working in the cities as migrant workers over five years. A total of 299 participants met the inclusion criteria in this study with participation rate of 84.5%. They were divided into three groups, HFG, DFPG and CG. Upon receiving their written consent, an in-person interview was conducted at the village clinics using a standardized and structured questionnaire to collect information on demographic factors, medical conditions, marriage status, and medication use including supplemental vitamins, reproductive history, smoking and alcohol consumption, the main source of heating and cooking fuel and dietary intakes. Each subject provided two 5 mL fasting blood samples and 50 mL of instant urine sample. Blood was collected in red top vacuum tubes, and placed immediately on ice. After centrifugation, serum and white blood cells were separated and frozen at -70℃ for subsequent analyses. All procedures were approved by the Institutional Review Board at Zhengzhou University, China.

2.2.Detection of fluoride exposure levels

The concentration of environmental fluoride exposure including air, drinking water, soil, vegetables, grain including wheat and corn, as well as fluoride levels in the urine samples were detected by fluoride ion selective electrode (Shanghai Exactitude Instrument Company, China).

2.3.Detection of serum hormone levels

All the serum samples were stored in a -70℃ freezer and had no thaws prior to assay for hormone levels. GnRH was determined by ELISA. LH, FSH, E_2 and T were detected using CLIA. The CLIA test kit and Chemiluminescence Apparatus were provided by Autobio Company (LUmo Luminometer, Autobio Labtec Instruments Co. Ltd., Zhengzhou, China). Each sample was run in duplicate and 10% of total samples were retested randomly. The intra- and inter-assay coefficients of variation were less than 10% for these assays.

2.4.Data analysis

The database was established using Epidata 3.0 software (Epidata 3.0 for windows, Epidata Association Odense, Denmark) and all the data was doubled entered into the database by different people. Kolmogorov-Smirnov test and Levene test were used to inspect the normality and homogeneity of variance of all data. Fluoride level in urine and hormone levels of LH, FSH and T in serum was logarithmically-transformed to achieve approximately normal distribution, and the transformed values were used in data analyses. Differences of fluoride in urine and hormone levels of LH, FSH and T in serum among different groups were examined by analysis of variance (ANOVA). Non-parametric test (Kruskal Wallis) was used to estimate the differences of serum GnRH and E_2 levels among different groups. Separate multivariate linear regression models were used to estimate the mean change in E_2 and T levels in different groups in relation to an increase of one standard deviation in serum levels of GnRH, FSH, LH and various personal characteristics including age, urine fluoride level and so on. . All statistical tests were two-sided with 0.05 as a cutoff for significance. Statistical analyses were performed using the SPSS software, version 12.0 (SPSS In., Chicago, USA).

3.Results

3.1.Distributions of select variables in different groups

As shown in Table 1, there were 299 local male residents were enrolled in the study (41 in HFG, 79 in DFPG and 179 in CG respectively). The mean age was 43.73 years in HFG, 40.94 years in DFPG and 41.03 years in CG. There was no statistical significance among the three groups (P>0.05). We compared the body weight, height, drink alcohol rate, smoking rate, the mean marriage age and the rate of

no pregnant history within one year without taking any contraceptive measures after marriage and no statistical significances were observed among the three groups (P>0.05 respectively). But prevalence of dental fluorosis in HFG and DFPG were higher compared with CG (P<0.05 respectively).

Table 1. Distributions of select variables in different groups

	HFG (n=41)	DFPG (n=79)	CG (n=179)
Age(years)*	43.73.00±9.64	40.94±8.83	41.03±8.74
P value	0.357	0.951	
Body weight*	69.18±10.25	71.41±10.47	70.70±11.32
P value	0.522	0.683	
Height*	167.64±5.85	168.30±6.54	168.39±5.38
P value	0.654	0.047	
Drink alcohol(n)#	44.4%	58.2%	48.6%
Yes	20	46	87
No	25	33	92
P value	0.739	0.177	
Smoking&	73.3%	68.4%	66.5%
Yes	33	54	119
No	12	25	60
P value	0.476	0.886	
Dental fluorosis^	77.8%	65.8%	20.7%
Yes	35	52	37
No	10	27	148
P value	**0.000**	**0.000**	
Marriage age*	22.37±2.56	23.14±3.08	23.43±3.60
P value	0.911	0.471	
No pregnant history within one year after marriage$	35.0%	29.8%	19.4%
Yes	26	52	137
No	14	20	33
P value	0.056	0.174	

* Values are means±SD

Drink alcohol means drinking at least once a week on the average

& Smoking means at least once a day and continuous smoking a month or more

^ Dental fluorosis were examined using Dean's method

$ The rate that one's wife was not pregnant when living together without taking any contraceptive measures

The two *P* values in each line represent HFG and DPFG compared with CG respectively

3.2.Determination result of environmental fluoride

The results of fluoride concentration in drinking water was shown in Table 2, which were 2.44 mg/L, 0.36 mg/L and 0.37 mg/L in HFG, DFPG and CG respectively, it was higher in HFG than that in DFPG and CG (P<0.05). No significant differences were observed between DFPG and CG (P>0.05). We also assayed environment fluoride include air, soil, vegetables and grain. No statistical significances were observed among the three groups (P>0.05 respectively).

Table 2. Water fluoride concentration (mg/L) of different areas

Group	n	$\bar{x}\pm S$	t	*P*
HFG	13	2.44±1.88		
DFPG	8	0.36±0.30	4.75	0.000**
CG	16	0.37±0.15	4.96	0.000*

*：HFG Vs CG；**：HFG Vs DFPG

3.3.Results of reproductive hormones and urine fluoride

Fluoride level in urine in different groups was: HFG> DFPG> CG, and there was significant difference in every two groups (P<0.05 respectively) (Tables 3-2). We compared levels of serum GnRH, FSH, LH, E_2, and T in all the participants from the six villages (Tables 3-1 and Tables 3-2). Compared with HFG and CG, the serum level of FSH was significantly lower in DFPG although all of them were within the ranges considered normal (P<0.05 respectively) (Tables 3-2). However, no statistical significance was observed between HFG and CG (P>0.05). Serum level of E_2 in DFPG was slightly higher compared with HFG and CG (P<0.05 respectively). No significant differences were observed among HFG, DFPG and CG for serum GnRH, LH, or T (P>0.05 respectively) (Tables 3-1 and Tables 3-2).

Tables 3-1. Reproductive hormone levels in individuals (Median (P_{25},P_{75})) in DFPG, HFG and CG

Group	n	GnRH (ng/mL)	E_2 (ng/ml)
CG	179	23.51(20.24, 27.21)	28.99(20.24, 33.92)
DFPG	79	21.45(24.07, 26.62)	33.67(29.25, 42.98)
HFG	41	23.58(21.72, 26.85)	29.17(20.01, 44.53)
x^2		1.039	9.611
P		0.595	**0.008**

Tables 3-2. Urine fluoride and reproductive hormone levels in individuals ($\bar{x}\pm S$) from DFPG, HFG and CG

Group	n	Urine F (mg/L)	LH (mIU/ml)	FSH (mIU/ml)	T (ng/ml)
CG	179	1.00±0.48	6.51±4.95	9.57±6.27	4.29±1.30
DFPG	79	1.37±0.67	6.48±5.14	7.82±7.29	4.41±1.36
HFG	41	2.64±1.40	6.37±4.87	10.20±9.68	4.45±1.21
t		104.318	0.048	4.857	0.396
P		**0.000**	0.954	**0.008**	0.674

3.4.Association of E_2, T with serum GnRH, FSH, LH and personal characteristics

Serum levels of E_2 in CG were associated with LH (r=0.343, P=0.000), age (r=0.195, P=0.015), and inversely associated with serum FSH (r=-0.237, P=0.003) (Table 4), whereas no significant association was observed for serum E_2 level in DFPG and HFG (Table 5 and Table 6). However, serum T in HFG was inversely associated with serum FSH level (r=-0.503, P=0.047) (Table 6).

Table 4. Separate multivariate linear regression models of E_2 and T with HPG hormones, Age and Urine F in CG

		β	t	*P*
E_2				
	GnRH	-0.063	-0.902	0.368
	LH	0.343	4.655	**0.000**
	FSH	-0.237	-2.976	**0.003**
	T	0.012	0.165	0.869
	Age	0.195	2.459	**0.015**
	Urine F	-0.101	-1.429	0.155
T				
	GnRH	0.056	0.754	0.452
	LH	-0.049	-0.592	0.554
	FSH	-0.123	-1.430	0.155
	E_2	0.013	0.165	0.869
	Age	-0.153	-1.805	0.073
	Urine F	0.007	0.091	0.928

Table 5. Separate multivariate linear regression models of E_2 and T with HPG hormones, Age and Urine F in DFPG

		β	t	*P*
E_2				
	GnRH	0.065	0.522	0.603
	LH	-0.085	-0.531	0.597
	FSH	-0.068	-0.432	0.667
	T	0.093	0.713	0.478
	Age	0.101	0.793	0.430
	Urine F	-0.017	-0.144	0.886
T				
	GnRH	-0.154	-1.368	0.176
	LH	-0.280	-1.933	0.057
	FSH	0.000	0.001	0.999
	E_2	0.079	0.713	0.478
	Age	-0.227	-1.976	0.052
	Urine F	0.072	0.656	0.514

Table 6. Separate multivariate linear regression models of E_2 and T with HPG hormones, age and urine F in HFG

		β	t	*P*
E_2				
	GnRH	-0.011	-0.065	0.948
	LH	0.007	0.027	0.978
	FSH	0.161	0.607	0.548
	T	-0.095	-0.541	0.592
	Age	0.229	1.326	0.194
	Urine F	-0.098	-0.595	0.556
T				
	GnRH	-0.144	-0.926	0.361
	LH	0.253	1.019	0.315
	FSH	-0.503	-2.062	**0.047**
	E_2	-0.090	-0.541	0.592
	Age	-0.121	-0.709	0.483
	Urine F	-0.092	-0.579	0.567

4.Discussion

Endemic fluorosis is a major public health concern in China due to the excessive consumption of fluoride through drinking water, brick tea, and coal-burning. Most of the fluorosis cases in Henan province are caused by the high concentration of fluoride in drinking water. In view of the influence of tea and coal consumption against chronic fluorosis, the fluoride levels in indoor and outdoor air, vegetables and crops were determined in the six villages. We found that most of the families cooked the meal using wheat straw and cornstalk as the sources of energy both in endemic fluorosis villages and control villages because of relatively undeveloped economy. The fluoride levels in indoor and outdoor air were all lower than the national standard and also no differences were found in different villages. Fluoride level in grain, vegetables also had no significant differences in all six villages. The results above showed that consumption of fluoride in drinking water is the major exposure pathway in the investigated villages. As for the fluoride concentration in urine, it was lower in male from DFPG compared with that form HFG, but higher compared with that form CG (P<0.05 respectively). It suggested that body fluoride level still remains relatively higher even if the defluoridation project has been implemented for two years. As we know, about half of the absorbed fluoride is quickly incorporated into developing bone and teeth, where nearly all of the body's fluoride is found (Padula and Macmillan, 2005). The absorbed fluoride by the skeleton is most efficient in children and decreases with age (Maudsley et al., 2004), but this process can continue up to age 55 (Merviel et al., 2005). Considering that endemic fluorosis is a public health issue in China and that the weight of the evidence in human beings and animals associates fluoride exposure with reproductive effects, we decided to assess these effects in male residents living in endemic fluorosis villages including villages which have been implemented defluoridation project for two years. Moreover, the present study was performed in a population exposed to fluoride levels lower than those previously reported in experimental or epidemiological investigations.

The activity of the hypothalamic-pituitary-gonadal (HPG) axis is controlled by GnRH (Filicori et al., 1984). GnRH is a hypothalamic neuronal secretory decapeptide that plays a pivotal role in mammalian reproduction (Ramakrishnappa et al., 2005). The result showed that there was no

significant difference in serum GnRH level in each group, similar to the previous study result (Ma et al., 2008). Because the process of hormone regulation is very complicated, GnRH aslo widely exists in reproductive tissues which extra-hypothalamic-pituitary axis such as didymus, prostate, ovary, mucous membrane, placenta and so on (Xu et al., 2010). Thus, GnRH may be maintained the normal level in serum by some pathways of regulation.

The LH and FSH, secreted by the pituitary gonadotropes, are dimeric protein hormones and act on the gonad in a sequential and synergistic manner to initiate sexual maturation and maintain cyclic reproductive function (Crawford et al., 2009; Wu et al., 1991). Serum FSH and LH were determined in all of the subjects in this study. It is interesting that FSH serum level of individuals in DFPG was significantly lower than that in HFG and CG, while no statistical significance was found between HFG and CG. Considering the government gave priority to the villages with high fluoride level in drinking water (most exceeding 4.0 mg/L), fluoride has been accumulated in vivo for several decades and the impact of fluoride on human body may still exist although the urine fluoride concentration was slightly lower compared with HFG after implementation of defluoridation project. The higher prevalence of dental fluorosis in DFPG also suggested that the higher fluoride concentration in drinking water before implement of defluoridation oproject. Chen et al. believed that testicle injury appears firstly the change of FSH level and then implicate interstitial cell and subsequently make the change of LH level (Chen et al., 2002). But we did not find any significant difference in serum LH level among of different groups. Regulation of LH and FSH hormones is facilitated through a complex interplay of multiple mechanisms not only including a direct action of hypothalamic GnRH, but also direct and indirect actions of gonadal-derived steroids and peptides (Wu et al., 1991), the change of FSH in DFPG may be not enough to influence the LH level under that condition with fluoride concentration in drinking water around the level of this study. In contrast, Deogracias et al. observed the increase of serum FSH in high-fluoride-exposed group (HFEG) (Ortiz et al., 2003). But in his study, HFEG was defined as working in a fluoride industry that produced fluorhydric acid and aluminum fluoride besides exposed to fluoride in drinking water (3.0 mg/L). It is more easily to absorb the gaseous fluoride by respiratory system, so different exposure way and the higher exposure concentration may be responsible for the different result. Similar to pituitary hormones FSH in this study, serum levels of gonadal steroid hormones E_2 in DFPG individuals were significant difference compared with HFG and CG, while no statistical significance was found between HFG and CG. But E_2, is exactly opposite to FSH, the concentration was increased in DFPG. It suggests that E_2 can feedback negatively on the pituitary hormone, such as FSH. PAK et al. believed that gonadal steroid hormones, such as T and E_2, can feedback negatively on the hypothalamus and/or anterior pituitary to regulate reproduction in mammals (Pak et al., 2001). Melnyk et al. also observed in their animal experiment that E_2 treatments were associated with decreases in mean FSH concentration (Melnyk et al., 1992). The findings of Kemeter et al. suggested that not only the E_2 but also other steroids and/or nonsteroidal ovarian inhibiting factors could inhibit FSH and LH and both FSH and LH are negatively correlated with E_2 in female monkeys (Kemeter et al., 1984). But if the effect of fluoride on male reproductive hormones is as endocrine disruptor, it is unclear so far. Both Deogracias (Ortiz et al., 2003) and our results suggest the existence of a fluoride-induced endocrine disruption over the hypothalamic- pituitary-testis axis.

In this study, we found Serum levels of E_2 in CG were associated with LH, age, and negatively associated with FSH, whereas no significant association was observed for serum E_2 in DFPG and HFG. It suggests that the normal regulatory relationship between gonadal steroid hormone and anterior pituitary hormone may be affected when long-term excessive intake fluoride in drinking water even if the fluoride concentration in drinking water has decreased to normal (<1.0 mg/L) by defluoridation project for two years. We did not observe any correlation between urine fluoride and any of all the selected reproductive hormones after controlling for age. Deogracias et al. also did not find any correlation between urine fluoride and same hormones in the individuals exposed to fluoride in drinking water and individuals exposed to fluoride both in drinking water and in occupational environment (Ortiz et al., 2003). The results above suggest that long-term exposure to fluoride in drinking water may modify the relations of reproductive hormones of hypothalamic-pituitary-testis axis and this modification may not be directly correlated with urine fluoride level.

5.Conclusion

This study provide the preliminary evidence that long-term exposure to fluoride in drinking water may induce endocrine disruption over the hypothalamic-pituitary-testis axis by affecting regulation of reproductive hormone levels. There has some limitations in this study, such as relative fewer

samples in the HFG, thus chance finding cannot be excluded. Cluster sampling method and detailed questionnaire were used to make up and avoid the chance finding. Further limitation of this study is we did not have enough power to detect dose-response/or effect relationship between fluoride level and reproductive hormones. Further studies should be based on larger samples, and should explore in greater detail the dose-response/or effect relationship between fluoride concentration in drinking water and reproductive hormones in male living in endemic fluoride areas. We have conducted a population-based study in several endemic fluorosis villages with different fluoride concentration in drinking water under the support by national natural science foundation of China and it is still under study.

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Effluent Quality Index and the Regression Equations and Correlation Coefficients its with the Physico-Chemical Parameters in the West Karun Agro-Industry

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Abstract: In this research, Amir kabir and mirza koochak khan's agro-industry in west side of karoon river were considered and original drain of each unit was selected and at three station, beginning of the drain (first station), middle of the drain (second station) and end of the drain (third station), nine selected units parameters including (oxygen solution, temperature, Ph, BOD_5 COD, phosphate nitrate, Ec, Tarbidity) were measured. For a year, above reviews were performed in each season at three station of original drain of both units agro-ingustry. Then by using customary methods, a qualitative index of Sewage was measured at three stations of each unit's drain for every season. Finally annual qualitative index was measured for sewage of each unit. After statistic analysis of results, It is observed that: we had been faced to reduction of sewage's qualtative index along with original drains of both units of agro- industry from first station towards second station and finally third station. It means that. in all seasons in both drains, the quality of ending drain of sewage was always lower than that in beginning of drain. Also during the different seasons of year, meaningful changes were observed in sewage quality of original drainages of both units. Sewage qualitative index was reduced in both units during autumn and winter. In research, it was observed that this fact can be because of not only the sometime cultivation season of sugar can's cuttings and start of harvesting in different farms but because of operation including fertilizing and spraying , in another word with starting the season harvesting of cane's production farms of sugar cane also are starting to act , so the sewage of mentioned units directly is shed to original drain. All of these factors cause a full of sewage's qualitative index in meaningful limit during autumn and winter forwards spring and summer. In this research , correlation coefficients between sewage's qualitative index and each one of chemical physical nine units parameters were measure and based on those coefficients , simple Regresion equations between sewage's qualitative index and each factors were written , also multilateral RegResion relation between sewage's qualitative index and all factors were presented.
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Key words: Effluent quality index, physico chemical parameters, Agro-Industry

1.Introduction

In IRAN, sugar cane Agro- industries are the oldest of these units because of the old record of sugar plants cultiration such as sugar cane and beet and also because of the proper climate conditions of khuzestan province. have been already a notice center regarding this situationand along recent two decades in spite of construction and development of seven units of sugar cane agro- industry over the province, repeatedly the sugar cane's sub cultivation level has been increased in area and so that dependent industries and farms also are developing. Although this factc auses employment and economical development, but unfortunately it also causes expanded environmental pollutions in different parts of climate and soil. It is noticed that sugar cane is a plant with high water use, so sugar cane agronomy will be followed high rate of drains. Sewage resulted from farms of suger cane production and dependent industries also must be added.

Agricultural development to meet the growing demand for food, is inevitable. According to reports, the Food and Agriculture (FAO 2002) people suffering from hunger are quantitatively and qualitatively.The three air pollution, water and soil can be seen and today, have caused environmental crises.EPA studies indicate that annual industrial and agricultural effluent to surface water shed area, times the dangerous pollutants into aquatic form above, although the quality of surface waters including rivers and wetlands case, but studies done Qualitative evaluation and research so far on the status of agricultural and industrial wastewater has been done. Thus, knowledge of the status of the current effluent quality, predicting the likely effects of their discharges to water resources, and environmental management strategies in order to improve the effluent quality, conduct research of this kind is necessary.

The research on sugarcane agro waste units, including Amir Kabir and Mirza small West Karoon Khan took to achieve the following objectives:

1 The physicochemical parameters measured in the effluent West Karun Agro-Industry

2 effluent quality index in West Karun Agro-Industry.

2. Materials and Methods

The names of both culture and industry in West Amir Kabir and Mirza Kochakkhan Karoon River in Ahwaz and Khorramshahr are located 45 km road. The drainage system of open drainage is discharged to HOROLAZIM. In this experiment, three stations in the drainage were considered:

The first station (early drainage), the second station (the drain) and the third station (end drain). Then during the year as a seasonal sampling was conducted. 9 was used to measure factors. Mean values for three months each season, as these factors were considered, measured temperature, PH, dissolved oxygen, turbidity and electrical conductivity at the sampling location and by portable devices carried by the effluent samples in clean plastic containers 500 and 1000 volumes by ml of cold (4 °C) or chemical solutions to established and used to measure nitrate, phosphate, residual oxygen remaining biological and chemical oxygen was transferred to the laboratory. After examination during the four seasons, with the help of diagrams raw results of qualitative factors and Table 9, the effluent quality index was calculated at each station in different seasons.

Overall quality indicators in four seasons, the annual index will result. Finally, by performing statistical analysis (tests, ANOVA, Duncan, simple regression and multiple regression) and MINITAB software and EXCELL results were analyzed and evaluated.

3. Results

3.1. The results of physico-chemical parameters measured in Amirkabir unit was as follows

Dissolved oxygen during all seasons from the beginning to the end of the drainage decreased. The maximum amount of dissolved oxygen in the first station (early drainage) and in winter to the 6/8 mg per liter and the lowest dissolved oxygen levels in the third station (end drain) in summer to the 2/4 mg per liter were observed.

Furthermore, the amount of dissolved oxygen in winter with an average 26.8 mg maximum in summer and 73.4 mg was the minimum.

Effluent temperature changes significantly during drainage. The highest temperature in the third station (end drain) was observed. Duncan test, two groups of spring and summer had the highest average temperature in the first class and second class with the lowest in autumn and winter temperatures were observed.

PH changes during drainage from the beginning to the end of the drainage was not significant, but with the Duncan test for different seasons, the average of two classes of PH rate was observed. The first class consists of autumn, winter and spring and summer was the second class.

Table 1: Agro-industry effluent quality index Amirkabir

Season state	spring	sumer	Autumn	Winter
1	42.47	41.44	38.17	40.23
2	41.66	38.22	37.45	39.33
3	39.86	37.28	36.87	38.18
Mean	41.33	38.98	37.49	39.24

In the study of biochemical oxygen remaining in all seasons throughout the drainage, increasing trend was observed. Maximum BOD_5, the third station in autumn and minimum in summer was the first station.

In addition, Duncan test at different seasons of the mean BOD5, showed a mean of three classes. First class fall (maximum) second class winter and spring and summer were in third grade.

The rate of oxygen chemical residues in all seasons during the main drainage to the drain end, the increase was seen. Lowest COD COD highest in spring and autumn in the first station was at the third station.

Table 2: Agro-Industry Effluent quality index Mirza Kochakkhan

Season state	spring	sumer	Autumn	Winter
1	43.44	44.22	39.39	40.19
2	43.63	42.86	38.47	39.14
3	41.92	39.91	38.24	38.43
Mean	42.99	42.33	38.70	39.25

Duncan test for the year showed an average rate of COD in three groups. The first autumn, the second group in winter and spring and summer were the third group. Electrical conductivity during drainage was consistently increased in all seasons, the highest electrical conductivity at the end of drainage (Station III) was observed. Duncan test for different seasons of the electrical conductivity showed a mean of three classes. First class summer, spring and in the second

class, third class, autumn and winter, which showed the lowest electrical conductivity.

Drainage of nitrate during the growth was observed in all seasons. The highest nitrate in winter was the first station. In addition, Duncan test for nitrate levels in different seasons showed the class average. The first class in spring and summer and autumn and winter were the second class.Summer (the highest rate of phosphate), spring, autumn and winter (low phosphate levels), the turbidity in the main drain and the highest turbidity showed an increasing trend in the third station (end drain) in summer and lowest turbidity in the first station (early drainage) was observed in winter.

The Duncan test showed three classes mean for Kdrvt during the year, in first class during the summer (highest turbidity) in the second class, third class in autumn and spring and summer (with the lowest turbidity) was observed.

Table 3: Evaluation of regression equations and correlation coefficients (r^2) between quality indicators and physico-chemical parameters (Parameters= x and qualitative indicators= y)

X	PH	EC	turbidity
WQI	56.2-2.13x y= r^2 = %4.8	37.7+ 0.00106x y= r^2 = %3.7	38.4+0.188x y= r^2 = %3.2

X	DO	T	Nit
WQI	-0.764x y= 44.7 = %30.5 r^2	34.2+ 0.262x y= r^2 = %34.2	y=38+0.504x r^2 = %.13.9

X	phosphate	BOD_5	COD
WQI	38.4-34/6X y= = %16.9 r^2	41.8+ 0.678X y= r^2 = %46.5	42.6+0.273X y= r^2 = %47.7

3.2. Physico-chemical factors measured in units Mirza Kochakkhan results were as follows

Spring and summer (minimum DO) showed effluent temperature) and the second class in autumn and winter (minimum temperature of effluent) were shown.the trend was increasing.

Duncan test showed three classes of first-class autumn (maximum amount of COD), second class, third class winter and spring and summer (lowest COD) showed. Electrical conductivity during drainage Duncan test also showed an increasing trend in average three classes, first class summer (maximum electrical conductivity), second class, third class in spring and autumn and winter (minimum level of electrical conductivity) were shown.

Nitrate and phosphate showed an increasing trend during drainage, Duncan test for nitrate seasons of spring and summer showed two classes (maximum) in autumn and winter (minimum nitrate) of phosphate was observed that the mean of four classes, respectively, in summer (Most phosphate), spring, fall and late winter (low phosphate) showed.

Turbidity levels increased during the drainage of the face lift and drainage at the end of the turbidity maximum (station III) was observed. Duncan test of means showed three classes of first-class summer (highest turbidity), Class II, Class III in the spring and autumn and winter (lowest turbidity) was observed.

Results of qualitative indicators on both units and the culture industry showed that the quality index had decreased during the drainage process. During the season, there were no significant differences in changes in quality indicators, so that the highest quality of qualitative indicators in the spring and summer and lowest in autumn and winter. Between two cultures in Amir Kabir and Mirza Kochakkhan of annual effluent quality index was not significantly different.

4. Discussion

Qualitative analysis of variance in each agro during different seasons was significant at 1% level. The main reason for this change, direct or indirect effects of climatic conditions on the physicochemical factors. For example, temperature change, the change in water temperature and the change in the amount of dissolved oxygen.Seasonal fluctuations in rainfall on factors such as EC and turbidity was effective.

In addition, the beginning of autumn, the harvest started sugarcane, sugar factories, as well as the activity starts.

On the other hand, the new culture and the whole operation is performed in the autumn planting and harvesting, causing severe loss of agricultural waste is a qualitative indicator.Using correlation coefficients between physico-chemical factors 9 and the effluent quality was observed that temperature, dissolved oxygen, COD and BOD_5 having the highest correlation coefficients, were the most effective factors in the effluent quality index change.

Furthermore, simple regression equations for individual factors as independent variables and

dependent variables were presented as indicators of effluent quality:

$$WQI=62.6+0.42PH-0.003EC+0.017Turb-0.066Do-0.29T-1.96Nit+100Phos+0.15BOD_5-0.13COD$$

PH = pH
EC = electrical conductivity
Turb = turbidity
DO = dissolved oxygen
T = temperature
Nit = nitrate
Phos = phosphate
BOD_5 = residual biochemical oxygen
COD = chemical oxygen remains

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EVALUATION OF MINIMAL PROSTATE CANCER IN NEEDLE BIOPSY SPECIMENS USING AMACR (P504S), P63 AND KI67

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ABSTRACT: Background: Prostate cancer is a major health problem throughout the developed world. Immunohistochemistry plays a very important role in the diagnosis of minimal prostatic adenocarcinoma and to exclude one of its benign mimickers**,** but it should always be interpreted in the context of the H&E appearances**.** In some cases of minimal prostate cancer morphologic features do not allow a diagnosis of carcinoma. In such situation, the presence of AMACR and the absence of basal cell marker (p63) confirm the presence of prostate cancer. In addition,Ki-67 a proliferating marker have a strong relationship with Gleason's grading, which has an important relationship with the prognosis of prostate cancer. **The aim:** Assessing the usefulness of immunohistochemical analysis with Alpha-methylacyl-CoA racemase (AMACR) and p63 to confirm the diagnosis of minimal prostate cancer. Also , detection of the prognostic role of ki67 in minimal prostate cancer detection and its association with p63 expression in prostate cancer. **Methods:** 50 needle biopsy specimens, including 30 with small foci of prostatic adenocarcinoma and 20 benign prostate (≤ 1mm or <5% of needle core tissue) were stained immunohistochemically with AMACR, P63 and Ki67 antibodies. **Results:** Of 30 cases of small foci of prostatic adenocarcinoma, 27 (90%) expressed AMACR; all malignant glands were negative for basal cell staining p63 (nuclear stain). All benign glands were recognized easily by basal cell marker (p63) positivity. There was focal positive staining with AMACR in 2 benign cases showing atrophy. A statistical significant correlation was observed between ki67 expression and increased Gleason's grade (p=0.02). Cytoplasmic expression of p63 was high in high grade prostate cancer, and it was associated with higher frequency of ki67 positive cells in prostatic adenocarcinoma. **Conclusions:** Immunostaining with the p504s and p63 could improve the diagnostic performance and helped in avoid carrying out new biopsies in small foci of prostatic carcinoma detection. An important relationship with the prognosis of prostate cancer was noticed through the strong relationship of Ki-67 marker with Gleason's grading. Therefore, we propose that this marker can be applied along with other prostate cancer prognostic factors.

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Key words: Prostate cancer, AMACR(P504S),P63 gene, KI67 marker, needle biopsy

1.INTRODUCTION

Prostate cancer incidence increased in most high-income countries, the increased detection was due to more frequent digital rectal examination, PSA, incidental diagnosis due to the increasing use of transurethral resection of the prostate (TURP) and developments in diagnostic techniques such as transrectal ultrasound (TRUS) imaging and fine needle biopsies. (Cremers et al. 2010).

The diagnosis of prostatic adenocarcinoma, especially in needle biopsy samples, can occasionally be challenging, either because they only show small foci of prostatic adenocarcinoma, or because of the difficulty in distinguishing prostatic carcinoma from benign mimickers (Hameed and Humphrey, 2006). The difficulty in the diagnosis of prostatic adenocarcinoma is mostly seen with minimal (limited<1mm) carcinoma in needle tissue (Thorson and Humphrey 2000).

Many major and minor histologic features important for the diagnosis of minimal prostatic carcinoma should be assessed specifically at low- and high-power magnification. The first of the major criteria is an infiltrative growth pattern which frequently presents as the presence of small malignant glands between larger, more complex (and often paler), benign glands. This is because the invading glands usually don't elicit a desmoplastic or inflammatory response, which characterizes many other types of invasive carcinoma. The second most common pattern of infiltration was a haphazard or disorderly arrangement of glands, with random dispersion of glands in stroma, without availability of benign glands as a reference point. On occasion, the invasive glands formed a column spanning the width

of the needle core, uncommon patterns of growth are cords of cells, single cells and cribriform glands (Epstein, 1995).

These infiltrative growth patterns are a hallmark of moderately to poorly differentiated; Gleason's score 5 to 6 adenocarcinoma of the prostate and were found in 82% of the minimal carcinoma (Thorson et al., 1998).

It was proposed that well-differentiated, Gleason's score 2 to 4 adenocarcinoma should not be diagnosed in prostate needle biopsy tissue (Epstein, 2000).Since in the vast majority of cases, this represent an under grading of the carcinoma in the whole gland (Thorson et al., 1998).

In a very small minority of cases, there are well differentiated, Gleason's score 2 to 4 carcinoma that are by definition, well circumscribed closely packed, pale, small acini (Epstein, 1995).

The second of the major criteria is absence of basal cells in the atypical glands (Cleary et al., 1983). Nuclear atypia in the form of nuclear enlargement and nucleolar enlargement is the third of the major criteria (Thorson et al., 1998). The major criteria don't include a quantitative threshold
for the number of glands required to establish a diagnosis of malignant neoplasm. Most urologic pathologist believed that 3 glands constituted typical lowest numeric cutoff (Grignon, 1998).

Minor diagnostic criteria are intraluminal wispy blue mucin, pink amorphous secretions, mitotic figures, intraluminal crystalloids, adjacent high-grade PIN, amphophilic cytoplasm and nuclear hyperchromasia. These minor diagnostic attributes are not specific for carcinoma but are useful for prompting in-depth study of the glands harboring these changes, with a view toward assessment of the aforementioned major diagnostic criteria (Algaba et al., 1996).

P63 gene is expressed in the regenerative epithelial compartment of several organs, and shares extensive homology with p53 (Yang et al., 1998). Prostate requires p63 expression for its development and it is expressed like in breast, in myoepithelial cells surrounding normal acinar glands; therefore p63 is routinely used to evaluate the presence of normal basal cells thus distinguishing between benign and malignant glands (Reis-Filho et al., 2003; Hameed et al., 2005).

In adenocarcinoma, p63 tends to be under-expressed (Di Como et al., 2002) and in prostate cancer specifically, negative immunohistochemical staining of p63 is clinically useful tool for identifying benign mimickers (Signoretti et al., 2000). Other studies have identified p63 as important in signatures of advanced disease, with lower expression associated with disease progression and the development of lethal prostate cancer (Bismar et al. 2006; Mucci et al. 2008).

Alpha-methylacyl-CoA racemase (AMACR), formerly known as P504s, is a mitochondrial and peroxisomal enzyme involved in the beta-oxidation of branched fatty acids and bile acid intermediates (Ferdinandusse et al., 2000). AMACR is a marker that selectively labels adenocarcinoma of the prostate and it is proposed as a positive marker in prostatic adenocarcinoma (Molinié et al.,2006).

Several benign mimickers of prostatic adenocarcinoma, including atrophy, atypical adenomatous hyperplasia, crowded benign glands, nephrogenic adenoma and mesonephric hyperplasia can stain negatively with basal cell markers. Although the absence of staining is in most cases usually only focally seen in scattered glands, a negative basal cell marker immune-stain alone does not exclude a diagnosis of benignancy. AMACR expression can also be identified in high-grade PIN, prostatic atrophy, atypical adenomatous hyperplasia and benign prostatic glands, and accordingly a diagnosis of prostatic adenocarcinoma should not be based solely on a positive AMACR immunostain, and basal cell markers should always be run with AMACR. The use of AMACR and basal cell markers can greatly facilitate the distinction between prostatic adenocarcinoma and its benign mimickers, especially when only limited tissue is available for staining (Hameed and Humphrey, 2006).

The Ki67 is a nuclear protein and it is the most widely recognized marker of proliferating cells. The antigen detected by ki67 antibody is localized primarily in nucleoli and is present only in proliferating cells. Its content increases during the S and G2-phases .The antigen appears to be degraded after mitosis, thus it could not be detected in resting (G0) cells (Revelos et al., 2005). Many studies have shown that Ki67 is associated with increased tumor aggressiveness and metastases (Cowen et al., 2002).

We aimed in this study to confirm or rule out small focal prostatic carcinoma in limited biopsy material using AMACR and p63.Also, detection of ki67 as a prognostic role in limited carcinoma and its correlation with p63 expression in prostate cancer.

2.MATERIALS AND METHODS:

2.1.Materials:

2.1.1.Subjects:

Patients (carcinoma group)with age range from from 52 to 81 years (mean = 69.5±8.2)and control group with age range from 49 to 82 years (mean = 65.4±10.1) obtained from the department of pathology, Faculty of Medicine, Zagazig University during the period from 2010 to 2012.
were chosed for this study.

- ***Criteria of choosing cases***:

Serum PSA level before biopsy ranged 2.3 to 21 ng/ml (mean 8.02 ng/ml), and from 4 to 150 ng/ml (mean 37.02 ng/ml) for the control and carcinoma groups, respectively.

2.1.2.Samples:

A total of 50 prostate needle biopsy specimens, including 30 prostate needle biopsy specimens with small foci (≤ 1 mm or less than 5% of needle core tissue) of prostatic adenocarcinoma and 20 benign prostates

The diagnosis of prostate cancer was established from:

* Examination of multiple levels of H&E-stained sections and was confirmed by absence of basal cell staining (p63) and/or positivity for AMACR (P504S). All radical prostatectomy specimens from cases with a small focus of prostate carcinoma in needle biopsy specimens showed residual prostate cancer. No false-positive cases were found in follow-up radical prostatectomy specimens.

*Morphological evaluation:

Thirteen specimens of prostatic adenocarcinomas were intermediate grade Gleason (5-7) and 17 were high grade Gleason (8-10).

2.2.Methods:

2.2.1.Immunohistochemical Analysis

Immunohistochemical staining was carried out using streptoavidin-biotin immunoperoxidase technique (Dako-cytomation, CA). Three to five micrometer thick sections, cut from formalin fixed paraffin embedded blocks, were deparaffinized in Xylene and rehydrated in graded alcohol. The mounted sections were immersed in ready to use Dako target retrieval solution (PH 6.0), then boiled in this solution in a microwave for 20 min and then washed in phosphate buffer saline (pH 7.3).Then blocking of endogenous peroxidase activity by 6% H_2O_2 in methanol was attained. The slides were then incubated over night using a polyclonal anti-AMACR antibody (1:2000 dilution; DakoCytomation), Ki-67 antibody (clone MIB-1, 1:50 dilution, overnight incubation; DakoCytomation) and incubated with a 1:600 dilution of the 4A4 mouse monoclonal antibody (Lab Vision Corporation, Santa Cruz), which binds to all isoforms of p63. After a buffer rinse, bound antibodies were detected with the DAKO Envision System. Slides were counterstained with hematoxylin, and rinsed again. The slides were allowed to air dry and were cover slipped with permanent mounting media. Negative controls, in which the primary antibodies were replaced by PBS, were carried out for each primary antibody. Squamous cell carcinoma for p63 and human tonsillar tissue for ki-67 were used as positive controls. For AMACR, prostate carcinoma was used as positive internal control.

- *Immunohistochemical Evaluation:*

Evaluation of ki67::

Each slide was evaluated at×40 magnification in order to find areas with maximum positive nuclei. Then these areas were examined at ×400 magnification and the percentage of positive nuclei to total nuclei was calculated. In this study, at least 500 cells were counted and only the cells that were definitely positive were considered. The tumors were divided into five groups regarding the percentage of positive cells. Cases in which the percentage of stained cells was less than or equal to 2% were considered negative. Cases with Ki-67 index of less than or equal to25% were considered 1+, 26-50% as 2+, 51-75% as 3+ and 76-100% as 4+ (Minner et al., 2011).

- *Evaluation of AMACR and P63:*

All glandular tissue that was seen on the whole needle biopsy section was taken into consideration for each case. The percentage of glands (extensiveness) that stained for the immunohistochemical markers (AMACR and P63) was evaluated in a semiquantitative fashion using the following scale: negative, <10%, 10%-50%, 50%-90%, and >90% as previously described (Abrahams et al., 2002; Diaz et al., 2000). The intensity of the P63 was classified as negative, weak, moderate, and strong (Shah et al., 2002). The AMACR staining intensity was graded as negative, weak (weak non-granular cytoplasmic staining), moderate (granular staining with weak or moderate intensity), and strong (granular staining with strong intensity) (Zhou et al., 2004).

2.2.2.Statistical analysis

The results of the study were statistically analyzed using SPSS version15 statistical program. Data were expressed as mean± SD for quantitative variables, numbers and percentage. For categorical variables, student t test was used. For statistical analysis of Gleason's grading Spearman's statistical test was used. $P< 0.05$ was considered the significant limit.

3.RESULTS

3.1.Staining results with AMACR and p63

AMACR expression in malignant glands had much more extensive and intensive staining results than benign glands ($P<0.001$). Prostatic carcinoma showed a brown cytoplasmic granular staining pattern of AMACR in the malignant glands and cells (Fig 2B, 3C1, 3C2, 4C). Out of 30 cases of small foci of prostatic carcinoma 27 (90%) expressed AMACR (p504s), of 27 cases, AMACR positivity was detected in more than 90% of the malignant glands in 22, 50% to 90% of malignant glands in 3, and 10% to 50% of malignant glands in 2 (Table1). Eighty six percent of

the positive samples with AMACR had moderate to strong staining intensity (Table2). All benign glands adjacent to the malignant glands were recognized by absence or very low level of AMACR expression. There was focal positive staining with AMACR in 2 benign cases showing atrophy. Out of 30 cases of adenocarcinoma,one case showed weak focal p63 nuclear staining.It may represent out-pouching from high-grade PIN or alternatively, flat, high grade PIN (Table1).

Table (1): Extensiveness of AMACR & p63immunohistochemical staining in malignant glands.

	AMACR		P63 (nuclear stain)	
	NO.	%	NO.	%
>90%	22	73.3	0	0.0
50-90%	3	10	0	0.0
10-50	2	6.7	1	3.3
<10%	0	0.0	0	0.0
0%	3	10	29	96.6
Total	30	100	30	100

Table (2): Intensity of immunohistochemical staining in malignant glands.

	AMACR		P63 (nuclear stain)	
	NO.	%	NO.	%
Negative	3	10	29	96.6
Weak	1	3.3	1	3.3
Moderate	11	36.7	0	0.0
Strong	15	50	0	0.0
Total	30	100	30	100

Benign glands adjacent to cancer were identified in 23 cases. Among these 23 cases, none showed positive staining for AMACR in benign glands. Virtually all cells in the basal layer of the epithelium in normal glands stained strongly for p63. The staining was confined exclusively to the nuclei of basal epithelial cells. No staining was observed in the secretary epithelial cells or in the stroma (Table3, 4) (Fig 1B, 1C).

Table (3):Immunohistochemical staining extensiveness of benign glands in carcinoma plus control groups.

	AMACR		P63 (nuclear stain)	
	NO.	%	NO.	%
>90%	0	0.0	39	90.7
50-90%	0	0.0	3	7
10-50	2	4.6	1	2.3
<10%	0	0.0	0	0.0
0%	41	95.4	0	0.0
Total	43	100	43	100

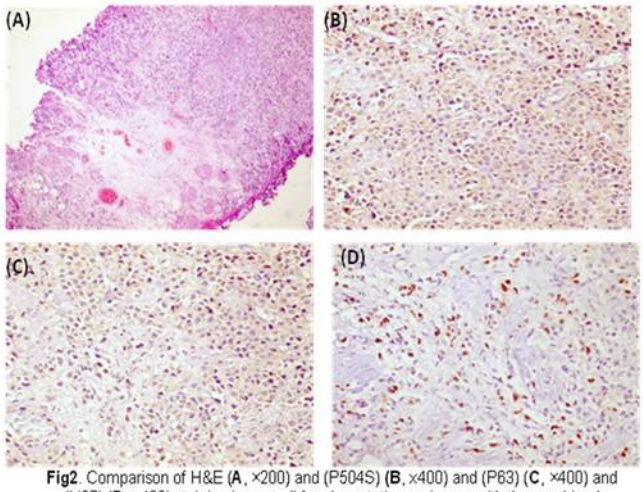

Fig2. Comparison of H&E (**A**, ×200) and (P504S) (**B**, x400) and (P63) (**C**, ×400) and (ki67) (**D**, ×400) staining in a small focal prostatic carcinoma with the combined Gleason's grade 10.**B**, tumor cells show a brown cytoplasmic granular staining (α-methylacyl coenzyme A racemase/P504S). **C** tumor cells show p63 cytoplasmic stain. **D** tumor cells show dark brown nuclear stain of ki67

Cytoplasmic staining for p63 was observed in tumor cells, which is a rare
expression pattern for p63 protein which is normally absent in prostate adenocarcinoma and that usually exhibits strong nuclear staining in basal cells of benign prostate gland. We observed higher levels of cytoplasmic p63 expresison in high grade prostatic adenocarcinoma, also higher levels of cytoplasmic p63 were associated with higher frequency of Ki-67 positive cells. But it was statistically un significant (P = 0.076) (Fig 2C, 3B).

Table (4):Immunohistochemical staining intensity of benign glands in carcinoma plus control groups.

	AMACR		P63 (nuclear stain)	
	NO.	%	NO.	%
Negative	40	93.1	0	0.0
Weak	2	4.6	0	0.0
Moderate	1	2.3	2	4.6
Strong	0	0.0	41	95.4
Total	43	100	43	100

Table (5): Frequency of the Ki-67 labeling index in relation to differentiation and Gleason's grade

Ki-67 labeling index	Gleason grade				Sum
	Poorly differentiated tumors		Moderately differentiated tumors		
	NO.	%	NO.	%	
<2	1	5.9	8	61.9	9
<25	8	47.1	4	30.8	12
26-50	3	17.6	1	7.7	4
51-75	2	11.8	0	0.0	2
76-100	3	17.6	0	0.0	3
Total	17	100	13	100	30

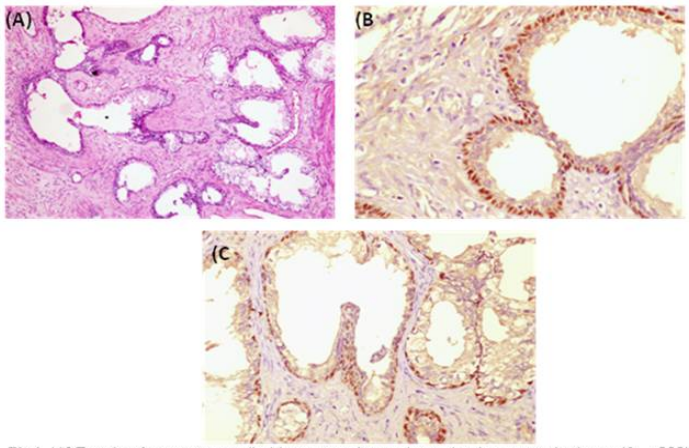

Fig1. H&E stain of prostate needle biopsy specimen shows benign prostatic tissue (**A**, ×200). Immune expression of **p63** in benign glands, positive expression is shown as brown circumferential nuclear **p63** stain in benign glands (immunoperoxidase stain). (**B, C,** ×400**).**

3.2.Ki-67 immunoreactivity results:

In studied specimens of benign prostatic tissue, only (1/20) specimen (5%) was positive for Ki-67 immunostaining. It has been shown that Ki-67 is significantly up-regulated in prostate cancer (P = 0.023) as compared with benign prostatic lesions (Fig 3D).In poorly differentiated carcinoma, one case (5.9%) was negative, whereas 8 cases (47.1%) were +1, 3 case (17.6%) +2, two cases (4.8%) 3+, and three cases (17.6%) 4+ (Table 5). Of 13 cases of moderately differentiated tumors eight cases (61.5%) were negative, while 4 cases (30.8%) were indexed as 1+ and one case (7.7%) as 2+. Consequently a statistical significant correlation was observed between Ki-67 positivity and increased Gleason's grade (P = 0.02) (Table 5).

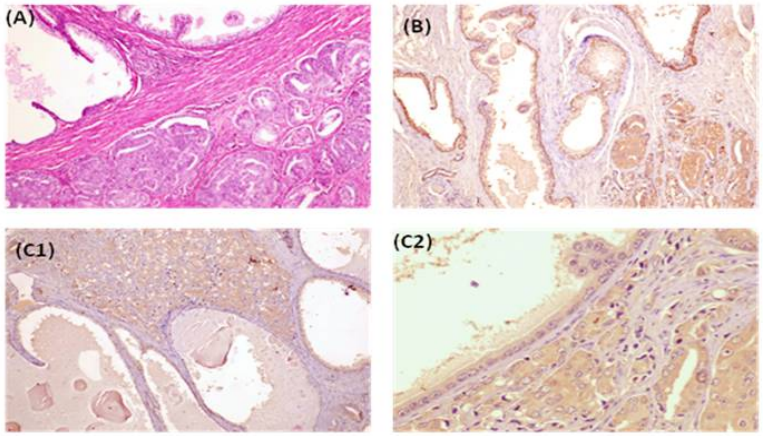

Fig3. Cluster of adenocarcinoma glands with the combined Gleason's grade8 invading between benign glands H&E (**A**, ×200). **P63**staining in a small focal prostatic carcinoma, the tumor glands show brown p63 cytoplasmic staining, whereas adjacent benign glands show dark brown nuclear staining of basal cells (**B**, ×200).**AMACR** expression in benign and malignant glands (immunoperoxidase stain, **C1** ×200) Brown color shows strong positive reaction in malignant glands whereas benign glands among malignant glands show complete negativity. (**C2** ×400) shows strong positive reaction in malignant glands and also in adjacent high grade PIN

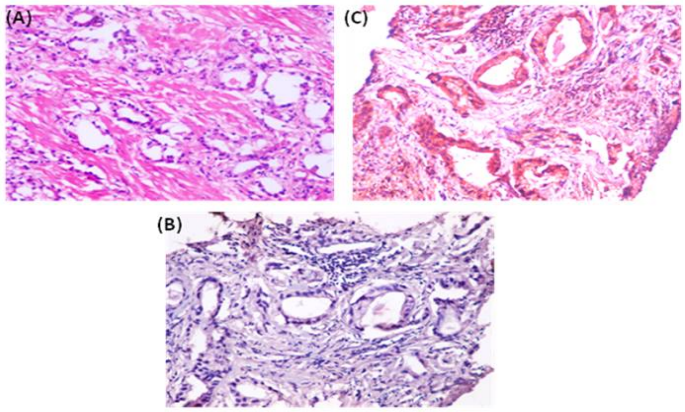

Fig4: A-Minimal carcinoma in prostatic smooth muscle stroma, without adjacent benign glands as reference points to assess infiltration, with the combined Gleason's grade6 (H&Ex200). **(B**, x200) **p63** immunostaining shows no reactivity in malignant glands. (**C**, x200) **AMACR** immunostaining shows a strong cytoplasmic reaction.

4.DISCUSSION

Prostate needle biopsy is the preferred method for diagnosing of early prostate cancer. It has low morbidity and provides specific information on the grade and extent of the tumor.

However, histologic confirmation of prostate carcinoma sometimes remains challenging in biopsy samples. The difficulty with needle biopsy stems not only from the small amount of tissue available for histological examination, but also from the fact that biopsies often identify only a few malignant glands (small focus carcinomas) or several histological benign mimics of cancer (Molinié et al. 2006). Diagnostic difficulty in indeterminate cases concerns 1.5%–9% of prostate biopsy (Iczkowski et al. 2002).

AMACR is used as a confirmatory stain for prostate cancer in conjunction with morphology and a basal cell–specific marker. Using AMACR as a positive marker alone might be misleading because weak expression of AMACR might be seen in benign glands and expression of AMACR is seen in high grade prostatic intraepithelial neoplasia (PIN) (Jiang et al. 2001) and atypical adenomatous hyperplasia (AAH) (Yang et al. 2002). Therefore, using AMACR as a positive marker along with basal cell-specific negative marker (p63) will enhance the diagnostic accuracy in minimal prostate cancer and reduce the chance of misdiagnosis (Srigley, 2004; Epstein, 2004).

In our study, only 3 cases of carcinoma group (10%) showed negative immune reaction with AMACR, and 83.3% of malignant glands showed >50% staining. Eighty six percent of the positive samples with AMACR had moderate to strong staining intensity. We also detected focal positive immune-stain with AMACR in two benign cases showing atrophy. This is due to the fact that the reverse transcriptase-polymerase chain reaction and quantitative IHC have shown that benign prostate epithelium also expresses AMACR mRNA and protein, respectively, but at very low level (Rubin et al. 2002).We consider only circumferential, diffuse or apical, granular, cytoplasmic staining of luminal cells that can be identified at x100 magnification as positive, while the adjacent benign prostatic glands should not show more than weak, partial (non-circumferential) staining as described in study done by Hameed and Humphrey (2006).

Jiang et al.,(2002) found that strong immune reaction for AMACR was consistently present in 94.5% of cases of prostate carcinoma and high-grade prostatic intraepithelial neoplasia. However, contrary to prostate carcinoma no expression was detected in most of the cases of benign hyperplasia. In another study, 71% of cancer cases showed positive immune-staining with AMACR, but variable intensities and percentages of cells were present. About 71%–100% of prostatic adenocarcinoma stained with AMACR (Yang et al., 2002; Jiang et al., 2002; Beach et al. 2002; Boran et al. 2011). In contrast to prostatic adenocarcinoma, about 0%–21% of benign prostatic glands stains for AMACR (Beach et al. 2002;Hameed et al., 2005; Boran et al. 2011).

Some variants of prostatic adenocarcinoma, including the atrophic, foamy gland and pseudohyperplastic variants can be AMACR negative. The sensitivity of AMACR in detecting these variants was found to be 70% (Farinola and Epstein 2004), 68% (Zhou et al., 2003) and 77% (Zhou et al., 2003) respectively. Accordingly, one should not render a diagnosis of benignancy based solely on a negative AMACR immune-stain (Hameed and Humphrey 2006).

Invasive prostatic adenocarcinoma lacks basal cells so using IHC to confirm their diffuse absence

in suspicious foci is a very useful diagnostic tool. Several studies showed that p63 is selectively expressed by basal cells in normal prostate gland. (Signoretti et al., 2000; parsons et al., 2001;Weinstein et al., 2002; Davis et al., 2002; Molinié et al., 2006) found that there was persistent basal cell staining with p63 in 1%–100% of atrophic and benign lesions, and a total absence of basal cells after p63 staining in prostate cancer. In another study, 97% of prostate tumors were completely negative for p63, and only a small percentage of p63 positive cells were detected (Signoretti et al., 2000).

Only complete absence of basal cell staining in all of the glands in a particular focus of concern, will support a diagnosis of carcinoma, as almost all of the mimkers of prostatic carcinoma can, at least focally in some glands, be negative with basal cell marker immune-stain (p63). In our study, there was only one subject with malignant glands that had positive p63 reaction in outer basal cells. It may represent out pouching from high grade PIN or flat, high grade PIN. All benign glands in our study showed strong p63 nuclear stain in outer basal cell layer, but no stain was observed in the secretory epithelial cells or in the stroma.

The expression of high levels of p63 exclusively in epithelial basal cells and the complete lack of prostate development in p63 null mice indicates that p63 expression is involved in the control of prostate growth and differentiation. Moreover, it strongly emphasizes the hypothesis that the basal cells represent prostate stem cells. Loss of p63 expression in secretory epithilum appears related to the process of differentiation as basal cells progress into the overlying epithelium and develops into secretory cell (parsons et al., 2001).

When small atypical glands identified by routine H&E staining are negative for basal cell markers and positive for AMACR/P504s, a malignant diagnosis is established. The main value of AMACR immune-staining is that it appears to provide additional diagnostic value beyond that of a negative basal cell marker immune-stain (Browne et al., 2004). Also there might be other explanations for a negative basal cell immunostaining, including the type of marker used as well as the fixative and antigen-retrieval methods used for the specimen (Varma et al., 1999).

Differences in p63 expression are associated with cancer progression or a poor prognosis for several cancer sites, including over-expression in the ovaries and oral squamous cell carcinoma (Lo Muzio et al., 2005;Marchini et al., 2008), down-regulated expression in the upper urinary tract and prostate (Zigeuner et al., 2004;Bismar et al.,2006;Mucci et al.,2008) and aberrant cytoplasmic expression in lung adenocarcinoma (Narahashi et al., 2006).

In our study, there was a predominantly cytoplasmic staining for p63 positive tumor cells, which is a rare expression pattern for p63 protein which is normally absent in prostate adenocarcinoma and that usually exhibits strong nuclear staining in basal cells of benign prostate gland. This expression was high in high grade prostate cancer, also higher levels of cytoplasmic p63 were associated with higher frequency of ki67 positive cells in prostatic adenocarcinoma, but it was statistically insignificant ($p=0.076$) this in contrast to Parsons et al.,(2001) who found that the majority of prostate adenocarcinomas do not express p63 except some tumor cells in high grade prostatic carcinomas representing less than 1% of cells in those carcinoma specimens showed very weak nuclear staining and there was a strong correlation between the expression of p63 in these tumor cells and Gleason grade ($p<0.0001$).

Dhillon et al.,(2009)found that significant association between cytoplasmic expression of p63 in prostate tumor tissue at the time of diagnosis and fatal prostate cancer and higher levels of cytoplasmic p63 were also significantly associated with increased proliferative activity (ki67) ($p=0.0026$). This difference in the significance of the result may be due to small number of our cases but Dhillon et al.,(2009) study were conducted on 298 men.

Our findings are in contrast with two studies done by Bismar et al.; (2006) and Mucci et al., (2008) that reported an inverse association between p63 expression (as part of a genetic signature) and prostate cancer progression. Bismar et al., (2006) generated 12-gene signature for aggressive prostate cancer that included p63 based on its under-expression in metastatic cancer compared to benign tissue and localized disease.

The nuclear localization of p63 is essential for its role as a transcription factor. Similar to p53, alterations in nuclear-cytoplasmic shuttling may lead to cellular mislocalization, which disrupts regulation of cell cycle checkpoints and apoptosis, contributing to the initiation or progression of cancer (Hood and Silver 2000;Fabbro and Henderson 2003). In patients with lung cancer, inflammatory breast carcinoma and colorectal carcinoma, the cytoplasmic sequestration of p53 is associated with metastasis and poor long-term survival (Moll et al., 1996). The localization shift may arise from disruptions in the nuclear transport pathway (Hood and Silver 2000), such as those mediated by the murine double minute-2 gene (Mdm2) where laboratory data show that p63-induced apoptosis is reduced when Mdm2 exports two isoforms of p63 (TAp63α and TAp63γ) from the nucleus to inhibit their transcription and pro-apoptotic activity (Kadakia et al., 2001). In our study, higher levels of

cytoplasmic p63 are associated with increased proliferation, this is in agreement with Dhillon et al.,(2009) who stated that, the mis-localization and imbalance in p63 isoforms may alter p63 stability and function and thereby disrupt cell cycle arrest and apoptosis, which may have prognostic significance for cytoplasmic sequestration of p63 and the progression of prostate cancer.

The management of patients with prostate cancer depends on an accurate assessment of the biological potential of the tumor. Unfortunately, the current examination techniques are mostly inadequate for correct clinical staging, and assessment of the tumor grade may vary due to subjectivity of the observer. Therefore, the search for additional prognosticators of the cancer behavior is the subject of intense ongoing investigation. Ki-67 is one of the most reliable markers of cell proliferation. Ki-67 was up-regulated in prostate cancer and PIN and was associated with Gleason grades. High ki-67 expression was a predictor of poor prognosis after radical prostatectomy (Nikoleishvili et al., 2008).

In this current study, the Ki67 was significantly up-regulated in prostate cancer ($P<0.023$) as compared with benign prostatic tissue. This finding is in agreement with Nikoleishvili et al., (2008) who found that this marker was highly expressed in prostate cancer as compared with BPH ($P=0.019$).

Ki-67 marker was positive in 21 out of 30 tumors in current study (70%). Forty nine percent of poorly differentiated tumors and 38.5% of the moderately differentiated tumors were positive for Ki-67 in this study. This is in agreement with Nilsson et al.,(1988) who showed a significant correlation between positive cases of Ki-67 and tumor cell differentiation. The present study also showed a statistical significant correlation between Ki-67 marker and increased Gleason's grading with increased number of stained cells ($P=0.02$) and this is in consistent with Madani et al., (2011).

In conclusion, immunohistochemical analysis with an AMACR (P504s) and p63 provides a simple and easy assay that can be used as a routine test, which overcomes the problems of studying limited carcinoma in prostate needle biopsies and increase its diagnostic accuracy. The diagnosis of these small foci of prostate cancer in needle biopsy specimens is one of the major diagnostic challenges in surgical pathology. Ki-67 marker was shown to have a strong relationship with Gleason's grading, which has an important relationship with the prognosis of prostate cancer. Cytoplasmic expression of p63 was high in high grade prostate cancer. Also it was associated with higher frequency of ki67 positive cells in prostatic adenocarcinoma. The mislocalized cytoplasmic expression of p63 was associated with higher proliferative activity, and may suggest an oncogenic role in prostate cancer progression.

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Paradoxical TRAIL Activity in Acute Promyelocytic Leukemia (APL) Cell Line AP-1060

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Abstract: TRAIL as a member of TNF ligands is involved in the anti-tumor mechanisms by its cytotoxic activity inducing apoptosis, and has been explored as a tumor target reagent in the therapy of different tumors. In acute promyelocytic leukemia (APL), TRAIL was used to be token as a crucial molecule that was up-regulated by retinoic acid and induced the blast cell to differentiation and apoptosis. However, our findings indicated that AP1060 cell, an APL cell line, produced a high level of TRAIL, and its growth and proliferation depended on the auto-secreted TRAIL. Furthermore, the above phenomenon may be related to the TRAIL-mediated NF-kB activation. It suggests that there may be a new therapy approach to the subtype of APL.
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Keywords: TRAIL; acute promyelocytic leukemia; retinoic acid; induced differentiation

1. Introduction

TNF-related apoptosis-inducing ligand (TRAIL) belongs to the superfamily of TNF ligands and is composed of 281 amino acids and has characteristics of a type II transmembrane protein. TRAIL has five death receptors including TRAIL-R1 (DR4), TRAIL-R2 (DR5), osteoprotegerin, and two decoy receptors. TRAIL is not only required for natural killer cell-mediated immune-surveillance against the tumor progression and metastasis (Takeda 2002), but also for Fur and dendritic cell-mediated innate and adaptive immunity in suppression of tumor progression (Schmaltz 2002, Taieb 2006). Alpdogan TRAIL induces apoptosis in tumor cells by binding to the death receptors DR4 and DR5 (Sheridan 1997). Once trimeric TRAIL cross-links with the receptor DR4 or DR5, there is an aggregation of the death domains, which leads to recruitment of the adaptor molecule FADD and activation of caspase 8-mediated cascade. Caspase 8 is responsible for initiating activation of caspase 3, ultimately committing tumor cells to undergo apoptosis (Kischkel 2000, Rudner 2005). In some cases, the mitochondrial type II pathway is involved in TRAIL-induced apoptosis (Song 2008). TRAIL-R3 (DcR1) and TRAIL-R4 (DcR2), which do not contain a cytoplasmic domain (DcR1) or contain a truncated death domain (DcR2), functions as a TRAIL-neutralizing decoy-receptor to protect normal cells from TRAIL-induced apoptosis in physiological mechanisms (Avi Ashkenazi 1999).

Unlike its other homologous ligands such as TNF-A and FasL, TRAIL exerts a cytotoxic activity against the majority of tumor cell lines, while it spares normal tissues (Kelley and Shankar 2004, Younes 2003). In mouse, the TRAIL deficiency made mice suffer from a greater than 25% increased risk of developing lymphoid malignancies after 500 days of age or enhances lymph node metastasis of squamous cell carcinoma (Zerafa 2005, Grosse-Wilde 2008). During the last few years, the anti-tumor activity of TRAIL has been reported in hematologic malignancies, including multiple myeloma cells and Philadelphia chromosome-positive leukemia in which it was shown that TRAIL was able to induce apoptosis (Chen 2001, Uno 2003). In acute promyelocytic leukemia (APL), Studies have documented that APL blasts are sensitive to TRAIL-mediated apoptosis and that retinoic acid induces TRAIL expression and thereafter killing of the leukemic cells (Altucci 2001). A phenomenon that AML blasts, including APL blasts, are resistant to TRAIL-mediated apoptosis is seemingly related to the expression of TRAIL deco receptor on these cells (Riccioni 2005). However, Soucek reported that a combination treatment with all-trans retinoic acid (ATRA) and TGF-beta1 led to the enhancement of ATRA-induced suppression of cell proliferation, which is accompanied by inhibition of ATRA-induced apoptosis and increasing of anti-apoptotic molecules (c-FLIP(L) and Mcl-1) in human leukemia HL-60 cells (Soucek 2006).

We found that AP1060 cell, an acute promyelocytic leukemia cell line (Sun 2004), highly expressed TRAIL during the characterization of this cell line that was developed from a multiple-relapse patient clinically-resistant to both ATRA and arsenic trioxide (ATO). In this tentative study, we observed the effects of auto-secreted TRAIL on AP1060 cell.

2. Material and Methods

Cell Culture: AP1060 cell line was established from the patient who had relapsed for the fourth time after achieving an initial, transient clinical response

on ATO therapy. The cells were incubated in Iscove's modification of Dulbecco's medium (IMDM, Invitrogen, USA) containing 10% FBS and 10ng/ml G-CSF (Amgen, Thousand Oaks, CA, USA). HL60, NB4 and Jurkat cells were maintained in RPMI-1640 Medium supplemented with 10% fetal bovine serum (FBS) and penicillin/streptomycin (Invitrogen, USA). The incubator was set at 37°C and 5% carbon dioxide (CO_2).

All-trans Retinoic acid (ATRA, Sigma, USA) dissolved in ethanol to a concentration of 1mM and stored at -20. The dose of the induced differentiation was referred to Y Sun et al.[19] The percentage of viable and non-viable cells was determined by the exclusion or uptake, respectively, of the vital trypan blue (Life Technologies) from/by a minimum of 200 cells manually counted using a hemocytometer under microscopic observation. Specific inhibition of TRAIL-induced cell death was performed using TRAIL and DcR1 neutralizing monoclonal antibody (R&D, USA), and the respective no-neutralizing antibodies were used as control. The evaluation of cytologic features by Wright's stains and the performance of the nitroblue tetrazolium (NBT) test.

RNase Protection Assays: RNA was extracted from the cultured cells using Trizol (Invitrogen, USA). The RNase protection was performed with 5ug RNA with the human apoptosis detection kits (hApo2, hApo3d and hApo5, Parmingen, USA) as specified by the manufacturer. In brief, RNA was hybridized overnight with the vitro-translated 32P-labeled probes. Following hybridization, samples were treated with RNases A and T1 plus proteinase K, phenol chloroform extracted, and ethanol precipitated. The protected fragments were resolved by electrophoresis on a 6% acryl amide-urea gel and exposed on a Phosphor Imager screen (Molecular Dynamics, Inc. USA) for12 hours at -80℃ to quantify the intensity of the bands. Relative amounts of message were corrected for RNA loading by comparison with the GAPDH band intensity for each sample.

Western blot analysis: The total protein was prepared from the control cells and treated cells with RIP buffer （150 mM NaCl, 1.0% NP-40, 0.5% sodium deoxycholate, 0.1% SDS, 50 mM Tris, pH 8.0 and 1x Roche protease inhibitor cocktail. Equal amounts of protein were electrophoresed in 10% polyacryl amide gels containing 0.1% SDS and then transferred to nitro-cellulose membranes (Bio-Rad Laboratories, Hercules, CA). The membranes were probed with an antibody directed against the TRAIL (1:1000 antibody dilution), and antibody binding was detected with the ECL chemiluminescence system (Amersham Life Science Inc., Arlington Heights, IL).

Evaluation of apoptosis: Sub G1 cells were detected by Propidium iodide (PI). Briefly, 10^6 cells were washed and fixed in a suspension of 70% ethanol on ice and stored at -20°C overnight. Following fixation, the cells were centrifuged and resuspended in 1 mL of Hank's balanced salt solution (HBSS). One ml of 0.2 M Na2HPO3 - 0.1 M citric acid buffer, pH 7.8, was then added and incubated at room temperature for 10 min. After centrifugation, 1 mL of HBSS containing 20 mg/ml of propidium iodide and 5 Kunitz units of DNase-free RNase was added to the cells, followed by incubation for 30 min at room temperature. The TUNEL (terminal deoxynucleo-tidyl transferase-mediated deoxyuridine triphosphate [dUTP] nick-end labeling) assay was performed by use of the cell death detection kit (Invitrogen, USA). DNA strand breaks generated by cleavage of genomic DNA during apoptosis can be identified in this assay by labeling free 3-OH termini with fluorescein isothiocyanate (FITC) -conjugated dUTP. Within 1 hour, the cells were analyzed at 488nm in a FACSCAN cytometer (Becton Dickinson Labware).

Electrophoretic mobility shift assay (EMSA): Nuclear extracts were prepared from AP1060 cells as previously described (Haas 1998), in which protein concentration 1 to 5mg/mL determined by the bicinchoninic acid protein assay (Pierce Chemical Co). The oligo nucleotide containing the prototypical NF-kB binding site from murine Igk, 5' -CA-GAGGGACTTTCCGAGA-3', was radio-labeled with [^{32}P]dCTP (>3000Ci/mmol, Amersham). The specificity of binding was verified using excessively unlabeled consensus oligonucleotide (x10) corresponding to the above sequence as a competitor in the binding reaction. The DNA binding reaction contained 5ug protein and 10 cpm radio-labeled DNA probes was performed as described (Haas 1998). Following incubation, the samples were loaded onto a 5% native polyacrylamide gel (acrylamide/ bisacrylamide at 40:1 in 0.3x TBE) for electrophoresis resolution. The intensity of each protein/DNA complex was determined by using a Molecular Dynamics Phosphor Imager.

3. Results

Highly expressed TRAIL in AP1060 cell: In order to explore the mechanism of AP1060 cell immortalization, we examined the expression level of the apoptosis molecules in the different myeloid cell lines (NB4, HL60 and AP1060), which belong to human promyelocytic leukemia cells and could been induced to differentiation with Retinoic acid, by apoptosis detection kits. It was found AP1060 cell expressed high level of TRAIL and significant level of its receptors (DcR1, DcR4 and DcR5) comparing

with NB4 and HL60 (Fig.1). A great amount of TRAIL was also detected in AP1060 cell cultural medium with immune-precipitation, and no mutation was detected in its cDNA (data not shown).

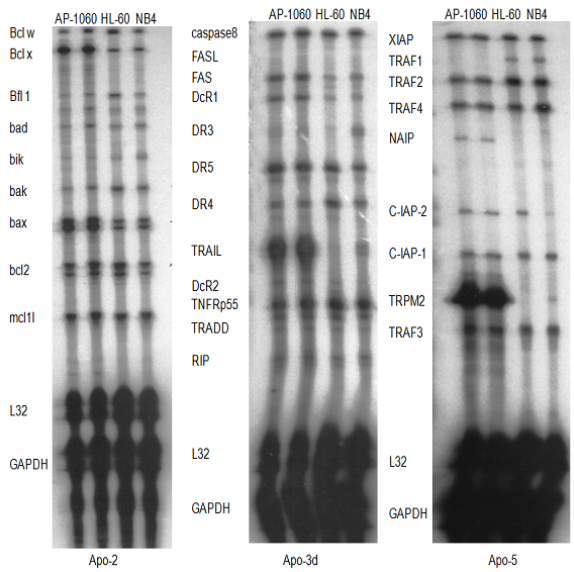

Fig 1. Expression of the apoptosis gene set. Cells were cultured for 72 h in the presence of medium. Total RNA from growing cells was analyzed for distinct mRNA species using multi-probe RNase protection assay system with the Apo2, hAPO3d and Apo5 multi-probe template set. Free probe and other single-strand dRNA molecules were digested with RNases. The RNase-protected probes were purified, resolved on denaturing polyacrylamide gels, and imaged by autoradiography. Bands of house-keeping genes (L32 and GAPDH) were included for normalizing signals. The high expression of TRAIL presented in AP1060 comparing with NB4 and HL60 both of which could be induced to differentiation with ATRA.

Down-regulation of TRAIL by Retinoic acid: It was commonly reported that TRAIL was increased in both of transcriptional and protein level in ATRA-induced differentiation. We also observed the mRNA expression of TRAIL by RNase-protected probes in Apo3d set. The cells were treated with 100 nM ATRA and 1 uM ATRA which was able to stimulate the terminal differentiation of virtually 100% of cells into neutrophilic granulocytes in both of NB4 and AP1060 cells. ATRA treatment sharply decreased the expression of TRAIL, and did not significantly modulate the expression of TRAIL receptors (Fig. 2). We did not encounter the imagine that the TRAIL was up-regulated by ATRA treatment in both of AP1060 and NB4 cells. The TRAIL manifested same pattern at protein level in ATRA treated AP1060 and NB4 cells (Fig. 3).

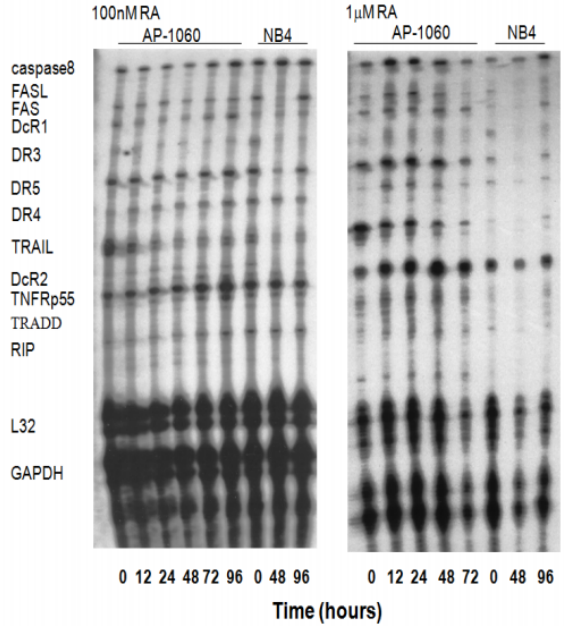

Fig 2. Analysis of possible genes involved in apoptosis during the induced differentiation with Retinoic acid. 100nM and 1uM ATRA stimulated cells to differentiate into 100% neutrophilic granulocytes which was determined with nitroblue tetrazolium (NBT) test. The expression of TRAIL was dramatically down-regulated by ATRA treatment in AP1060 cell.

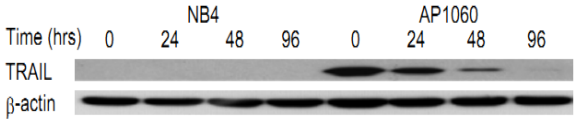

Fig 3. TRAIL protein expression is consistent with mRNA levels. Cells were treated with 1 uM ATRA to be induced into terminal differentiation. 100 ug total protein per lane was loaded for electrophoresis. After 96 hours induction, the trace of TRAIL was seen in AP1060 cell.

Bio-activity of TRAIL: It was known the Jurkat cell, a lymphocytic leukemia cell line, was very sensitive to TRAIL, which could efficiently induce the cell apoptosis, and used for the evaluation of TRAIL activity. 2.5 x 10^5/ml AP1060 cells were incubated for 48 hours, and the supernatant was collected as the condition medium for TRAIL activity

assay. The 2.5 x 10^5 /ml cells were plated in the above condition medium. 24 hours later, The viable and dead cells were counted by Trypan blue staining. The percentage of dead cells in the condition medium was more than 15% contrast to 3.2% in control medium.

Inhibition of proliferation by Neutralizing TRAIL: AP1060 cells were cultured in the medium supplemented with TRAIL-neutralizing monoclonal antibody as indicated in following figure 4. The cell growth was obviously inhibited by neutralization of TRAIL with its specific antibody, which corresponded to the increasing dose of the antibody. Respectively, we did not observed significant inhibition effect on AP1060 cell by neutralizing DcR1 with its specific antibody (Fig. 4 B). The AP1060 cells, which were cultured for 5 days in the neutralization treatment and control, were stained with Wright's, and presented in figure 5.

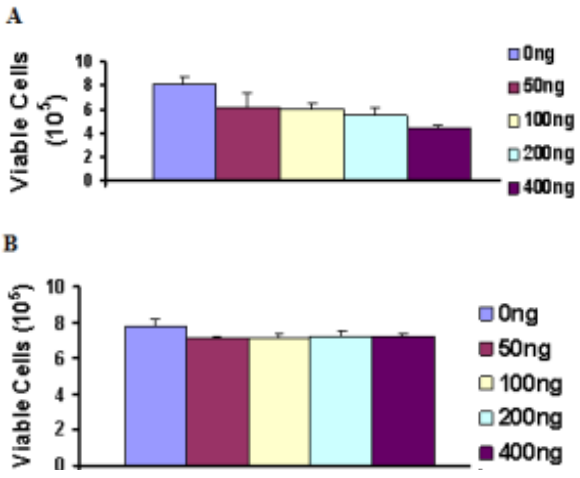

Fig 4. Neutralizing Tests. A. Inhibition of cell proliferation by Neutralizing TRAIL. B. The cell growth did not be significantly affected by the neutralization of DcR1. 2.5 x10^5 AP1060 cells were seeded in IMDM containing 10% FBS, 20ng/ml G-CSF and the indicated dose of neutralizing monoclonal antibodies. 400ng respective no-neutralizing antibodies were added in control. All cells were cultured with antibody for 5 days. The viable cell was determined by Trypan blue.

Apoptosis induced by neutralizing TRAIL: Cell cycle was detected by Flow cytometer to analyze the cause of the inhibition of AP1060 proliferation as described in Methods. AP1060 cells were treated with TRAIL neutralizing antibody for 5 days and stained in propidium iodide, and analyzed for cell cycle distribution flowcytometrically using CFlow® software. The population of the cells in sub-G1 phase is increasing with the successively incremental dose of neutralizing TRAIL antibody comparing with the control that was treated with 400 ng/ml no-neutralizing TRAIL antibody (Fig. 5). These results were confirmed by TUNEL assay. The percentage of the cells containing FITC labeled DNA fragments was raising in TRAIL neutralizing antibody treatment, which was consistent with the its sub-G1 cell cycle distribution (Fig. 6). Simultaneously, we did not find any significant effect of neutralizing DcR1 on AP1060 cell (Fig. 5). It indicated that the treatment with TRAIL neutralizing antibody made AP1060 cell undergo apoptosis.

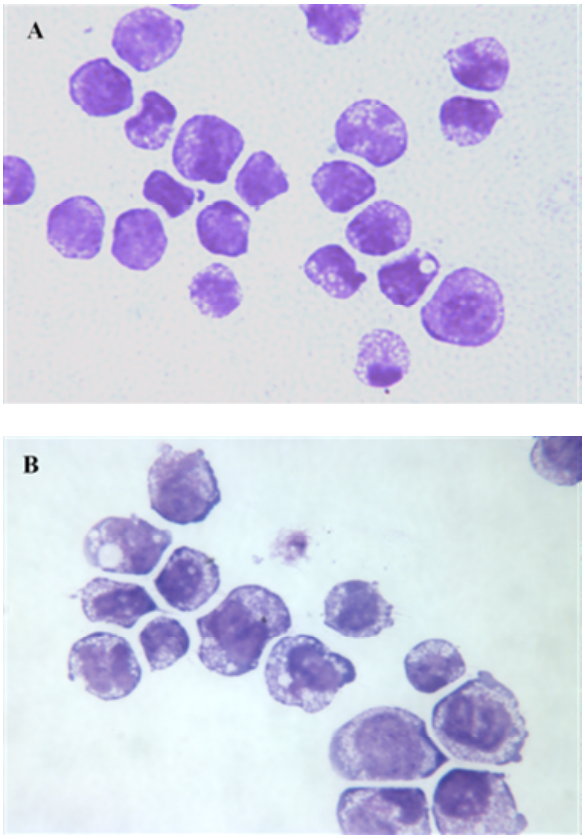

Figure 5. AP1060 cells cultured for 5 days. A. treated with TRAIL no-neutralizing antibody (400ng/ml). B. treated with TRAIL neutralizing antibody (400ng/ml).

Modulation of NF-kB activation by neutralizing TRAIL: 2.5 x 10^5 cells were plated and incubated respectively with 400 ng/ml TRAIL neutralizing or no-neutralizing antibody for 96 hours. The translocation of NF-kB was measured by EMSA as described in methods and presented as Figure 7. It was indicated that the activity of NF-kB was markedly inhibited following the treatment of TRAIL neutralizing antibody.

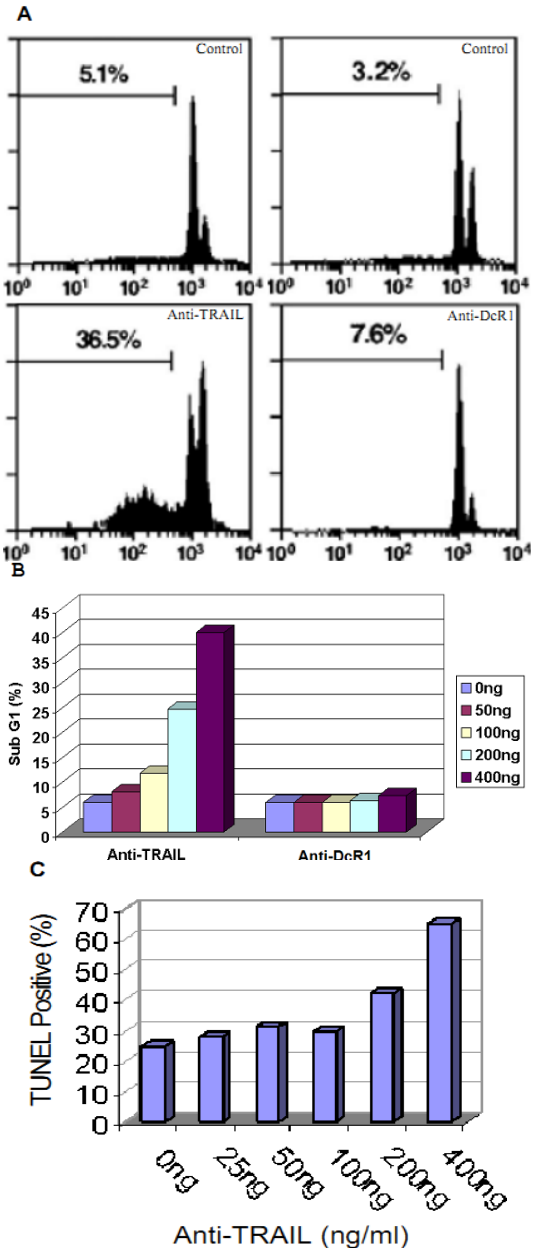

Figure 6. Flow cytometric cell cycle analysis demonstrates sub-G1 accumulation after the treatment with TRAIL neutralizing antibody. The cells were cultured with the indicated neutralizing antibodies (ng/ml). The no-neutralizing antibodies (400ng/ml) were used in controls. A. DNA Histograms of cell cycle (from single experiment). The relative number of cells displaying an apoptotic, sub-G1 DNA content, is given between the marker bars. Cells were treated respectively with 400 ng/ml antibody. B. Dose response. Apoptosis was determined by flow-cytometric detection of nuclear DNA fragmentation. C. TUNEL assay. Bars in B and C represent the mean of cells displaying a sub-G1 DNA or FICT labeled DNA content from three independent experiments.

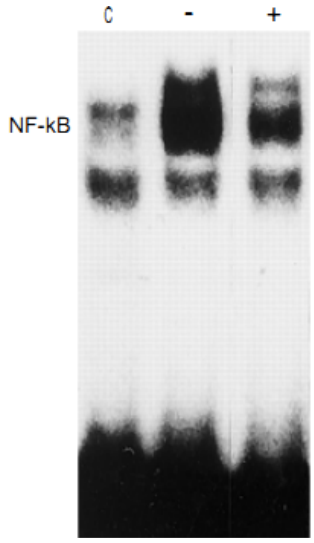

Figure 7. Inhibition of NF-kB activation by neutralizing TRAIL. Nuclear extracts were prepared from AP1060 cells incubated for 96 hours with TRAIL no-neutralizing antibody (-) and neutralizing antibody (+). The excessively competent reaction was presented as control (c). The presence of NF-kB was determined by EMSA using a radio-labeled probe containing the prototypical NF-kB site.

4. Discussions

The retinoic acid therapy has been established over twenty years and is only successful model in which the patient is cured by inducing the malignant cells to differentiate terminally. A variety of studies about the cellular and molecular characters during the RA-induced differentiation has been carried out to explore it mechanism. It is used to be believed that Retinoic acid-induced apoptosis in acute promyelocytic leukemia cells is mediated by TRAIL that is induced by retinoic acid (Altucci 2001). However, our findings suggest that TRAIL may play a different role in RA-induced differentiation.

The myeloid leukemia is commonly characterized by an arrest of granulocytic differentiation and failure of apoptosis. It was early indicated that the initiation of differentiation required the transcriptional activation of specific genes leading to proliferation arrest and cell cycle exit (Rousselot 1994). The evidence that TRAIL was highly expressed and dramatically down-regulated after retinoic acid treatment was found not only in AP1060 cell line but also in APL specimens (Riccioni 2005). It does not support the hypothesis that TRAIL is related to the ATRA-induced differentiation and apoptosis of APL cells. In addition, our previous data of the genes expression profile of RA-induced differentiation of AP1060

presented an intricate gene-reprogramming network in which deregulated growth, increased cell survival, processed differentiation were hallmarks (Sun 2011). For example, the inhibition molecules of apoptosis such as BCL related protein 2, were up-regulated while the apoptosis-inducing molecules (TRAIL, SIAH1) were down-regulated. It is consistent with the results of the experiments that were conducted in HL60 cells with the combination treatment of retinoic acid and TGF-β (Soucek 2006).

Our results indicated that 1060 cell expressed a wild type and bio-effective TRAIL. It is interesting how APL1060 cell could escaped from the TRAIL attack. Many studies have reported that the expression of TRAIL decoy receptors is involved in the mechanisms of resistance of several tumor cell types to TRAIL, including breast cancer, myeloma and osteosarcoma (De Almodovar 2004, Shipman and Bouralexis 2003). Ricconio also ascribed such resistant phenomenon in APL cells to the expression of death decoy receptors (Riccioni 2005). However, in this study the protection of decoy receptors had not been wiped off by neutralizing DcR1 that expressed mainly in AP1060 cells. Furthermore, it was more surprising that AP1060 cells underwent a apoptosis after the treatment with TRAIL neutralizing antibody. It implies that the auto-secreted TRAIL is necessary for the growth and proliferation of AP1060 cells. In fact, it was reported that TRAIL could stimulate the proliferation of leukemia cells and activated NF-κB (Ehrhardt 2003, Degli-Esposti 1997). NF-kB activation is mediated not only by the truncated decoy receptor TRAIL-R4 (DcR2), but also by the death receptors, TRAIL-R1 and -R2 (Degli-Esposti 1997, Harper 2001). Here, the result of EMSA showed that the activation of NF-kb was inhibited by a treatment with TRAIL neutralizing antibody. Therefore the activation of NF-kB leads the transcription of genes that antagonize the death signaling pathway. In other hand, the activation of NF-kB mediated by TRAIL-R1 and TRAIL-R2 depended on the inhibition of apical Caspases (caspase 8 and FADD) (Ehrhardt 2003, Harper 2001). Sensitivity to TRAIL-induced apoptosis was also modulated by inhibition or activation of NF-κB (Harper 2001). We wonder whether the deficiency of Caspase-8 or dysfunction of FADD may harbor in AP1060 cell line and offer it a bio-feature that TRAIL could not kill it but stimulate its proliferation.

In cancer therapy, the apoptosis resistance of tumors is a commonly challenging issue whatever in physiological self-defending system or chemotherapy including APL-retinoid-induced differentiation. Our findings enlighten us to explore the clinical relevant features of APL and the differential strategies of therapy.

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The Relationship between Principal's Emotional Intelligence and Leadership Styles in Primary Schools

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Abstract: There are various studies done on relationship between emotional intelligence and leadership, only a few of them were took part in educational organizations by focusing on view point of the Bass & Avolio's leadership styles and Bar-On's theory of emotional intelligence. This research made an attempt to prepare an empirical research-evidence for supporting the proposed link between school principal's emotional intelligence and leadership styles. Collected data (n=268) were analyzed by using. The Pearson's correlation coefficient. The result indicated statistically significant relationship between principal's emotional intelligence and leadership styles. This significant relationship resulted that the higher level of the principal's emotional intelligence is linked to the transformational leadership style. In contrast, transactional and laissiez-fair leadership styles were not positive associated with emotional intelligence. Consequently, school programmer should be focused on the especial training methods to enhance principal's emotional intelligence level whereby they can develop the transformational leadership style qualities. These development qualities concerns about transforming the present condition and followers requirements.

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Keywords: Emotional intelligence, Transformational, Transactional, Laissez-faire, Principals, School

1. Introduction

In the 21th century, a significant amount of research and attention has been given to identifying relationship between emotional intelligence regarding, leadership (Dulewicz & Higgs, 2003) and leadership styles (Boss, 1990; Golman, 1998). Emotional intelligence has gained much popularity as an absolute necessity for effective leadership (Sosik & Megerian, 1999; Anand & Udaya Suriyan, 2010).

Effective leaders have a quality that sets them apart from ineffective leadership; they have the ability to understand why people do what they do when they do it (Hartley, 2004). This ability is called emotional intelligence. Emotional intelligence is so important in the work environment because the ability to gauge oneself and one's coworkers emotionally fosters the necessary social skills to succeed in a professional context (Dong & Howard, 2006; Ruestow, 2008). Human service leaders with a high level of emotional intelligence demonstrated less subjective stress and better physical and psychological well-being (Ogińska-Bulik, 2005). In addition, researchers have revealed that managers with high emotional intelligence obtain results from employees that are beyond expectations, developing and using talents crucial for organizational effectiveness (Barbuto & Burbach, 2006; Anand & UdayaSuriyan, 2010) .With this in mind, and the increased popularity of emotional intelligence, researchers began to study the connection between emotional intelligence and leadership effectiveness (Golman, 1998; Golman, Boyatzis & McKee, 2002; and Caruso & Salovey, 2002). Dulewicz and Higgs (2003) identified common emotional intelligence elements that have been linked to effective leadership characteristics. Moreover, close to ninety percent of success in leadership positions is attributable to emotional intelligence (Chen, Jacobs, and Spencer, 1998: Anand & UdayaSuriyan, 2010). The leadership characteristics have been described by Boss (1990a, b) into three well-known styles of leadership; Laissez-faire, transactional, and transformational leadership. Any leader can use any style, and a good mix that is customized to the situation is generally the most effective approach.

According to McColl-Kennedy (2002) the style of leaders is a considerable function as a particular important in achieving organizational goals. Leadership style refers to a leader's behavior (Boss, 1999). The leader's style is also considerable important in being able to evoke performance among subordinates (Barling, Weber, & Kelloway, 1996; Zacharatos, Barling & Kelloway, 2000; Berson, Shamair, Avolio & Popper, 2001) and as playing a key role in developing effective behaviors for mentors (McColl-Kennedy, 2002). In an organization, Leaders expected to feel and display emotion (McColl-Kennedy, 2002) and emotional intelligence contributes to effective leadership in organizations (George, 2000). Emotionally

intelligence leaders know how to manage their disruptive emotions so that they can keep their focus, thinking clearly under pressure (Golman, 2002). By studying the relationship between emotional intelligence and leadership styles, research aimed to contribute to the leadership literature and to test emotional intelligence applications for leadership.

Carmeli (2003) pointed out that the literature suggests that managerial skills in general and emotional intelligence in particular, play a significant role in the success of senior managers in workplace.

Chi and colleagues (2007) have been aimed to study the relationship among leadership styles and emotional intelligence affected to salespeople's job performance. Results showed that emotional intelligence of salespeople was moderating the relationship between leadership styles and job performance. However, the relation between leadership styles and job performance that moderated by emotional intelligence was significant negative correlate, can assumed that salespeople need to be more recognize and concentrate by leader as used transformational leadership styles, not only exchanged reward to attain the goals as transactional leadership styles.

Connelly and Ruark (2010) have been argued that leader emotional displays are important to consider both within and outside of transformational/charismatic paradigms and must look beyond positive and negative affect. Accordingly, they examined the effects of emotion valence as moderated by leadership style (transformational vs. transactional) on follower satisfaction, evaluations of the leader, and creative task performance. Findings showed differential effects of positive and negative emotions for different leader styles for evaluations of transformational leadership and leader effectiveness and for follower performance. Additionally, positive emotions with higher-activating potential resulted in more desirable outcomes than those lower in activating potential, but the reverse was true for negative emotions.

Charbonneau and Nicol (2002) investigated the relationship between emotional intelligence and leadership in adolescents group. Their study results demonstrated that some aspects of emotional intelligence are associated with leadership in adolescents. They concluded that managers who scored high on the Bar-on (1997)'s emotional intelligence inventory were perceived by their subordinates as displaying more transformational leadership behaviors.

Mandell & Pherwani (2003) examined the predictive relationship between emotional intelligence and transformational leadership style. A significant predictive relationship ($p \leq .05$) was found between transformational leadership style and emotional intelligence. Lastly, no significant difference ($p > .05$) was found in the transformational leadership scores of male and female managers.

Palmer and his colleagues (2001) stated, emotional intelligence has fast become popular as a means for identifying potentially effective leaders and as a tool for nurturing effective leadership skills. Their findings indicate that EI may be an underlying competency of transformational leadership style.

Barling and colleagues (2000) Investigated whether emotional intelligence (EQ) is associated with use of transformational leadership in managers. They showed that three aspects of transformational leadership (i.e. idealized influence, inspirational motivation, and individualized consideration) and constructive transactions differed according to level of emotional intelligence. In contrast, active and passive management and laissez faire management were not associated with emotional intelligence.

Goleman and colleagues (2002) have argued that emotional intelligence is crucial component of leadership effectiveness, particularly as leader deal with teams. Emotionally intelligent leaders serve as a transformational influence over team members.

Sivanathan and Fekken (2002) resulted that the followers perceived leaders with high emotional intelligence as more effective and transformational. They found that emotional intelligence conceptually and empirically linked to transformational leadership behaviors. Hence, they concluded that having high emotional intelligence increased one's transformational leadership behaviors.

2. Material and method

This study employed a correlational cross-sectional quantitative survey design as it defines the relationship between the primary school principal's emotional intelligence and leadership styles. According to Smith and Glass (1987), such an approach provides the researcher with information regarding not only the direction of the relationship between two variables, but also the magnitude of the relationship.

The sample population of research consisted of all primary school (337 schools)'s principals in Golestan-Iran during 2010-2011 school years. Principals were identified as individuals who worked within educational organization and lead teachers. About 300 principals considered as real sample size. Then, based on real sample size and proportional fraction of principals of the cities within area study, principal sample sizes of the cities were computed.

The quantitative data for the study was gathered utilizing; 1) the Emotional Quotient Inventory (EQi) (Bar-On, 1997) to ascertain the emotional intelligence of the school Principals, 2) the

leadership behaviors (Bass, 1990a,b) to ascertain the well-known styles of principal's leadership. Then, collected data (n=268; male=126, female=142) were used in the analysis process. The Pearson's correlation coefficient and Guilford's rule of thumb (r < .20; negligible, r = .20 to .40; Low, r = .40 to .70; moderate, r = .70 to .9; high, r > .90; very high relationship) were used for determining the strength and direction (positive or negative) of the relationships between two metric variables such as; IV= principals' emotional intelligence and DV=leadership styles. Pearson's method and Guilford's rule no indication is reflected on the significance of the relationship. Hence, t-test statistical method applied to analysis differences between groups of subjects.

3. Results

The result of the statistical analysis of the hypothesis testing done with the aim of defining the relationship between emotional intelligence-its sub-scales and different leadership styles-its sub-scales are stated as follows.

Table 1 shows the results from testing of the hypothesis of the research by using the Pearson's statistical method. The result indicated a statistically significant relationship between principal's emotional intelligence and leadership styles at 99% ($p \leq .01$) of the confidence level. This significant relationship is positive-high magnitude, between principal's emotional intelligence and transformational leadership style (r=.765**, $p<.01$), negative-moderate magnitude between principal's emotional intelligence and transactional leadership style (r= -.555**, $p<.01$), and negative–low relationship between principal's emotional intelligence and laissez - faire style (r= -.334**, $p<.01$). It is concluded that most of the components of emotional intelligence have significant moderate-positive relationship with the transformational leadership components (Table 1). Findings is confirmed the Megerian and Sosik (1996)'s theoretical linkage between emotional intelligence and transformational leadership style. In addition, it is supported by Robbins (2001) and Anand (2010) "an important component of transformational theories of leadership is the emotionally appealing aspect of leader behaviour". Moreover, Results is also supported by Charbonneau (2002) and Sivanathan and Fekken (2002) and Goleman and colleagues (2002)'s study that principals who got high emotional intelligence were displayed more transformational leadership behaviors. In contrast, active and passive management and laissez faire management were not associated with emotional intelligence. In other words, the emotional intelligence components and total (TEQ) has significant negative correlation with transactional components and laissez-faire leadership styles. This research finding is supported by Barling and colleagues (2000), "Management-by-exception active and passive and laissez faire management were not associated with emotional intelligence".

Finally, the result of the analysis indicated that the higher level of the principal's emotional intelligence is linked to the principal's transformational leadership style and principals with lower level of emotional intelligence are linked to laissez-faire style of leadership. In other word, a principal who shows a high level of emotional intelligence more like to display positive response to transformational style of leadership. In addition, the emotional intelligence level helps transformational principals to think positive in their attitude which in turn makes them to feel comfortable with their work relationships. Moreover, the good relationship with more experience makes the principals to express their thoughts and ideas clearly.

Table 1: Pearson's correlation coefficient between principal's emotional intelligence (EQi) and different leadership styles

Subscales Emotional intelligence Leadership styles	IP	IAP	SM	AD	GM	TEQ
Idealized Influence (attributed)	.506**	.502**	.557**	.478**	.453**	.670**
Idealized Influence (behavior)	.458**	.439**	.410**	.393**	.386**	.554**
Inspirational Motivation	.435**	.419**	.451**	.412**	.380**	.558**
Intellectual stimulation	.439**	.441**	.473**	.409**	.412**	.580**
Individualized consideration	.442**	.400**	.499**	.438**	.519**	.613**
Transformational Leadership	.585**	.570**	.616**	.547**	.553**	.765**
Contingent reward	-.351**	-.340**	-.204**	-.312**	-.295**	-.397**
Management-by-exception (active)	-.338**	-.313**	-.400**	-.255**	-.365**	-.447**
Management-by-exception (passive)	-.226**	-.197**	-.299**	-.166**	-.235**	-.301**
Transactional leadership	-.445**	-.414**	-.432**	-.358**	-.433**	-.555**
Laissez - Faire	-.264**	-.395**	-.204**	-.207**	-.183**	-.334**

IP=interpersonal, IAP=intrapersonal, SM=stress management, AD=adaptability, GM=general mood, TEQ=Total Emotional Intelligence, **=correlation is significant at .01 level

4. Conclusion

The aim of this study is to determine the relationship between the emotional intelligence level of primary school principals and their different leadership styles. Although it is stated that there is an important relationship between emotional intelligence and leadership behaviors, any research trying to determine this relationship for primary school principals has not been made. Therefore, it is important to determine the relationship between these two variables in schools. Principals are individuals who worked within educational organization and lead teachers. In addition, the principal is typically the person held accountable for all decisions within a school. Moreover, there is relationship between the effective leadership behaviors and academic success of school. Accordingly, success is higher in schools where principal's emotional intelligence is developed. One of the important subjects affected by emotional intelligence is behavioral styles of leaders. According to the results got from this research, as long as the level of primary school principal's emotional intelligence and its sub-scales increase, it becomes an increase tendency also in transformational leadership style. In other word, principals with high level of emotional intelligence would like to behave transformational styles and with low level of emotional intelligence would like to show transactional and laissez – faire styles in their behaviors as a leader of schools. Results of this research is supported by Charbonneau (2002)'s study that principals who scored high on the Bar-on's emotional intelligence were perceived by their subordinates as displaying more transformational leadership behaviors. Consequently, principals working in the schools need the emotional intelligence skills to work more effectively to important knowledge to their teachers as well as to maintain a cordial relationship with others in the school. This gives a training idea about the relevance of emotional intelligence and leadership styles in the schools. Therefore, educational programmers should be thinking about the especial training methods to enhance level of the principals' emotional Intelligence whereby they can improve leadership qualities.

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Synthesis of Ursolic Acid Derivatives and Research on Their Cytotoxic Activities

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Abstract: In order to search for effective hepatic protectant and antineoplastic drugs, methods using ursolic acid as the lead compound, eighteen novel compounds were designed and synthesized by modified at the C-3 and the C-28 positions of ursolic acid (UA). The structures of the derivatives were confirmed by IR, MS, ^{1}HNMR,^{13}C NMR andelemental analysis. Effects of the derivatives on in vitro growth of 3 cell lines (HeLa, $SKOV_3$ and BGC-823) were determined by MTT method. The results show that compound 9and11 has high antineoplastic activity. So the results of this thesis will benefit the further investigating on the modification and anti-tumor activity of pentacyclic triterpenes.
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Keywords: ursolic acid derivatives; hepatoprotective effect; anti-tumor activity

1. Introduction

Ursolic acid (UA) , a pentacyclic triterpenoid which is a member of cyclosqualenoid family, derived from berries, leaves, flowers, and fruits of medicinal plants such as *Etiobotrya japonica*, *Rosmarinus officinalis* and *Glecheoma hederaceae*[1]. Ursolic acid is really a versatile compound which possesses anti-tumor[2,3], anti-inflammatory[4,5], anti-HIV[6] and anti-angiogenic effects[7] in chick chorioallantotic membrane (CAM). Several of these effects of ursolic acid are mediated through suppression of the expression of lipoxygenase ,COX-2, MMP-9[8], and iNOS, some of which are genes regulated by NF-κB[9]. In addition, UA could act on almost all steps in the whole cancer process: initiation, promotion, progression and metastasis.

It was proved that the ester functionality at C-3 or a hydrogen donor group at C-3 and/or C-28 is necessary for the cytotoxic activity[0]. Based on above analysis, eighteen UA derivatives have been designed and synthesized. Most of the derivatives have an ester functionality at C-3. And some of them have been evaluated against three cancer call lines (HeLa, $SKOV_3$ and BGC-823).The results show that compound9and11has antineoplastic activity against HeLa,$SKOV_3$and BGC-823.

2. Experimental

2.1 General

UA (98%) was purchased from China Chengdu Scholar Bio-Tech.Co., Ltd. Unless otherwise indicated other reagents were all analytical grade, purchased from commercial suppliers and used without further purification. The melting points were determined on an electrically heated X-4 digital visual melting point apparatus and are uncorrected. IR spectra were recorded on a ThermoNicolet 470FT spectrometer. ^{1}H-NMR and^{13}C-NMR spectra were measured on Bruker ARX-300MHz spectrometer at room temperature, with TMS as the internal standard. ESI-MS were determined with Thermo-Finnigan LCQ equipment.

2.2 Methyl N-[3β-butyryloxyl-urs-12-en-28-oyl]-2-amino-3-(4`-hydroxy)-phenyl propionate (4)

To a stirred solution of UA(100mg, 0.22mmol) in THF(40mL) and a small amount of DMAP, was added n-butyric anhydride (139.20mg, 4.4mmol) at room temperature. TLC method was used to decide the end of reaction. The crude material was concentrated by vacuum distillation. The solids was added ethanol (3mL) and concentrate the solution, this was done twice. Dissolve the solid in warm methanol then the solution was added ether (6-10mL) to the solution would give a precipitate. The mixture was filtered through Buchner funnel dried at room temperature to

give raw compound 2.

The raw compound 2 was purified by silica gel chromatography with a gradient elution of petroleum ether/ ethyl acetate (6:1, v/v), to yield compound 2 (92.9mg, yield: 80.2%); m.p.183~184°C; ^{1}H NMR(300MHz, $CDCl_3$):δ 5.23 (s, 1H, H-12), 4.50 (1H, t-like, H-3), 2.31~2.26 (m, 2H, -CH_2CO), 2.19 (d, 1H, J=11.4 Hz, H-18), 1.08 (s, 3H, CH3), 0.97~0.92 (s, 9H, CH_3×3), 0.86～0.85 (s, 9H, CH_3×3), 0.78 (s, 3H, CH_3); IR (KBr): 3440, 2927, 1732, 1692, 1460, 1384, 1255, 1186cm-1; ESI-MS m/z: 525.5 (M-H)- Elemental analysis (%, found) C, 77.68 (77.52); H,10.28 (10.33).

Compound 2 (80mg, 0.1519mmol) was dissolved in CH_2Cl_2 (3mL), the oxalyl chloride was added and stirred for 24h. Concentrate the crude material by vacuum distillation, then residue was dissolved in cyclohexane (2mL) to eliminate the unreacted oxalyl chloride. This was done twice to give compound 3. Compound 3 was dissolved in CH_2Cl_2 (2mL) and alkalified to pH 9-10 with Et_3N. The mixture solution was stirred for 5 min and appropriate amino (0.6076mmol) was added at room temperature. The reaction completion was decided by TLC. The mixture was evaporated under reduced pressure to remove CH_2Cl_2, water (3mL) was added and acidified with 2M HCl to pH 3 to doposit the solid. Filtered the mixture, the filter cake was wash by water and dried to give white powder.

Compound 3(80mg ,0.1519mmol) was dissolved in CH_2Cl_2 (2mL) and alkalified to pH 9-10 with Et_3N. The mixture solution was stirred for 5 min and L-tyrosine methyl ester hydrochloride (140.77mg, 0.6076mmol) was added in at room temperature. The reaction completion was decided by TLC. The mixture was evaporated under reduced pressure to remove CH_2Cl_2, water (3mL) was added and acidified with 2M HCl to pH 3 to doposit the solid. Filtered the mixture, the filter cake was wash by water and dried to give white powder.The residue was purified by silica gel chromatography with a gradient elution of petroleum ether/acetone (3:1, v/v), to yield compound 4(43.4mg, yield: 40.6%); m.p.118-121°C; ^{1}H NMR (300MHz, $CDCl_3$):δ6.733~6.966 (m, 4H, Ph-H) ,6.426 (d, 1H, NH), 5.272 (t-like, 1H, H-12), 4.483 (m, 1H, H-3), 4.691 (m, 1H, NHCH), 3.678 (s, 3H, OCH_3), 3.042~3.075 (m, 1H, Ph- CHa) , 2.937~2.970 (m, 1H, Ph- CHb) , 2.289 (s, 3H, CH_3CO), 1.057 (s, 3H, CH_3) , 0.946 (m, 6H, CH_3×2), 0.899 (s, 3H, CH3), 0.840 (m ,9H, CH_3×3), 0.606 (s, 3H, CH_3); ESI-MS m/z: 704.3 (M+H)+. Elemental analysis (%, found) C, 76.37 (76.40); H,9.57 (9.69); N, 2.14 (2.07).

2.3 General procedure for the synthesis of N-[3β-acetoxy-urs-12-en-28-oyl]-amine compounds 5,6,7,8,9

To a stirred solution of UA(200mg, 0.44mmol) in THF(20mL) and a small amount of DMAP was added acetic anhydride (1347.1mg, 13.2mmol) at room temperature. TLC method was used to decide the end of reaction. The crude material was concentrated by vacuum distillation. The solids were dispersed in water, then acidified to pH 3-4 with 2M HCl. The mixture was filtered through Buchner funnel, washed with water to neutrality, and dried at room temperature to give raw compound 2.

The raw compound 2 was purified by silica gel chromatography with a gradient elution of petroleum ether/ ethyl acetate (10:1, v/v), to yield compound 2 (185.4mg, yield: 84.5%); m.p.285~288°C; ^{1}H NMR (300MHz, CDCl3):δ 5.23 (s, 1H, H-12), 4.50 (t, 1H, H-3), 2.20 (d-like, 1H, H-18), 2.04 (s, 3H, CH_3CO), 1.07 (s, 3H, CH_3), 0.95 (s, 6H, CH_3×2), 0.86~0.81 (m, 9H, CH_3×3), 0.76 (s, 3H, CH_3); IR (KBr): 3445, 2926, 1735,1694, 1460, 1383, 1246 cm-1. Elemental analysis (%, found) C, 77.23 (77.06); H,10.11 (11.10).

Compound 3 was reacted with oxalyl chloride following the above steps (see 3.2)

2.3.1N-[3β-acetoxy-urs-12-en-28-oyl]-3-aminopropanol acetate (5)

Compound 3 (80mg , 0.1519mmol) was reacted with 3-aminopropanol (45.63mg, 0.6076mmol) following the method of compound 4. The residue was purified by silica gel chromatography with a gradient elution of petroleum ether/acetone(3:1,v/v), to yield N-[3β-butyryloxyl-urs-12-en-28-oyl]-3-aminopropanol (37.3mg, yield: 42.0%); m.p.103-107°C; ^{1}H NMR (300MHz, $CDCl_3$):δ 6.219 (m, 1H, NH), 5.311 (m, 1H, H-12), 4.500 (t, 1H, H-3), 3.586 (m, 2H, CH_2OH) , 3.523(m, 1H, NHCHa), 3.103 (m, 1H, NHCHb), 2.277 (m, 2H, CH_2CO), 1.090 (s, 3H, CH_3) , 0.944 (s ,9H,

CH_3×3) , 0.864 (m, 9H, CH_3×3); ESI-MS m/z: 584.8 (M+H)+. Elemental analysis (%, found) C, 78.36 (78.43); H,10.80 (10.91); N, 2.29 (2.41).

To a stirred solution of N-[3β-acetoxy-urs-12-en-28-oyl]-3-aminopropanol (102.2mg, 0.1839mmol) in THF(40mL) and a small amount of DMAP, was added acetic anhydride (0.5629g, 5.516mmol) at room temperature following the general steps. The residue was purified by silica gel chromatography with a gradient elution of petroleum ether/ ethyl acetate (6:1, v/v), to yield compound 5 (76.4mg, yield: 69.5%); m.p.83-85°C; ^{1}H NMR(300MHz, $CDCl_3$): δ6.069 (br, 1H, NH), 5.327 (s, 1H, H-12), 4.492 (t-like, 1H, H-3), 4.107(m, 2H, CH_2OCOCH_3), 3.387～3.420 (m, 1H, NHCHa), 3.062～3.115 (m, 1H, NHCHb), 2.071 (s, 3H, $OCOCH_3$), 2.052 (s, 3H, CH_3COO), 1.092 (s, 3H, CH_3), 0.857 (m, 9H, CH_3×3), 0.949 (s, 6H, CH_3×2), 0.766 (s, 3H, CH_3); ESI-MS: 598.9 (M+H)+.

2.3.2N-[3β- acetoxy-urs-12-en-28-oyl]-4'-morpholinopiperidine (6)

Compound 3(100mg,0.20mmol)was reacted with 4-(Piperidin-4-yl)morpholine (102.4mg, 0.6mmol) following the general steps. The residue was purified by silica gel chromatography with a gradient elution of petroleum ether/acetone (10:1, v/v), to yield compound 6 (59.6mg, yield: 45.8%); m.p.120-122°C; ^{1}H NMR(300MHz, $CDCl_3$): δ5.218 (s, 1H, H-12), 4.499(t, 1H, H-3), 3.71 (br, 1H, NHCH), 2.047 (s, 3H, CH_3CO), 1.09 (s, 3H, CH_3), 0.96 (s, 6H, CH_3×2), 0.88～0.86 (m, 9H, CH_3×3), 0.83 (s, 3H, CH_3); ESI-MS: 651.8$(M+H)^+$.

2.3.3N-[3β- acetoxy-urs-12-en-28-oyl]-5'-methyl-2'- thiozolamine (7)

Compound 3 (80mg , 0.1604mmol) was reacted with 2-amino-5-methylthiazole (54.94mg, 0.4812mmol) following the general steps. The residue was purified by silica gel chromatography with a gradient elution of petroleum ether/ ethyl acetate (2:1, v/v), to yield compound 7 (28.8mg, yield: 30.2%); m.p.203-205°C; ^{1}H NMR(300MHz, $CDCl_3$): δ6.840 (s, 1H, NH), 6.840 (s, 1H, Ar-H), 5.228~5.505(m, 1H, H-12), 4.496 (m, 1H, H-3), 2.052 (s, 3H, CH_3CO), 1.083 (s, 3H, CH_3), 0.962 (s, 6H, CH_3×2), 0.870～0.849 (m, 12H, CH_3×4), 0.823 (s, 3H, CH_3); ESI-MS: 595.4$(M+H)^+$.

2.3.4N-[3β- acetoxy-urs-12-en-28-oyl]- 4'-methyl piperazine (8)

Compound 3 (100mg , 0.20mmol) was reacted with N-methyl piperazine (60.10mg, 0.60mmol) following the general steps. The residue was purified by silica gel chromatography with a gradient elution of petroleum ether/ acetone (3:1, v/v), to yield compound 8(51.9mg, yield: 44.7%); m.p.91-93°C; ^{1}H NMR(300MHz, $CDCl_3$): δ5.221 (s, 1H, H-12), 4.497 (t, 1H, H-3), 3.634 (brs, 3H, NCH_3), 2.155～2.399 (br, 8H, N(CH_2×4))， 2.049 (s, 3H, CH_3CO), 1.072 (s, 3H, CH_3), 0.940 (s, 6H, CH_3×2), 0.853 (m, 9H, CH_3×3), 0.745 (s, 3H, CH_3); ESI-MS: 581.8$(M+H)^+$， 582.8$(M+2H)^+$.

2.3.5 N-[3β-acetoxy-urs-12-en-28-oyl]-morpholine (9)

Compound 3 (145mg , 0.29mmol) was reacted with morpholine (69 mg ,0.88 mmol)following the general steps. The residue was purified by silica gel chromatography with a gradient elution of petroleum ether/ acetone (6:1, v/v), to yield compound 9(65 mg, 52.0%): mp 174～177℃; ^{1}H NMR(600MHz, $CDCl_3$): δ5.22 (s, 1H, H-12), 4.51 ~ 4.48 (t-like, 1H, H-3), 3.64 ~ 3.59 (m, 8H, N(CH_2CH_2)2O), 2.04 (s, 3H, CH_3CO), 1.07 (s, 3H, CH_3), 0.98 (s, 3H, CH_3), 0.94(d, 3H, J=6.2 Hz, CH_3), 0.89 (s, 3H, CH_3), 0.87(d, 3H, J=6.2 Hz, CH_3), 0.84 (s, 3H, CH_3), 0.75 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$, 75 MHz): δ175.1(C-28), 170.7 (CH_3COO), 138.3(C-13), 124.8(C-12) 80.6(C-3), 66.6(CH_2OCH_2), 55.0(C-5), 54.6(C-18), 48.2 (CH_2NCH_2), 47.2(C-17), 45.8(C-9), 41.8(C-14), 39.1 (C-8), 38.4(C-19) 38.4(C-20), 37.9(C-4), 37.9(C-1), 37.3(C-10), 36.6(C-22), 32.97(C-7), 30.15(C-21), 28.1 (C-23), 27.8 (C-15), 27.1(C-2), 24.9(C-16), 23.2(C-27), 23.1(C-11), 21.0 (CH_3CO), 20.9(C-30), 17.4(C-6), 17.1(C-29), 16.5 (C-26), 16.4(C-24), 15.2(C-25).IR (KBr): 3431, 2921, 1733, 1637, 1452, 1376, 1248, 1117 cm^{-1}..ESI-MS: 568.5$(M+H)^+$.

2.3.6 N-[3β-propionylox-urs-12-en-28-oyl]-morpholine (10)

3β-propionyloxy-urs-12-ene-28-oic acid was prepared from ursolic acid 1 (50 mg, 0.11 mmol) and propionic anhydride under the similar conditions as preparing Compound 2. The solid was purified by flash column chromatography [petroleum ether/ethyl acetate(6:1)] to give an amorphous solid (19.5 mg, 45.8%).

According to the same method as

compound 9, compound 10 was prepared from 3β-propionyloxy-urs-12-ene-28-oic acid (100 mg, 0.20 mmol) and morpholine (69 mg, 0.88 mmol) through 3-O- propionylursolyl chloride 7. The solid was purified by flash column chromatography [petroleum ether/ ethyl acetate(6:1)] to give 10 as an amorphous solid (36.2mg, 31.1%): mp129 ~ 131℃; ^{1}H NMR(300MHz, $CDCl_3$): δ5.22 (s, 1H, H-12), 4.50(t, 1H, J=7.9Hz, H-3), 3.64 (br, 8H, $N(CH_2CH_2)_2O$), 2.32 (q, 2H, J=5.7Hz, CH_2CO), 1.14 (d, 3H, J=7.6Hz CH_3CH_2COO), 1.08 (s, 3H, CH_3), 0.97(s, 3H, CH_3), 0.94(d, J=6.2Hz, CH_3), 0.89(s, 3H, CH_3), 0.86(d, J=6.2Hz, CH_3), 0.83(s, 3H, CH_3), 0.75(s, 3H, CH_3); ESI-MS: 582.7 (M+H)+.

2.3.7 N-[3β- butyroxy -urs-12-en-28-oyl]-morpholine (11)

Compound 3 (100 mg, 0.19 mmol) reacted with morpholine(66.2 mg, 0.76 mmol) following the general steps. The residue was purified by silica gel chromatography with a gradient elution of petroleum ether/ acetone (6:1, v/v), to yield compound 11(17.1mg, 15.1%): mp243 ~ 257℃; ^{1}H NMR(300MHz, CDCl3): δ5.22 (s, 1H, H-12), 4.50 (t-like, 1H, H-3), 3.64 (br, 8H, $N(CH_2CH_2)_2O$), 2.28(t, 2H, J=7.2Hz, CH_2CO), 1.08 (s, 3H, CH_3), 0.97(s, 3H, CH_3), 0.95(d, J=6.4Hz, CH_3), 0.91(s, 3H, CH_3), 0.89(d, J=6.4Hz, CH_3) 0.86(s, 3H, CH_3), 0.83(s, 3H, CH_3), 0.75 (s, 3H, CH_3); IR(KBr): 3436, 2965, 1731, 1637, 1458, 1384, 1184, 1119cm^{-1} ESI-MS: 596.5(M+H)+.

2.3.8N-[3β-hydroxy-urs-12-en-28-oyl]-morpholine (12*)

A mixture of 9 (40mg, 0.07mmol) and 4 N sodium hydroide (0.2 mL) in a 1:1 mixture of CH_3OH and THF(2 mL) was stirred at 40℃ for 12 h . After concentrated under reduced pressure, the residue was dispersed by water, then filtered and the filter cake was washed with water to pH 7, evaporated in vacuo to give white solid. The solid was purified by flash column chromatography [petroleum ether/ ethyl acetate(6:1)] to give compond 12* as an amorphous solid (34.8 mg, 93.3%): mp 126 ~ 129℃; 1H NMR (600MHz, $CDCl_3$): δ5.22 (s, 1H, H-12), 3.64 ~ 3.59 (m, 8H, $N(CH_2CH_2)_2O$), 3.21 ~ 3.20 (m, 1H, H-3), 1.08 (s, 3H, CH_3), 0.99 (s, 3H, CH_3), 0.95 (d, 3H, J=6.2Hz, CH_3), 0.91 (s, 3H, CH_3), 0.88 (d, 3H, J=6.3Hz, CH_3), 0.78 (s, 3H, CH_3), 0.75 (s, 3H, CH_3); ^{13}C NMR (75MHz, $CDCl_3$): δ175.4 (CONH), 138.6 (C-13), 125.8 (C-12), 79.0 (C-3), 66.8 (CH_2OCH_2); IR(KBr): 3449, 2925, 1628, 1455, 1395, 1116cm^{-1} ESI-MS: 526.4 (M+H)+.

2.3.9N-[3β-ethoxy-urs-12-en-28-oyl]-morpholine (13)

To a solution of comppund 12* (80mg, 0.15mmol) in anhydrous DMF (3 mL) were added sodium hydride(100 mg) and ethyl bromide (0.5 mL) with stirring at room temperature, and the mixture was heated at 70℃ for 3 h. After removal of DMF under reduced pressure, the residue was dispersed by water, then acidified to pH 4 ~ 5 with dilute hydrochloric acid, filtered and the filter cake was washed with water to pH 7, evaporated in vacuo to give white solid. The solid was purified by column chromatography. [petroleum ether/ ethyl acetate(6:1)] to give compound 13 as an amorphous solid (36.5 mg, 43.3%): mp 197 ~ 199℃; ^{13}C NMR (75MHz, $CDCl_3$): δ175.4(C-28), 138.6(C-13), 125.5(C-12), 86.7(C-3), 66.9(CH_2OCH_2), 65.2 (OCH_2CH_3), 55.8(C-5), 54.9 (C-18), 48.5(CH_2NCH_2), 47.6(C-17), 46.1(C-9), 42.1 (C-14), 39.5 (C-8), 38.7(C-19), 38.7(C-20), 37.0(C-4), 37.0(C-1), 34.3(C-10), 34.3(C-22), 33.1(C-7), 30.5 C-21), 29.7(C-23), 28.2(C-15), 27.1(C-2), 23.8(C-16), 23.3(C-27), 23.2(C-11), 21.2 (C-30), 18.3(C-6), 17.4 (C-29), 16.9(C-26), 16.5(CH_3CH_2O), 15.6(C-24), 15.4 (C-25). IR (KBr): 3431, 2923, 1623, 1459, 1387, 1118cm^{-1}; ESI-MS: 630.6(M+H)+.

2.3.10N-[3β-propoxy-urs-12-en-28-oyl]-morpholine (14)

Following the procedure described for compound 13, compound 12* (80mg, 0.15 mmol) and propyl bromide(0.5 mL) led to crude compound 14. The solid was purified by flash column chromato -graphy [petroleum ether/ ethyl acetate(6:1)] to give compund 14 as an amorphous solid (32.1 mg,37.8%): mp 206 ~ 210℃; ^{1}H NMR(300MHz, CDCl3): δ5.22 (s, 1H, H-12), 3.64 (br, 8H, $N(CH_2CH_2)_2O$), 3.64 (br, 1H, $CH_3CH_2CH_aO$), 3.23 ~ 3.20 (m, 1H, $CH_3CH_2CH_bO$), 2.74 ~ 2.72 (m, 1H, H-3), 1.08 (s, 3H, CH_3), 0.98(s, 3H, CH_3), 0.96(d, J=6.4Hz, CH_3), 0.90(s, 3H, CH_3), 0.87(d, J=6.4Hz, CH_3), 0.85(s, 3H, CH_3), 0.83(s, 3H, CH_3), 0.77 (s, 3H, CH_3), 0.69 (s, 3H, CH_3); IR (KBr): 3424, 2925,

1627, 1458, 1384, 1118 cm^{-1}; ESI-MS: 568.5(M+H)+.

2.3.11N-[3β-benzyloxy-urs-12-en-28-oyl]-morpholine (15)

Compound 15 an amorphous solid was prepared from compound 12*(80mg, 0.15 mmol) and benzyl bromide (0.5 mL) under the similar conditions as preparing compound 13 (yield 39.6%):mp 60 ~ 62℃; ^1H NMR(300MHz, $CDCl_3$): δ7.34 ~ 7.29 (m, 5H, Ar-H), 5.22 (s, 1H, H-12), 4.67 (d, 1H, J=11.9Hz, $PhCH_a$-O), 4.43(d, 1H, J=11.9Hz, $PhCH_b$-O), 3.64(br, 8H, $N(CH_2CH_2)_2O$), 2.93 ~ 2.91(m, 1H, H-3), 1.07 (s, 3H, CH_3), 0.99 (s, 3H, CH_3), 0.96(d, 3H, J=6.3Hz, CH_3), 0.93 (s, 3H, CH_3), 0.87(d, 3H, J=6.3Hz, CH_3), 0.84 (s, 3H, CH_3),. ESI-MS: 616.5(M+H)+.

2.3.12N-[3β-benzoyloxy-urs-12-en-28-oyl]-morpholine (16)

To a solution of 12*(80 mg, 0.15 mmol) in THF (4 mL) were added benzoyl chloride (0.05 mL) and a small amount of DMAP, and the mixture was heated at reflux for 3 h. After removal of THF under reduced pressure, the residue was added water(3 mL) and CH_2Cl_2(3 mL),then acidified to pH 8 ~ 9 with 10% aqueous K_2CO_3, standing and the layers were separated. The organic layer was washed with saturated NaCl, dried over MgSO4, and concentrated. The residue was purified by flash column chromatography [petroleum ether/ethyl acetate(8:1)] to give compound 16 as an amorphous solid (16.4 mg, 17.1%):mp 243 ~ 257℃; ^{13}C NMR(75MHz, $CDCl_3$): δ175.4 (CONH), 166.3 (PhCO), 133.5 (C-13), 132.7 (C-4`), 130.9 (C-1`), 129.5 (C-2`, 6`), 128.4, 128.3 (C-3`, 5`), 125.2 (C-12), 81.6 (C-3), 66.8 (CH_2OCH_2); IR(KBr): 3415, 2925, 1717, 1636, 1453, 1392, 1274, 1115, 712cm^{-1}. ESI-MS: 630.6(M+H)+.

2.4 General procedure for the synthesis of 3-Oxo-urs-12-en-28-oic acid ester compounds 18,19,20

To a stirred solution of UA(100mg, 0.22mmol) in DMF(4mL) and a modicum of anhydrous potassium carbonate (60mg), bromoalkane (139.20mg, 4.4mmol) was dropped slowly to this mixture at room temperature. TLC method was used to decide the end of reaction. The crude material was concentrated by vacuum distillation. After dilution with ethyl acetate (4mL×3), the mixture was washed with saturated NaCl solution (2mL). The organic phase was dried over anhydrous magnesium sulfate. Filtration and evaporation of solvent at reduced pressure gave compound17. A solution of pyridinium chlorochromate (PCC, 142mg, 0,6639mmol) in CH_2Cl_2 was added dropwise to compound 17 in CH_2Cl_2 and refluxed for 2h. Filtered it through Buchner filter by using filter paper and silica gel. The filter cake was wash by water and ethyl acetate (7mL×3). The organic phase was dried over anhydrous magnesium sulfate. Filtration and evaporation of solvent an reduced pressure gave a while solid, which was purified by silica gel chromatography.

2.4.1 3-Oxo-urs-12-en-28-oic acid propyl ester (18)

UA (100mg, 0.22mol) was reacted with bromopropane (108.23mg, 0.88mmol) following the general steps to give compound 17 (93.0mg, yield:84. 9%), ^1H NMR(300MHz, $CDCl_3$): δ 5.302 (s, 1H, H-12), 3.947 (t, 2H, $OCH_2C_2H_5$)，3.200 (brs, 1H, H-3)，2.20 (d, 1H, H-18), 1.075 (s, 6H, CH_3×2), 0.987 (s, 3H, CH_3), 0.943 (s, 3H, CH_3), 0.914 (d, 3H, CH_3), 0.864 (d, 3H, CH_3), 0.777 (s, 3H, CH_3). Compound 17 (110.2mg, 0.2213mmol) was reacted with PCC (142mg, 0.6639mmol) following the general steps. The residue was purified by silica gel chromatography with a gradient elution of petroleum ether/ ethyl acetate (24:1, v/v), to yield compound 18 (91.8mg, yield: 82.8%); m.p.105-107°C; ^1H NMR(300MHz, $CDCl_3$): δ 5.260 (s, 1H, H-12), 3.951 (t, 2H, $OCH_2C_2H_5$)，2.510 (m, 1H, H-2a), 2.400 (m, 1H, H-2b), 2.20 (d, 1H, H-18), 1.080 (s, 6H, CH_3×2), 1.038 (d, 3H, CH_3), 0.943 (s, 3H, CH_3), 0.938 (s, 3H, CH_3), 0.867 (d, 3H, CH_3), 0.802 (s, 3H, CH_3).

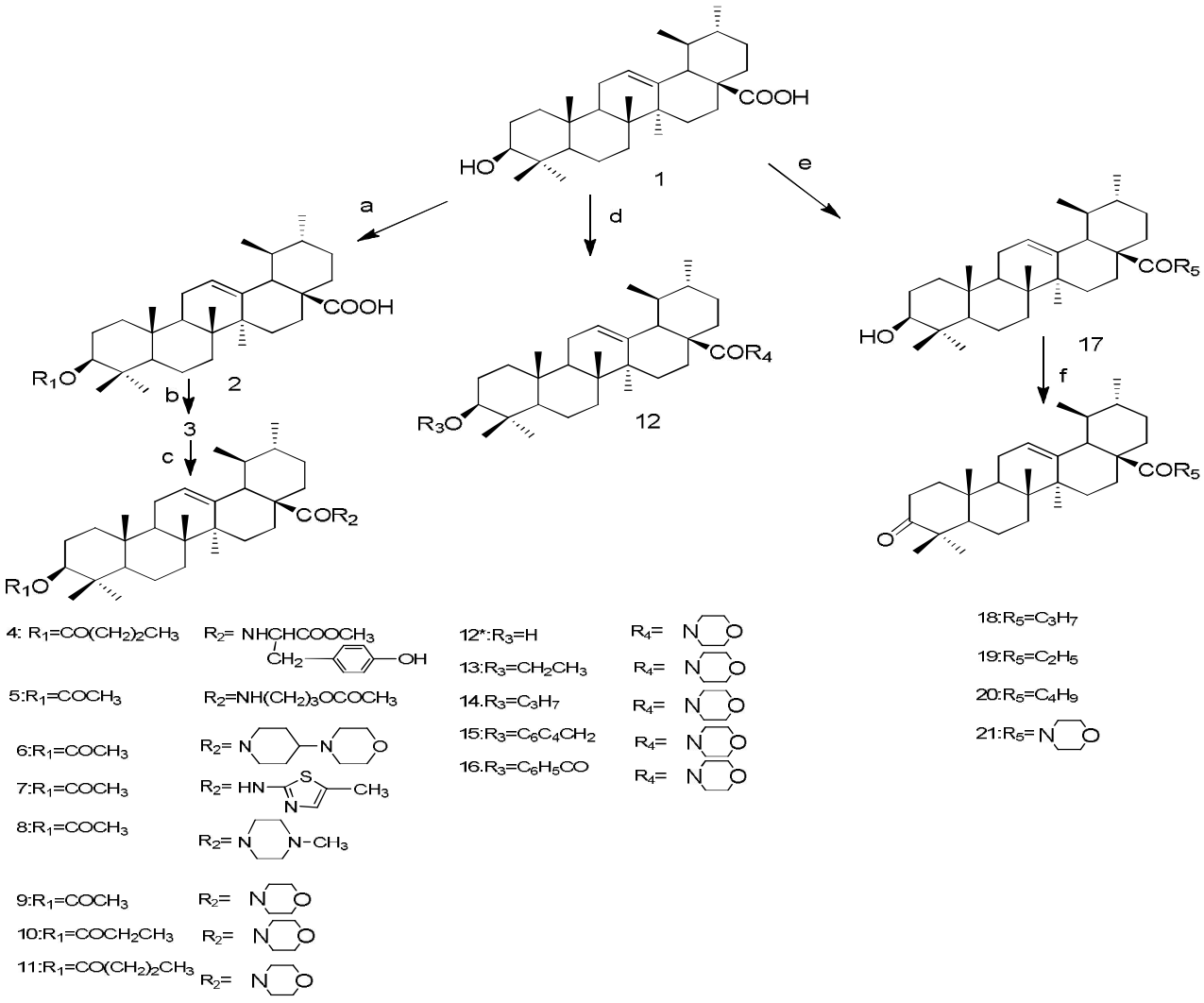
2.4.2. 3-Oxo-urs-12-en-28-oic acid ethyl ester (19)

UA (100mg, 0.22mmol)was reacted with bromoethane (95.9mg, 0.88mmol) following the general steps to give compound 17 (85.8mg, yield: 80.56%), IR (KBr): 3446, 2927, 2871，1724,1456, 1384, 1230 cm^{-1}. Compound 17 (102.9mg, 0.2126mmol) was reacted with PCC (137.5mg, 0.6378mmol) following the general steps. The residue was purified by silica gel chromatography with a gradient elution of petroleum ether/ ethyl acetate (25:1, v/v), to yield compound 19 (77.08mg, yield: 75.1%); m.p.89-89°C; IR(KBr):2975, 2924, 1705, 1454, 1384 cm^{-1}.

2.4.3 3-Oxo-urs-12-en-28-oic acid butyl ester (20)

UA (200mg, 0.44mmol) was reacted

with n-butyl bromide (241.2mg, 1.76mmol) to give compound 17 (193.6mg, yield:85.8%), IR (KBr): 3550, 2928, 1717, 1459, 1382, 1200, 1182cm^{-1}.Compound 17 (154.3mg, 0.3009mmol) was reacted with PCC (259.4mg, 1.2036mmol) following the general steps. The residue was purified by silica gel chromatography with a gradient elution of petroleum ether/ ethyl acetate (20:1, v/v), to yield compound20 (102.8mg, yield: 66.8%); m.p.139-140°C; IR(KBr): 2929, 1720, 1457, 1382, 1271 cm^{-1}.



Scheme 1. Synthesis of Ursolic acid derivatives

2.4.4 N-[3- Oxo-urs-12-en-28-oyl]- morpholine (21)

To a solution of 12*(44 mg, 0.084 mmol) in THF (2 mL) and CH_2Cl_2 (15 mL)were added celite (150 mg),PDC (99.1 mg, 0.336 mmol). The mixture was stirred at room temperature for 2h,filtered and the filter cake was washed with CH_2Cl_2 (2 ×5 mL), then evaporated in vacuo to give white solid. The solid was purified by flash column chromatography [petroleum ether/ ethyl acetate(4:1)] to give compound 21 as an amorphous solid (6.7 mg, 15.2%): mp 106～108℃; ^{1}H NMR(300MHz, CDCl3): δ5.25 (s,1H, H-12), 3.64 (br 8H, NH(CH_2CH_2)$_2$O), 1.09(s, 3H, CH_3), 1.06(s, 3H, CH_3),1.04(s, 3H, CH_3), 1.00(d, 3H, J=6.3, CH_3), 0.95 (s, 3H, CH_3), 0.87 (d, 3H, J=6.3, CH_3), 0.81 (s, 3H, CH_3); ESI-MS: 524.5 (M+H)+.

3. Results and discussion

3.1 Synthesis of UA derivatives

The paper focused on ursolic acid as a lead compound, 18 derivatives were designed and synthesized by three routes. These structure modification in the three routes were all done at the positons C-3 and C-28.

Compound 2 was synthesized through reaction of UA with n-butyric anhydride, acetic anhydride or propionic anhydride in the presence of DMAP; Afterward, oxalyl chloride was added to a solution of compound 2 in methylene dichloride to give compound 3. Then compound 3 was treated with corresponding amines in the presence of triethylamine to give compound 4-11. Ethers **13-15** prepared from **12*** with ethyl bromide, propyl bromide and benzyl bromide in presence of sodium hydride in anhydrous DMF, respectively. Similarly, compound **16** was synthesized from **12*** with benzoyl chloride by using the same method of preparing compound **2**.UA was reacted with appropriate bromoalkane or morpholine in the presence of potassium carbonate in DMF then these compounds were oxidized by PCC or PDC, to give compounds 18-21. (Scheme 1)

Reagents and conditions: (a) $(CH_3CH_2CH_2CO)_2O$, $(C_3H_7O)_2O$ or Ac_2O, DMAP, THF, r.t.; (b) CH_2Cl_2, $(COCl)_2$, r.t.; (c) Et_3N, HR_2, r.t.; (d) C_2H_5Br or C_3H_7Br or $C_6H_5CH_2Br$ or PhCOCl; (e) R_5Br, K_2CO_3, DMF; (f)PCC, CH_2Cl_2.

All the target compounds were purified by a silica gel column with petroleum ether/ethyl acetate or petroleum ether/acetone as eluents. Their structures were characterized by means of mp, IR, MS, ^{1}HNMR, ^{13}CNMR and elemental analysis.

3.2 Bioactivity

Some of antitumor activities of compounds were evaluated in vitro using the MTT method against HeLa, BGC-823 and SKOV3 cell lines with UA as the positive control. The results are summarized in Table 1.

Table 1. Inhibitory activity of ursolic acid and its derivatives on different cancer cell proliferation

Comp.	$IC_{50}(\mu mol \cdot L^{-1})$[a]		
	HeLa	SKOV3	BGC-823
1	>10	>10	>10
4	>100	>100	>100
5	>10	>10	9.20±
6	>100	>100	>100
7	>100	>100	>100
8	>10	>10	>10
9	2.16±0.26	nt[b]	>10
10	>10	>10	>10
11	>10	>10	9.7±1.01
12*	>10	nt	nt
13	>10	>10	nt
14	>10	>10	>10
15	>10	>10	cell growth
16	>10	>10	nt
18	nt	nt	nt
19	nt	nt	nt
20	nt	nt	nt
21	>10	>10	nt
VP-16[c]	2.37±0.99	5.62±2.11	5.01±2.85

[a] The agent concentration that inhibited HeLa cells growth by 50%;
[b] Not tested;
[c] Etoposide.

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Tourism and its history

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Abstract: Tourism has many definitions that most of them are surveyed in economical aspect. Tourism is described as a social activity that includes human behavior, using resources, interaction with other people, economic and environment. Tourism industry is one of important phenomenon in three last millennium, in half past century with increasing growth has more influences in economical dynamism and growth and cultural interactions between countries. As, most of scientists know this century as tourism century. In last year's, tourism is known as a industry without smoke and more income in universal business and also important element in improving and adjusting commerce balance and payment balance in most of countries. This industry is considered as a unique industry and has more fans. considerable growth in tourism in fifty last year, shows abundant importance of economical and social aspect of this phenomenon.so in this research, in addition to defining tourism basics and different definitions, we say symbols of this industry to recognizing different types and knowing existent capacities in this industry, use this without smoke industry optimally. Method of this research is library studies.
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Keywords: Tourism, Development, Approach

1. Introduction

Travel from one place to another place is a problem that always is considered by people. Although tourism is affected by different agents such as security, facilities and different journey incentives. This phenomenon has been existed between people. Tourism theory results from differences in social structure and if there are two different things in where ever, undoubtedly understanding will be created for other kind. So, these attractions are incentives for journey and movement. If we want to know tourism role in economical and cultural development in country, we should have previous recognition from international tourism industry and methods. These recognitions are generalized to some extent and are accepted by most of people. Although theorists such as "john lee" changed some of these viewpoints (Law, C. M. 1992).

1.1. Tourism definition:

Tourism is introduced for the first time in 1811 Gregorian year in English magazine (sporting magazine). tourism consists of two parts : " tour " word means circulating, journey, travel and trip and suffix " ism" that points to literal, religious and philosophical thought or institution. So, tourism means an institution that it's thinking base rely on travel and journey. In Webster dictionary, tourism is a travel that is done to a destination and then return to his / her residential place. (Mowforth, M. and Munt, I. (2003). in Longman dictionary, tourism means recreation and journey for entertainment. In larros dictionary, tourism means journey for recreation (pleasure or satisfaction). Greece stem of this word is "tour nest ". That has been entered from Greece to Spain, then to France and then entered to English. in fourteenth Gregorian century, " tour " word means " shift " or " service duration ", in fifteenth century means " circulating movement " and in seventeenth century means " take a trip to around " and was used as " tourist " and " tourism" in nineteenth and eighteenth century. Tourism not only in English and French languages, but also in most of live languages in world has similar conception with a little difference. in French language, tour in according to shift, means " movement ", " journey " and " circulation ". This circulation can be around world or around an axis. (Oppermann, M. and Chon, K. S. (1997)). This word has stem in Latin word (turns) that means circulating, going and coming between destination and starting point that has been entered from Greek to Spanish and French and at last has been entered to English. Also, tourism word and "tourism "and "tourist" expressions were used by nations society for the first time in 1937. in Persian words dictionary, tourism is defined as below : in different points of world, traveling and recognition, journey is done for recreation and amusement and a trip that traveler go to destination and then return to his or her residential place. In first definitions, has been emphasized on distance dimension and travelers are categorized according to their distance from their residential location. American tourism national commission (1973) in tourism definition inside of country considers fifty miles that consist of all of journeys except travel for working. (Pearce, D. G. (1979)). First writings about tourism concentrate on

historical dimensions of this phenomenon in tourism and journey encyclopedia. By passing times, usual and specialized definitions of tourism have been changed. (SETE (1993)). Although first definitions emphasize on tourism as an economical producer, last definitions cover complete and extended concept. Probably, new viewpoints are created because of human knowledge increase in tourism dimensions; because it has been proved that tourism is multi dimension and so complex phenomenon. (Poria Yaniv.et.al.2003). In 1993 march, United Nations statistics commission accepted presented definition by tourism world organization. According to this definition, tourism means: set of personal activities that travel to places out of their living and working place for recreation and amusement and doing other activities and don't remain in that place more than one year. This definition considers tourism demand. Definitions that consider tourism presentation, concentrate on presented products and services. Although definitions related to demand dimension rely on tourist behavior and what they want and search (Harry G. Matthews. 1991). in other definition of tourism world organization, defined tourism as below : tourism is activities that a person do in trip and in a location out of his or her ordinary place in a condition that this travel isn't more than one year and is done with recreation, rest, sport, visiting relatives and families, working, mission, participating in seminars and conferences, treatment, studying and research, religious activities, business or these kinds of activities. According to Murphy, one of well-known definitions is a definition that presented by United Nations organization conference about travel and tourism in 1963 and then accepted by travel formal international syndicate that was the base for tourism world meeting. In other definition, tourism is a provisional movement to a location out of person's living or working place. As travelers do some activities in that duration in destination and special facilities are provided for their needs. Holden describes tourism as social activity that consist of human behavior, using resources, interaction with other people, economic and environment. This definition can be known as conceptual development in using natural resources and interaction with environment. morgen rout relies on tourist consumer role in tourism definition and he believes tourism is a travel that people far from their living place provisionally to prepare their living, cultural and personal needs as cultural and economical services and goods consumer. (rezvani, 1374). In geographical dimension, tourism is spending holidays or recreations that need night absent in ordinary residential location. (skinner, 1999 ; 280) tourism in social dimension can convert world to worldwide village according to extensive cultural and economical communications. In addition to society's national awareness, can provide suitable base about living style, beliefs and customs for linking and strengthening of people and their more correlation. According to social dimension, tourism definition is a common season between native habitants ordinary living and unusual tourism living. koltmann in his definition concentrates on distance dimension and economical aspects. he defines tourism a short-term trip that begin from one point and then return to that point and visit different places in travel duration according to special program and tourist spend more exchange costs (Michael, 1989). or in another effort, by considering experiment dimension and conscious selection and according to authority, tourism is provisional and mandatory travel that is shaped for using novel views and new experiments in partly long journey(according to distance dimension). Also, tourism is activities that are hold in attracting and lodger process between tourists, travel agencies, starting point countries, host government and local people. in sociology viewpoint, tourism (circulating, traveling, journeys, and tourism) is a ancient phenomenon and has more importance in new shapes and dimensions and in economical and social and improving cultures. This definition shows that tourist is a person that visits a country except his or her residential countries, in every reason despite working that is paid for it. A visitor is a tourist that resides in a night or a passenger does his visit in daytimes. But this definition has a defect and this defect is inattentive to internal tourists that have big volume of tourists. So, technical and correct definition is a definition that is presented by Britain tourism meeting: "tourism is a provisional and short term movement to places to out of locations that reside or work ordinary and consist of activities that do in their residential duration and also consist of every movement such as daily visits and journeys. the other definitions, tourism is translated and its Arabic meaning is journey and spending persons holidays in a place except permanent residential location for pleasuring. Journey in far pasts (or in underdeveloped countries) is considered as luxurious activity and is done with traditional methods by amateur people.

In tourism definition, all of agents that affect tourism should be considered. These agents are:

A) Tourist
B) Services suppliers and goods that are needed for tourism.
C) Government: governmental politics about tourism.
D) Host society: dictionaries and incentives, tourism attractions.

According to different views and different definitions about tourism can find two viewpoints between these definitions and also, extract two dimensions, so tourism has two main economical and social dimensions. Its economical effects can be seen in national and international level in gaining exchange income, balancing income promotion, attracting funds and using it in healthy financing, creating jobs and using dull income and preventing from exchange exit. In new notion ism for tourism, two main thinking are created: first, a thinking that is called political and economical thinking. This thinking viewpoint relies on this hypothesis that tourism has close relations with historical and cultural backgrounds and economical dependencies and colonialism patterns. The other thinking corresponds to economical aspects of tourism. That is resulted from application processes. In this thinking emphasis is on economical importance of tourism industry for all of people and beneficiaries countries and efficiencies improving methods and minimizing its losses effects. This application viewpoint is optimistic and believes that most of economical problems can be solved by good management and suitable criterions.

2.1. Political and economical approach:

According to this approach, creating tourism industry is influenced by determined political and economical agents and the other aspects don't have more importance. Among these cases can pointed to facilities varieties in destinations, different kinds of holidays or even tourist feeling about journey experience. Total political and economical analyses about tourism effects are with negative viewpoint. Despite of considerable economical benefits for poor countries cause poor stability and inequality.

3.1. Application approach:

An analyzed and extended method in tourism that approved in economical aspects, consist of tourism divided to main element or three stages:

One stage is dynamic that consist of movement to destination and return from it. Second stage is static that consist of residential and third stage explain basic economical and physical and social and cultural effects on environment. These cases are surveyed by alister matison and jefry wall as a complex of related parts in tourism. although its economical values have feedback links in all of system and this shows that tourism is a system or in other words tourism consist of balanced or related that this interaction give totality to tourism subject and also give them hierarchy.

4.1. Tourism history:

People journeys in far pasts was done for obtaining food and prevent from danger and etc that they went to places with suitable conditions and weather that gradually business incentives and goods exchange were added to above reasons and by extending ancient kingdom territory, governmental formal trips were begun. In government duration, kingdom families in Egypt, journeys were done by recreation and business incentives. Tourism history in Europe corresponded to pilgrims trips in middle centuries. contebory great church pilgrims in England knew their trips as a religious trips and also knew it as a experiment in their weekend. pilgrims began activities that can see them today, such as : souvenirs from journeys, accounts in other countries banks (that in middle centuries lambards and Jacobs international networks were their founders) and also using different kinds of shipping (such as Santiago and compestella shrine pilgrims used England vine ships in middle centuries to transform vine to vigo port in Spain.) Also, pilgrims have important place in tourism industry. In sixteenth century in England, holding great tours was so prevalent. Eighteenth century was the gold era for great tours. Noblemen and gentlemen were sent to around Europe tours for achieving experiments. In west and especially among Muslims, pilgrimage tourism has important place and in the beginning of Islam, Muslims went to Hajj. Ancient Greece did great considerable activities for extending journeys and tourism. in that time, journeys were done through sea channels and people benefit from journeys and visiting ancient cities and ancient celebrations and Olympic matches, as in 170 Gregorian year (guest director) book was issued that consisted of ten volumes. In roman kingdom, journeys and tourism domain had great extension. For example, Romans went to journeys for participating in sport or religious or visiting new and historical places in Egypt and Greece or for shopping, Egypt converted to a place for selling and buying and recreation place for gentlemen. Also, in ancient china and Japan, gentlemen and their guests had trips to good weather regions and other visual places for recreation in summer. In middle centuries era, (Gregorian fifth to fourteenth centuries) business and journeys were unimportant and albeit journeys were done by permissions from Christ church for pilgrimage. In the end of that era, journeys converted to an organized and dense phenomenon, as the great networks of charity organizations and high classes in society persuade this kind of journeys. These kinds of solely religious journeys found social and recreational identity and etc. in that time, in second half of Gregorian thirteenth century, marc polo wrote a book about his trip to Asia and this book is considered as the most important informational reference for west about living that time east living. in 1975 Gregorian year "serjan medvil wrote journey book about journey explanation to near and

far points of world and translated to several languages. First tourists (pilgrims caravan) that was social, was done in fifteenth century and someone were responsible for these organized social journeys by accepting complete services for Jerusalem pilgrimage. In renaissance era (fourteenth to seventeenth century), most of journeys was done with gaining knowledge and experiments. For the first time, journey permission was issued in first Elizabeth era (England princess) that had three years credibility. In that period, journey caravan were created with organized structure and was named "grand tour that tourists had journeys for achieving knowledge, new experiments and familiarity with different customs with those tours. As tomas fognet in 1778 Gregorian year issued trip director book (grand tour) and was considered as the most expensive book in its duration. In industrial revolution (1750 – 1850) social recreations was created. Deep social and economical changes in that era, cause social changes and changes in jobs and extending middle class in society. And this class had more situations for journey and recreation. Usually these journeys were done in weekends. In the end of nineteenth century workers had yearly holidays and spend their holidays near seas. In that era, some of eras that was especially for wealthy class recreations, were extended and new installments were created and prepared essential space for journeys and tourism for other classes. Our land Iran, with ancient and stable culture and consisted of different tribes and nations and different weathers and also having historical and cultural and natural attractions is ready for this activity.

As before revolution dawn, in Iran, different cultures with unique characteristics were created.as west historians said, governors had lessons that should learn from them. These characteristics, converted Iran to one of attractions between world nations for internal and external tourist. Because of great historical and cultural and natural endowments, this country has been noted by alien tourists. According to historians such as gezanfono herodet, Greeks and Romans that had journeys for business and trips to Iran and had interactions and communications with Iranians. after Islam advent, famous tourist such as *ebnehoghel, yaghot hamavi, moqadasi, ebne batote, abodalf,* had journeys to Iran and wrote their different visits from Iran and other countries in famous books such as *soratolarz, ahsanoltaghasim fe marefatealeghlim, majomlbadlan and etc.* European tourist and non Muslims that had trips to Iran were *benyamin todelay, marc polo, o driks, joseph barbaro, pitter dolavaleh, zan sharden, engilber tekempefer, taverneih, antonne* sherly, madam *div lofoa, henry rener dalmani, pier loti, ozen flanden, lorens kel hart, goerge and etal.* new technology such as airplane lines, computers and satellite communications and etc cause living, working, games and recreations methods change in contemporary era. Because of different reasons technology progress causes journeys and tourism increase between different classes in society. Because of this phenomenon could increase promotion time and increase incomes, reinforce communications and present effective methods of transportations. As tourism and journeys are considered as one of great resources of income. It is accepted that tourism industry consists of extensive activities. Tourism consists of parts and subparts like transportations, attractions, reception and rest places, hand artifacts and other similar activities. These parts are main supporters of product and services for people and tourism groups that leave their homes for spending holidays and weekends. This phenomenon is considered as the biggest cultural and social and environmental activity that prepares international tourists familiarity background with different cultural experiments. tourism consist of activities that have effects in production, marketing and consuming sections and abundant products such as automobile rent, hotel, artifacts and etc. Today, new jobs are considered as the biggest result of tourism industry. New jobs candidates need new skills and educations. Providing education costs and tourist projects need approved budget.

5.1. Different kinds of tourism:

Tourism have different and extensive kinds and can divide according to different agents that we point to some ofthem, in below :

Vans smith named six different tourism kinds as below:

1. Ethnic tourism: These kinds of tourism is done for surveying native people living method. ethnic tourism purpose recognizing different ethnics and participating in their experiments.
2. Artistic tourism: artistic tourism wants to recognize other nation's arts and awareness of them.
3. Historical tourism: this kind of tourism is called legacy tourism and visit museums and places and historical constructions and today, and allocated most part of tourism to itself.
4. Nature- oriented tourism: this kind of tourism emphasize on natural and environmental attractions and going to beautiful nature and pleasuring from natural attractions.
5. Recreational tourism : this kind of tourism rely on participating in sport activities, using hot mineral water streams, sun bath, and also resting in a quiet and beautiful place.

6. Working tourism: main purpose of this kind of tourism isn't resting or recreational activities. But doing part of work and journey. Specification of this kind of tourism is participating in conferences, meetings or scientific, research and specialized seminars.

Kinds of tourism are according to below:

1. Recreational tourism
2. Treatment tourism
3. Cultural tourism
4. Social tourism
5. Sport tourism
6. Religious and pilgrimage tourism
7. Commercial and business tourism
8. Political tourism

The other kinds of tourism can be divided according to place: a) tourism according to destination: 1. Rural tourism 2. Urban tourism. 3. Tribe and ethnic tourism 4. Tourism in nature 5. Coastal and sea tourism 6. Mountain tourism 7. Space and air tourism 8. Environmental tourism (forest, mountain, desert, depth of sea, agricultural places, caves and etc) 9. Electronic tourism 10. War tourism. Similarity between all of tourisms is human agent that leave their main living place to create variety and progress in other place.

2. Discussions

Tourism progress and develop day by day and in every year, millions of people and tourism with different customs and preferences trip from one place to place in world and affect universal economic completely and cause to be an important resource for economic development and financial balance in one hand and by creating different jobs and improving income level of different classes of social life, affect tourist countries on the other hand. Economical, social, cultural and ecologic influences and creating different jobs is the most important income of tourism extension in world. In a realistic viewpoint, its role in development and growth of a country is considerable and have undeniable role in improving living level quality and affect their economic and culture. Tourism results are so complex and sometimes inconsistent and symbolize in different dimensions unexpectedly. So, different dimensions identity and aspects should be analyzed. Although we can't deny positive aspects of tourism industry, it may be a tool for economical, human growth, peace promotion and international responsiveness between different ethnics.

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HIV/AIDS, Women Farmers, and Livelihood Activities: A Comparative Analysis of Productivity in Prevalent And Non-Prevalent Areas of Benue State, Nigeria

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Abstract: The study was carried out in Benue State of Nigeria by the use of questionnaires administered to 200 women farmers in HIV prevalent and non prevalent areas. Frequencies and percentages were used to explain descriptive data while t-test was used to analyse the inferential statistics. The awareness and knowledge of HIV among women farmers was average, and there was no behavioural change towards sex as almost all respondents shy away from the use of condoms. There is significant difference in farm size in the prevalent areas ($t = 2.982$ $P < 0.05$) as compared to non prevalent areas. The non-prevalent areas had a higher mean score than the prevalent areas. The results obtained from the study showed that there was a general decrease of agricultural productivity in HIV prevalent areas than non-prevalent areas. In conclusion, the two study areas, which represent the prevalent and non-prevalent areas, HIV was established to be high in both transmission and prevalence. Over 70% of the respondents are sexually active, the study indicates that the rate of promiscuity is high and among the respondents as unmarried residents has had at least 3 sexual partners. The knowledge and use of condoms is low. Only 7% and 6% of respondents in non-prevalent and prevalent areas have used condoms. The epidemic has severely affected and worsens the economic situation within the respondents, and has therefore placed demands on women who are both agricultural workers and caretakers.

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Introduction

Agriculture is the largest sector in most African economies of which women are active participants. With the tripartite burden of child bearing, domestic chores and agricultural activities, women are sandwiched between several constraints in the production process, this include official underestimation of female agricultural labour, lack of inputs, lack of mobility and time, lack of healthy and living conditions, lack of health education and access to affordable health care facilities. Benue state, a state generally acclaimed by observers as Nigeria's 'Food Basket', is involved in the production of crops such as Yams, Cassava, Soya beans, Maize, Rice, Millet and Groundnut; livestock and fisheries. NAERLS and PCU (2001) estimate the land area ('000ha) devoted to yam to be (224.05), Rice (135.86), Soybean (82.00), Cowpea (26.40), Sorghum (110.9), while production estimates ('000 metric tonnes) for yam is (2789.4), Cassava (3554.35), Soybean (159.90), Maize (135.16), Rice (271.71), Millet (64.37) and G/nut (343.68) respectively.

Agricultural commodity prices (N/kg) for yam tuber (58.34), Maize (57.61), Rice (63.78), Soybean (51.43), Groundnut (56.67), Fish (277.5). The state will soon lose the name 'Food Basket 'because of the presence of HIV/AIDS, which is spreading fast in the state and affecting farmer's productivity. According to FMH (2001), Benue state has the highest HIV prevalence rate in the Country (13.5%). Women are agricultural workers, and account for a large portion of agricultural production in the state. They are actively involved in agriculture activities such as planting, weeding, harvesting and processing. The scourge of HIV/ AIDS is threatening this farming activity, which is very rampant in this part of the country. HIV/AIDS will have adverse effects on agriculture, including loss of labour supply and remittance income. The loss of farm workers at the crucial periods of planting and harvesting can significantly reduce the size of harvests. Individuals and their families feel the economic effects of HIV/AIDS first. The household impacts begin as soon as a member of the house starts to suffer from HIV related illnesses. Ayoola (1992) states the problem in this way: The decline in population has resulted in loss of labour force affecting especially agricultural based economies in areas of crops and livestock production. This illness also result in family members of the sick spending more time taking care of the sick which could have been spent in other more production activities. The finances of such families are also hard-hit, resulting not only from reduced productivity but also from money spent on medical bills taking care of the sick, which could have been re-invested into agricultural income generating activities. While it is logical that effect of HIV is negative on farmer productivity, there is paucity of empirical data. Hence, it would be important that this study proffer answers to the questions of

productivity, knowledge, perception of risks, preventive behavioural pattern, on HIV/AIDS by women farmers in the study areas. General objective of this study is to compare women farmers' productivity in HIV prevalent and non-prevalent areas of Benue state.

Materials and Methods

The study area is Benue state of Nigeria, which was created in 1976. The population of the state according to 1991 census is 2, 753, and 077 people: and it is located on longitudes 7.56 degree and 10.00 degree East and latitude 6.37 degree and 8.11 degree North. It is bounded in the North and West by Nassarawa and Taraba states respectively. In the East and South East by Kogi and Enugu states, and in the South by Ebonyi and cross River States. The mean annual rainfall is 2000 mm with its peaks during the months of June and September. There are two major tribes namely Idoma and Tiv. The people are predominantly farmers and are engaged in the production of crops such as Yams, rice, soybeans, maize, cassava pepper and millet. They are also engaged in livestock production such as sheep, goats and poultry. FMH (2001) report showed that Benue state has the highest HIV prevalence in the country. The target population of the study is all women farmers in Benue state.

Benue state of Nigeria was chosen for this study because of the prevalence of HIV in the State. The state was divided into prevalent and non-prevalent areas. Based on FMH (2001), the prevalent areas are Gboko, Otukpo and I high while the non-prevalent areas are Oju, Aliade and Apa. The prevalent area has 75 villages with Gboko having 25 villages, Otukpo 30 and Ihugh 20 respectively. For the prevalent areas, three villages were selected randomly from Gboko, Otukpo and 2 from Ihugh to give eight villages. The non-prevalent areas have 35 villages in Apa, 15 in Aliade and 20 in Oju to give 70 villages. Two villages from Oju, two from Aliade and four from Apa were randomly selected to give eight villages. Ten Percent of women from the selected villages of prevalent and non-prevalent areas were selected and interviewed. The instruments for collecting data for the study were questionnaires. They were highly structured to make responding to the questions less cumbersome for both interviewers and the respondents. Two hundred respondents were interviewed. The researcher drew up interview schedules and four field's enumerators who were trained in interview techniques; and had to translate questions into the local languages administered them. Data from the respondents were subjected to both descriptive statistics such as frequencies and percentages were used to describe variables and their occurrence in the population for data organization and presentation.

Results and Discussion

The result on the demographic characteristics of women studied shows that majority are below thirty years (44% from non-prevalent areas and 41% from prevalent areas); married (64% in non-prevalent and 32% in prevalent areas). In addition, majority are Christians (96% in non-prevalent areas and 98% in prevalent areas) cultivate farmlands that are between 1-5 plots (58% for both non-prevalent and prevalent areas) and without formal education (59% in non-prevalent area 50% in prevalent areas).

Majority of women farmers (97% in non-prevalent areas and 93% of respondents in prevalent areas) have heard about diseases that can be transmitted through sexual intercourse. A level of some awareness has been created on AIDS. This is probably due to the facts that AIDs is one of the most discussed issues today. The radio is still the most effective means of communicating messages on HIV/AIDS. Majority of respondents (97%) in the non prevalent areas admitted to have heard about AIDS campaign through the radio, 3% did not respond; while in the prevalent areas, 88% said their awareness was through the radio, 2% did not respond. This high awareness is because several farmers own the transistor radio. Among the respondents, 3% in the non prevalent areas have heard about AIDS through television, 97% have not heard through the television while in prevalent areas 18% respondents have heard through television and 82% admitted not to have heard about AIDs on television. The large proportion who has not heard through T.V may be because most rural areas are without electricity.

Only few respondents (3%) in the non-prevalent areas agreed to have heard about AIDs from health worker, as much as 97% did not while 12% of respondents in the prevalent areas admitted to have heard about it through health workers, only 88% did not. Most respondents did not hear from health workers because they did not associate with community health centers because of lack of drugs and qualified staff. While more than half (85%) of respondents in non-prevalent areas did not respond, only 15% heard about AIDS through school/teachers. In the prevalent areas, 83% did not respond, 17% heard about AIDs through school. The little awareness is because school enrollment is very low, particularly among girls who receive limited education. While 59% of respondents in the non-prevalent areas did not respond, 57 percent of respondents in prevalent areas did not respond. The number of respondents who first heard about AIDS

through friends/relatives was 41% in non-prevalent areas, while it was 43% in the prevalent areas.

Very few women farmers heard about AIDS in the church as most of them could have heard from other sources before. Those who heard about AIDS in the church could probably be during funeral church services for victims; this accounts for 9% in the non-prevalent areas and 19% in the prevalent areas. Ninety one percent of respondents in the non-prevalent areas did not respond. Likewise, 81% in the prevalent areas did not respond. Very few respondents have heard about AIDS in their work place. This accounts for 17% of respondents in the non-prevalent areas and 10% in prevalent areas.

The result on mode of transmission of HIV/AIDs among the respondents shows that almost all the respondents (91%) in the non-prevalent areas agreed that AIDS can be contacted through sexual intercourse, 9% did not respond. In the prevalent areas, 88% of respondents admitted that AIDs could be contacted through sexual intercourse, only 12% did not respond. Half (51%) of respondents in the non prevalent areas have never used condoms during sexual intercourse, 6% have used condoms, 43% did not respond while in the prevalent areas, as high as 80% of respondents have never used condoms, 5% have used condoms, 25% did not respond. Most of the respondents are not using condoms not because of the traditional belief that women should produce as many children as possible, but these rural women have not seen condoms before. There should be enlightenment campaign about the importance of condoms; Drama play such as "Condom Masquerade Dance" to raise and sustain their awareness to adopt condom usage for safer sex (SWAAN 1999). Blood transfusion is considered the source of HIV risk for a sizeable proportion of rural dwellers. About 34% of respondents in the non-prevalent areas perceived that AIDs could be transmitted through blood transfusion, 66% of respondents did not respond. On the other hand, 32% of respondents in the prevalent areas agreed that it could be transmitted through blood transfusion, 68% did not respond. Thirty three percent of respondents in non-prevalent areas perceived that AIDS could be contacted by injection syringes by not using sterilized needles. In addition, 67% did not respond while in the prevalent areas, 40% perceived that the virus could be contacted through needles, 60% not respond. Breast-feeding by HIV infected mothers' carries a significant risk of transmission. According to PPBA (2001), many children are infected parentally, that is they receive the infection from their mothers during pregnancy, at the time of birth or through breast-feeding. Respondents were asked if AIDs could be transmitted from mother to child. Ninety five percent of respondents in the non-prevalent areas did not agree that AIDS could be transmitted from mother to child; 5% admitted that AIDs could be transmitted from mother to child. In the prevalent areas, 97% of respondents did not believe that a child through the mother could contact AIDS; only 3% admitted it could be transmitted from mother to child. Sharp objects used for circumcision can cause the spread of HIV/AIDs if not properly sterilized. In the non-prevalent areas 6% of respondents perceived that AIDs could be contacted by circumcision; while in the prevalent areas only 10% agreed that AIDS could be contacted through circumcision. The sources who agreed with this transmission method are the few educated farmers. In the non-prevalent areas, 94& of respondents did not respond, also, 90% of respondents in the prevalent areas did not respond.

Women farmers who have heard of AIDS were asked of their personal risk of contacting HIV/AIDS. They were asked to classify their risk as small or great. The data revealed that 75% of respondents in the non-prevalent areas said their chance of contacting AIDS is small, whereas, in the prevalent areas, 80% of respondents said their chance of getting HIV is small. On the other hand, while 6% of respondents in non-prevalent areas think their chance of getting AIDS is great; likewise, 12% of respondents in prevalent areas also think their chance of contacting AIDS is great or high. The reasons given by respondents for their perception of high risk of contacting AIDS shows that most of the respondents (60%) in non-prevalent areas believe they are at great risk because they do not use condoms, while in the prevalent areas; the number was as high as 65%. In the non-prevalent areas, 25% of respondents believe they are at great risk because their spouse has other sex partners. The same number of respondents reported prostitutes as the source of their risk because their partners frequent prostitutes. In the prevalent areas, the proportion was 30%. Respondents said they were at great risk because of using unsterilized needles for injection, these accounted for 9% in the non-prevalent areas while it was 3% in the prevalent areas. Respondents who agreed that they are at great risk because of untreated blood in the non-prevalent areas were 6% while it was 2% in the prevalent areas. NDHS (1999) stated that blood transfusion and injections are considered the source of HIV risk for sizeable proportion of women and men in Nigeria.

Respondents who said their chances of contacting AIDS was small, were asked the reasons why they felt so. About 65 percent of respondents in non-prevalent areas said their risk of contacting the virus was small because they keep only one sex partner. While in the prevalent areas, the proportion

was 67%. About 15% of respondents in the non-prevalent areas stopped sex while in the prevalent were 5%. Seven percent of respondents used condoms in the non-prevalent areas while it was 6% in the prevalent areas.

Behavioural Change

Respondents who have heard of HIV/AIDS were asked whether they have changed their sexual behaviour since they heard about the disease. About 43% of respondents in the non-prevalent areas have not changed their sexual behaviour since hearing about AIDS while 66% of respondents in prevalent areas also have not changed their sexual behaviour. Though some of them wished they could change their sexual behaviour since they believed their greatest risk of becoming infected with HIV comes from their spouses, but could not because they are powerless in such matters. This agrees with Piot (2002) that gender inequalities make the impact of HIV fall harder on women because they lack complete control over their lives as they were taught to be obedient to men. Fifteen percent of respondents in non-prevalent areas stopped sex, while 5% of respondents in prevalent tare as also stopped sex. Seven percent of respondents began using condoms in the non-prevalent areas while in the prevalent areas the proportion was 6%. Maintaining one sex partner, 13% of respondents in non-prevalent areas maintained single partners; it was 12% in the prevalent areas. Seven percent of respondents ensure safe injection and blood transfusion in the non-prevalent areas while it was 6% in the prevalent areas.

Condoms are a warranty but not a total guarantee. Knowledge about condoms is unpopular among respondents. Eighty-seven percent of respondents in the non-prevalent areas have never used condoms; while 84% of respondents in prevalent areas have never used condoms too. Seven percent of respondents in non-prevalent areas have used condoms to prevent AIDS; while in the prevalent areas, 6% have used condoms too. 6 percent and 10% of respondents in both non-prevalent and prevalent areas did not respond to the question. Seventy-five percent of respondents in non-prevalent areas did not receive money, gifts or favours in return for sex during the previous year. In the prevalent areas, it was 86%. Majority of respondents in this category are the married women. About 25 percent of respondents in the non-prevalent areas have received money or favours for sex; while in the prevalent areas, it accounted for 14% of respondents. Prominent among those who have received money and gifts for sex were unmarried and formerly married women. Significant difference in farm size was found between non-prevalent and prevalent areas. ($t=2.982$, $P < 0.05$). The mean score (6.1100) of respondents in the non-prevalent areas is higher than the mean score (4.5950) of respondents in the prevalent areas. Conversely, there was no significant difference between the amounts realized from farm produce in the non-prevalent and prevalent areas respectively. ($t = 1.24$, $P > 0.05$). The mean score of amount realized in the non-prevalent areas is (103551.1) higher than the mean score (89735.0) of the prevalent areas. The study has clearly shown that farming as an enterprise was dominated by women aged 30 - 50 years with low literacy level, who are engaged in other income generating activities. Awareness and knowledge of HIV/AIDS among women farmers were average, and radio was cited as the major source of information; but no respondent admitted suffering from HIV/AIDS. There was a decline in agricultural productivity in the prevalent areas identified. Most respondents have not changed their sexual behaviour since hearing about AIDS. There is therefore the need to direct the campaign on HIV/AIDS

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Prioritizing the Factors Affecting the Management of Cold Water Fish Farming (Case Study: Qazvin Province)

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Abstract: Today, fish farming and breeding is considered as one of the most significant sources of protein supply in the world so that we can observe the remarkable improvements in this field. Artificial methods of fish breeding began in China and dates back to 3000 years ago. However, though the precedence of breeding fish in a pool is long, the artificial reproduction is a new experience. The first step in artificial fertilization was taken by a German scientist named Ludwick Jacobi. Despite the long experience of fish farming in the world, in Iran, it has been started in 1972 by reproducing Acipenseridae and in 1959 by reproducing rainbow trout. Industrial and economical growth as well as the need for supplying food for the growing population and the higher quality of fish protein compared to that of other kinds of meat has caused an increasing interest in the fish and fishing in the seas and water resources and has made it an important source of attracting foreign currency. Iran should also take the same actions considering the current policies. Therefore, reproduction of cold water fish should be taken into consideration as one of the most significant issues in this regard and all kinds of facilities should be provided for its optimal application[3]. There is a high potential in national waters of Iran in this regard few of which are currently being used. One reason for this is the lack of technical knowledge and required experience in fish farms management. Most farmers who engage in this occupation have suffered losses or have not used the available facilities properly due to the lack of sufficient technical and scientific knowledge. Regarding the above-mentioned issues, this study aims to signify the effective factors in the production of cold water fish farms and prioritize them based on decision making methods of AHP[4].

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Keywords: Prioritizing, Management, Fish Farming, AHP

Statement of the Problem:

Cold water fish farms have highly been operated and their production in the unit of area is remarkably higher than that of comparable farms in Iran. This indicates that the destination is not known to the managers of such farms. In other words, strategic purposes, ideals and, in some cases, operational purposes have been defined and considered. Currently, the organizations established for reproducing cold water fish do not know where to go. In case the managers of these farms know the factors effective in the operation of their farms and consider their preference and priority in their planning, they would have a much better function[5].

Factors which are considered effective in management of cold water fish farms are, on one hand, social, cultural, legal, technological, competitive, economical events and trends which can bring profit or loss for the farms in the future. On the other hand, management, marketing, operational activities are also among the factors affecting the management of fish farms.

These factors are generally borrowed from the studies carried out by others and the content of books and scientific articles but the question remains whether the same factors are effective in the farms of Qazvin Province or not. These factors are of different degrees of significance, hence this study signifies the general as well as particular factors which affect the cold water fish farm management in the Province of Qazvin through asking questions from the experts in fishery organization, professionals and farm owners of the province and then prioritizes them based on Analytical Hypothetic Process. Prioritizing the factors affecting the management of cold water fish farm management means considering the preference of one over another in the process of the operation, application of which brings about a better economical result. This issue results in gaining higher profits in return to the expenditure spent.

Significance of the Study

This study is significant and essential in several aspects. Some of these issues are explained below:

Allocation of Limited Resources

The global population has currently reached above 5 billion and considering the annual production of 4 billions of tons of food, the annual per capita portion would be 8 tons which means less than 2.2 kilograms a day and this would be possible only when a fair distribution is practiced worldwide which, of course, is not. 98% of the food is prepared

through agriculture and the remaining 2% from water resources. Farming lands are limited and include 3 to 5% of the earth; while 71% water-covered part of the earth is not properly used. Considering the existing limitations and the impossibility of the improvement of agricultural lands, man should think of supplying the food from water resources. One way, is to reproduce the fish and this needs a desirable appropriate management all over the world[6].

National Policymakers

Concerning the frequent recommendations of the physicians to consume fish frequently, the policy makers have set the expansion of fish production of different species at a national level and have issued various kinds of certificates and licenses in this regard. Gaining knowledge about the factors affecting the management of such farms helps the policymakers to plan much more appropriate and accurate plans so that the production would conform to the needs of society and this be done on time.

Fishery Organization of the Province

It is necessary for the Fishery organization of the province to know based on what parameters to evaluate the function of the farms and with what degree of significance to consider which factors as more determining. This research can help this organization in some respects.

Farm Owners of the Province

It is important for the farm owners of the province to know which factor is preferred in the management of the farms and results in a better functioning. Rather than the instances mentioned above, the following issues should also be taken into consideration in discussing the significance of the study.

Purposes of the Study

The main purpose of this study is to determine the priority of the effective factors in the management of cold water fish farms of the Province of Qazvin. This would be carried out through the following steps:

- Determining the primary factors;
- Determining the secondary factors;
- Prioritizing through Analytical Hierarchy Process (AHP)[6].

Other than the main purpose of the study which is to be achieved through this study there are other secondary purposes which result from this study. These purposes include:

- **Scholarly Purposes:** the result of this study can promote the knowledge of national and provincial policymakers as well as the farm owners; these results can also be applied in the next studies.
- **Applicable Purposes:** the results of this study can be used in the following cases:

1. Future programs and plans at a national level by the Ministry of Construction Crusade and Fishery Management Organization;
2. Future provincial plans for the development of fish farms;
3. (By the farm owners) for the improvement of the products.

Research Questions:

This study deals with two questions:

Q1: What are the effective factors in the management of cold water fish farms in the Province of Qazvin?

Q2: Which level of preference can be given to different effective factors in the management of cold water fish farms of Qazvin Province?

Operational Definition of Key Terms:

Several terms have been applied in this study every one of which is defined in this section. The definitions of the key terms of the next chapters are provided in this section to have coherence in the definitions provided.

- Aquatic species farming: includes the farming of the living beings such as the fish, oyster, crustacean, and aquatic plants[7].
- Farming: is interfering in the process of reproduction and breeding of the aquatic species with the purpose of promoting the production including reservation, nutrition, protection against the hunters, etc. Every cold water fish farm consists of several pools, related installations and administrative establishments.
- Farm Management: if management is considered as the ability to utilize the resources in the best and the most profitable manner, then it can be specifically described about the farms. The farm management plans to help the activity of farming through its appropriate decisions for the purpose of maximizing the revenues according to the purposes of administer or the family (farm owners).
- Farming Plan: includes drafting the method or the structure of the organization and using the available resources for the considered farm. Taking action without having a plan means the decision makingin an accidental, haphazard manner.
- The factors of production are the four main factors including: land, labor, capital, management.
- Marketing of the products of aquatic species farming includes the result of the function of all acts of production and service of aquatic species farming from the beginning point to the delivery of the product to the consumer[8].

Statistical Community

The statistical community includes all individuals and objects, etc. which share at least one guild. The statistical community can be either limited or unlimited. With regard to the title of the study, it is evident that the researcher seeks to explore the

effective factors in the management of cold water fish farms in Qazvin Province based on the opinions of the experts. The experts of the statistical community include a limited community as follows:

- The owners of cold water fish farms of Qazvin Province who, considering the requirements of receiving the license for establishing a fish farm can possess the qualifications required in this study to call them experts.
- Specialists of the Fishery Organization of Qazvin Province who are qualified as specialized individuals in the area of issuing license, establishment, breeding and function of the fish farm.

The individuals who are qualified based on these two clauses include 32 individuals whose judgments as technicians of the province in the scope of the study have been applied.

The qualifications of these individuals regarding individual specifications are as follows:

1. 22 , i.e. 68.75%, of the experts were among the specialists of the Fishery Organization of the province and 10 or 31.25% of them were among the managers or the experts of cold water fish farms of the province.
2. Among the experts, 21 or 65.62% had a bachelor degree, 10 or 31.25% had a master degree and just one individual with the portion of 3.125% had a PhD degree. It should be added that the experts of the farms all had a bachelor degree and higher degrees belonged to the specialists from the Fishery Organization.
3. 15 persons, i.e. 48.87% of the experts, had 5-10 year record of service and 17, i.e. 53.125%, had served more than 10 years in the area of fish farms or Fishery Organization.
4. All experts are male.

Statistical Sample:

Regarding the fact that AHP method[9] has been used in this study and the judgments of the experts are significant, this study sets to use the judgments of the experts of the statistical community instead of sampling, and related questionnaires have been completed by all experts. In other words, in this study instead of sampling, a census is used.

Domain of Study:

Spatial Domain of the study

This study has been performed within the boundaries of Qazvin Province including all fish farms and fishery management of the province. The Province of Qazvin with the climatic conditions mentioned before is on of the regions which are suitable for developing the farming of fish in Iran and has the privilege of easy access ways and other features which make it a suitable place for fish farming.

Temporal Domain of the Study

This study has been carried out within the years 2004-2005. All the activities of data collection and related analyses have been completed within 15 months.

Subject Domain of the Study

The subject of the study is to define and prioritize the effective elements in managing cold water fish farms in the province of Qazvin using AHP based on the judgments of the experts in the province.

Design of the Study:

Regarding the scientific classification of the studies, this study is of applied type. In definition, an applied research seeks to achieve a scientific destination and focuses on the prosperity and welfare of the public and the desirability of the activity. The findings of applied studies are to a high degree time- and space- dependent.

The method used in this study is a survey method. A survey includes the observation of the phenomena with the purpose of giving meaning to several aspects of the data collected. Surveys include two phases:

Close and careful observation of the parameters that are to be investigated in the society;

Collecting data and giving meaning to what has been observed.

In other words, a survey is a research process that is implemented to collect data on the subjects such as what a group of people know, what they think, or what they do. A survey has 3 objectives:

1. Description – discovering the existing realities or what exists.
2. Explanation – using terms in order to convey your purpose to the others.
3. Discovering – investigation.

Relying on the definition of a survey, this study has first considered the numerous effective factors in the management of cold water fish farming in order to describe the present situation. When the factors were explored through field study or judgment of the experts, the analysis and prioritization was performed using an AHP method [9]. Therefore the research method in this study can be defined as a survey with a descriptive-analytical purpose. Since the exploration of the factors is of little effect, exploration can not be directly described as a purpose.

Concerning the direct definitions given about descriptive, analytic, and explorative research in the literature on research methodology, the present study can be described as a descriptive-analytic research.

Instrumentations

The instrumentations used in this study for collecting data were 4 questionnaires used in Analytical Hierarchy Process:

1. First questionnaire – this questionnaire includes one question in which the experts have been asked to present up to 10 major factors effective in the management of fish farms without giving them any priority.
2. Second questionnaire – the major effective factors were extracted from the first questionnaire and after assigning the factors which had the highest frequency in the judgments given by the experts, these factors were included in the second questionnaire and presented to the experts. In this questionnaire, the experts were asked to exclude from the provided list those factors they assumed as irrelevant and add to the list those factors they assumed the list lacks while they can be considered as effective factors. This questionnaire contained two questions.
3. Third questionnaire – after the extraction of the main effective factors from the questionnaire, in this questionnaire the experts were asked to present the secondary factors or parameters for each main factor, the experts had to present one parameter or more.
4. Fourth questionnaire – the next step was to draw a hierarchical tree for the problem based on the data collected from the third questionnaire. In the fourth questionnaire, the experts were asked to compare each factor or parameter with a higher factor as a pair. In every comparison made, the expert specified the preference they made between two parameters or factors.

Variables of the Study

The variables of the study are as follows: dependent variable, the management of cold water fish farms is the dependent variable; and independent variable, the factors effective in the management of cold water fish farms or the parameters of prioritization of the model .

The study mainly deals with qualitative variables; hence, in order to record them, we can take advantages of non-numerical methods and then convert them based on Saaty's scale. The scale used in this study is a rank-based or ordered scale of AHP model.

Validity and Reliability

Before using the instrument, its validity and the reliability should be established scientifically. The instrument is valid if it measures the quality it has been designed to measure. It is reliable if it measures consistently.

In order to explain the validity of the questionnaires, considering the following two issues would be sufficient:

- AHP questionnaires, in many works, have been used as a way of obtaining a hierarchical tree and are nearly accepted as the standard of this method by all researches of this field of study.
- These four questionnaires have been distributed only when they have been approved by some faculty members and their validity has been declared.

For the approval of the reliability of the questionnaires, the consistency of the components has been checked. In AHP, a consistency test has been systematically performed between the answers through the application of software; if the consistency rate of each table of pair comparisons is more than 0.1, it would be said that there is no consistency in the judgments and the comparisons should be repeated. Therefore, after each conclusion, the consistency rate is calculated by the software and presented below each result.

AHP Model of Research

Analytical Hierarchy Process (AHP) was first introduced and used by Professor Thomas Al Saaty (the Professor of Pitsburc University), and the first book on this issue was published by him in 1980. AHP is a method of decision making. It is used when the decision is to be made among several options or several qualitative and quantitative factors. Just as we can differentiate physical relations and measure them – for example meter for length and second for time – so abstract relations can be differentiated. Feelings and perceptions permit us to develop the relation between the elements of a problem and determine which element has the most influence on the desired solution[10].

In dealing with real and tangible subjects, such as repairing a car, the degree of the effect of a variable is understood through our faculties, for example hearing the voice of the defective function of the engine or seeing the leakage, etc.

The process of measuring the effect of the priorities is performed to solve the problems. Therefore, in order to determine the degree of the effect from various components of a system, some measurements should be exercised based on a criterion with its units such as gram, second, meter and dollar. However, these criteria limit the nature of the ideas that we can examine.

Social, political and other qualitative factors can not be evaluated in a rational and acceptable way in terms of physical measurement. (However, in dealing with such issues there is a method that enables us to measure intangible qualities as well.)

AHP or Analytical Hierarchy Process is an analytic approach that enables us to measure intangible and impalpable characteristics as well and overcome the problems of decision making.

AHP is a demonstrable and comprehensible method without the complexities of other methods of decision making.

AHP is a method which relies on the mathematic(6) knowledge which generally includes all the thoughts related to a problem. This method is constructed upon the experiences of the user and is completed and supported by the explanation and interpretation of the judgments so that finally a proper understanding and a comprehensive perspective to the problem would be achieved.

In AHP, after the definition of the significant and essential factors, the attempt is made to create a rational balance among various effective criteria and their influence on each other be understood before the final decision and through pair comparison.

In this paper attempt is made to present the components of AHP and the way of its application in a quite simplistic manner[10].

The Advantages of AHP

- Being simple and applicable;
- Compiling and systematizing the very mental process of decision making and as a result, facilitating a proper and accurate judgment;
- Flexibility with regard to different problems and in various grounds;
- Creating appropriate environment and conditions for the improvement of the definitions and supervision through discussion in a decision making group (combining, analysis and adjusting the contradictions among them);
- The possibility of analyzing the sensitivity of the results and examination with low costs;
- Doing the numerical calculations and specifying the priority of the options and alternatives based on numerical values (a method for measuring qualitative values in the form of figures);
- The access of the manager to the data related to the evaluation standards and determining the percentage of the confidence in the data and information obtained by the decision maker (by a fixed rate or comprehensiveness) and the weight of each of the criteria as a side advantage of the method.

Hence, AHP consists of the principles which include:

1. ***Designing a Hierarchy***
   The description of a complicated problem, about which the decision should be made as a hierarchy for which it's better to use a tree called the tree of decision making and is a graphic description of the problem and is composed of three levels.

2. ***Determining the Priorities***
   Pair comparison of the factors in order to determine the relative significance of the factors in each level of the hierarchy.

3. ***Calculating the Results***
   Combining and integrating the comparisons made for the purpose of a general evaluation of the decision; in short, in this method, the decision maker or the decision making group assigns a goal and when the goal is specified as the subject of the decision making, various options or alternatives which are significant in the process of decision making are listed and then various criteria are compared in pairs among the options, after that based on a series of mathematic rules such as normalization we can determine the priority of the choice.

The Defects of AHP

This method applies too many mathematical operations and hence great care is needed to be made in the application of this method. Moreover, too many phases should be carried out for the performance of the operations; however, the application of the software has removed these defects as well.

Applicable Example of AHP Model (Finding the location for chain store)

Imagine that we are going to select a location among three alternatives of A, B and C in order to establish a store. Four criteria of culture of the people, income, population, and residential area are involved. The solution of this problem can be explained through the following steps:

- building a hierarchy;
- estimating the weight;
- system consistency.

The first step is to provide a graphic presentation of the problem in which the objective, criteria and the alternatives are illustrated.

Estimating the weight

In AHP, the elements of each level are compared with their counterpart elements in the higher level in a binary Format and their weight is calculated; such weights are called relative weight. Then, through combining the relative weights, the final weight which is called the absolute weight, is determined for each alternative.

First, the locations are separately compared regarding culture, income, population, residential area and easy communication and the weight of each is specified regarding these criteria.

In such comparisons, the decision makers would use verbal judgments. These judgments are transformed into quantitative amounts from 1 to 9 which are presented in the following table:

A. Now, the locations regarding culture are compared and their values regarding this criterion are estimated. Imagine that the decision maker selects the preference of A to B between equal and moderately preferred, and then the value of the judgment is 2. However, if the preference of location A to location C is between very strongly preferred and extremely

preferred, then the numerical value of this judgment would be 8; and if the preference of B to C is between strong and very strong, the value would be 6. This judgment can be shown as illustrated in table 2.

- In comparison of pairs, the preference of each element to itself equals one.
- If the preference of A to B equals 2, the preference of B to A would be ½.

B. The weight of each alternative of pair comparison matrix is estimated through approximate methods

First step: the values of each column are added.

Second step: Each element in the pair comparison matrix is divided to the total of its column so that the pair comparison matrix would be normalized.

Third step: The estimation of the average of the elements of each row.

Hence, observe that regarding culture, location A (with the preference of 0.593) is the best location. Moreover, based on the estimations made above, the locations are now compared regarding income, population and the residential area and their weight are estimated regarding these criteria. When all these calculations have been carried out for locations A, B and C, based on an arithmetic mean method, the results of the calculations are presented in table.

The results of the above table can be explained in the following way: Location A is the best regarding culture, income, population and residential area; however, for example location B might be the best regarding income, and location C might be the best regarding population. The final decision should be made when the weight of each criterion is determined.

After calculating the weight of the locations in relation to all constraints, the weight of the criteria should be determined; in other words the portion of eachh one of the criteria in specifying the best location should be determined. To do this, the criteria should be compared in pairs. The results are presented in table 8. The weight of the criteria is estimated through arithmetical average method which is displayed in the following way:

Culture: 0.355
Income: 0.359
Population: 0.169
Residential area: 0.117

As can be observed, the criterion of income possesses the largest weight.

C) The estimation of the final weight of the locations

Now that the weight of the criteria is estimated with regard to the objective and the weight of the locations is estimated with regard to the criteria, it's the time for the way of combining these weights to be explained for the estimation of the final weight.

The following table displays the weight of the locations with regard to the criteria which have previously been calculated and would be briefly explained in the following way.

Then, regarding the relative weights estimated, the final weight for each alternative would be obtained in the following way.

Final weight of location A = (0.355*0.593) + (0.395*0.702) + (0.169*0.740) + (0.117*0.629) = 0.660

Final weight of location B = (0.355*0.341) + (0.359*0213) + (0.169*0.113) + (0.117*0.629) = 0.289

Final weight of location C = (0.355*0.066) + (0.395*0.085) + (0.169*0.147) + (0.117*0.629) = 0.151

Therefore the preference of the locations would be as follows: As can be observed, location A is the best choice.

Data Analysis and Interpretation of Research Tests

Research Question:

What are the effective factors in the management of cold water fish farms in the Province of Qazvin?

In order to find the answer to this question, three first questionnaires of this research were designed and the data were collected using these questionnaires.

In questionnaire (1) the specialists (the experts in research) were asked to signify the ten major factors effective in management of cold water fish farms in the Province of Qazvin. After collecting the questionnaires and extracting the factors indicated in it, the following results were obtained.

After the collection of the data of questionnaire (1), questionnaire (2) was designed and distributed. In this questionnaire, ten major factors about which there was a high degree of agreement, was presented to the experts and asked to write in the first column the factors which are essential but are not included among these ten factor, and in the second column exclude those factors which are redundant from among the factors presented by the researcher.

The data collected from questionnaire (2) were extracted and the results are as follows in table 12.

Regarding the judgments of the experts in questionnaire (2), 14 percent alteration was observed in the factors in comparison to questionnaire (1).

In questionnaire (3) the results obtained from questionnaires (1) and (2) were presented to the experts and they were asked to signify the sub-parameters related to each factor (major parameter)

which were classified in three groups. The results of the completion of questionnaire (3) by the experts are demonstrated in the following tables.

Research Question 2:

What is the priority of the effective factors in the management of cold water fish farms of the Province of Qazvin?

When the questions were distributed and the answers to questionnaires (1), (2) and (3) were gathered, questionnaire 4 (the last one) was compiled based on the data collected from three previous questionnaires and then were delivered to the experts to be completed.

In order to answer the questionnaires of the last questionnaire based on AHP method, the experts needed to be informed about the basic issues, then the questionnaire which included:

- The diagram of hierarchical tree of decision making;
- Questionnaire with Saaty spectrum.

Was distributed and completed by the experts.

The data collected from the questionnaire was entered in the file of Expert Choice Software the results obtained from the analysis of the data in the format of AHP group decision making, in which a geometric mean relation was used, are demonstrated in the following tables.

When the estimations were made, the weights of the major parameters were compared with the purpose and the result was as is presented in table 16.

Results

Efficient attention to the management of cold water fish farms can result in changes and new directions in the industry of aquatic species farming. This study seeks to specify and prioritize the effective factors in the management of cold water fish farms in the province of Qazvin. After the determination of these factors and the designing of the related hierarchy, the results obtained from prioritizations were as follows:

1. First, three major factors of economic(5) factors, technical-engineering factors and management factors were compared with each other and then they were prioritized. The result of this prioritization was as follows:

- Management factors at the first level of priority;
- Technical-engineering factors at the second level of priority;
- Economical factors at the third level of priority.

This means that management factors with the significance ratio of 47% are to be considered as the most significant effective factors in the management of fish farms. Technical factors with 39% and economical ones with 14% come next. It should be noted that based on the opinions of the experts, focusing on management factors are more significant or in other words, in order to come to a higher degree of exploitation and a better production, the role of management factors should be taken into consideration in all phases of production.

2. Parameters or the secondary factors related to economical factors included: marketing, access to the regional power supply, the facilities of local investment, economic access to skilled manpower and the transportation facilities. Based on the judgments of the experts the levels of priority were classified as follows:

- First level of priority is given to local facilities of investment;
- Second level of priority is given to the transportation facilities;
- Third level of priority to the access to the regional power supply;
- Fourth level of priority to economical access to skilled manpower;
- Fifth level of priority to marketing.

It should also be mentioned that the three first priorities possess 70% of significance and the two last factors only possess 21% of significance.

3. Among three factors of secondary position considering the major factor of technical-engineering nature it was observed that:

- Design and construction of the pools with 42% significance were at the first level of priority;
- Location with 33% at the second priority;
- Safety and emergency with 25% at third level of priority.

This indicates that in several phases of construction to exploitation of cold water fish farming the highest attention should be directed toward design and structure of the pools. This factor can also be classified into five secondary factors which can be prioritized in the following way (the prioritization of third level of parameters):

- Constructing a diverting dam with the significance ratio of 29%;
- Observing the gradient of the pool with the significance ratio of 24%;
- Appropriate deposit cleaner with the significance ratio of 20%;
- Protection wall with the significance ratio of 15%;
- Appropriate concrete canal with significance ratio of 12%.

Location is in the next level of significance. It is with 8 secondary factors which can be prioritized as follows:

- Estimating the critical discharge of the upper regions;

- Appropriate temperature of water in the place of establishment;
- The length of the period of muddiness in the place of establishment;
- Distance from the market;
- Distance from the farms of upper regions;
- Distance from the access road;
- Observing the side distance from river;
- Distance from the food supplies.

The factor of safety and emergency is placed at the third level with 5 secondary factors which can be prioritized as follows:

- Reflective pumps;
- Pumping wells;
- Ventilating pumps;
- Alarm systems of the interruption of water flow;
- Emergency power supply;

4. There are 9 secondary factors regarding the major factor of management which can be prioritized as follows:

- Specialty and experience;
- Training courses;
- Considering weather reports;
- Using guide-tables;
- Observing hygienic and quarantine issues;
- Observing the weight of release;
- Using modern methods of improving the production in the unit of area;

The numbers of times of separation;

- Recording the conditions of the farm during the period of farming.

5. After the prioritization of secondary factors with regard to each major factor, the final phase of prioritization was carried out. This phase included the prioritization of each one of the 32 secondary factors with regard to other factors. The priorities are as follows:

If the priorities of factors are observed as determined above, we can claim that the farm would be productive and its management would be efficient.

Other Results of the Study

In addition to what was directly obtained from the analyses, some other issues are concluded during the study which can not be assumed as the direct implications of the findings of the research and are mainly considered as implicit results of the study:

- Water is a gift of God, and reserving water and preventing it from being wasted is one of the issues that have frequently been focused. In modern management of farms, the fact that water is non-renewal has been taken into great consideration. It is necessary for us to take this fact into consideration in our country.
- The manager should not merely care about marketing to the purchasing phase; he/she should also attempt to encourage the consumption of fish regarding new findings about the significant role of the aquatic species in nutrition, for example stressing the content of Omega 3 in fish.
- Modern methods of farming in enclosed waters should also be taken into consideration and the management methods other nations should be used and defined as models of application.

Two groups of suggestions are presented in this paper; the first group is derived from the results of this study and the second group is derived from the personal studies and experiences of the researcher.

Suggestions Derived from the Study

- The managers of the Fishery Organization of the Province and the officials of cold water fish farms should take the prioritization of the factors in consideration in the management of general affairs and improvement of their farms in order to have productive farms.
- The officials of the farms should plan their activities based on the priority of each secondary factor and its role in programming the establishment, production and utilization, and maintenance.
- Before issuing the license for the establishment of new farm, the provided prioritization can be observed.
- In examining the conditions of each farm for the improvement of the management, the status of each factor that is to be changed should be taken into consideration.

Suggestions of the Researcher

Here, in addition to the suggestions derived from this study, there are some other suggestions made by the researcher which are not directly resulted from the present study but can be regarded as the implications of the study:

- It is suggested that other factors be taken into consideration beside and parallel to the management factors but with a different degree of significance, so that it would be possible to have a productive farm and plan for the optimization of the management of farm affairs.
- It is suggested that the man power who are to be employed in the farm be qualified enough to help the management of farm affairs in a proper manner.

- It is suggested that the future developments of the farms in the province be managed according to the prioritization offered so that exploitation of the resources be directed toward optimization.
- Allocating the credits of the province to cold water fish farms based on the prioritization suggested here as the criteria of evaluation of farm conditions with different levels of significance.

Suggestions for Further Research

Regarding the limitations faced in the accomplishment of this study it is suggested that the following subjects be studied by other researchers:

- Investigation of the effective factors in warm water fish farming in the province of Qazvin;
- Comparative study of management of cold water fish farms in different provinces;
- Determination of the level of significance of the effective factors in the management of cold water fish farms in the province of Qazvin compare with other factors;
- Investigation and accomplishment of 5S in the cold water fish farms of the province of Qazvin;
- Investigation of the results of the application of this prioritization in the improvement of the quality of products.

Limitations of the Study

Here the limitations faced by the researcher in the process of this study are presented.

a) Dispersion of the farms around the province and the difficulty of contacting their managers;
b) Non-cooperation of some of the experts of the Fishery Organization and farm owners;
c) Lack of sufficient literature on the management of fish farms;
d) The problems the experts had in completion of the questionnaires especially questionnaire (4) which required high degrees of skill and proficiency;
e) Based on the analysis made, the factors have not been totally independent from each other and some degrees of dependence exist among them. In order to adjust this problem all respondents were informed and asked to fill in the questionnaires bearing such dependences in mind. This can not totally exclude the negative influence of the dependence between the priorities; however, it can cover them to a significant degree. This limitation can be observed in several articles and many authors assume the aforementioned solution as the most effective approach with regard to issues with limited numbers experts available.
f) The error resulted from the act of making the figures of geometric mean round may affect the results achieved from the software. In this study, the figures are rounded to 3 decimal places.

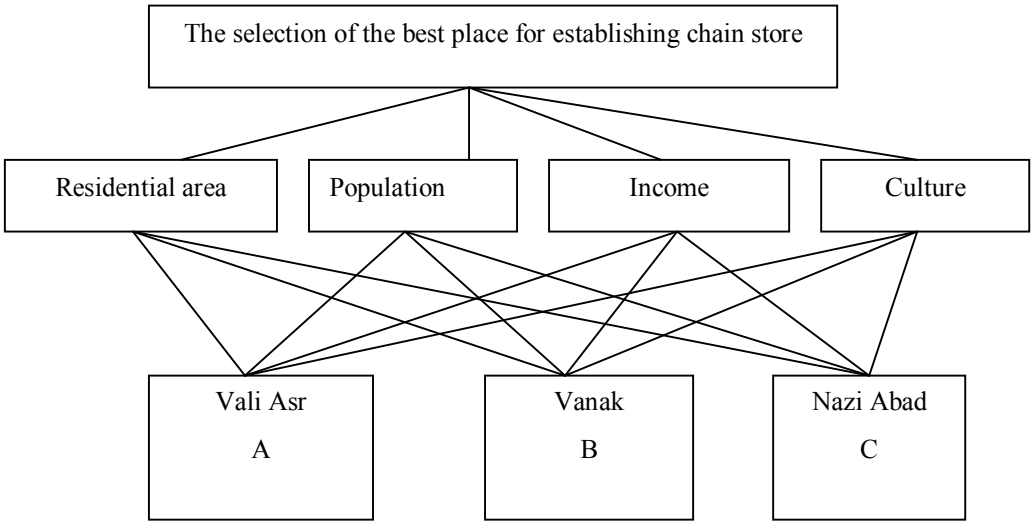

Fig 1. The hierarchy of the selection of a location for chain store

Table 1. The value of the preferences for pair comparisons

Degree of significance	Definition	Description
1	Equally preferred	Both activities play an equal role in achieving the desired destination
2	Moderately preferred	The experience and the judgment support one of the activities to some degree
5	Strongly preferred	The experience and the judgment strongly support one of the activities
7	Very strongly preferred	One of the activities is focused much more than other activities and its prevalence is evident in practice
9	Extremely preferred	Evident preference of one activity over another; the highest possible order is verified
2,4,6,8	For the states where the degree of significance is between the above-mentioned values	The preferences within the above-mentioned intervals

Table 2. Comparison of locations

Culture	Location A	Location B	Location C
Location A	1	2	8
Location B	½	1	6
Location C	1/8	1/6	1

Table 3. Comparison of locations

Culture	Location A	Location B	Location C
Location A	1	2	8
Location B	½	1	6
Location C	1/8	1/6	1
Total of each column	13/8	19/6	15

Table 4. Comparison of locations

Culture	Location A	Location B	Location C
Location A	8/13	12/19	8/15
Location B	4/13	6/19	6/15
Location C	1/13	1/19	1/15

Table 5. Comparison of locations

Culture	Location A	Location B	Location C	The average of the row
Location A	0.615	0.632	0.533	0.593
Location B	0.308	0.316	0.316	0.341
Location C	0.077	0.053	0.053	0.066
Total	1.000	1.000	1.000	1.000

Table 6. Comparison of Culture, Income, Population and Residential area

	Culture	Income	Population	Residential area
Location A	0.593	0.702	0.740	0.629
Location B	0.341	0.213	0.113	0.250
Location C	0.066	0.085	0.147	0.121

Table 7. Comparison of Culture, Income, Population and Residential area

	Culture	Income	Population	Residential area	Average of each line
Culture	0.414	0.321	0.284	1.400	0.355
Income	0.414	0.321	0.636	0.067	0.359
Population	0.104	0.036	0.070	0.466	0.169
Residential area	0.068	0.322	0.010	0.067	0.117
Total of each line	1.000	1.000	1.000	1.000	----

Table 8. Comparison of Culture, Income, Population and Residential area

	Culture	Income	Population	Residential area
Location A	0.593	0.702	0.740	0.629
Location B	0.341	0.213	0.113	0.250
Location C	0.066	0.085	0.147	0.121

Table 9. preference of the locations

Final weight	Location	Preference
0.660	A	1
0.289	B	2
0.151	C	3

Table 10. results

Rank	Factor	Frequency of the responses
1	Management of the farm	23
2	Training courses	19
3	Location	18
4	Estimating the food change index	18
5	Design and construction of pools	17
6	Technical and engineering factors	15
7	The number of times of separation	12
8	Taking economic factors into consideration	12
9	Safety and emergency	10
10	Using modern methods of production development in the unit of area	8

Table 11. the result of questionnaire (2)

The judgments of the experts		**Factor**	**Rank**	**Factor**
Non-effective	**Effective**	Management of the farm	1	**Existing factors**
		Training courses	2	
		Location	3	
		Estimating the food change index	4	
		Design and construction of pools	5	
		Technical and engineering factors	6	
		The number of times of separation	7	
		Taking economic factors into consideration	8	
		Safety and emergency	9	

		Using modern methods of production development in the unit of area	10	
Frequency: 9		Marketing	1	**Factors added**
Frequency: 7		Proper access	2	
Frequency: 5		The facilities of regional investment	3	
Frequency: 5		Obtaining the deposits	4	
Frequency: 4		Water discharge	5	

Table 12. the result of first group of parameters from questionnaire (3)

Major factor (parameter)	Sub-parameters	The frequency of the judgment of the experts
Economical factors	Marketing	14
	Access to the regional electricity power supply	17
	The facilities of regional investment	11
	Economical availability to the skilled manpower	18
	Transportation facilities	19

Table13. the result of the second group of sub-parameters from the major technical-engineering parameters of questionnaire

Frequency	Sub-parameter	Factor
16	Construction of the diverting dam	**Design and structure of the pools**
18	Having an appropriate concrete canal	
15	Having proper deposit cleaning	
22	Observing the gradient of the bottom of the pools and height and the relation of the length to width	
19	Having the protective wall	
27	To have pumping wells	**Security and emergency considerations**
28	To have emergency power supply	
31	To have reflective pumps	
30	To have ventilation pumps	
27	To have alarm systems for the interruption of water flow	
18	Observing the side distance from the river	**Mechanical**
29	Estimation of the critical discharge for upper regions	
26	The length of the muddiness in the establishment location	
22	Having a proper temperature in the establishment	
17	Distance from that access road	
20	Distance from the market	
19	Distance from food supplies	
22	Distance from the farms of the upper regions	

Table14 – The result of the third group of sub-parameters from questionnaire (3)

Frequency	Sub-parameter	Major factor (parameter)
17	Specialty and experience	**Management**
18	Training courses	
22	Using guide-tables	
29	Observing hygienic and quarantine principles	
31	Observing the releasing weight	
30	The numbers of times of separation	
29	Considering weather reports	
18	Using modern methods for the improvement of production in the unit of area	
15	Recording the details about the farm in the farming period	

Table 15. The geometrical mean of pair comparison of the first level

	Management	Economical	Technical and engineering
Management	1	4.615	
Economical		1	4.764
Technical and engineering	3.681		1

Table 16. the result of prioritization

Priority	Normalized weight	Main parameters
1	0.47	Management factors
3	0.14	Economical factors
2	0.39	Technical and engineering

Table 17. Priority of The effective factor in farm management

Priority	The effective factor in farm management
1	Local facilities of investment
2	Construction of deviational dam
3	Reflective pumps
4	Observation of the gradient of the bottom of the pool
5	Transportation facilities
6	Estimation of critical parameter of the upper region
7	Pumping wells
8	Access to the regional power supply
9	Specialty and experience
10	Appropriate water temperature in the location
11	Appropriate deposit cleaner
12	Ventilating pumps
13	Training courses
14	The length of the period of muddiness in the location
15	Alarming systems for the interruption in the flow of water
16	Considering the weather report
17	Protection wall
18	Economical access to skilled manpower
19	Emergency power supply
20	Appropriate concrete canal

21	Distance from market
22	Application of guide-tables
23	Observation of hygienic and quarantine issues
24	Distance from the farms of the upper region
25	Marketing
26	Observation of the weight of release
27	Distance from access road
28	Application of modern methods for the improvement of production in the unit of area
29	Observation of the side distance from the river
30	Numbers of times of separation
31	Distance from food supplies
32	Recording the farm conditions during the period of farming

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Object Oriented Metrics for Prototype based Languages

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Abstract: Prototype (classless) based object oriented programming approach has several advantages for representing default knowledge and dynamically modifying concepts over traditional class based languages. Many modern languages like C#, JavaScript and others are in part or completely utilizing astounding features of prototypes. With this growing interest in adoption of prototypes a sheer need is emerging to redesign software metrics for prototype based languages. These paper highlights issues for prototype based software metrics for object oriented programming.

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Keywords: Software life cycle, software complexity, design metrics, prototype object modeling

1. Introduction

Espousing of prototype based features such as delegates in part or completely in modern languages like C#, JavaScript led towards consideration for prototype based approach for object oriented programming. When compared to class-based languages, prototype-based languages are conceptually simpler, and have many other characteristics that make them suitable especially to the development of evolving, exploratory and distributed software systems. Significant work has previously been done and many languages have already been designed implementing prototypes: SELF [1-4], KEVO [5-6], AGORA [7], GARNET [8-9], MOOSTRAP [10-11], CECIL [12], OMEGA [13] and NEWTON-SCRIPT [11] are such languages which represent another view of the object oriented programming. This approach is advantageous in sense that one does not need to rely so much on advance categorization and classification, instead the focus should be to make the concepts in the problem domain as tangible and intuitive as possible. In turn prototypes may give rise to a broad spectrum of interesting technical, conceptual and philosophical issues. Although interest in espousing prototypical features is increasing, yet no serious attempt has been made towards designing software metrics for prototype based languages till now. For class based approaches various software metrics related to quality assurance have been proposed in the past and are still being proposed but none of them explicitly discuss prototype based language.

Many metrics have been proposed explicitly in context of class based object-oriented programming such as class, coupling, cohesion, inheritance, information hiding and polymorphism. As the development of object-oriented software is rising, more and more metrics are required for object-oriented languages but the applicability of metrics developed previously are mostly limited to requirement, design and implementation phase instead of data representation and nature of problems. According to Moreau [10-11] traditional metrics are inappropriate for OO systems for several reasons. First, the assumptions relating program size and programmer productivity in structured systems do not apply directly to OO systems. Second, the traditional metrics do not address the structural aspects of OO systems. Third, the computation of the system's complexity as the sum of the complexity of the components is not appropriate for OO systems. The Significance of software measurement along-with the increasing interest in prototypical features leads towards reconsideration of software metrics and to answers the questions associated with the effectiveness of applying traditional software metrics to OO systems.

Functionally object oriented metrics can be divided into three categories from prototype perspective:

1. Some metrics needs to be redesigned for prototype based languages.
2. Some metrics needs no change as some features of class based and prototypes based languages are similar.
3. For some metrics old day's structural languages metrics can be used.

4. Some metrics are no longer needed for prototype based languages.
5. Some new metrics needs to be presented for novel features of prototypes.

Table 1: Comparison of Object Oriented and Prototype languages [13]

No.	Features	Cass-Based Techniques	Prototype based techniques
1	Basis	Mathematical concept – set of knowledge representation	Knowledge representation through object observation
2	Object Modeling Parameters	(i) defined an object by distinct parameters structure and state (ii) Object is not defined in an incremental fashion	(i)defines an object by a single parameter prototype and doesn't differentiate between structure (or meta data) and state (or data) of the object.
3	Organization of objects of a system	Objects are organized into a hierarchical structure, called a class lattice	Objects are not organized in any hierarchical structure – no class-latice
4	Tracing of changes to a specific object	Not possible	Possible since each change to an object is stored in a separate prototype
5	Knowledge sharing mechanism	Inheritance mechanism, and it is static mechanism	Delegation mechanism and it is dynamic mechanism
6	Fixation of Message Passing Pattern	Message passing pattern is fixed at compile time	Message passing pattern is fixed at run time
7	Retention control while message passing	Control remains with the self class while the search goes to the next super class	Control is passed to the next prototype with the search delegation
8	Flexibility and Efficiency	(i) In case of simple inheritance and single parent delegation, both mechanism are equally powerful (ii) Otherwise it is less flexible and less powerful than the delegation mechanism (iii) Efficiency is predictable	(i)In case of simple inheritance and single parent delegation, both mechanism are equally powerful (ii)More flexible and powerful than the inheritance mechanism (iii)Efficiency is not predictable.

Type 1. Metrics to be redesigned: In the traditional object oriented systems knowledge sharing between object and classes is typically done by a mechanism called inheritance, initially used by the language Simula and later adopted by most of the modern object oriented languages. Every class contains a common behavior for a set of objects along-with the description of what characteristics are allowed to vary among objects. It is important to note that all instances of a class share the same behavior, but can maintain unique values for a set of state variables pre-declared by the class. There are a number of metrics available for object oriented systems to deal with inheritance. Some of such metrics are Attribute Inheritance Factor (AIF) [11], FAN-IN [9], Method Inheritance Factor (MIF) [2] and Number of Methods Inherited (NMI) [12]. Here AIF counts the ratio of the sum of inherited attributes in all classes of the system under consideration to the total number of available attributes for all classes. FIN is the number of classes from which a class is derived and high values indicate excessive use of multiple inheritances. MIF is the ratio of the sum of the inherited methods in all classes to the total number of available methods for all classes and NMI measures the number of methods a class inherits.

Type 2: While on the other hand prototypical approach for sharing knowledge in object oriented systems is based on an alternative mechanism called delegation, appearing in several languages [7], [5],and [8]. In this approach the distinction between classes and instances is removed in sense that any object can serve as a prototype. To create an object that shares knowledge with a prototype, an extension object is constructed, which contains a list of its prototypes which may be shared with other objects. When an extension object receives a message, it first attempts to respond to the message using the behavior

stored in its personal part. If the object's personal characteristics are not relevant for answering the message, the object forwards the message on to the prototypes to see if one can respond to the message. This process of forwarding is called delegating the message. Keeping in mind this difference of sharing knowledge among objects and classes the traditional metrics being used for inheritance needs to be changed with more sophisticated metrics for delegation. For instance the prototypical version for above mentioned metrics can be designed such as in AIF instead of counting the ratio of inherited attributes/objects, number of delegated objects can be calculated. FIN is the number of classes from which a class is derived while in class-less languages prototype is used instead of classes so FIN can be redesigned for number of prototypes from which other prototypes are delegated. Similarly a prototypical version of MIF and NMI can also be designed.

Type3: Source Lines of Code (SLOC or LOC) is one of the most widely used sizing metrics in industry and literature. Size is one of the most important attributes of a software product. It is not only the key indicator of software cost and time but also a base unit to derive other metrics for project status and software quality measurement. According to [7] survey on cost estimation approaches, size metric is used as an essential input for most of cost estimation models. SLOC is the traditional and the most popular sizing metric. Its long-standing tradition is due to the fact that SLOC is the direct result of programming work. In the early age of software development, most of software cost was spent on programming, and SLOC emerged as the most perceivable indicator of software cost. Unfortunately, SLOC has a number of shortcomings [3]. One significant deficiency is the lack of precise and methodical guideline for determining what SLOC means. Another feature lacking in SLOC counts, reducing its usefulness as an effective size measure for understanding a piece of code is that it doesn't account for complexity of a line of code.

These ambiguities of complexity between difference lines of code is addressed in the new metric of S/C (size/Complexity) described by Pant [3]. The S/C measure is based on the notion that, in a high-order programming language, decision making and iterative statements are normally more complex than assignment statements. This metric count's one for simple statements, one for each binder and one for each simple predicate. It also takes into account the number of mental paths within the control flow structure and allows for nested structures. Contrary to this notion prototype languages donot take advantage of such complex or nested structure instead all statements in the languages are used just like simple message passing statement and functionally same behavior is followed by most statements in prototype languages. This analysis implies that in prototype languages there is no need to use the enhanced version of SLOC and the older version of this metric can easily be deployed to measure size of the program as each statement uses equal level of complexity.

Type 4 Metrics: There are many metrics in used to measure cohesion and complexity of classes such as attribute hiding factor[4], class cohesion[5] and class entropy complexity[6] etc. Hereby attribute hiding factor measures the ratio of the sum of inherited attributes in all system classes under consideration to the total number of available classes attributes. While class cohesion measures relations between classes and class entropy complexity measures the complexity of classes based on their information content. Dony, Malenfant & Cointe [2] outlined two primary arguments in favor of prototype-based object oriented programming. First, it is easier to figure out concrete examples before generalizing concepts into abstract definitions. Second, classes add unnecessary constraints by preventing the customization of individual object instances as well as the inheritance of member data values. Although the first issue tends to be more of a philosophical or process-oriented distinction, the second issue regarding class constraints deals with a potential limitation in the available programming constructs that support the abstraction and encapsulation of concerns in software. In order to remove this limitation there is a sheer need to use classless approach which entails to omit the use of metrics used for measuring classes.

Type 5 Metrics: One of the notable feature of prototype based languages is that the object system are dynamic in that the objects can be created, updated and destroyed during program execution and even the type of values can be changed [8,9]. Every object is self-describing and can be changed independently and each change to an object is stored in a separate prototype. In class based systems any object cannot be created or associated with class at run time. Although this might seem handy, it also means that you have to be very careful when you update a prototype object. You have to know the object dependency graph of the objects derived from the prototype object, in order to do a safe update. The object systems also support a part-owner hierarchy, by which objects can be grouped together [9]. For instance, the graphics in a

window are added as *parts* of the window.

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Analysis of an Edge-Core Joint Node in OBS Networks

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Abstract: Optical burst switching (OBS) has been introduced as a short term implementable solution for future all-optical networks. Its performance evaluation received a considerable attention and does not seem to be weaken. In prevalent studies, an OBS network is strictly divided into edge and core domains, while practically for future all-optical mesh network deployments; this is not valid for most of nodes. In these networks, not only a few but probably majority of the nodes will have to combine both functionalities to provide flexible operation. Such nodes are termed herein joint edge-core OBS nodes. In this paper, an architecture of a joint edge-core OBS node is studied and a new scheduling algorithm have been proposed to multiplex the local assembled traffic along with the transit traffic. Thorough simulations have been done on benchmark networks to show the significance of edge scheduling in joint edge-core OBS nodes.
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Keywords: Optical burst switching, Joint edge-core node

In a prevalent number of OBS performance studies, it is taken for granted that an OBS network is strictly divided into the edge and the core part. This means that the network consists of the nodes only assembling packet traffic into bursts (edge nodes) and the nodes only switching the burst traffic along the transmission path (core nodes), see Fig. 1a. However, this assumption cannot be valid for practical future deployments in dynamically reconfigurable networks with mesh topology. In these networks, not only a few but probably majority of the nodes will have to combine both functionalities to provide flexible operation, as shown in Fig. 1b. Such nodes are termed herein joint edge-core OBS nodes. The Fig. 2 explains the difference between two kind of networks, the conventional and network consists of joint OBS nodes in addition with pure edge or core nodes. A significant challenge in their design is that the local traffic must be multiplexed on output wavelengths channels with the transit traffic cutting through the node and a mechanism is needed to control the channel. Otherwise, a high local load may cause high losses of the external traffic and, vice versa, too intensive transit traffic may greatly delay the transmission of locally assembled bursts. Both phenomenadegrade the performance of OBS. However, the increase of losses for transit traffic is expected to have more adverse effects. Burst.

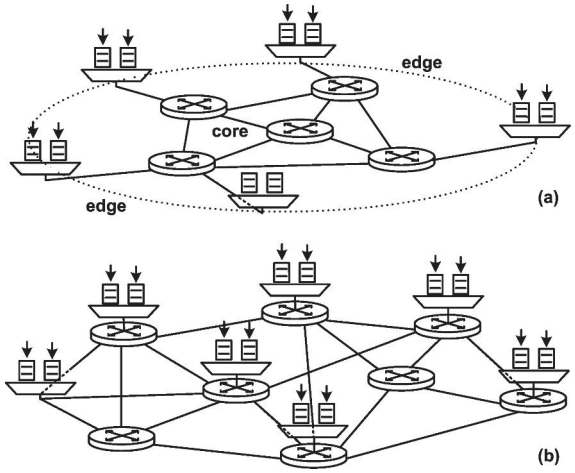

Fig. 1. OBS network with (a) separation of edge and core parts (b)joint node

Bursts that are lost not only waste the reserved path bandwidth but also invoke the retransmission and reordering delays in higher network layers. These effects are well-documented in numerous studies on Transmission Control Protocol over OBS, see for example [4], [3], [5] and references therein. On the contrary, waiting in the transmission buffer, even when excessive, is easier to monitor and does not propagate out of OBS layer to such an extent, thus assuring more stability to particular end-to- end packet flows.

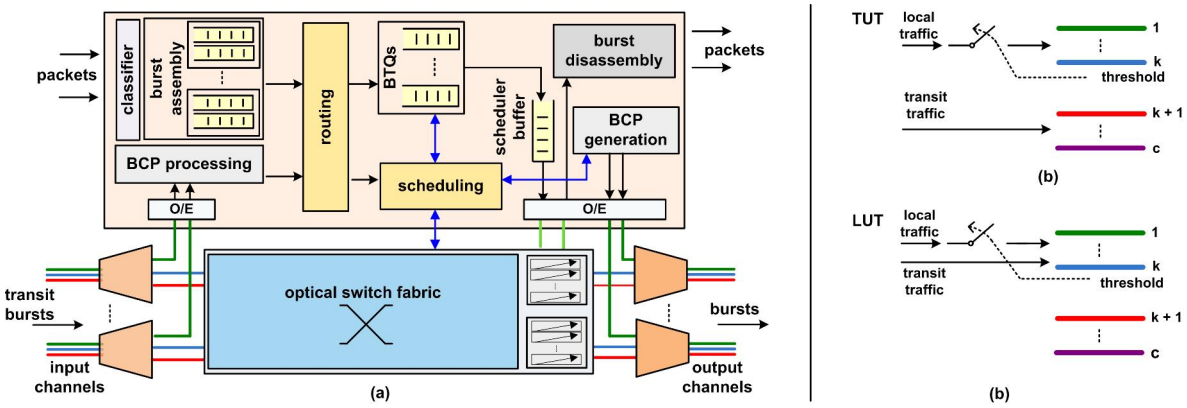

Fig. 2. Architecture of the joint OBS node

Therefore when contention for the channels occurs, transit traffic should be prioritized with no doubt. The issue of multiplexing of local and transit traffic in OBS has not been a subject of analysis. In several simulation studies, this fact was indeed assumed, the blocking of transit and vice-versa. The study gives many insights into the performance issues involved in the real implementation of OBS nodes.

2. Materials and Methods

2.1 Architecture of Joint Edge-Core Node

Edge-core joint node of OBS network is a combination of both edge and core nodes. It can assemble data bursts (DB) with the edge node functions and forward the transit bursts to the next node with the core node function. Figure 2 shows the complete architecture of ECJN. Packets arriving from different traffic sources are classified according to their traffic class and destination address and distributed into corresponding assembly queues. The bursts are assembled in edge node, according to size, time for hybrid based burst assembly mechanisms. The locally assembled bursts are then forwarded to burst transmission queues (BTQs), where they are buffered electronically and wait for the availability of the output channel. When the scheduling module finds output channel, it reserves the channel and forwards burst control packet (BCP) to the destination node, and forwards the burst from BTQ to the scheduler buffer, where the bursts wait electronically until their transmission time is reached. On the other hand, while performing the functions of the core node, the transit traffic (BCP) arrives at routing module, if the destination of arriving bursts is current node, the bursts are forwarded to the burst disassembly module, which disassembles the bursts into IP packets and forwards IP packets to their destinations. If transit bursts need to be forwarded to the next nodes, information is sent to the scheduling module, which looks for the availability to reserve output wavelength channel. If channel is found after wavelength conversion (if required), the wavelength is reserved for incoming bursts, otherwise the burst is dropped. Components of edge-core joint node are discussed in detail. Components of edge-core joint node are discussed in detail.

1) Classifier: The classifier receives the incoming packets from different packet sources, retrieves the packet header to extract destination, class of service and quality of service information and forwards the packet to the relevant assembler queue.

2) Burst Assembly: The burst assembly in a joint node is similar to the burst assembly in an edge node. After sorting of packets into assembler queues by the classifier, the burst assembly process starts from the arrival of first packet in each queue. The number of assembler queues depends upon the number of destination nodes in the network, and class of service. There exists one queue for each class of service for every destination node. The bursts are assembled according to size, time or hybrid based burst assembly technique.

3) Routing: The routing module in a joint node has to perform both edge and core node functions. As an edge node, when a new burst arrives, the routing module selects the most feasible path for the burst's destination, from the existing pre-calculated paths, or calculates a new path on each burst arrival. The decision of selection or calculation depends upon the adopted routing strategy. After the routing decision, the burst is forwarded to the scheduler module of the selected path's output link. In case of arrival of control packet of transit burst, while performing the function of core node's routing module, next hop of the burst is retrieved from the routing table and the control packet is forwarded to the scheduler module of next hop's output link. In case that the destination of transit burst is current node, the incoming burst is

forwarded to the burst disassembly module, where the packets in the burst are disassembled and forwarded to the respective packet destinations.

4) Burst Transmission Queues (BTQs): The locally assembled bursts dispatched from routing module to the scheduler module arrive into the burst transmission queues (BTQs) while the scheduler module searches for output wavelength for scheduling. Ideally there should be an infinite buffer availability for the delay traffic, but a higher load of transit traffic results in the loss of the locally assembled bursts in case the buer is full. Whereas, the transit bursts have no buering facility available. BTQs are further explained in channel scheduling in Section 2.2.

5) Scheduling: Scheduling in a joint node is a combination of scheduling in an edge node and the core node. The core node operates in all optical domain, at the arrival of a control packet, the scheduler module searches for the time slot to fit the burst on the output wavelength channel. If the wavelength is found possibly with the need of wavelength conversion), the output wavelength is reserved for arriving burst, starting from arrival time of the burst. Otherwise, if channel is not found, the burst is dropped due to the unavailability of optical buffers. At most, fiber delay line (FDL) components may be used but FDLs do not operate similar to the electronic buffers. As discussed in Section 4.1.4, the local burst data remains in the electronic domain during scheduling, therefore, rather than dropping the bursts, it is possible to buffer them until channel is available. The scheduler module searches for availability of channel, when it is found, the burst from the front of the queue is forwarded to the scheduler buffer, and channel is reserved for the considered burst after its offset time. Channel scheduling for the joint node is explained in detail in Section 2.2.

6) Buffers: In the joint node, there are three locations which require electronic buffers.

- Assembly queues
- Burst transmission queues
- Scheduler buffer

The assembly queues themselves are buffers, packets are delayed until burst completion according to the burst assembly algorithm implemented. Burst transmission queues are the queues for local bursts, and scheduler buffer is the queue where local bursts are delayed from their control packet generation to the actual transmission.

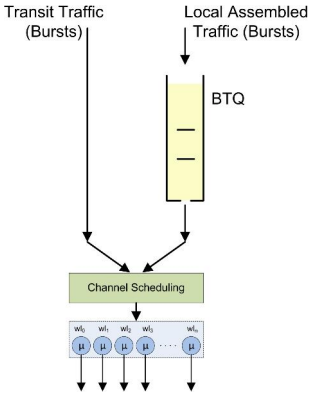

Fig. 3. Queueing diagram

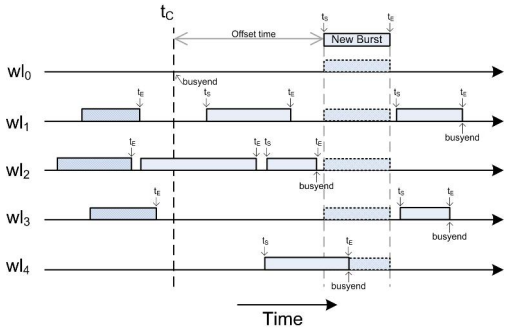

Fig. 4. Channel scheduling

7) Control Packet Processing and Generation: The control packet for the transit bursts is received after opto-electrical conversion, and forwarded to the routing module. Control packet generation module generates the control packet for the local assembled burst after the wavelength reservation by scheduling unit. This control packet contains necessary information of preceding burst, for example, burst arrival time, burst length, burst destination, selected path index and class of service. The primary function of the control packet is to reserve the resources in advance for the incoming burst.

2.2 Channel Scheduling

Channel scheduling is used to allocate suitable output wavelength channels to incoming bursts. Scheduling in an egdge node is different than scheduling in the core node. The edge node is capable of buffering bursts in electronic domain if there is no output wavelength channel available. Whereas, the core node operates in the optical domain, and if scheduling algorithm fails to find any output wavelength channel for the burst, the burst is simply dropped. Fiber delay lines are proposed to be used as buffers in the core node, but they work differently than queueing buffers. Channel scheduling in a joint node is different than both edge and core node. In a

joint node, locally assembled traffic has to compete with transit traffic for output wavelengths. Similarly, the transit bursts coming from previous nodes have to compete for output resources with local traffic. The transit bursts are dealt as loss traffic and therefore, the burst is dropped if the scheduling algorithm remains unble to find a time slot for the burst to fit on output channel. While, the joint node is capable of buffering local assembled bursts. Figure 3 describes the queueing model of input traffic in a joint node. Arrivals of transit traffic go directly to wavelength channels, whereas, local bursts have buffers available.

A new burst scheduling algorithm composite edge core scheduling with void filling (CECS-VF) is proposed to schedule both local and transit bursts. This algorithm is based on LAUC-VF, but it also takes into account burst transmission queues where local assembled bursts are waiting.

Algorithm II.1: BURSTARRIVAL(B_i)

if LocalBurst

then
- Insert burst into BTQ
- $B_i \leftarrow$ Front burst of BTQ
- Find ch using LAUC-VF
- **if** Ch Found
  - **then**
    - Reserve ch
    - Pop B_i from BTQ
    - $B_i$forwarded to Scheduler Buffer
    - CALL CHAVAILABLE() at $t_E[B_i]$
    - Update statistics
  - **else** B_i stays in BTQ

else if TransitBurst

then
- Find ch using LAUC-VF
- **if** ChFound
  - **then**
    - Reserve ch
    - CALL CHAVAILABLE() at $t_E[B_i]$
    - Update statistics
  - **else**
    - Drop the burst B_i
    - Update statistics

2.3 Composite Edge/Core Scheduling (CECS-VF)

The CECS-VF performs a composite scheduling of both local and transit bursts. This scheduling algorithm is different from general edge scheduler in the sense that it searches and reserves wavelength when there is the time to transmit burst control packet (BCP). EPMV-VF proposed in [ ], is an edge scheduler algorithm which reserves wavelengths in advance prior to BCP transmission time. And therefore, it keeps track of burst control packet time tcp, and transmits BCP when tcp occurs. This practice if implemented in composite scheduling technique will give priority to local traffic, and increase the loss rate of transit traffic. The offset time of local burst is already calculated in router module according to number of hops this burst have to travel, therefore, when the burst arrives, the scheduler finds for the available time slot for this burst to fit on the wavelength channel. The CECS-VF algorithm is explained in Algorithm II.1 and Algorithm II.2.

CECS-VF, whenever there is an arrival, either local burst or control packet for transit burst, the BURSTARRIVAL method is called. The burst received from local router module, is inserted into BTQ, then the reference of the burst at front of the queue is retrieved. The scheduler module searches for availability of output channel after offset time of burst from burst start time tS[Bi] to burst end time t_E[Bi] using LAUC-VF algorithm. As shown in the Figure 4, channel is searched for the time slot of burst size in time after current time plus pre calculated offset time of the burst. Five possibilities can occur, there can be no burst on the channel, burst can be scheduled in the void and contention may occur. In case of contention, the channel is supposed to be unavailable. If no channel is found for this time slot, the burst remains into BTQ. If a channel is found, it is reserved, BCP for the burst Bi is transmitted, and burst is moved from BTQ to scheduler buffer and statistics are updated. One more event is scheduled at the end time of scheduled burst t_E[B_i], i.e., CHANNEL AVAILABLE. This event tells the scheduler that there is void available at this moment and output wavelength channel is available for scheduling. When scheduling the local burst, all output wavelength channels are traversed for availability, and selected according to LAUC-VF.

Algorithm II.2: CHAVAILABLE( )

if BTQ $!= NULL$

then
- $B_i \leftarrow$ Front burst of BTQ
- Find ch using LAUC-VF
- **if** ChFound
  - **then**
    - Reserve ch
    - Pop B_i from BTQ
    - B_i forwarded to Scheduler Buffer
    - CALL CHAVAILABLE() at $t_E[B_i]$
    - Update statistics
  - **else** B_i stays in BTQ

The CHANNELAVAILABLE is called whenever there is availability of void on output wavelength channel. If there are bursts in the BTQ, this module performs same functions as the scheduling of local burst is performed.

3. Results and Discussion

3.1 Simulator

This section presents an overview of OBS simulator designed to facilitate the study of OBS networks. The simulator is built upon a general network simulator IKNSim was originally developed in 2004 [8], many protocols were added over the years to aid in the research work. The current version of the simulator is referred to as IBKSim. This simulator is capable of simulating tiny queueing models, single network nodes and able to asses the performance of bigger networks. Its modular structure provides high degree of flexibility and object oriented design approach facilitates extension on the development part. A detailed description about the simulator is beyond the scope of this document, and only fundamental concepts are covered here needed to simulate OBS networks. For the basic concept of IBKSim and the use of XML as configuration and logging language the readers are referred to [9]. The architecture of optical burst switched (OBS) networks has complex structure and components of OBS network have high degree of freedom in traffic pattern, arriving packet distribution, assembly techniques, service class, routing protocols, scheduling algorithms, buffering requirements, burst size, offset time settings and signaling protocols. So far, a vast literature on OBS is there and analytical modeling providing insights to individual components of OBS networks, but might scarcely cope with multiple factors that may arise in complete network scenarios [10]. Therefore, there coexists a need of simulation tools to evaluate the performance of complex network architectures such as OBS. The OBS module of IBKSim takes XML configuration file as input and generates logging file in the form of XML file. In the configuration file the user can describe the whole simulation scenario, such as, run time of simulation or number of specific events the simulation has to run, network, nodes, components within the nodes, links between nodes and internal links of components within a node. Figure 5. demonstrates a typical simulation flow of OBS network. After the start of simulation, packet sources connected to edge nodes start generating packets using packet size distribution and generate rate given in input configuration file. Packets are forwarded to burst assembler module which assembles packets into bursts according to given assembly scheme. Assembled bursts are forwarded to routing module, which looks up the routing table to forward the burst to relevant scheduler or the sink in case current node is destination for the burst. Scheduler module searches for the output wavelength, if found, the burst is forwarded to the routing module of next node. Simulation lasts until the input simulation time or the number of time the specific event is occurred. OBS is mainly composed of edge router and core router, which further contain many components having number of internal connections. Both edge and core router, and main components are discussed below.

Joint Edge-Core Components: The joint edge-core node (JECN), assembles the data bursts performing the function of edge router, and forwards transit bursts to the next nodes while performing the function of core router. Components in the simulation node of joint node are shown in 2. Packets arrived from different packet sources are assembled into bursts which are forwarded to the edge scheduler after routing decision by routing module. Transit bursts are received by routing module as well, which are also forwarded to scheduler module. The scheduler module of joint node differentiates between locally assembled bursts and transit burst, buffers the local bursts in BTQs, whereas, transit bursts are scheduled without buffering. Forwarded bursts are sent to the routing module of the next node, which after routing decision, forwarded to the channel scheduler.

3.2 Discussion

In this section, the behavior of ECJN is analyzed in terms of mixing of local and transit traffic using simulation environment described in Chapter 3. It is worth mentioning that higher rate of transit traffic causes increase in waiting time of local bursts in the burst transmission queues (BTQs), and vice versa, higher rate of local traffic causes higher burst loss of transit traffic.

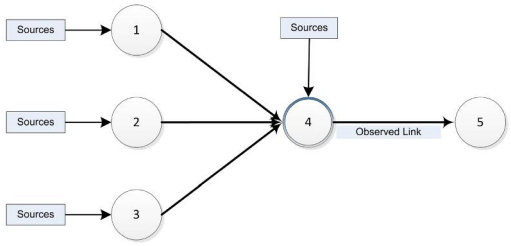

Fig. 5. sim Network

Simulation Setup: Simulations of edge-core joint node are performed on a 5-node sample topology presented in Figure 5. In this topology, node 1 ,2, 3

are ingress edge nodes and node 4 is a joint node. All of the four nodes generate bursts which are destined to egress node 5. The local bursts assembled at joint node 4 compete for output wavelengths with transit bursts arriving from previous nodes. Figure 5: 5-node topology, with node 4 as a joint node. Packet arrivals to the nodes are Poisson with exponentially generated mean packet length of 40 kbits. Bursts are assembled using hybrid based burst assembly mechanism with time threshold of 100 micro second and size threshold of 1 Mbits. CECSVF explained in previously is used for wavelength scheduling. Burst transmission queues (BTQs) can buffer upto 1000 bursts. Following assumptions are taken:

- The number of wavelengths on each link is 4 with 10 Gbits capacity.
- Full wavelength conversion is available at node 4.
- No re-attempt is performed when a connection is blocked.

Calculation of offered network load and blocking probabilities are calculated using standard procedure. Total simulation time is divided into 20 intervals plus a warm-up or transient period and 95 % confidence interval is used to determine the accuracy of the output. 0:1 million bursts are generated for each simulation interval. Figure 6 plots the waiting time of local bursts as a function of offered transit load while the local load is fixed at 0:2, 0:3 and 0:5. It is observed that with the increase in transit load, the waiting time of the local bursts increases. Similar behavior is shown in Figure 7, which plots the BTQ size for local bursts. Figure 8 draws the mean blocking probability of transit bursts as a function of local load. It can be observed that with the increase in local traffic load, transit traffic is affected badly in terms of burst blocking probability. The results show that if both local and transit traffic has equal priority, both will effect the traffic of other. The usage of wavelengths can be restricted for both local and transit traffic to achieve a bargain between mean waiting time of local traffic and the loss rate of transit traffic. Mixing of local and transit traffic is studied in to control the output channel sharing by extending the basic mixed loss-delay queueing models and are solved by Markov chain techniques.

4. Conclusion

This paper introduced the requirement of an edgecore joint node (ECJN) in optical burst switched (OBS) networks. Architecture of ECJN is described in detail with functionalities of each module. A new scheduling algorithm, composite edge-core scheduling with void filling (CECS-VF) for multiplexing both local and transit traffic is proposed and the buffer requirements for the ECJN is discussed. The effect of high transit load on local assembled bursts, and effect of high local load on transit traffic is analyzed through simulations.

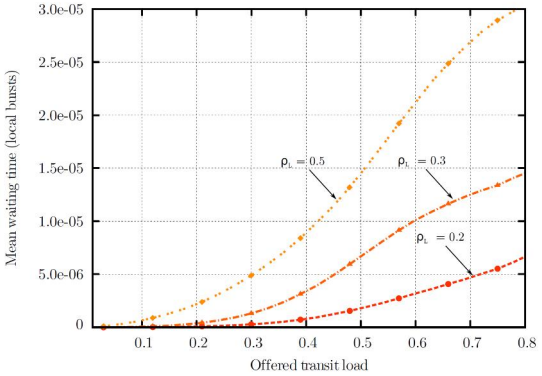

Fig. 6. Waiting time of local bursts as a function of offered transit load

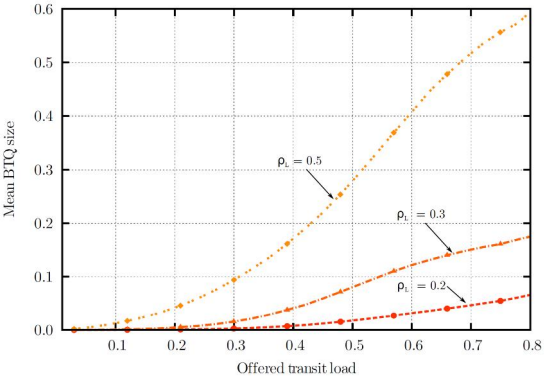

Fig. 7. Mean BTQ size as a function of offered transit load

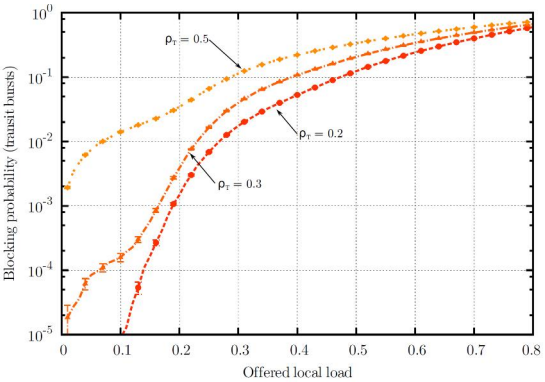

Fig. 8. Mean blocking probability of transit bursts as a function of offered local load

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Modelling Scheduling Systems with Exhaustive Priority Service

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Abstract: Scheduling systems are an integral part of edge nodes in modern communication systems. These nodes aggregate the incoming traffic flows and groomed traffic is sent to core network. The aggregation is done on the basis of scheduling criteria. In this work, we have analyzed a scheduling system where high priority traffic is exhaustively served. The modelling approach we have used is Markov chain modelling. It is assumed that buffers available are of finite nature. Important performance measures such as blocking and waiting probabilities and mean flow time have been analyzed to give an insight into the system behavior. To prove the accuracy of analytical modelling, all results have been simulated.
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Keywords: Optical burst switching, Joint edge-core node

1. Introduction:

In modern communication networks, edge node's major functionality is to provide a fair service to all incoming flows which are using edge nodes as access points. This fairness is achieved using some scheduling mechanism. A lot of scheduling policies have been proposed and it depends upon the particular scenario which policy suits the system requirements. There are several mathematical treatments possible to model a scheduling system. In general, such a system is many queue single server queueing system. Many results from queueing literature can be extended to analyze such a system. It is very helpful to categorize the research in this context. The recent survey has been given by [35]. This categorization is based on type of service, switch over rates, and the system size. There are other categorizations, presented by [25]. The most common types of service are exhaustive, non-exhaustive or gated service. In exhaustive service there are also many flavors. In general, in exhaustive service, the server provides service to a particular queue till the queue is empty. In a gated service system, the server switches to a queue and serves exhaustively only those customers which were present in the queue at switch-over time. In non-exhaustive systems, the server serves only one customer in a queue but queues can be polled in many different fashion like in a cyclic manner or some pre-defined order [13]. The switchover time is also an important parameter. It is the time taken by server to switch to another queue after service completion. This time is usually very small as compared to service time and is ignored in many studies; however this can affect the performance of a scheduling system where it cannot be ignored in comparison with service time. The capacity of system is also an important parameter to take into account. Most of studies have assumed infinite queue capacities for modelling such scheduling systems. However, real systems always have finite capacities, therefore queues with finite capacities should be considered for true performance evaluations. Blocking probability, which is an important performance measure in real networks is only applicable when system has limited capacity. In literature, first study of multi-queue single server was done by [19]. The first study on communication networks was done in early 70s in order to model the time-sharing systems. Leibowitz in [16] first studied a cyclic queueing system with constant switch over times. Exhaustive and gated service with null switch-over times have been analyzed by Cooper and Murray [5], [6]. Bux and Truong [3] gave a very general analysis for exhaustive service discipline with any number of queues. Models with asymmetric service were also presented by many authors like Lee [15]. He presented an analysis for two-queue system. Lee analyzed one queue for exhaustive service and limited discipline was used for the other queue. Kuehn [13] analyzed the round-robin queueing system. He derived results for GI/G/1 queues based on cylic service time and general switch-over rates. He also extended the results for batch service and re-transmission with constant bit-error rate [14]. All studies discussed above used infinite queue capacity. The models with exhaustive and non-exhaustive service discipline with finite queue capacity are not so common in the

literature. Single buffer systems were first discussed by Chung [4] and Takine [29], [30], [31]. Magalhaes in [20] used M/M/1/1 queues for a multi-queue system. Titenko [32] calculated moments of the waiting time for single buffer multi-queue system. Takagi [26] used M/G/1/n for finding Laplace transforms of cycle times with exhaustive service. Tran-Gia have done several studies in this regard [33], [34]. He used imbedded Markov chain for analysis of a non-exhaustive queueing system with finite buffers. In [1] authors presented a markov chain analysis of cyclic finite queue system, with non-zero switch-over times. A very good extension of early work was presented by Takagi [27]. In addition [35] covers a good overview of available models. Closed-form solutions have been given by some authors but mostly these results are available for single-buffer systems. There are several ways to give priority to involved queues in a scheduling system [25], which includes schemes using more visits to higher priority queues or exhaustive/gated service of higher priority queues. These schemes are known as queue priority schemes. Manfield [21], [22] studied one exhaustive queue and n - 1 limited service queues. Message priority schemes are another form of priority schemes, in which priority is done within one queue [25]. A priority scheme in which the highest priority is served while visit to a queue was analyzed by [37], Karvelas and Leon-Garcia [12]. Regarding application areas, Nagle in [24] proposed and analyzed fairqueueing system which is mapable to many scheduling algorithms without any priority considerations. Ibe and Trivedi [11] purposed stochastic Petri Net models for exhaustive, gated and limited service queue scheduling discipline. Takagi [28] proposed three main areas in communication networks where polling models can be used for modelling scheduling policies. Bruneel and Kim [2], Grillo [9] and Levy [17] analyzed several examples of communication networks including ATM. In late 90s, people also studied many recent systems using multiqueue models like [38] and [36]. They analyzed polling systems involved in communications over IEEE 802.11 WLANs. Bluetooth systems were studied using multi-queue polling models by [23]. In this paper an exhaustive priority scheduling system with non-negligible switchover time and queues with finite capacity is considered. The paper is originally presented in [10] and it is extended here with more results. and discussions. Additionally, background of the work is properly extended to have reference for future research. The system is a multi-queue single server system and is evaluated with very high switch-over rate as compared to the service rate; therefore its effect is ignored when switch-over happens with a service in the current queue. However, when the server switches without any service the switch-over time has to be taken into account. The main quantities of interest are the mean number of customers, the mean waiting time, and the mean blocking in a queue.

2. Materials and Methods:

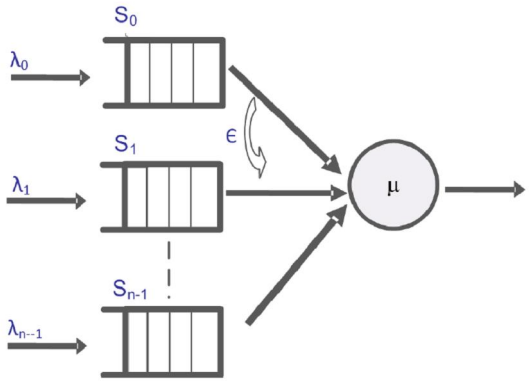

Fig. 1. Model for queueing system

An n-queue single server system is used model scheduling problem under consideration. The size of queues is represented by s_i where (i = 0, 1, …, n - 1). Single server provides the service with exponentially distributed service time which is represented by μ_i and it is the same for all types of customers. The arrival rate in different queues is shown by λi where index i serves for the same purpose as above. The server switches from one queue to the next and switch-over time is only taken into account when the server jumps to the next queue from an empty queue. It means that we are ignoring switchover time when a service takes place and server jumps to the next queue with a service in the current queue. Switchover rate is represented by ε. and it is also considered a single parameters for all queues. The queues are numbered ; 0; 1; 2 … :n - 1. And descending order is used to show priority. It means a queue with number 0 has the highest priority. The working of system is explained below. The server starts with the queue 0 and service all available customers exhaustively if queue is not empty, after the highest queue is empty it jumps over to the next queue in priority and serves one customer from it. After serving one customer it again polls the queue 0 for any available customer. If a queue is empty, the server switches to the next queue with delay of switch over time. The server keeps on going down to priority if all high priority queues are empty. As a thumb rule the

server jumps to the queue 0 after serving one in any queue and it goes down to lower priority queue if higher priority queues are empty. The switch over time is only used when the server switches over without serving any from the current queue.

2.1 Analysis of Model

To analyze the model consisting of a single server and multiple priority queues as described in the last section above, we take a vector T = [Q_1;Q_2; … ;Q_n; i], where i is used as queue index and is also used in diagram to show the queue being served. The process involved here is modelled then with the help of a Markov Chain. For an n-queue system n + 1 parameters are used to represent a state of the Markov process in the steady state. To explain better using Fig. 2, we can draw the state diagram for a two queue system. Three parameters Q_1,Q_2,L are used, to represent the state as shown in Fig. First parameter describes the number of customers in queue in the first queue, second parameter represents the number of customers in the second queue and the last one describes the state of server. L = 1 shows first queue is being served which is having capacity s_1 and L = 2 shows the server is busy with queue 1. First two parameters range upto s_i +1, where si is the queue size and 1 shows an extra customer in with server. The state space is having three-dimensions and it is visualized as shown in Fig. The state space shown actually helps to understand the flow of process, however, it cannot be extended for more queues. Nevertheless, the same rules can be used to formulate the problem in computer programs to extend the Markov chain for large number of queues. The symmetry of the system also helps to generalize the rule of evolution of Markov process involved. Using state space, the state probabilities p can be calculated by solving a system of linear equations given by:

$$PQ = 0 \tag{1}$$

Where P = [p_1, p_2, …], is probability vector and Q is an infinitesimal generator matrix given by

$$Q = \begin{bmatrix} -2\lambda & 0 & 0 & \epsilon & 0 & \cdots \\ 0 & -2\lambda & \epsilon & 0 & 0 & \cdots \\ 0 & 0 & -(2\lambda+2\epsilon) & \epsilon & 0 & \cdots \\ 0 & 0 & \epsilon & -(2\lambda+2\epsilon) & 0 & \cdots \\ \lambda & 0 & \lambda & 0 & -(2\lambda+\epsilon) & \cdots \\ 0 & \lambda & 0 & \lambda & \epsilon & \cdots \\ \vdots & \vdots & \vdots & \vdots & \vdots & \ddots \end{bmatrix}$$

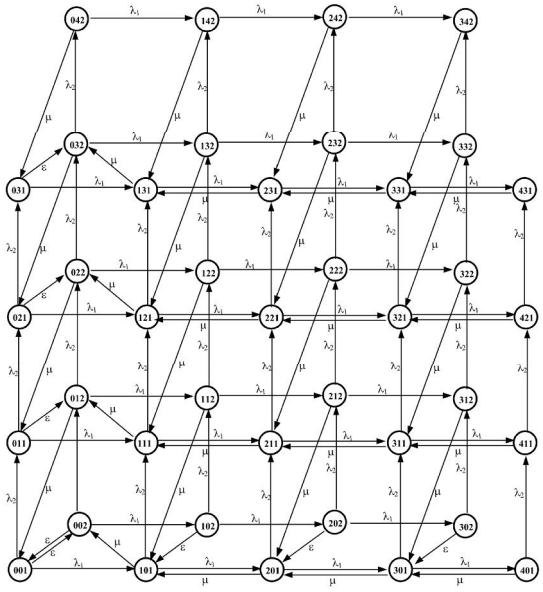

Fig. 2. State space of a system with two queues

Using state probabilities, the mean number of customers in a queue and the mean number of customers in the system can be found using equations below. Mean number of customers in the system:

$$E[X] = \sum_{i=0}^{s+1} i p_i \tag{2}$$

Mean number of customers in the queue:

$$E[Q] = \sum_{i=0}^{s+1} (i-1) p_i \tag{3}$$

From above equations using Little's law [18], it can be found Mean flow time (time spent in system)

$$E[T_w] = \frac{E[Q]}{\lambda} \tag{4}$$

Mean waiting time (time spent in queue):

$$E[T_f] = \frac{E[X]}{\lambda} \tag{5}$$

State space is easy to extend to form a generalized system for n-queues. It requires now n + 1 parameters to represent a state. It is not at all easy to draw state diagram for large number of queues, however, the same construction rules evaluated for two dimensional state diagram may be extended to formulate a system of linear equations for higher dimensions. The various performance measures can also be given in a general form as shown by equation given below. The mean number in the system and the

mean number in queue are given by

$$E[X_1] = \sum_{i_n=0}^{s_n} \cdots \sum_{i_2=0}^{s_2} \sum_{i_1=0}^{s_1+1} i_1 P(i_1, i_2, \cdots, i_n, 1) + \sum_{i_n=0}^{s_n} \cdots \sum_{i_2=0}^{s_2+1} \sum_{i_1=0}^{s_1} i_1 P(i_1, i_2, \cdots, i_n, 2) + \cdots + \sum_{i_n=0}^{s_n+1} \cdots \sum_{i_2=0}^{s_2} \sum_{i_1=0}^{s_1} i_1 P(i_1, i_2, \cdots, i_n, n)$$

$$E[Q_1] = \sum_{i_n=0}^{s_n} \cdots \sum_{i_2=0}^{s_2} \sum_{i_1=2}^{s_1+1} (i_1 - 1) P(i_1, i_2, \cdots, i_n, 1) + \sum_{i_n=0}^{s_n} \cdots \sum_{i_2=0}^{s_2+1} \sum_{i_1=1}^{s_1} i_1 P(i_1, i_2, \cdots, i_n, 2) + \cdots + \sum_{i_n=0}^{s_n+1} \cdots \sum_{i_2=0}^{s_2} \sum_{i_1=1}^{s_1} i_1 P(i_1, i_2, \cdots, i_n, n) \quad (7)$$

The waiting probability can be calculated using equation 8, which sums up all state probabilities, where a queue is neither empty nor full and blocking probability can be calculated by summing up all the state probabilities where a queue is full as given in equation 9.

$$P_{w_1} = \sum_{i_n=0}^{s_n} \cdots \sum_{i_2=0}^{s_2} \sum_{i_1=1}^{s_1} P(i_1, i_2, \cdots, i_n, 1) + \sum_{i_n=0}^{s_n} \cdots \sum_{i_2=0}^{s_2+1} \sum_{i_1=0}^{s_1-1} P(i_1, i_2, \cdots, i_n, 2) + \cdots + \sum_{i_n=0}^{s_n+1} \cdots \sum_{i_2=0}^{s_2} \sum_{i_1=0}^{s_1-1} P(i_1, i_2, \cdots, i_n, n) \quad (8)$$

$$P_{b_1} = \sum_{i_n=0}^{s_n} \cdots \sum_{i_2=0}^{s_2} P(s_1 + 1, i_2, \cdots, i_n, 1) + \sum_{i_n=0}^{s_n} \cdots \sum_{i_2=0}^{s_2+1} P(s_1, i_2, \cdots, i_n, 2) + \cdots + \sum_{i_n=0}^{s_n+1} \cdots \sum_{i_2=0}^{s_2} P(s_1, i_2, \cdots, i_n, n) \quad (9)$$

3. Results and Discussion:

Various characteristics measures have been used to analyze the behaviour of presented system. Mean queue sizes, flow time, waiting and blocking probabilities have been studied using different arrival rates or total load to the system in this section. Additionally, the effect of arrival rates of lower priority queues on high priority queue has been demonstrated to show the validity of exhaustive priority scheduling. The model assumes ignorable switch over times as compared to service time. Maximum queue sizes for all the plots are same and equal to 10. For clarity and ease of understanding, all figures except Fig. 3 and Fig. 4 are plotted using three priority classes. All results have been plotted with simulations points on analytical curves. Fig. 3 and Fig. 4 are using a scheduling system with four priority classes. Fig. 3 shows the mean number of customer in all queues for varying arrival rates. It is clear that the highest priority queue has minimum number for all arrival rates. The mean number of customers in all queues grows slowly for low arrival rate or total load, but increases rapidly after a certain load for low priority queues which is different for different queues. However, the high priority queues continues to show the same steady behaviour. The low priority queues reaches to the saturation point, which is approximately equal to 1. The behaviour of exhaustive priority discipline is quite clear from the plot. The same conclusions can be drawn from Fig. 4. Here, all high priorities experience comparative blocking probabilities and low priorities approach to 1 much earlier for all different arrival rates. In Fig. 5. the mean flow time is plotted against varying arrival rates in all queues of the priority system. It is quite clear that low priorities face comparatively high flow time. The customer which are blocked are not included in the calculations of flow time. Fig. 6 depicts the same kind of behaviour where the difference is only that now customers blocked have been included in calculations. In Fig. 7, the mean flow time has been plotted against varying arrival rates. The arrival rate of the highest priority queue is fixed and we can easily observe that increase of arrival rate in lower priority queues does not much influence the flow time of highest priority queue. Next in Fig. 8 the mean waiting probability has been plotted against varying arrival rates in all queues. The same behaviour is observed as the mean flow time plot discussed earlier. The waiting probability increases and after a certain saturation point it tends to decrease for all queues. The decrease in lowest priority queue is most rapid, and the reason of that is the sharp increase in blocking probability which resultantly reduces the mean waiting time of waiting customers. Fig. 9 shows that only increasing the arrival rate in lower priority classes has negligible effect on highest priority which validates the exhaustive service scheduling again. In the last Fig. 10 switch over time has been plotted against blocking probabilities for a system of three queues with fixed arrival rate in all queues equal to 0.3. It can be seen that increasing the switch over time has very little effect on the performance measures. Definitely this can only be assumed if the chosen switch over rate is much higher than service rate.

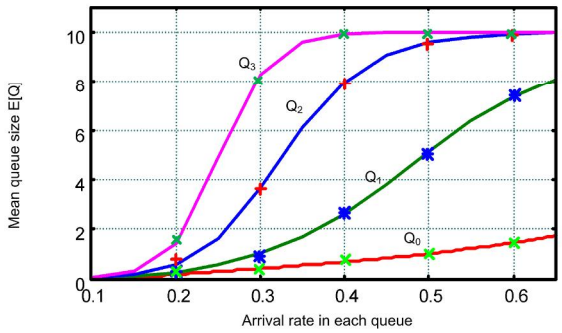

Fig. 3. Effect of varying all arrival rates on queue sizes with maximum queue capacity = 10 for a four-queue system.

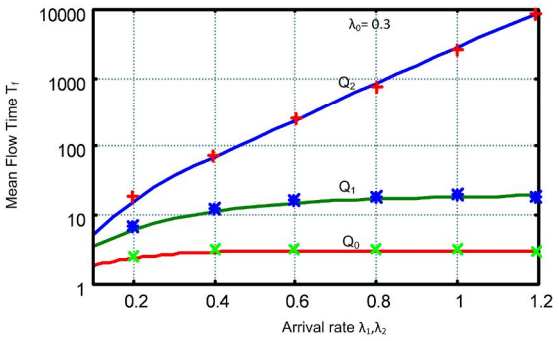

Fig. 7. Varying only λ_1; λ_2, mean flow time $E[T_f]$ of all queues with maximum queue capacity = 10 for a three-queue system. Blocked customers are not taken.

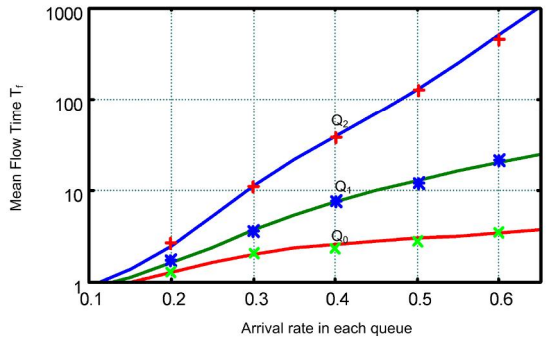

Fig. 5. Mean flow time E[Tf] with maximum queue capacity = 10 for a three-queue system vs arrival rate. all rates are varied simultaneously and blocked customers are not taken for mean flow time calculations.

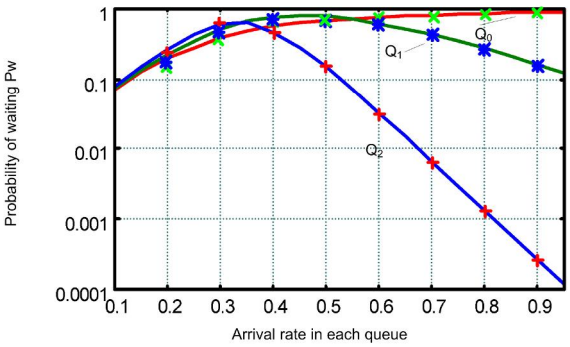

Fig. 8. Waiting probability Pw vs arrival rates where all rates are varied simultaneously with s = 10. Blocked customers are included in calculations.

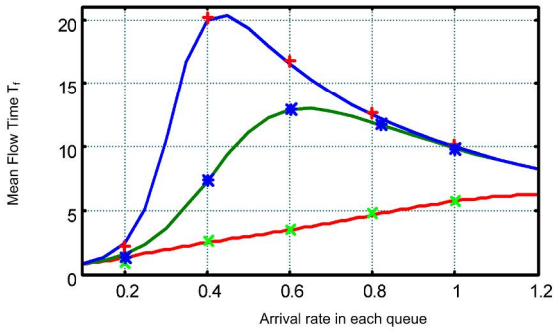

Fig. 6. Mean flow time E[Tf] vs arrival rates, where s = 10 for a three-queue system. Blocked customers are considered for mean flow
time.

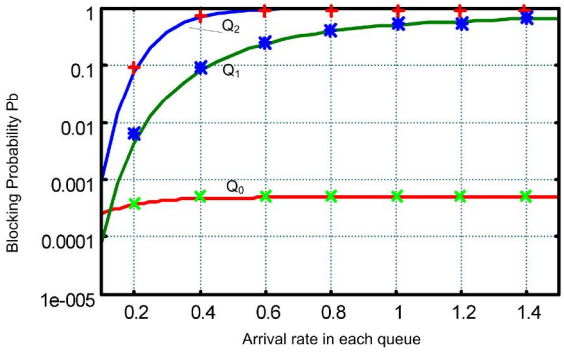

Fig. 9. Varying λ_1; λ_2, for Arrival rates vs blocking probability P_b s = 10 and system is a three-queue system.

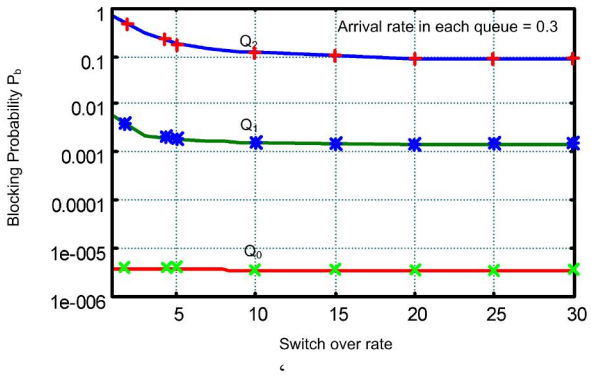

Fig. 10. Switch over time vs blocking probability P_b with maximum queue capacity = 10 and $\lambda_i = 0:3$; i = 0; 1; 2

4. Conclusion:

In this paper, a Markov chain model is introduced to model an exhaustive priority scheduling system where the involved buffers are limited and switch over time is only considerable when it happens with empty queues. The Markov chain is extended to a system with few queues but it is computationally not feasible to extend the analysis for very large number of queues. However, the analysis presented a real insight into the system behavior under consideration.

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The effects of some agricultural By-products on ruminal fermentation and apparent digestibility of Holstein dairy cow

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Abstract: This study was carried out to evaluate the effects of replacing alfalfa hay with ensiled mixed tomato and apple pomace (EMTAP) on ruminal fermentation and apparent digestibility of diets. Six multiparous Holstein dairy cows in mid lactation were used in 3×3 Latin square design and fed alfalfa hay plus concentrate mixture with three levels replacement with EMTAP (0, 15, 30%) during 63 days. Results showed that, differences between treatments were significant. Feeding EMTAP resulted in a significant higher acetic and propionic acids and total volatile fatty acids concentration and significant ($P<0.01$) lower rumen pH. The substitution of EMTAP in dairy cow diets is associated with a better DM, OM, CP and NDF digestibility. It was concluded that, EMTAP can efficiently replace up to 30% alfalfa hay. The nutritive value of tomato and apple pomace could be improved when they are used together (50:50) in dairy cows diet.
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Keywords: agricultural By-products; tomato pomace; apple pomace; dairy cow.

1. Introduction

Shortage of inexpensive feed resources often impose major constrains on the promotion of animal production. Considerable quantity of agro-industrial by-products are generated every year in most developed and developing countries. Introduction of alternative feedstuffs is an interesting challenge for animal nutritionists and it could overcome the problems of environmental and production costs. Ruminants which are able in converting waste items in to useful products such as meat, milk and skin, offers a feasible solution of using by-products and preventing pollution (Oni et al., 2008). Tomato and apple pomace are two alternative by-products that obtained from tomato paste and apple juice industrial production, respectively. These by-products are produced in huge amount annually. The chemical composition of final pomace is linked to the morphology of the original feed stock and the extraction technique used. Although tomato and apple pomace are varying from nutrient density, effective processing can improve their nutritive value. According to NRC, (2001) apple pomace (AP) is very low in protein (6.4% protein on DM basis), it also serves as a useful energy source because of high content of soluble carbohydrate for ruminants. Researches conducted on AP (Rumsey, 1978; Fontenot et al., 1977), showed that AP supplemented with natural proteins was comparable to protein enriched corn silage. In contrast, Elloitt et al. (1981) demonstrated that, tomato pomace (TP) have the potential to be a good source of protein, however its energy source may be limited due to the high fiber content.Previous researches reported different results from feeding TP and AP. The complementary composition of AP (low protein concentration) (Alibes et al., 1984; NRC, 2001; Pirmohammadi et al., 2006) and TP (high protein content) (Fondevila et al., 1994; Del Valle et al., 2006; Weiss et al., 1997) suggest to use those by-products together. Our previous observations (unpublished data) showed that processed TP with AP (ratio of 50:50) had more palatability and digestibility than processing with urea, wheat straw, NaCl and NaOH for sheep. The aim of the present study is to evaluate the effect of ensiled mixed tomato and apple pomace (EMTAP) on ruminal fermentation and diet digestibility in Holstein dairy cows.

2. Material and methods

2.1 Tomato and apple pomace and silage preparation

Fresh experimental samples of tomato pomace (TP) and apple pomace (AP) were collected from several factories in Urmia city (Iran). TP and AP were mixed together (50:50 on DM basis) and ensiled without any additive in a trench silo on a concrete floor. The mixed TP and AP silage (EMTAP) was sealed for 55 days, next fed as TMR diets in three levels replacement of alfalfa hay. Chemical composition of TP, AP and EMTAP was determined using the method suggested by AOAC (2000). Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were determined using method of Van Soest et al. (1991).

2.2 Dairy cow management

Six multiparous dairy Holstein cows were used in a 3×3 Latin square experimental design with

three 3-wk periods. They were kept in individual concrete tie-stalls and had free access to drinking water at all times. The daily TMR diets were given in two equal feeds at 08:00 and 20:00 h to provide approximately 10% feed refusal each day (as-fed basis). Feed refusal were removed and weighed before feed offered at 08:00 h. Body weight was recorded prior to morning feeding on 2 consecutive days at the beginning and at finish of each period. The experimental periods lasted 21 d, including 14 d of adaptation and 7th d of sampling and data collection. During the last 7th d of the experimental period collection and sampling of TMR diets, feed refusal, rumen fluid, blood, feces and urine were performed. Normal herd management practices were followed during the experiment. Daily feed intakes and milk production were recorded for individual cows throughout the experiment.

2.3 Treatments and digestibility study

The dietary treatments consisted of three levels replacement of alfalfa with EMTAP and include diet 1 (control or 0% EMTAP), 2(15% EMTAP) and 3(30% EMTAP) on DM basis. The diets were formulated according to the NRC (2001) guidelines. Ingredients and chemical composition of the diets are reported in Table 1. To evaluate the diet's nutritive value, the apparent digestibility was determined at 4th, 5th and 6th -day of each sampling period. Total feces were collected individually from each animal, sub sampled and stored (-20°C) for the subsequent laboratory analysis.

2.4 Data collection, analytical methods and laboratory analyses

Samples of rumen contents were collected on 6th day of each sampling period, 3 h post feeding by esophageal tube connected to vacuum pump. The first 20-30 ml of ruminal fluid obtained was discarded prior to collecting samples to minimize the saliva contamination. Ruminal contents were squeezed through two layers of cheesecloth and immediately analyzed for pH using a Schott Titrator Titroline easy pH-meter. To inhibit microbial growth and NH_3 volatilization the pH value was maintained less than 2.0. Five milliliters of strained rumen fluid were preserved by adding 1 ml of 25% H_2PO_4 to determine volatile fatty acids (VFA), and 10 ml of filtrate were preserved by adding1 ml of 1% H_2SO_4 to determine ammonia-N. Samples were frozen at 20°C and subsequently analyzed for ammonia-N using micro Kjeldahl method, (according to AOAC 2000 recommendations) and VFA concentrations (acetic, propionic, butyric, Valeric, Isovaleric) by gas chromatography (mark Philips, Varian 3700, serial number pu4410, Varian Specialties Ltd., Brockville, Ontario, Canada) with a 15-m (0.53 mm i.d.) fused silica column (DBFFAP column; J and W Scientific, Folsom, CA) in Animal Nutrition Laboratory in Tehran University. Feces were collected and extracted through cheesecloth into a clean beaker; the urine samples were taken via vulva stimulation. Feces and urine pH was measured using a Schott Titrator Titroline easy pH-meter. For the estimation of DM intake and apparent digestibility, sample of feed offered, rejected feeds and feces were dried (60°C for 48 h), weighed and sub sampled to be later analyzed for chemical composition. The dried samples were grounded through a 1-mm screen and were assayed for DM, OM, (AOAC, 2000; #930.15) and CP (AOAC, 2000; #990.03). The organic matter (OM) was calculated as weight loss upon ash. Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were analyzed according to Van Soest et al., (1991). Dry matter intake was calculated as the difference between DM offered and refused.

2.5 Experimental design and statistical analysis

Data collected from DM, OM and CP digestibility and pH (rumen, urine and feces) were statistically analyzed using the GLM procedure (SAS. 1998, Inst. Inc., Cary, NC). Level of significance was α = 0.05, and the Tukey test was used to test for all pairwise comparisons among means. The model used for this analyze was:

$$Y_{ijk} = \mu + T_i + C_j + P_k + \varepsilon_{ijk}$$

where Y is dependent variable, μ is the overall mean, T is treatment effect (i = 1, 2, 3 EMTPA levels), C is cow effect (j= 1 to 6), P is period effect (k= 1, 2, 3) and ε is random residual error term. Remaining data from VFA concentration and ammonia-N were analyzed as unbalanced completely randomized design. GLM procedure and Duncan test were used to compare differences between treatments.

Elevation of plasma glucose, elevation of plasma cholesterol, and immunosuppression of humoral and cell-mediated immune responses (Taxton and Siegel. 1970).

3. Results and discussion

3.1 Ruminal fermentations

Concentration of total VFA profile, ammonia-N and pH in ruminal fluid at 3 h post feeding were used to observe the ruminal fermentation pattern (Table 2). Results from analysis of rumen fluids indicated that ruminal pH and VFA concentrations were different ($p<0.05$) among diets but ammonia-N was not affected. Data obtained showed that both levels of EMTAP resulted in higher acetic, propionic, acetic: propionic ratio and total VFA concentrations, but in lower ruminal pH ($P<0.01$). These trends are similar to those described by Rumsey, 1978; who worked on supplementation of AP with non protein nitrogen (NPN) in gestating

beef cows and fistulated steers, and Chumpawadee and Pimpa (2009), who worked on effect of some non forage fiber sources such as TP in beef cattle nutrition. The rate and extent of carbohydrate degradation in the rumen affected VFA production. Rumsey (1978) reported that feeding AP was associated with a slightly reduction of ruminal pH, a higher acetic to propionic acid ratio and lower proportions of ruminal branched-chain fatty acids. Same results were also observed by Oltjen et al. (1977) when compared AP with corn silage. Due to the high content of acids components of EMTAP (pH: 3.5), especially malic and citric acid (NRC, 2001) a decrease of rumen pH was observed. At the same time its high amount of pectin (AP 15 and TP 7.55 %) can represents a substrate for rumen bacteria to produce acetate (Del Valle et al., 2006). Concerning TP, present results are comparing with findings reported by Chumpawadee and Pimpa (2009), those authors observed that total VFA concentrations and ruminal acetate of animal fed TP were higher ($P<0.05$) compared with other (rice straw, palm meal, dried brawer gain, soybean hull) diets. The authors conclude that these results are due to high carbohydrate fraction in TP.

Table 1. Ingredients and nutrient composition of experimental diets (DM basis)

	Diets (EMTAP levels)		
	1	**2**	**3**
Ingredients	0%EMTAP	15%EMTAP	30%EMTAP
Alfalfa hay	45.67	33.35	18.41
EMTAP‡	0	15	30
Soy bean meal	10.25	10.23	9.92
Barley	37.96	37.99	38.2
Fat (Oil plant)	0	0.57	0.99
Wheat bran	5.4	2.09	1.54
Caco3	0.22	0.27	0.44
Premix†	0.5	0.5	0.5
Nutrient compositions		**(% based DM)**	
DM	98	78.3	63.1
NEL (mcal/kg)	1.54	1.58	1.62
CP	15.4	15.5	15.5
NDF	35.4	35.2	35.1
ADF	21.4	23.1	24.3
Calcium	0.6	0.6	0.5
Phosphorus	0.4	0.4	0.4
Concentrate	54.33	51.65	51.59
Forage	45.67	48.35	48.41

‡EMTAP, ensiled mixed tomato and apple pomace; DM, dry matter; NEL, net energy for lactation; CP, crude protein; NDF, neutral detergent fiber; ADF, acid detergent fiber. †Premix supplied (on a concentrate DM basis): 400.000 IU of vitamin A/kg, 100.000 IU of vitamin D3/kg, 100 mg of vitamin E/kg, 219 mg/kg of Mn, 69 mg/kg of Zn, 116 mg/kg of Fe, 23 mg/kg of Cu, 1.8 mg/kg of I, 0.6 mg/kg of Co, and 0.46 mg/kg of Se.

3.2 Digestibility study

Mean values of diets digestibility are shown in Table 3. The results showed that DM and OM digestibility tended significantly ($P<0.05$) to increase with add EMTAP in the diet. It was showed by several studies (Ibrahem and Alwash, 1983; Ojeda and Torrealba, 2001) that TP can improve the nutritional value of the diet, due to its content in more digestible protein (61.2 %) and ether extract (86.3 %). Rumsey (1978) reported that AP is similar to corn silage for total digestible nutrients content as pectin, pentosans and ether extract.(already reported before) Generally, the diets containing EMTAP respect to the presence of more nitrogen free extract (NFE), appreciable quantities of soluble carbohydrates (NRC, 2001; Hang and Woodams, 1986, Rumsey 1978) and pectin (Del Valle et al., 2006) in AP and TP, may lead to higher digestibility of DM and OM in control diet.

Table 2. Volatile fatty acids, ammonia-N and pH of rumen content, feces and urine pH in cows fed diets with different content of EMTAP.

	Diets (EMTAP Levels) ‡				
	1	**2**	**3**		
	0% EMTAP	15% EMTAP	30% EMTAP		
Concentrations				**S.E.M**	**P value**
Total VFA, mmol/L	68.15^{c}	83.7^{b}	91.24^{a}	0.56	<0.01
Acetic, mmol/L	40.1^{b}	51.75^{a}	53.15^{a}	0.29	<0.01
Propionic, mmol/L	18.55^{b}	20.2^{b}	26.2^{a}	0.35	0.04
Butyric, mmol/L	8.6	9	9.55	0.18	0.25
Valeric, mmol/L.	0.7	1.55	2.55	0.36	0.25
Isovaleric, mmol/L	0.2	0.3	0.65	0.08	0.21
Acetic: Propionic, mmol/L	2.03^{b}	2.16^{b}	2.57^{a}	0.05	0.04
Ammonia-N, mmol/L	6.98	7.3	7.45	0.11	0.35
pH variations					
Rumen	6.55^{a}	6.31^{b}	6.01^{c}	0.04	<0.01
Faeces	7.3^{a}	6.65^{b}	6.59^{b}	0.12	<0.01
Urine.	8.5	8.28	8.23	0.09	0.19

‡Diets,1= (control or 0% EMTAP); 2= (15% EMTAP); 3= (30% EMTAP); S.E.M = standard error of means; a,b,c Means in the rows with different superscripts are significantly different (P<0.05).

Table 3. Nutrient digestibility and dry matter intake of cows fed diets differing in ratio of EMTAP.

	Diets (EMTAP Levels) ‡				
	1	**2**	**3**		
	0% EMTAP	15% EMTAP	30% EMTAP		
Item				**S.E.M**	**P value**
DM intake (kg/d)	21.3^{b}	23.7^{ab}	24.5^{a}	0.68	0.02
DM	64.24^{b}	66.59^{a}	66.84^{a}	0.6	0.03
OM	68.30^{b}	70.36^{a}	70.21^{ab}	0.5	0.04
CP	66.19	66.25	66.24	0.14	0.94
NDF	58.02	59.01	58.60	0.27	0.08

‡Diets; 1= (control or 0% EMTAP); 2= (15% EMTAP); 3= (30% EMTAP); DM intake = dry matter intake (kg/d);S.E.M = standard error of means

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Influence of economic world crisis in tourism negative growth in Tajikistan with short look at tourism destinations

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Abstract: Tourism industry has been confronted with challenges like other activities and economical circles parallel with economic world crisis and different industries and jobs like tourism are goal and cause severe decline and downfall, this is right when according to WTO (tourism world organization) report, tourism is one of main economic activities after oil industries and constructing automobiles. And researchers declare in surveys that tourism industry include more than eleven percent of total world national gross production. Tourism world costs expenditures are 2000 million dollar and countries share has been declined after economic world crisis of these numbers. This problem causes to influences on Tajikistan that has different tourism destinations such as remedial hot water resources and tourist journeys for remedy and recovering, is one of main purposes of tourism of this country. As formal statistics of tourism organizations in Tajikistan country shows negative growth of tourism entrance statistics to this country.

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Keywords: Tourism industry, remedial tourism, tourism organizations, economic world crisis, Tajikistan.

1. Introduction

Tourism in ancient soviet countries after collapsing in first years of Tajikistan autonomy after 1991 Gregorian year usually has positive growth and tourists in all parts of world, after collapsing ancient soviet irony walls, they interested in to have a journey to fifteen soviet countries and being familiar with people, culture and tourism destination of these independent countries, and came back to their countries with valuable experiments of journey, republic of Tajikistan also is one of historical countries and most important Persian language in middle Asia and mavaonahr countries (countries between sir sea and amo sea). this country has seven million people population and about 143100 square kilometer perimeter and also has neighbors such as Uzbekistan, Afghanistan And has hundred natural rivers, mountains, jungles, natural hot waters, different traditional arts and national and historical museums. Remedial tourism is one of income creating resources in Tajikistan. Remedial tourism, today in tourism markets has been allocated growing section of market to itself. (Bookman, Milica Z &Bookmam, Karla R, 2006). seven categories of these agents are influenced in remedial tourism affluence in international level : decrease in patients remedial cost, better quality of remedial services, shortening of prospect queue, increase in number of insurance incapable remedies in west countries, and special remedies. (Woodman, Josef, 2006).

1.1. Tourism industry in economic viewpoint

Economists know tourism industry as employment and capital production industry, and pay attention to this industry, so they believe that tourism correct development causes exchange increase in economic aspect. Economic science scientists believe that if a country can use special potentials in tourism destination in countries, these economic wheels will move. Host region is called to definite limitations in society that in addition to residential population and social and economic life, tourism focus is settled in it. (Ritchie, J. R. Brent. Et.al.2011)

2.1. Tourism economic benefits

Exchange entrance to country, employment in most of cases, retailer market dynamism, airport activity and transportation system, financial increase, increase in native productions and artifacts, increase in private section financing (in constructing hotels and other related services) and creating thousand employment situation and new entrepreneurship. “Mack gahan”, believes that thirty six percent of companies and industries profitability depends on their specifications and capabilities.

3.1. Looking at tourism destinations in Tajikistan

Different tourism destinations in Tajikistan and its originality can be surveyed in different dimensions, in the center of Dushanbe capital of republic of Tajikistan, we can name Tajikistan national museum and teacher kamaledin Behzad, valuable complex that ancient and antique and costly

things are kept in it, several art gallery and traditional marketing shops, artificial seas beside Height hotel and zoo with different species of animals and creatures and donated bears from Russia, hundreds rivers and seas coasts that are suitable for swimming in summer, Tajikistan national library and ancient building of national library that was named hakim abolqasem ferdosi, beautiful squares with huge statues from soviet times and autonomy era like esmaeel samani square, sadredine eyni, ebnesina and statues that were hanged on walls and they were Tajik writers such as khiam, jami, chekhof, lahoti and other famous writers. Tajikistan country with special natural and continental specifications, traditional and art buildings for tourism proponents, is a good destination and also is so important in international level because of geographical specifications and settled in Europe in east of Asia road (silk road). And on the other hand, properties can be reached from nature directly. (Such as natural resources) and processes converts this properties to outputs (such as products and services) for selling and exposing in market. But exploiting from different focal capabilities of tourism needs complete perception of economical and social systems of host region in one hand, and tourist's needs and wants recognition and their coordinating with host region infrastructure on the other hand. different regions and more important cities of ancient saqad province, capital of khojand city in the north of Tajikistan that have historical specifications, sea, dam, powerhouse from soviet times, ancient museum, qaraqom sea that was constructed between three Tajikistan, Uzbekistan and ** countries about fifty years ago. also, khojand museum have hundreds historical arts and ancient goods and international airport of khojand accepts flights even from Moscow and also has historical mosque (moslehedin) and historical market (Thursday market) and nature tourism probability (with fishing). Different regions of tourism and kolab city that emam ali rahman is president of this ancient city and also has airport and international flights from Russia. the other considerable regions are ecotourism destinations in Tajikistan and natural jungles beside rivers especially near some cities, with hunting probability of extinct animal species and special birds that some o European tourism are interested to this regions. Tourism is a complex process that is consists of national and international development agents and involved groups with government politics, programming and legislation. (Peterson, T.Guiden.et.al.2004) in addition to this, tens sport and international matches are hold annually in tens sport field that are interested by sport tourists, admissible excavating projects that are done by universities, are considered by ancient and historical tourists. qolbeh park and cable car in the center of doshanbe city also is considered by war tourists as a place of soviet war victors against Hitler German army has pictures museum and local historical arts that every year soldiers and armies pay regards(Bojanic, D.C. (1991).

4.1. Surveying economic crisis on Tajikistan tourism

According to increasing growth tourist in more than one decade after republic of Tajikistan autonomy from ancient soviet, transforming destination experiments is more important in tourism industry in more aspects, and also transforming a positive sample from destination has considerable important, because selecting destination priorities is influenced by people perception ideality of these destinations extensively, and despite of first fifteen years of autonomy of these republic, according to economic universal crisis, increasing oil price, increasing unemployment in world level, decreasing income per capita, entrance tourism declined from 2008 years to now gradually to Tajikistan (Tefler, David and Sharply, Richard, (2008).

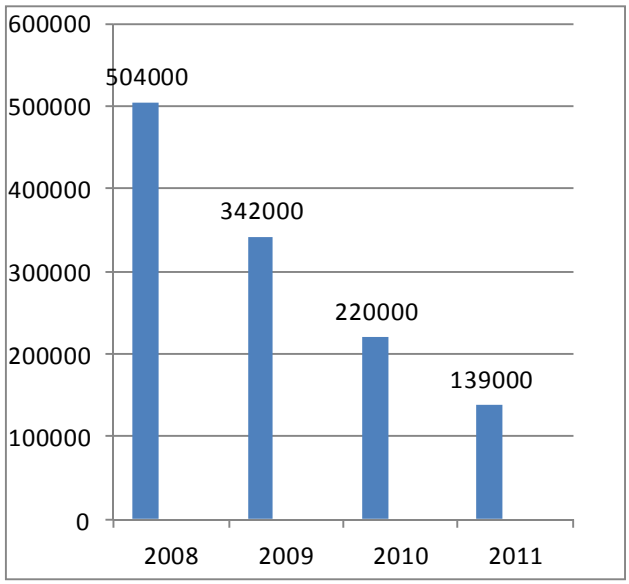

Figure1: Foreign passengers table to Tajikistan with negative growth to 35 percent in 2008 to 2011 Gregorian years

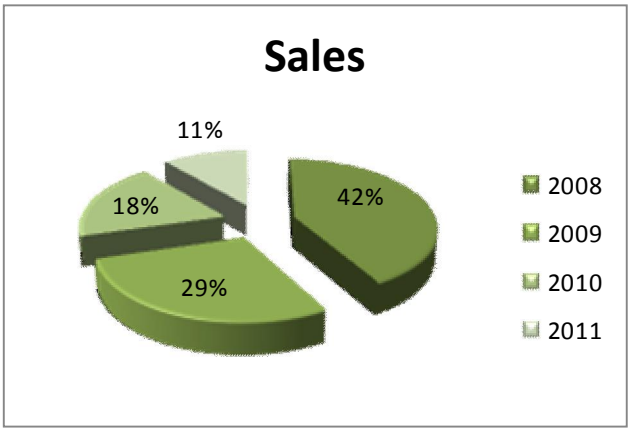

Figure2: Tourism entrance Circle diagram to Tajikistan from 2008 Gregorian year to first six month of 2011 Gregorian year

2. Conclusion:

Tajikistan country, has been placed in a special region in middle Asia, is a country that has more abilities and capabilities, and more proponents take a trip in natural tourism, historical tourism, remedial tourism, sport tourism, war tourism, religious tourism and educational tourism section. Tajikistan for recovering declining entrance tourist to this country increase tourism facilities such as developing roads, transportation vehicles for tourists, reducing hotels fee, teaching officers, and internal tour leaders and sending tourism section specialists for reviewing different points in successful countries in tourism industry with successful strategies against rival countries with governmental budget devotion about study and executive and infrastructure affairs, takes steps in this country's industry future that in addition to creating new jobs restricts work force recent migration to Russia and other countries. By increasing gross internal production (GDP) and gross national production (GNP) causes increasing income per capita in Tajikistan country. According to that ninety three percent of Tajikistan land is mountain, so can converted to Swiss in middle Asia in tourism aspect. And in addition can cause tourism activity in nature and mountain climbing, and hold mountain climbing international championship period in one period according to communist famous summit that was named in soviet times and today had been named esmaiel samani in Sassanid government, with tourism attraction incentive.

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Review of the status of children's rights in the Islamic Republic of Iran's legal and judicial sources

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Abstract: Considering the fact that children are among the most vulnerable communities and since they can't overcome adversity due to their young age and intellectual and physical immaturity, placing priority on their basic and natural rights is something obvious and is the natural and legal authorities' responsibility. Since all human beings are subject to the rights and freedoms enshrined in the constitution without considering their race, color, gender, language, religion, and so on, children must have particular rights because they make the country's future so that their welfare, liberty, happiness, and beliefs must be formally recognized and necessary facilities and equipments must be properly and healthily provided for their physical, mental, ethical and social growth in a healthy free environment. Children need adequate family and social security and special support including certain care before and after birth. Even children who are intellectually and physically disabled need special care and attention together with proper training appropriate to their disability status.
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Keywords: Child – Children Rights - Legal Sources - Islamic Republic of Iran

1. Introduction

Considering the fact that children are among the most vulnerable communities, there is no doubt that they can't overcome adversity due to their young age and intellectual and physical immaturity. Therefore, placing priority on their basic and natural rights is something obvious and is the natural and legal authorities' responsibility. Since all human beings are subject to the rights and freedoms enshrined in the constitution without considering their race, color, gender, language, religion, and so on, children must have particular rights because they make the country's future so that their welfare, liberty, happiness, and beliefs must be formally recognized and necessary facilities and equipments must be properly and healthily provided for their physical, mental, ethical and social growth in a healthy free environment. Children need adequate family and social security and special support including certain care before and after birth. Even children who are intellectually and physically disabled need special care and attention together with proper training appropriate to their disability status. The basic principle that children need love and affection has been proved by psychologists. If a child is not faced with love and affection since birth, his personality won't grow perfectly or moderately and is prepared to move toward devious way and social disorder in future. Thus, in first step, parents must provide and environment full of love and affection with ethical, spiritual and material security for their children and most importantly, a child shouldn't be separated from his or her mother except in very exceptional cases. In the second step the society and related authorities are responsible to provide a relaxing environment for children and their families without any concern, and to take good care of poor children and children without any family. If governments provide material help for poor families whose children need welfare, they not only won't lose anything, but also will benefit from these children as country's wealth who will make up any loss in future and since the field of their moral, cultural and mental growth has been provided, they will make a healthy, peaceful society. That's why it is recommended that children must benefit from free but compulsory education. Legal system reflects the rights and duties of human beings in a society, and its supreme goal is to establish law and public order. So that everyone who doesn't respect other people's privacy and dignity will be punished and no abnormality or illegal behavior is tolerated in defending their rights. But depending on his physical and mental conditions, a child has some special rights in addition to the legal rights of every human being, which make it necessary for the laws to support them which is essential for their growth and development. With the formation of modern international law, child rights appeared in this system. Convention on "the Rights of the Child" was passed by the UN General Assembly in November 20, 1989 and different countries joined the convention on "the Rights of the Child" by signing this treaty.

Islamic Republic of Iran's accession to the treaty, citing the seventy-second principle of constitution, was realized in March, 1993 with the approval of Parliament and Guardian Council. Of course, in top of the single article of this law it is stated that the provisions of the Convention in any case and at any

time must not be in conflict with domestic laws and Islamic criteria.

Article I of the convention on the Rights of the Child, defines child as all human beings under the age of 18.Thus, according to this convention everyone under18, is called a child and is supported by this treaty. In Iranian legal system, the child is looked at differently by the convention. The legal systems of different countries and consequently according to the convention of Child Rights, a child .i.e. anyone under 18, is acquitted of any responsibility. But Iranian legislator, in Article 49 of the Islamic Penal Code adopted in 1991, states that: children are acquitted of criminal charges if they commit crimes. The first note of article 49 also defines child as: a person who hasn't reached religious maturity yet. Iranian legislator, in the first note of article 1210 of civil code which has been modified in 1991, defines the age of religious maturity as: Maturity age for boys is 15 and for girls are 9 of Arabic lunar year. Considering the above mentioned articles and with regard to the contents of Child Rights Convention and top part of single article of Iran's accession to international convention of child rights, which declares that the provisions of the convention must not be in conflict with domestic laws and Islamic criteria, in any case and at any time, and also based on the rules of Iran Legal system in superiority of domestic laws to international laws and treaties, it can be inferred that In Iran In case of committing crime. Children are just acquitted of criminal charges and that religious maturity is the age of criminal charge and responsibility in Iran. However, in international law system, everyone under the age of 18 is considered as a child and therefore is free from any responsibilities and charges (Abrahamian, Ervand.2008).

Posing lack of responsibility to a child takes the right of agreeing to definite or indefinite contracts away from him according to the world convention of child rights. It includes marriage as a kind of contract.

However, according to article 1210 of civil code, children under legal age can get married with their parent permission; unfortunately, some forced marriages at young ages, especially among girls who live in rural and nomadic areas are based on this law which causes social abnormal consequences in the future. World convention of child rights in several cases, while supporting children rights and expressing governments duties in ensuring the realization of these rights, has forbidden exploitation, sexual abuse, child trafficking, torture, execution, illegal arrest and detention of children in various forms. It also supports and protects them entirely against narcotics, social reconstruction, social insurance and services, abuse, plays and leisure time, military service, custody and education, freedom of opinion and expression, individual and group freedom, preservation of privacy, right to live, the right to use a lawyer, personal identity, citizenship and the rights related to early life and name selection. A look at Islam attitude and holy prophet, Muhammad's (Peace be upon him) and infallible Imams' lifestyles and religious orders and instructions indicates their support of child legal rights. Arabic pre-Islamic ignorance considered a female's birth a shame and embarrasses for her father and had legitimized the innocent girls burying alive.

But prophet's lifestyle which was inspired by Islamic instructions, not only saved innocent girl from being buried alive, but also granted her the right of life and even the right to receive inheritance from parents as half much as her brother. It seems that the rights which Islam has considered for a child deserves more learning and decent audiences. But the limited circle of thoughts and ideas has been incapable of introducing the significance and honor of religious instructions to the world within the context of time and space and simultaneous with the progress of human knowledge. Examples of such limited cycle could be observed in the formation of the laws ruling Iranian law system in relation to infants and children which hasn't reached adequate development and efficiency over time. Of course, the significance of new Islamic penal code, which is approved of by qualified authorities and will soon replace the previous one with a legal notice, must not be ignored. The new law, in a precious step about the age of criminal charge and responsibility, has distinguished mental and physical maturity from each other and has classified children in three age groups of up to 12, 12-15, and 15-18. Iranian legislator has also dropped the separation of girls and boys for punishment in new penal code, and has defined child as anybody under the age of 18. In fact, legislator has realized the content of the first article of child rights convention by proper modification of law, which defines child as all human beings under 18 and therefore Iranian law system in support of children is in accordance with article 37 of the world convention of child rights about the prohibition of child execution. An affair which can lead to the honor of human rights in Iranian society and will promote the image of Iranian law system appropriately in international level. Child abuse refers to any deeds or abandoned deeds which cause physical or mental hurt and which has some lasting impacts on a child. Unfortunately, this disgusting phenomenon has been one of the issues of Iranian society recently which makes it necessary to investigate it (Frost, Nick. 2004). In World Health Organization in definition of child abuse, it has remarkable and noticeable sides. According to this organization definition, child abuse is any physical or mental harm or threat to a child's physical or mental health and prosperity and welfare

by parents or anyone who is responsible for looking after him. Based on this definition, child abuse can be studied in four physical, psychological, neglect and abuse dimensions. There are various reasons for this immoral phenomenon to appear the most important among which are:

Parents' unawareness of children rights, economic and financial problems and inability to meet children's needs in family environment, addiction and its consequences, behavioral disorders and mental diseases, family problems such as divorce, problems between couples, children physical problems such as enuresis and like which mentally cause stress for parents, educational problems such as not meeting children educational needs, or children inability in achieving their parents expected successes and goals. Therefore, it must be admitted that child abuse could have negative consequences for both the child in future and the society. If serious pathological studies were done in the field of criminology on some criminals such as the murderer of children in Pakdasht in early 2001, it would be quite clear that the murderer's main incentive for murdering 26 innocent children after being raped was due to the fact that he had been raped when he was 9. It was the most tragic and brutal murder of children in the history of Iran. Unfortunately, sometimes it is observed that an addicted father, or irresponsible mother, or a step parent without ethical or moral principles, commits crime against children which irritates public thoughts and consciousness and poses some questions on how to deal with such offenders legally. We must accept the fact that our legislators, unfortunately, have not identified any limit on how to defend children's rights and stuff like this, and if there are any laws, their implementation and effectiveness are not guaranteed and cannot be responsive to current conditions. Although the law of protecting children and teenagers, adopted by the Islamic parliament in 2001, has somehow defended the rights of children and has explicitly stated in article 1 that people under 18, are supported by laws, it is not comprehensive enough to respond to public conscience and to defend children rights. The above mentioned law, which was passed in 9 articles, has forbidden any children abuse in its second article, but has not mentioned any instances of abuse or at least has not adapted it to WHO's definition of child abuse. It seems that the stipulation of this law articles in determining punishment for perpetrators of child abuse is a good deed, but it can't be a strong basis for preserving children rights in relation to child abuse.

Article five of this law, classifies child abuse as public crime which does not require a private plaintiff and the prosecutor can cite it to be raised in court, leading to the issue of verdict against criminals. Comparative study of child abuse in terms of some developed countries laws shows that those who violate children's rights encounter serious punishments. In Germany if someone who abuses children is over 25, they will be made sterile by the verdict of the court and medical commission. In Sweden, France and Spain similar legal citations are seen for child abusing that can be effective deterrent.

Public law is a practical knowledge which determines the rights and duties of governments and people. Since it is the government duty to defend children's rights, it is proper that this branch of law, with considering all aspects and noticing experts' opinions in children affairs, propose a legislation that will pass and implement a comprehensive law about children rights so that human-oriented notion and protecting human rights will become practical in comprehensive plan of our country's development and disgusting and immoral events such as child abuse and violation of innocent children's rights will disappear in Iranian civilized and sophisticated society and the way will be paved for next generations to grow and promote better and to form a better and prettier country.

According to Iran laws, a child is a person who has not entirely grown physically and mentally to participate in social and family affairs. Therefore, it is believed that in a certain age children will grow naturally and intellectually and will be prepared physically and mentally to take part in social and family life. According to Iranian civil code, childhood ends for girls when they are 9 and for boys when they are 15 in lunar year. But this age is not the same in all legal and political affairs and the legislator has, in fact, determined a certain age, with regard to the child's understanding and recognition abilities and the importance of the matter and his action, which is actually the end of childhood for that certain issue. As it was mentioned before, physical and intellectual growth (full maturity) in all children is not the same and depends on different factors such as nutrition, environment, heredity and the way of maintenance and custody. At first and by a quick view of laws it seems that Iranian legislator considers the beginning of childhood from a child's birth, but according to the rules and regulations about protecting and taking care of fetus, which exist in Islamic laws and the laws of Iran which are derived from Islamic jurisprudence, we might believe that the beginning of childhood according to Iranian laws is another time rather than a child's birth date.

In article (957) of Civil Code, Iranian legislator has stated that a fetus which has been defined as "Haml" owns civil rights provided that he is born alive. Therefore, although Iranian legislator generally believes that a person's capability and qualification

for having certain rights is from the moment of his birth and will end with his death, it has applied this potentiality to the period before birth and has emphasized that a fetus is entitled to civil rights since the time of formation. The fact that a fetus inherits from his dead parents in case of being formed at the time of his parent's death and being born alive, confirms the fact that according to Iranian laws, the child's life and his childhood begins from the time when the fetus is formed(Harvey, Colin J. 2005). According to Iranian Laws, childhood begins from the time when fetus is formed. So it's necessary to determine the end of this period which is the end of children's immaturity and dependence and the deterioration of domination and authority of their parents and other guardians. After the establishment of the Islamic Republic of Iran and the necessity of harmonizing customary laws with religious norms, thanks to the legislature's decisions, some of the laws were demolished and new laws were passed instead, and or some of the former laws were reformed including some articles of Civil Code. Reforming article (1210) of Civil Code and adding Note "1" to it in 1982 in one example. In this Note it is stated that:

"The age to reach puberty is 15 for a boy and 9 for a girl in lunar year." Thus, in terms of Civil Code a girl who is 9 or a boy who is 15 in lunar year, is mature and can do all legal deeds and transactions and their criminal responsibility begins from this time. For instance, if a 10-year-old girl is involved with theft, she will be tried and punished based on the same law that a 40-year-old guy would be. Therefore, it is permissible to sentence a 9-year-old girl to death under the Iranian laws. Shiite famous jurists determine the age of criminal charges as 15 for boys and 9 for girls. The most important explanation for criminal responsibility is narrated by Hamze Ebne Hamra which was mentioned before. This statement (Hadith), is quoted in the book of Kafi with a little difference in context in comparison to former ones. Of course it seems that since these two statements (hadith) are quoted by the same Imam and narrated by Ebne Mahout, they have the same origin. The study of the origin of Hamze Ebne Hamran's narration indicates that those of Ahmad Ebne Mohammed Ben Essa Alghasri and Abdol Aziz Alabdi are too weak. Hamze Ebne Hamran which is in the document of Hamran's Hadith, hasn't been approved by any of exalted scholars, either. However, only Shahid Sani has approved of this narration.

There are different disagreeing opinions against the opinion of Shiite's famous scholars which, although a little different from each other, all disagree with the famous scholars' opinions. In many articles of the convention, it has been emphasized to support children's rights. In second article of this convention it is stressed that all member countries, must respect and guarantee the rights which are considered by this convention for all children who live in their judicial realm without any discrimination of race, color, religion, language, political ideas and beliefs, nationality, ethnic and social status, property, disability, birth and or other personal characteristics of their parents or their legal guardians. They will also take all measures to ensure protection of children against all forms of discrimination and punishment, based on the status, activities, opinion expression and or beliefs of children's parents, legal guardians or family members. The significance of children's interests in this convention is such that in all measures related to children which are taken by public or private social welfare institutions, courts, administrative authorities, or legal organizations, it is one of the most important considerations and the member countries make a commitment to guarantee the required protection and care of children's welfare with regard to the rights and duties of their parents, guardians and whoever legally responsible for them. In this regard, appropriate legal and administrative measures will be taken; moreover, the engaged countries will also promise that institutions, services, and facilities provided for children care and protection will be in accord with the standards determined by qualified authorities especially in safety, health, the number of staff and the way of supervision and inspection(Bencomo, Clarisa. 2007). Above mentioned cases are among the duties of the government towards children which should be noticed in Iran, as well. A person under 18 who hangs out in the streets temporarily or repeatedly including a child who is still in touch with his family and has a shelter or one who knows the streets this home, and is in little or no touch with his family.

Thus in domestic laws and regulations the age of 18 and in some cases like the law of protecting women and children without any protector or guardian, which was passed in 1995, an older age is considered as the end of childhood which is due to intellectual growth. These points are actually in perfect harmony with the first part of article 1 of convention. Beside these cases, there is also the criterion of religious maturity in the rules and regulations of the Islamic Republic of Iran. According to Note 1, Article 1210 of Civil Code, age of maturity is 15 for boys and 9 for girls in lunar year. Articles 49 and 59 of Islam penal code states that reaching to religious maturity is adequate for punishment to be exposed. Note 1 of Article 49 is the law of "religious maturity". An important point in this matter is that whether it means "yes" or "No". Disagreements in judicial procedure, ultimately led to the issue of Supreme Court verdicts No. 13 dated 1995 and No.8 dated 2000 and No. 7.1513 dated 2001 by

the law department of State Legislature which explicitly states that "age" doesn't matter in maturity and that it is possible to resort to other resorts to approve of a child growth. Verdict of procedure unity, No. 518 dated 1996, refers to this matter. In relation to marriage age, as well, religious maturity is applied to verify the capacity and capability of getting married. According to Article 1041 of Civil Code, the marriage of a girl before the age of 13 and that of the boy before the age of 15 in solar year depends on the permission of their guardian on condition of considering interests and with recognition of righteous court. At present, the age of marriage for boys and girls has actually increased in Iran particularly in large cities and cultural growth and development has gradually has prevented marriage under the age of 18 in small towns and rural areas. The above mentioned disagreements led to the modification of related laws in the new bill of Islamic penal code. In this bill, intellectual growth and gradual penal responsibility have been applied to verify children's penal responsibility and to determine social responses.

Article 90 of Islamic penal code, states that in crimes which require punishment or retaliation, whenever people under 18, do not comprehend the nature of the committed crime or there is doubt in the development or perfection of their wisdom, based on the crime, they will be sentenced to the punishments predicted in this chapter .

2. Conclusion:

Legal system reflects the rights and duties of human beings in a society, and its supreme goal is to establish law and public order. So that everyone who doesn't respect other people's privacy and dignity will be punished and no abnormality or illegal behavior is tolerated in defending their rights. But depending on his physical and mental conditions, a child has some special rights in addition to the legal rights of every human being, which make it necessary for the laws to support them which is essential for their growth and development.

With the formation of modern international law, child rights appeared in this system. Convention on "the Rights of the Child" was passed by the UN General Assembly in November 20, 1989 and different countries joined the convention on "the Rights of the Child" by signing this treaty. Islamic Republic of Iran's accession to the treaty, citing the seventy-second principle of constitution, was realized in March, 1993 with the approval of Parliament and Guardian Council. Of course, in top of the single article of this law it is stated that the provisions of the Convention in any case and at any time must not be in conflict with domestic laws and Islamic criteria.

Article I of the convention on the Rights of the Child, defines child as all human beings under the age of 18.Thus, according to this convention everyone under18, is called a child and is supported by this treaty. In Iranian legal system, the child is looked at differently by the convention. The legal systems of different countries and consequently according to the convention of Child Rights, a child .i.e. anyone under 18, is acquitted of any responsibility. But Iranian legislator, in Article 49 of the Islamic Penal Code adopted in 1991, states that: children are acquitted of criminal charges if they commit crimes. The first note of article 49 also defines child as: a person who hasn't reached religious maturity yet. Iranian legislator, in the first note of article 1210 of civil code which has been modified in 1991, defines the age of religious maturity as: Maturity age for boys is 15 and for girls are 9 of Arabic lunar year. Considering the above mentioned articles and with regard to the contents of Child Rights Convention and top part of single article of Iran's accession to international convention of child rights, which declares that the provisions of the convention must not be in conflict with domestic laws and Islamic criteria, in any case and at any time, and also based on the rules of Iran Legal system in superiority of domestic laws to international laws and treaties, it can be inferred that In Iran In case of committing crime, Children are just acquitted of criminal charges and that religious maturity is the age of criminal charge and responsibility in Iran. However, in international law system, everyone under the age of 18 is considered as a child and therefore is free from any responsibilities and charges. Posing lack of responsibility to a child takes the right of agreeing to definite or indefinite contracts away from him according to the world convention of child rights. It includes marriage as a kind of contract. However, according to article 1210 of civil code, children under legal age can get married with their parent permission; unfortunately, some forced marriages at young ages, especially among girls who live in rural and nomadic areas are based on this law which causes social abnormal consequences in the future. World convention of child rights in several cases, while supporting children rights and expressing governments duties in ensuring the realization of these rights, has forbidden exploitation, sexual abuse, child trafficking, torture, execution, illegal arrest and detention of children in various forms. It also supports and protects them entirely against narcotics, social reconstruction, social insurance and services, abuse, plays and leisure time, military service, custody and education, freedom of opinion and expression, individual and group freedom, preservation of privacy, right to live, the right to use a lawyer, personal identity, citizenship and the rights related to early life

and name selection. A look at Islam attitude and holy prophet, Muhammad's (Peace be upon him) and infallible Imams' lifestyles and religious orders and instructions indicates their support of child legal rights. Arabic pre-Islamic ignorance considered a female's birth a shame and embarrasses for her father and had legitimized the innocent girls burying alive.

But prophet's lifestyle which was inspired by Islamic instructions, not only saved innocent girl from being buried alive, but also granted her the right of life and even the right to receive inheritance from parents as half much as her brother. It seems that the rights which Islam has considered for a child deserves more learning and decent audiences. But the limited circle of thoughts and ideas has been incapable of introducing the significance and honor of religious instructions to the world within the context of time and space and simultaneous with the progress of human knowledge. Examples of such limited cycle could be observed in the formation of the laws ruling Iranian law system in relation to infants and children which hasn't reached adequate development and efficiency over time. Of course, the significance of new Islamic penal code, which is approved of by qualified authorities and will soon replace the previous one with a legal notice, must not be ignored. The new law, in a precious step about the age of criminal charge and responsibility, has distinguished mental and physical maturity from each other and has classified children in three age groups of up to 12, 12-15, and 15-18. Iranian legislator has also dropped the separation of girls and boys for punishment in new penal code, and has defined child as anybody under the age of 18. In fact, legislator has realized the content of the first article of child rights convention by proper modification of law, which defines child as all human beings under 18 and therefore Iranian law system in support of children is in accordance with article 37 of the world convention of child rights about the prohibition of child execution. An affair which can lead to the honor of human rights in Iranian society and will promote the image of Iranian law system appropriately in international level. Child abuse refers to any deeds or abandoned deeds which cause physical or mental hurt and which has some lasting impacts on a child. Unfortunately, this disgusting phenomenon has been one of the issues of Iranian society recently which makes it necessary to investigate it. In World Health Organization in definition of child abuse, it has remarkable and noticeable sides. According to this organization definition, child abuse is any physical or mental harm or threat to a child's physical or mental health and prosperity and welfare by parents or anyone who is responsible for looking after him.

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Predicting test effectiveness using performance models in Life Science IT projects

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Abstract – Testing plays an important role in any project irrespective of the domain. The ability to test right before it reaches the intended customer matters. Test effectiveness is an important metric that tracks the ability of the testing team. The efficiency of a tester to cover all aspects of testing and ensuring 100% coverage determines the quality of the product. There could be number of factors that influence the test effectiveness. Organizations rely on the quality assurance team to strategize and plan the testing phase. Past experience in handling similar testing projects matter. Shifting left, the opportunity to be in pro-active mode helps to improve the efficiency. Predictive process performance models can be built for test effectiveness. This paper illustrates the process performance model to predict test effectiveness based on data from life science project in an organization.
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Keywords- process, performance, test effectiveness, defects, metrics

1. INTRODUCTION TO TEST EFFECTIVESS

Test effectiveness or bug finding effectiveness of the test set can be found by dividing the number of bugs found during testing divided by the number of bugs found in the product. It is a key metric that IT quality assurance team will track for every release or change request that the team works on. It primarily covers the aspects around how the customer requirements and specifications are satisfied based on the effort spent in developing the system. Identifying the number of defects during test execution in specific phase of the project is critical. Domain experience is an important factor to understand the application or system. This will help to build the appropriate test strategy.

Test effectiveness is a quality metric meaning how good is the product during testing. Test effectiveness is focused on the product quality and process quality as it is directly related to defects identified by the customer. It is the effectiveness to find the defects during core test execution across the different rounds of testing. It is applicable for any type of testing performed as part of the project life cycle. Each organization would interpret test effectiveness in different context. Some might include only production defects, some might include only accepted defects, and some might include rejected defects as well. The organizational definition should clearly articulate the operational definition and it should be explicitly mentioned in the statement of work.

2. PROCESS PERFORMANCE MODELS IN ORGANIZATIONS

Process Performance Models (PPM) is probabilistic, statistical and simulative in nature. It can predict interim and final outcome, it is a proactive measure of tracking the end goal instead of a reactive one. It can model the variation of factor and help us understand the predicted range or the variation of its outcomes. Mid-course correction can be made to achieve desired outcome. Interestingly, PPMs enable "What-if" analysis for project planning, dynamic re-planning and problem resolution during project execution. We can run "what if" exercises holding one or more values constant. We can see the effect of tradeoffs between schedule, effort, defects, staff and functionality

Software Engineering Institutes Capability Maturity Model

Integrated (CMMI) standard recommends five maturity levels. Levels 4 & 5 are defined as the high maturity levels. Process performance models are the base for high maturity. Quantitative project management is referred to in high maturity levels. Though the focus on metrics starts at Level 2 and Level 3 itself, statistical and quantitative management is covered in Level 4 & Level 5. Strong metrics handbook

across the different work types is a must for organizations. The ability to capture the metrics and analysis the metrics is the base to move to high maturity. Managing a project quantitatively involves predicting project outcomes bases on the project data.

PPB is the base for process performance model (PPM). The PPM describes the relationships among attributes of a process and its work products. It is used to predict a value of a critical outcome that cannot be measured until later in the project's life. For example, predicting the number of delivered defects. A high level business objective (Y) would have multiple next level business objective (y's). In turn, these small (y's) would have project level measurement objectives. These small (y's) are further drilled down to high level process measures (X).

In any testing project, the test strategy and test plan are the most important steps. Based on the project context there could be exclusive test strategy for functional testing and integration testing. At each phase, the test plan and test design plays an important role. The way the test scenarios are thought through and the test cases are written matter. The domain experience of the team is critical. Most of the large customers outsource development part to a vendor and testing to another vendor. This would ensure the transparency in findings defects. Using PPMs, if it is a testing project, and test effectiveness has to be predicted, then test case execution process would critically influence test effectiveness. Manager will have to look at past data to see the various parameters than influence test case review. For example it could be test conditions or test scenarios or combination of both. Upper and lower specification limits for each of these have to be arrived.

Typically process performance model are established to manage the Project Objective. PPM's are used by Project manager during planning phase and throughout Project management life cycle. PPM helps in proactively identifying risks in achieving project objective and identifying the action plan. Before implementing corrective action, Project Managers use PPM to see the impact on the objective. If there are no risks, we go ahead and implement corrective action.PPM is used to check the implementation effectiveness of Corrective action. The organization uses PPM and PPB for estimating, analyzing, and predicting the process performance associated with the processes in the organization defined process and identify areas that require improvements and innovations.

3. VARIABLES ASSOCIATED IN PREDICTION MODEL

Based on brain storming session with the project team in the organization the different parameters that influence test effectiveness in a testing project were looked at. The team shortlisted following factors to start with, domain experience, technical experience, test effectiveness, defects identified during testing phase, testing review efficiency and usage of tools. Operational definitions for these parameters were baselined and data was collected from projects in a particular account against these parameters. Linear regression was performed against the data to find out which are the key variables that influences the test effectiveness. After many trial and error methods the below three variables were established as the x factors.

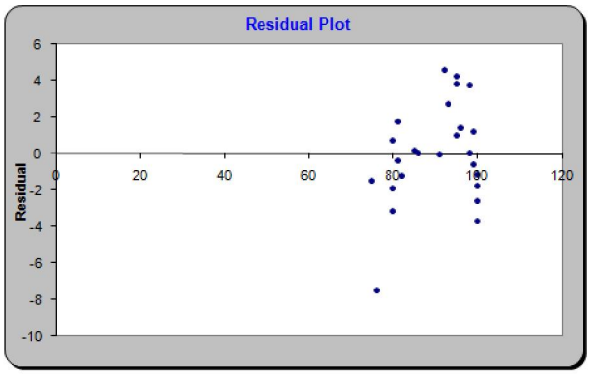
1. Y – Test effectiveness – Total number of defects identified by the testing team / (Total number of defects identified by the testing team + total number of defects indentified by the customer)
2. X1 – Life Science Domain experience – Average life science domain experience of the team, in person months
3. X3 – TCEDD – Test Case Execution Defect Density - Defects attributed to test execution, identified during test execution review against effort spent for test case execution.

4. TEST EFFECTIVENESS – REGRESSION EQUATION

The project data collated for the x and y factors are as shown in the Table 3.1. Data points from 25 projects in an organization were collected and considered for analysis. Projects factored in were similar in nature.

Y	X1	X3
Test Effectiveness (%)	Life Science Domain Experience (in months)	Test Case Execution Defect Density
95	30	0.620
99	40	0.950
80	10	0.110
81	15	0.150
96	35	0.800
99	45	0.950
85	22	0.250
91	34	0.450
100	50	0.990
100	52	1.000
95	31	0.890
86	25	0.250
81	10	0.110
75	5	0.005
100	48	1.000
98	34	0.800
80	16	0.300
95	32	0.500
92	24	0.450
100	55	1.000
93	32	0.450
98	42	0.890
76	13	0.450
82	23	0.030
80	19	0.050

Table 3.1 – Project data values



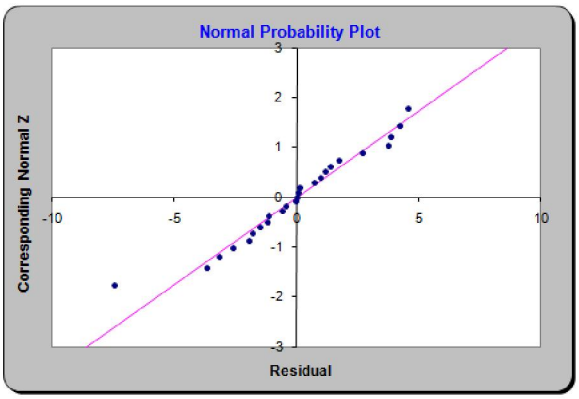


Figure 3.1 – Residual Plot

Mirror pattern is not found in Figure 3.1, Residual Plot and hence no heteroscedasticity is found. The normal probability plot is approximately linear. This would indicate that the normality assumption for the errors has not been violated.

The p value for test case execution defect density is 0.01 which is < 0.05, also the domain experience p value is 0.0009 which is also < 0.05, null hypothesis is not valid, which means the variables selected have an impact to test effectiveness.

Table 3.2 – Regression Equation

Intercept	Domain Experience (in months)	Test Case Execution Defect Density
74.63	0.3595	9.2573

As shown in Table 3.2, domain experience has a positive influence on test effectiveness. As the team's domain experience increases the test effectiveness increases. The influence of Test Case Execution Defect Density is positive. This means that when the values of Test Case Execution Defect Density are low the test effectiveness will be low and vice versa. The more the defects are identified the higher the test effectiveness.

5. TEST EFFECTIVENESS – COMPONENTS OF PREDICTION MODEL

Based on project data analyzed it is evident that test effectiveness is critically influenced by test case execution sub process and domain experience. Life science domain knowledge is referred to as domain experience. Organization base measurement group will provide the baseline data for these metrics. For test case execution sub process, the number of rounds of testing is considered as the parameter. First, second and third round of testing are the parameters factored. Metrics Council group will share the baseline values for these combinations. Baseline values will include lower specification limit (LSL), goal and upper specification limit (USL). The upper and lower specification limits for domain experience and test effectiveness defect density will be provided by the metrics group. Project team needs to choose the process that they would be following for test execution sub process. Project team will have to factor the average domain experience in the team. Based on the composition of sub process, project goal for TCEDD would be calculated. It is also important for the project team to justify why they have gone with a particular sub process and the rationale. Table 4.1 gives the sub process performance baseline for TCEDD. The values are represented by D1, D2, and D3 and so on. Organization Metrics team would have the actual baseline values for LSL, Goal and USL for these identified metrics. Based on the current project context, the parameters and rounds of testing chosen are shown in Table 4.2, Selected Sub process performance baseline.

Table 4.1 - Sub process performance baseline

Sub process	Metric	Parameter	LSL	Goal	USL
Test case execution review	TCEDD	1 round of testing	D1	D2	D3
Test case execution review	TCEDD	2 rounds of testing	E1	E2	E3
Test case execution review	TCEDD	3 rounds of testing	F1	F2	F3

Table 4.2 – Selected Sub process performance baseline

Sub process	Metric	Parameter	Goal	Comments
Test case execution review	TCEDD	3 rounds of testing	F2	

6. TEST EFFECTIVENESS MODEL – PRACTICAL USAGE

One of the current releases in design phase was considered for the practical usage of this model. The below steps will illustrate the prediction model.

1. X factors baseline data was used as input. Domain experience goal is 32 months with LSL of 15 months and USL of 51 months

2. Sub process performance baseline data was reviewed and based on the current project context the below selection was made. As shown in Table 5.1 the sub process test case execution review was selected. Based on the project usage, 3 rounds of testing were selected for test execution. The goal, upper specification limit and lower specification limit are chosen from organization baseline report.

Table 5.1 – Selected Sub process

Sub process	**Metric**	**Technology**	**LSL**	**Goal**	**USL**
Test case execution review	TCEDD	3 rounds of testing	0.32	**0.69**	1.24

3. Update the actual domain experience in the team and predict the test effectiveness. The predicted value is based on Monte Carlo simulation.

TableTable 5.2 – Predicted Test effectiveness

Average Domain Experience (in months)	TCEDD (Defects/ Personday)	Predicted Test effectiveness
32	0.50	90%

4. The data was compared against goal. The client goal for test effectiveness is 99% whereas the predicted value is 90%.
5. Perform what-if analysis and look at various combinations of the x factors and analyze the predicted test effectiveness based on these factors. Based on the project experience choose the one which is close to reality.
6. List down the assumptions considered when the final decision is made on the values of x factors. Ensure all the relevant assumptions are documented. As need be, the assumptions need to be validated with the relevant stakeholders before the baseline process.
7. Understand the deviation and prepare preventive action plan

Table 5.3 – Deviation Analysis

Expected client test effectiveness	Predicted test effectiveness	Preventive Action	Responsibility
99%	90%	List down the top three preventive action items	Project Manager

8. Estimated effort in person days for the project is 500 person days. Based on the predicted defect density and organizations standard effort distribution across phases, the defects that could be injected at each phase are predicted as show in Table 5.4

Table 5.4 – Predicted-Actual Defects phase wise

Phases	**Expected Injection**	**Actual Defects Captured**	**Remarks**
System test cases execution			
System integration test Execution			

9. Based on the actual data collated, keep updating Table 5.4 to compare the expected and actual defects captured. Based on the actual value in each phases, the predicted value for next phases are accordingly impacted. If there any specific inputs or considerations on the actual values, those are highlighted in the remarks column.
10. Prepare the detailed defect prevention plan. Against each phase, list down the defect type, defect cause, root cause, preventive action planned, responsible person, target date and the status. Defect types could be incorrect functionality or missing functionality or incorrect user interface or missing user interface. Defect causes could be lack of knowledge, missing information or incidental. Root cause should be as detailed as possible to plan for preventive and corrective action. 5-Why analysis can be used to identify the root causes. Defect prevention plan is an on- going document that need to be tracked very closely. It is meant both for planning and tracking defect prevention activities. This plan has to be revisited after completion of each stage. If defects detected during the completed stage fall under different defect types and defect causes not identified for preventing at that stage, then these new types need to be included in the on-going phases.

8. CONCLUSION

Most of the customers demand 100% test effectiveness. It is a service level agreement in the statement of work as initiated by the clients. A metric that needs to be tracked on monthly basis and deviations with corrective actions are to be reported. Certain customers even demand penalty clauses based on the criticality of the project. Given the importance of testing, the ability to predict test effectiveness using process performance models plays a major role. Test effectiveness is directly related to the product defects in turn to customer satisfaction. Capability Maturity Model V1.2 recommends use of quantitative models to predict project outcomes. The practical implementation

of test effectiveness process performance model in life science project was demonstrated. This is an on- going process which managers should use day in and out. Predicting project metrics and quantitatively managing by pro-actively taking preventive actions is the success factor. This illustration gave the practical applicability of test effectiveness model in life science testing projects thus helping to reduce residual defects.

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8/29/2-12

Breviscapine Reduces the Acute Lung Injury Induced by Left Heart Ischemic Reperfusion in Rat through inhibition of the Expression of IL-6 and ICAM-1

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Abstract: Objective To explore the effect of breviscapine on expression of IL-6 and ICAM-1 in rat with acute lung injury induced by left heart ischemic reperfusion， and the mechanism of breviscapine protecting respiratory function. **Methods** 60 rats were divided into 2 equal groups randomly: group 1 is treatment group (TG),group 2 is control group(CG).All rats were made left heart ischemia-reperfusion model by ligaturing the anterior descending branch 30min and loosing. Rats in this group were intravenous injected breviscapine ($10mg \cdot kg^{-1}$) when the myocardial ischemia had been for 10 minutes. While，the rats of CG were treated with normal saline. The same amount of rats in 2 groups were killed at 3 point in time: 30min after ligaturing (T1), 30min after loosing(T2) and 60min after loosing (T3).All rats were recorded and observed respiration curve with BL-420 biological signal collect and analysis system, measured the expression of interleukin 6 (IL-6)and inflammatory cell adhesion molecules-1 (ICAM-1) with Immunohistochemistry, measured the expression of IL-6 in peripheral blood and bronchial alveolarlavage fluid with Enzyme Linked Immunosorbent Assay methods, and measured the activity of myeloperoxidase (MPO) in lung tissue with colorimetry. **Results** In T1, T2 and T3, the level of the expression of IL-6 and ICAM-1 in CG were higher than those in TG, the activity of myeloperoxidase in lung tissue of CG was stronger than that of TG, the expressions of IL-6 of CG were higher than those of TG in peripheral blood and bronchial alveolarlavage fluid, and the amplitude and duration time of respiration curve of TG was higher than those of CG. The comparisons were great. **Conclusions** Breviscapine can inhibit the expression of ICAM-1, which means that leukocyte adhesiveness/aggregation and release reaction of can be reduced in the pulmonary circulation, the expression of IL-6 can be decreased, and inflammatory cascade response will be reduced to protect respiratory function.

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Key words： breviscapine; left heart ischemic reperfusion； acute lung injury； inflammatory cell adhesion molecules 1; interleukin 6

1. Introduction

Breviscapine is a commonly used drug in clinical medicine for various types of coronary heart disease presently. The correlation studies (LIU et al., 2010) of breviscapine pharmacological effect are mostly related to the following aspects: the inhibition of adhesion of platelet and erythrocyte, the decrease of the blood viscosity, and the dilating vessel. But the effect of breviscapine has not been systematically explored on expression of inflammatory factors in acute lung injury induced by left heart ischemic reperfusion. In our study, rat model of acute lung injury induced by left heart ischemic reperfusion is established, and being treated with breviscapine, to investigate the effect of breviscapine on expression of interleuki 6(IL-6) and inflammatory cell adhesion molecules 1(ICAM-1), to reveal the mechanism of breviscapine protective role of respiratory function against acute lung injury induced by left heart ischemic reperfusion, and to provide theoretical basis for the clinical application of breviscapine to treat acute respiratory dysfunction reduced by left ventricular dysfunction.

2. Materials and Methods

2.1 Laboratory animal

Sixty healthy rats of both sexes, weighting 250~350g, obtained from Experimental Animal Center of Zhengzhou University, were randomly divided into 2 groups: the first group (n=30) is treatment group (TG), these rats in TG were treated by breviscapine.The second group is control group(CG), these ones in CG were not treated by breviscapine.

2.2 Main reagents and instruments

Breviscapine injection (Feixia Pharmacology Company, Harbin, China). BL-420 biological signal collect and analysis system (Chengdu TME

Technology Company, Chengdu, China). IL-6 ELISA Kit for Detecting, IL-6 poly-clonal antibody, ICAM-I poly-clonal antibody, SP kit (Zhongshan Company, Beijing). Myeloperoxidase kit (Jiancheng Bioengineering Institute, Nanjing).

2.3 Methods

Experimental operation according to Reference(JI et al.,2011) : external jugular vein cannula was placed after the rats were anesthetized with 4% chloral hydrate （ 1ml·100g^{-1} ） , and then left anterior descending artery was ligated for 30min, then blood supply was restored, then left heart ischemia reperfusion injury was caused, and the rats of TG were treated with breviscapine injection （ 10mg·kg^{-1} ） through external jugular vein cannula when the myocardial ischemia had been for 10 minutes, however, the rats of CG were treated with normal saline. Rats' diaphragm was exposed before chest experimental operations were carried, and the diaphragm was connected with BL-420 biological signal collect and analysis system through needle electrode for recording respiratory curve. These respiratory curve data was retained to comparative analysis. 5ml Peripheral Blood were sampled through external jugular vein cannula in 30min after ischemia (T1), 30 min after reperfusion (T2) and 60min after reperfusion (T3), then rats were sacrificed, their lungs were took out, bronchial pulmonary alveolus was lavaged immediately, then 5ml bronchoalveolar lavage fluids (BALF) were obtained, the level of IL-6 were measured in BALF by Enzyme Linked Immunosorbent Assay methods. Inferior lobe of right lung tissures were divided into two parts, one part was fixed with 4% formaldehydum polymerisatum, and embedded by paraffin and sliced into 4 μm continuous sections for immunohistochemical staining to test the expression of IL-6 and ICAM-1, the other was homogenized to test the activity of Myeloperoxidase by colorimetric method. All data was retained and analysed using SPSS 11.0 statistical software, statistical methods include rank sum test and Independent t-test (size of test α=0.05).

3. Results

3.1 Comparision of expression IL-6 and ICAM-1 between two groups

The immune response products of IL-6, which are brown-yellow particles, were mainly located in the cytoplasm, these positive expressions of IL-6 protein in TG were obviously lower than those in CG at T1, T2 and T3 by rank sum test; The immune response products of ICAM-1, which are brown-yellow particles, were mainly located in the cytoplasm or membrane, these positive expressions of ICAM-1 protein in TG were obviously lower than those in CG at T1, T2 and T3 by rank sum test. The above results were showed in Tab.1 ~ Tab.2 and Fig.1~ Fig.2 (pictures of expression of IL-6 and ICAM-1 at T2 were selected and showed).

Table 1. Comparison of the expression of IL-6 between two groups(n=10)

Time	n	IL-6				Z	P
		-	+	++	+++		
T1							
TG	10	6	3	1	0	1.972	0.039
CG	10	4	3	3	0		
T2							
TG	10	5	3	1	1	2.161	0.027
CG	10	2	3	3	1		
T3							
TG	10	4	3	1	2	1.984	0.046
CG	10	2	2	4	2		

Table 2. Comparison of the expression of ICAM-1 between two groups(n=10)

Time	n	ICAM-1				Z	P
		-	+	++	+++		
T1							
TG	10	5	3	2	1	1.985	0.042
CG	10	3	3	3	1		
T2							
TG	10	4	4	1	1	1.962	0.047
CG	10	2	3	4	1		
T3							
TG	10	4	3	2	1	2.216	0.031
CG	10	2	3	3	2		

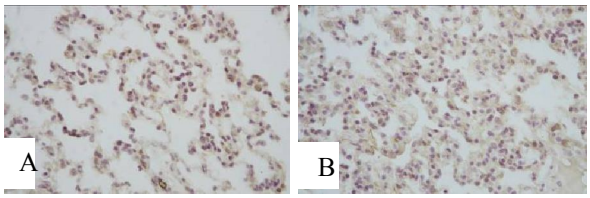

Figure 1. Comparison of expression of ICAM-1 in lung tissues (immunohistochemical chemical staining, ×400). A: expression of ICAM-1 in lung tissue at reperfusion 30min of TG rats.B: expression of ICAM-1 in lung tissue at reperfusion 30min of CG rats.

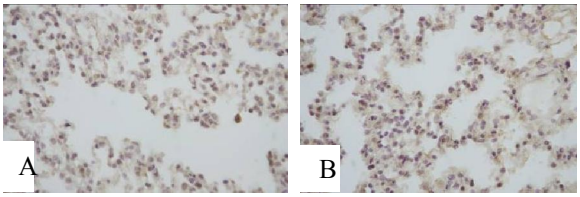

Figure 2. Comparison of expression of IL-6 (immunohistochemical chemical staining, ×400). A: expression of IL-6 in lung tissue at reperfusion 30min of TG rats. B: expression of IL-6 in lung tissue at reperfusion 30min of CG rats.

3.2 Comparison of the activity of pulmonary MPO between two groups The activity of pulmonary MPO in TG was significantly lower than that in CG at T1, T2 and T3 by independent t-test, which was showed in tab.3.

3.2 Comparison of the activity of pulmonary MPO between two groups

The activity of pulmonary MPO in TG was significantly lower than that in CG at T1, T2 and T3 by independent t-test, which was showed in tab.3.

3.3 Comparison of the level of IL-6 in peripheral blood and bronchial alveolarlavage fluid between two groups

Rats was obviously lower than that of CG rats at T1, T2 and T3 by independent t-test, which were showed in Table 4. The level of IL-6 in peripheral blood and BALF of TG was compared.

Table 3. Comparison of the activity of pulmonary MPO between two groups(n=10, $U \cdot g^{-1}$)

Group	MPO		
	T1	T2	T3
TG	54.27±4.12	63.62±3.27	67.64±4.36
CG	68.13±2.79	89.46±7.23	94.75±5.13
t	12.126	16.131	13.271
p	0.000	0.000	0.001

Table 4. Comparison of IL-6 in peripheral blood and bronchial alveolarlavage fluid between two groups (n=10，$pg \cdot ml^{-1}$)

Group	IL-6 in peripheral blood			IL-6 in BALF		
	T1	T2	T3	T1	T2	T3
TG	29.37±2.14	32.42±3.23	43.64± 4.31	11.21±1.04	14.89±2.53	19.25±3.19
CG	42.83±3.03	56.48±4.24	63.62±11.43	17.86±3.12	28.78±3.34	36.21±4.37
t	11.479	12.541	13.649	15.329	14.153	11.306
p	0.001	0.000	0.000	0.000	0.000	0.000

3.4 Comparison of respiration curve between two groups

The amplitude of respiration curve in TG was significantly higher than that in CG at T1,T2 and T3, the duration time of respiration curve in TG was obviously higher than that in CG at T1,T2 and T3, which were showed in tab.5.

Table 5. Comparison of respiration curve between two groups

Group	Amplitude(mv)			duration time (ms)		
	T1	T2	T3	T1	T2	T3
TG	159.93±4.31	147.65±7.12	141.36±3.45	375.00± 42.37	228.56±24.21	225.82±23. 16
CG	109.82±3.01	82.45±4.21	65.67±3.13	165.00±17.33	98.00±14.31	76.54± 9.14
t	15.160	22.735	19.273	18.017	14.524	15.182
p	0.001	0.001	0.000	0.000	0.000	0.000

4. Discussions

ICAM-1 protein is mainly located in the surface of endothelial cells (Yokomura et al., 2001), which was hardly expressed on most tissues of human under physiological condition(Porter et al., 2009). When the expression of ICAM-1 was increased because of pathologic factors on endothelial cells, interactions between ICAM-1 and integrin located on surface of granulocyte cell occurred, which caused aggregation, adhesion and release of leucocytes, and the cytokines were released immediately (Sun et al., 2011). The above process was molecular biological basis of inflammatory reaction(Lawson and Wolf, 2009). In our study, the expression of ICAM-1 in lung cells of TG rats was less than that of CG rats, which suggested that breviscapine might reduce the expression of ICAM-1.

MPO is the unique and stable reductase of neutrophil (Prokopowicz et al., 2012). The activity of MPO per unit weight of lung tissues, which is analyzed by colorimetric method, can reflect the number of neutrophils indirectly (Pawlus et al., 2010). The aggregation and adhesion of neutrophils result in inflammatory effectiveness in pulmonary circulation, which is considered as characteristic markers of early inflammatory injury (Gustapane et al., 2011). IL-6, which is mainly released by mononuclear macrophages, plays an important role in defense function, immune response, acute phase response, hematopoietic response, etc. In this study, the MPO activity of lung tissues of TG rats was significantly lower than that of CG rats, and the levels of IL-6 in peripheral blood and BALF of TG rats were obviously lower than those of CG rats, which suggested that

breviscapine might reduce the aggregation and adhesion of neutrophils, and the expression of pro-inflammatory cytokines IL-6. It is well known that the level of IL-6 in peripheral blood was considered as a marker to reflect tissues damage, so it is thought that breviscapine might reduce lung tissue damage after left heart ischemic reperfusion, and protect respiratory function (Ramirez et al., 2009). The above conclusion was supported by the experimental results that the amplitude and duration time of respiration curve of TG rats were significantly higher than those of CG rats in this study.

In a word, the mechanism of the effcet of breviscapine on acute lung injury induced by left heart ischemic reperfusion is that breviscapine can reduce the expression of ICAM-1, thus reduces the aggregation and adhesion of neutrophils, which reduces the expression of IL-6, and finally reduces the inflammatory injury in lung, and then respiratory function is protected.

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Evaluation of interleukin 8, 12 & 33 serum level in patients with chronic periodontitis, aggressive periodontitis and healthy subjects

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Abstract: Periodontitis is a multifactorial inflammatory disease characterized by destruction of tooth supporting tissues. Environmental, genetic and immune factors are involved in progress of the disease. Recently numerous studies have focused on the role of different cytokines in development of periodontitis.The aim of the present study was to estimate the serum level of IL-8, IL-12 and IL-33 in patients withchronic periodontitis, generalized aggressive periodontitis (GAP) and healthy individuals.A total of 96 subjects were included in the study of which 35 patients had chronic periodontitis, 26 patients had generalized aggressive periodontitis and 35 persons was healthy group. 3ml blood obtained from each person and serum samples separated. Level of IL-8, IL-12(p70) and IL-33 were determined by enzyme linked immunosorbent assay.Data analyzed with Kruskal-Wallis, Mann-Whitney testand SPSS Ver.16 software.The level of IL-12 increased significantly in chronic and aggressive patients than in health group ($p=0.001$). On the other hand, there was no significant difference at IL8 dose level between periodontitis patients and healthy group ($p>0.05$). The amount of IL-33 was no difference between patients and healthy group ($p>0.05$).There was a significant association between the level of IL-12 with chronic periodontitis and GAP.

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Key words: interleukin-8, interleukin-12, interleukin-33, chronic periodontitis, aggressive periodontitis

Introduction:

Periodontal disease is the most common inflammatory disease in human beings which is relatively hard to control.In some cases, in spite of satisfactoryoral hygiene, severe bone deterioration and generalized periodontitis occur. Other factors other than pathogen microorganisms of the oral cavity and accumulation of dental plaque are apparently involved in pathogenesis of this disease(1,6).

Periodontitis is the inflammation of periodontium that goes beyond the gums and deteriorates the dental connective tissue. In fact, microorganisms cause periodontitis, but the clinical manifestation of this disease (its severity and extent) depends on the reaction of the host to the extent of intrusion of the bacteria. Periodontium immune cells release proinflammatorymediators in response to periodontal pathogens and their endotoxins(1). Among the many immunity and inflammatory mediators known in the gingival crevicular fluid (GCF) cytokines have attracted special attention(2,8,9). Cytokines are a group of inflammatory mediators that are produced in response to microbes and antigens and contribute to the interaction of immune cells, and proliferation and distinction of lymphocytes. Cytokines are mainly produced by activated T cells and antigen-processing cells. They affect the T cells and are, in fact, the cause of proliferation of helper T cells, which are divided into Th1 (T helper cells 1) and Th2 (T helper cells 2) cells. Th1 activates the cell immune system by releasing cytokines while Th2 mediateshumoral immunity response (HIR) by releasing cytokines. Since the cytokines are mainly produced by leukocytes and also affect other leukocytes, they are known asinterleukins(3,7,10).

Production of cytokines (including interleukins) is stimulated by microbial factors and their contents are increased in the gingival crevicularfluid and tissues of patients with periodontitis. This phenomenon is more critical in patients with aggressive periodontitis. Measuring the content of these cytokines in a patientleads to a decisive diagnosis. Moreover, these

cytokines can also be considered as the target of medications intended to prevent progression of the diseases(4,11-14). Progression of aggressive periodontitis leaves irreversible effects, including premature tooth loss in early ages with a prevalence rate of 0.1%. If the disease is diagnosed early, its progression can be stopped by controlling the disease and prescribing medicines such as levamisole and colchicine(5,16-18). Since the level of all the interleukins (except interleukins 8, 12 and 33)has beenmeasured in the previous studies; the level of interleukin-12 has been only measured in chronic and aggressive periodontitis; no decisive relationship has been found between interleukin-33 and periodontitis;and neitherinterleukin-8 nor interleukin-33 has been analyzed in aggressive periodontitis; this study is an attempt to measure the serum content of the interleukins 8, 12, and 33 in patients with chronic periodontitis and generalized aggressive periodontitis that live inSistan and Baluchestan Province.

Methodos:

This is an observational case study. The statistical population includes patients with chronic and aggressive periodontitis who have visited the periodontics department of Zahedan Dentistry Faculty. The control group consists of individuals whovisited the dentistry faculty for other reasons anddid not suffer from periodontitis. Smokers, pregnant women, patients with any systemic diseases (such as diabetes or self-immune diseases), and patients with viral, bacterial, and fungal diseases; patients with anti-inflammatory and antibiotic diseases; and patients who had consumed anti-inflammatory drugs (e.g. aspirin or ibuprofen), antibiotics, immunosuppressive drugs (e.g. cyclosporine), drugs that increase the size of the gum (such as calcium channel blockers), and antiepileptic drugs for the last 6 months are excluded from this study. By using a simple sampling method 35 samples were obtained (based on the number of visits and their availability).

The information was analyzed using the SPSS software version 16 and the statistical analysis method introduced byKruskal-Wallis and Mann-Whitney, in order to examine the mean difference between the concentrations of serum interleukins 8, 12, and 33 in patients with chronic and aggressive periodontitis and healthy individuals.

26 patients with aggressive periodontitis, 35 patients with chronic periodontitis, and 35 healthy individuals that had visited the periodontics department of Zahedan Dentistry Faculty were selected. In this study, due to the rarity of patients with aggressive periodontitis (26 individuals) the required quorum was not achieved.

Smokers, pregnant women, patients with any systemic diseases (such as diabetes or self-immune diseases), and patients with viral, bacterial, and fungal diseases; patients with anti-inflammatory and antibiotic diseases; and patients who had consumed anti-inflammatory drugs (e.g. aspirin or ibuprofen), antibiotics, immunosuppressive drugs (e.g. cyclosporine), drugs that increase the size of the gums (such as calcium channel blockers), and antiepileptic drugs for the last 6 months are excluded from the study. A written explanation of the objective of the study was provided to each patient. This document, which included the ethical principles mentioned in the Declaration of Helsinki, was signed or fingered by the patients. The criteria for labeling the individuals as patients suffering from generalized aggressive periodontitis were as follows: generalized loss of proximal connections of at least 3 permanent teeth in addition to the first molars and incisors;pocket depth and clinical attachment loss (CAL) of more than or equal to 5 millimeters in these teeth; deterioration of the bone surrounding these teeth that can be observed in pre-optical radiographstaken by parallel techniques(1,19).

The criteria for recognizing patients with chronic periodontitis were as follows: pocket depth clinical attachment loss of more than or equal to 5 millimeters and in at least one tooth in the jaw quadrate bone; and manifestation of bone deterioration in radiographs. Moreover, the pocket depth and clinical attachment loss in healthy individuals had to be also less than or equal to ($\leq$) 3 millimeters(20,21,25). 3 millimeters of venous blood was extracted from each person. The samples were kept in typical tubes without anticoagulants. After separating clot from serum, the samples were centrifuged. The obtained serum was stored at a temperature of $-20^{\circ}C$. The cytokine content of the serum samples was measured by ELISA kits (Bioscience, San Diego, CA, USA). Using these kits, the levels of interleukin-8, interleukin-12 (P70), and interleukin-33 were measured. Protocols for preparing the kits and placing the serum samples into the plates were implemented according to the instructions of the manufacturer. After plotting the related standard curve, the concentration of the aforementioned interleukins was determined in terms of pg/ml based on the optical absorption capacity of ELISA plate wells by using an ELISA reader. Furthermore, treatment of the patients continued after sampling.

For the purpose of this study, students carried out periodontal examination of the participants, extracted blood samples from their veins, centrifuged the samples and sent them to the laboratory. Moreover, the samples were examined by the biochemical laboratory staff using the ELISA test method.

Results :

96 subjects (50 male and 46 female) participated in this study. This population was divided into 4 age classes

and 60.2% of the subjects belonged to the 20-30 age grade (Table 1).

Table 1: Frequency and percentage of the target population in terms of age class

age class / frequency	20-25	26-30	31-35	≥36	Total Number
Quantity	42	20	13	21	96
Percentage	43.8	20.8	13.5	21.9	100

The mean age of healthy individuals was equal to 27.6 ± 8.73, that of patients with chronic periodontitis was equal to 37.37 ± 8.701, and that of patients with aggressive periodontitis was equal to 23.69 ± 4.443. There was a significant statistical difference between the mean ages of the subject group and control group (P=0.001)(Table 2).

Table 2: The mean distribution and standard deviation of the ages of the subjects based on their health condition

Mean age / Group	Quantity	Mean	Standard deviation	P-Value (Kruskal-Wallis)
Healthy	35	27.6	8.73	0.001
Chronic	35	37.37	8.701	
Aggressive	26	23.69	4.443	

35 healthy subjects and 35 subjects with chronic periodontitis participated in this study (18 male and 17 female). The aggressive group consisted of 26 individuals with 14 male and 12 female participants.

In the current study, the mean concentration of serum interleukin-8 was $0.045 \pm 0.19\, pg/ml$ while the maximum concentration of this interleukin was $0.9\, pg/ml$. The statistical difference between these values was not significant compared to the groups containing patients with chronic and aggressive groups (P=0.172) (Table 3).

Table 3: Comparison of the level of serum interleukin-8 in healthy individuals and patients with periodontitis

Mean value / Group	Quantity	Mean value (pg/ml)	Standard deviation	Minimum serum level (pg/ml)	Maximum serum level (pg/ml)	P-Value (Kruksal-Wallis)
Healthy	35	0.045	0.19	0	0.9	0.172
Chronic	35	0	0	0	0	
Aggressive	26	0	0	0	0	

No significant difference was observed betweenthe levels of interleukin-8 in patients with chronic periodontitisand healthy subjects (P=0.154) [Mann-Whitney]. No significant difference was also observed between the levels of this interleukin in the aggressive and healthy groups (P=0.219) [Mann-Whitney]. There is no relation between the levels of interleukin-8 in groups with chronic and aggressive periodontitis (P=1) [Mann-Whitney].

The mean and standard deviation of interleukin-12 in patients with chronic periodontitis and patients with aggressive periodontitis were $0.82 \pm 1.172\, pg/ml$ and $0.85 \pm 0.623\, pg/ml$, respectively. The aforementioned values are larger than those of the healthy group with a mean and standard deviation of $0.194 \pm 0.502\, pg/ml$. This difference was statistically significant (P=0.001). The maximum amount of serum interleukin-12, which belonged to patients with chronic periodontitis, was 5.2pg/ml (Table 4).

Table 4: Comparison of the level of serum interleukin-12 in healthy individuals and patients with chronic periodontitis patients

Mean value / Group	Quantity	Mean value (pg/ml)	Standard deviation	Minimum serum level (pg/ml)	Maximum serum level (pg/ml)	P-Value (Kruksal-Wallis)
Healthy	35	0.194	0.5017	0	2.3	0.001
Chronic	35	0.82	1.172	0	5.2	
Aggressive	26	0.85	0.632	0	3	

Comparisons showed significant relations between the levels of serum interleukin-12 in patients with chronic periodontitis and healthy individuals and the levels of serum interleukin-12 in patients with aggressive periodontitis and healthy individuals (P=0.001) [Mann-Whitney]. However, no relation existed between the levels of serum interleukin-12 in the chronic and aggressive groups (P=0.119) [Mann-Whitney].

The mean and standard deviation of serum interleukin-33 in healthy individuals was $0.269 \pm 0.961 pg/ml$ and the maximum value for this group was $2.3 pg/ml$. On the other hand, in patients with chronic and aggressive periodontitis, the mean values and standard deviations were $0.431 \pm 1.058 pg/ml$ and $0.223 \pm 0.573 pg/ml$, respectively. However, no significant statistical difference was observed between these three groups (P=0.614)(Table 5).

Table 5: Comparison of the level of serum interleukin-33 in healthy individuals and patients with periodontitis

Mean value / Group	Quantity	Mean value (pg/ml)	Standard deviation	Minimum serum level (pg/ml)	Maximum serum level (pg/ml)	P-Value (Kruksal-Wallis)
Healthy	35	0.269	0.961	0	2.3	0.614
Chronic	35	0.431	1.058	0	5	
Aggressive	26	0.223	0.573	0	2	

According to the abovetable5, the level of serum interleukin-33 in chronic and aggressive periodontitis groups does not significantly differ from that of the healthy group (P>0.05) [Mann-Whitney].

Age-based comparison of the level of serum interleukin-12 in patients with chronic and aggressive periodontitis and healthy individuals indicated that a significant statistical difference existed among the age classes 20-25, 26-30, and 31-35 (P<-0/05). Among the 46 subjects that belonged to the 20-25 age group patients with chronic periodontitis had the maximum mean and standard deviation ($1.6 \pm 1.229 pg/ml$). The statistical results of the group that included 21 subjects with more than 36 years of age did not suggest a significant relation (P=0.335) (Table 6).

Table 6: Age-based comparison of the level of serum interleukin-12 in healthy individuals and patients with periodontitis

age class (year)	Group	Number	Mean value (pg/ml)	Standard deviation	P-Value (Kruskal-Wallis)
20-25	Healthy	19	0.337	0.648	0.002
	Chronic	1	3.8	0	
	Aggressive	22	0.768	0.484	
26-30	Healthy	9	0	0	0.001
	Chronic	8	0.25	0.316	
	Aggressive	3	1.6	1.229	
31-35	Healthy	4	0	0	0.023
	Chronic	9	1.156	1.654	
More than 36	Healthy	3	0.133	0.231	0.355
	Chronic	17	0.735	0.849	
	Aggressive	1	0.4	0	

Age-based comparisonof the levels of serum interleukin-33 in patients with (chronic and aggressive) periodontitis and healthy individuals indicates that no significant relation exists between the group grades (P>0.05) (Table 7).

Table 7: Age-based comparison of the level of serum interleukin-33 in healthy individuals and patients with periodontitis

age class (year)	Group	Quantity	Mean value (pg/ml)	Standard deviation	P-Value (Kruskal-Wallis)
20-25	Healthy	19	0.179	0.404	0.166
	Chronic	1	1.2	0	
	Aggressive	22	0.264	0.616	
26-30	Healthy	9	0.033	0.1	0.218
	Chronic	8	0.562	1.144	
	Aggressive	3	0	0	
31-35	Healthy	4	1.375	2.75	0.462
	Chronic	9	0.122	0.367	
More than 36	Healthy	3	0.067	0.115	0.848
	Chronic	17	0.488	1.28	
	Aggressive	1	0	0	

The results indicated that in the healthy and chronic periodontitis groups, the mean and standard deviation for men are more than women. That is to say, the mean value of men with chronic periodontitis is equal to $1.122 \pm 1.374\, pg/ml$. However, the female group with aggressive periodontitis had a mean and standard deviation of $1.042 \pm 0.773\, pg/ml$. The Kruskal-Wallis statistical test shows that there is no relation between the levels of interleukin-12 in all male and female groups (P>0.05).

The results of the study indicated that no relation exists between the level of serum interleukin-33 and gender in the participant groups (healthy, chronic, and aggressive) (P>0.05) [Kruskal-Wallis].

Discussion

amed, the level of inflammatory factors (including interleukins) is expected to be high(23,26).

Interleukin-8 belongs to the family of supergene interleukins-8 that includes small peptides with chemotactic activity of certain types of leukocytes. This interleukin is a proinflammatory cytokine that is released by a number of cells such as monocyte, lymphocyte, and endothelial cells. Interleukin-8 absorbs polymorphonuclear (PMN) cells into the inflamed region and contributes to the release of enzyme granules by these cells(22,24). According to the obtained results, the level of serum interleukin-8 in the healthy group showed a little growth, but the level of this interleukin was not increased for the chronic and aggressive groups.

This study was aimed at determining and comparing the levels of serum interleukins 8, 12, and 33 in patients with chronic and aggressive periodontitis, and healthy individuals. The levels of serum interleukins 8 and 33 in patients did not differ significantly from those of healthy individuals. However, the level of serum interleukin-12 in patients with increasing gum inflammation was more than that of the members of the healthy group.

Periodontal diseases manifest in the form of inflammatory-immune responses of the gum and periodontal tissues against periodontopathogenic bacteria, which are regulated by inflammatory cytokines. Factors other than the presence of pathogen microorganisms in the oral cavity and accumulation of dental plaque are apparently involved in the pathogenesis of this disease. Since in the periodontal diseases the periodontium is infl

This indicates the lack of relation between the level of this serum interleukin and destruction of periodontal tissues.

Interleukin-12 is known as a proinflammatory cytokine that is released by monocyte-macrophage cells in response to antigen stimulants (such as LPS) of gram-negative bacteria. IL12 causes the production of INFg by T cells and natural killer (NT) cells. In this study, the level of serum IL12 in the chronic and aggressive periodontitis groups was more than the level of IL12 in the healthy group. This indicates that a relation exists between the level of destruction of periodontal tissues and the increase in serum IL12. The increase in the level of IL12 follows the stimulation of dendritic cells by

P.gingivalis and A.actinomycetemcomitans bacteria. This increase plays an important role in the response of Th1 and production of INFg. INFg affects the monocyte-macrophage cells and causes the production of IL12, IL1, and INFg. These proinflammatory cytokines lead to the activation of osteoclasts and deterioration of bones by indirect stimulation of osteoblast cells(26).

In the study conducted by Orozco et al. (2006) the level of IL12 (P70) in the serum obtained from patients with periodontitis was extremely low. Furthermore, the increase in the inflammation would decrease the level of IL12. In the current study, the serum level of IL12 in the chronic group had a considerable growth compared to the level of IL12 in the healthy group. The level of IL12 in the aggressive group was also more than that of the healthy group. This difference was maybe causedby the small number of the individuals who had participated in the Orozco et al. research (10 patients with periodontitis) or the difference in the kits used for these two studies(27).

In the study carried out by Sanchez et al. (2011) the serum content of IL12 (P70) in the patients with aggressive periodontitis was higher than that of the patients with chronic periodontitis or healthy subjects(26). In the current study, the level of IL12 in the aggressive group was also extremely more than that of the healthy group.

In the research that was performed by Robati et al. (2011) in Iran (Ahwaz) no relation was found between the serum content of IL12 and aggressive periodontitis(21). This finding is contradictory to the results of our study. This difference can be attributed to the effect of various genetics, ethnicity, and races on the response of the immune system and the levels of interleukins.

Interleukin-33 is a proinflammatory cytokine that increases the production of other cytokines (such as interleukins 5 and 13) by Th2 cells(28). It also leads to the activation of basophils, mast cells, NK cells, and Th2 cells. No substantial difference was observed in the serum content of interleukin-33 in the chronic group and that of the healthy and aggressive groups. No relation was also found between the level of IL33 and chronic and aggressive periodontitis.

In the study performed by Bununeli et al. (2011) the plasma content of IL33 did not vary for the healthy and chronic groups. Therefore, these researchers concluded that the level of plasma IL33 cannot be useful in distinguishing patients with chronic periodontitis and healthy individuals(29). This result matches the results obtained from our study.

In the current study, the serum content of interleukins varied for different ages. In the 20-25, 26-30, and 31-35 age grades the level of IL12 was associated with periodontitis. However, in subjects with more than 36 years of age no significant difference was seen. This can be attributed to the low number of subjects forming the healthy and aggressive groups who belong to this age group. The level of serum IL33 in the group grades under study was not relevant to the level of serum IL33 in these three groups.

There was also no considerable difference between the level of IL12 in male and female members of the healthy, chronic, and aggressive groups. Gender-based comparison of the groups did not show a relation between the levels of IL33. This shows that gender does not affect the content of blood interleukins.

Conclusion:

In the current study, the serum content of interleukins 8 and 33 in patients with chronic and aggressive periodontitis was not significantly different from that of the healthy subjects. However, the level of interleukin-12 was increased with the increase in the inflammation of periodontal tissues of the chronic and aggressive groups. Therefore, there can be a relation between the level of serum interleukin-12 (as a biomarker) and the level of destruction of periodontal tissues. Gender cannot affect the serum content of these interleukins as well.It is recommended to conduct more researches in the future on the serum contents of interleukins 8, 12, and 33 and other inflammatory factors of periodontal diseases. Furthermore, factors such as smoking, systemic diseases, and their effects on the level of interleukins present in the serum and gingival crevicular fluid should also be studied in the future studies.

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Assessment of Land Degradation and Farm-Level Deforestation in the Niger Delta Region of Nigeria

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Abstract: Land degradation in the Niger Delta region of Nigeria has been aggravated by deforestation with resultant losses of vital biodiversity. In this paper, we provided an assessment of land degradation with drivers of deforestation in selected states in the Niger Delta. Data were collected with multi-stage sampling procedure and analyzed using descriptive and Tobit regression methods. Results show that average age of the farmers is 51.55 years with average years of education being 8 years. The farmers recognized a degraded land through the colour (20.79%), depth of the soil (4.4%) and performance of maize crop (71.73%). Deforestation is positively influenced by sex of the household heads, market distance, population per forest land, purchased land, sold land, fertile land and population density. Legislations for curtailing the pace of deforestation should be enforced with some hints on population control.

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Introduction

Land degradation is a decline in land quality caused by human activities and it is a major global issue during the 20th century and will remain high on the international agenda in the 21st century (Eswaran *et al*.2001). It is a pertinent problem confronting sustainable agricultural production in many tropical regions of the world. At least two distinct schools of thought have emerged regarding the prediction, severity, and impact of land degradation. One school believes that it is a serious global threat posing a major challenge to humans in terms of its adverse impact on biomass productivity and environmental quality (Pimentel *et al*., 1995; Dregne and Chou, 1994). Ecologists, soil scientists, and agronomists primarily support this argument. The second school, comprising primarily economists, believes that if land degradation is a severe issue, why is it that market forces have not taken care of it? Supporters argue that land managers (e.g. farmers) have vested interest in their land and will not let it degrade to the point that it is detrimental to their profits (Crosson, 1997).

The importance of land degradation among global environmental problems cannot be over-emphasized because of its impact on world food security and quality of the environment. High population density is not necessarily related to land degradation; it is what a population does to the land that determines the extent of degradation. People can be a major asset in reversing a trend towards degradation. However, they need to be healthy, politically and economically motivated to care for the land, as subsistence agriculture, poverty, and illiteracy can be important causes of land and environmental degradation (Eswaran *et al*. 2001).

Information on the economic impact of land degradation by different processes on a global scale is not available. Some information for local and regional scales is available and has been reviewed by Lal (1998). In Canada, for example, on-farm effects of land degradation were estimated to range from US$700 to US$915 million in 1984 (Girt, 1986). Land degradation can be considered in terms of the loss of actual or potential productivity or utility as a result of natural or anthropic factors; it is the decline in land quality or reduction in its productivity. In the context of productivity, land degradation results from a mismatch between land quality and land use (Beinroth *et al.,* 1994).

Niger Delta is one of the most richly endowed and yet one of the least developed regions in Nigeria. The rich flora and fauna of the area have supplied the immediate source of livelihood for the people of the region for many generations. For so long, the people lived in harmony and there was evident balance in the ecosystem. Flooding and riverbank or coastal erosion is among the major environmental hazards that the people face. However, the region is endowed with enormous natural resource. Part of a World Bank's report, following a visit to the Niger Delta in 1952 and 1953 declared that the region had great prospects to feed the entire population of the then West African sub-region and had sufficient commodities for export. Some of the produce highlighted by the report includes palm oil and cassava, which are in abundance in some parts of the area (Eswaran *et al*., 2001; Iyayi, 2004; Omofonmwan, and Odia, 2009).

Oil exploration and exploitation has over the last four decades had disastrous impacts on the physical

environment of the oil rich region. This massively threatens subsistence peasant economy, environment and the entire livelihood and basic survival of the people. It should be noted that while oil extraction has caused negative socio-economic and environmental problems in the Niger Delta, the Nigerian State has benefited immensely from petroleum since it was discovered in commercial quantities in 1956. On the other scale, when considered in respect of it's negative impact on the socio-economic life and the environment of the immediate oil bearing local communities and its inhabitants, it has left a balance sheet of ecological and socio-physical disaster (Aluko, 2004; Mathew, 2004). In the Niger Delta, the negative impact of oil exploration is visible in the life of the people.

Agriculture forms the most dominant economic activity of many communities in the area. The Federal Office of Statistics (FOS) in 1985 stated that crop farming and fishing activities account for about 90% of all forms of activities in the area. They also estimated that about 50%-68% of the active labour force is engaged in one form of agricultural activity or the other including fishing and farming. Agricultural technology has remained relatively unchanged over the years and over 90% of the farmers are subsistent farmers operating on traditional methods using basic tools.

Farming techniques still remained the use of land rotation or bush fallow system characterised by land and labour being the principal inputs of production. The organic farming technique widely used is highly susceptible to environmental changes affecting the soil, water and or deforestation because it is not technologically inspired, but rather land and labour intensive. Oil extraction and production has led to adverse environmental impact on the soil, forest and water of the Niger Delta. Various harmful and toxic organic compounds when introduced into the natural environment during oil extraction such as during seismic work, oil spill, gas flares and several other forms of pollution, changes the geo-chemical composition of the soil, river and other components of the environment. This in turn affects agriculture and leads to a drastic decline in output in both fishing and farming activities. This has ultimately affected peasant agriculture in a variety of ways, which ultimately have caused problems of environmental refugees (Aluko 2004; Uyigwe and Agho, 2007).

The peasants are very reactive to these changes because of unavailability of modern farming and fishing techniques to meet the challenges of a declining soil and marine resources. The drastic fall in output of agricultural production leads to intensive exploitation of other fertile land. The long run effect of this is land degradation and migration of peasant farmers to other rural and urban areas, where pressure is exerted on the often inadequate and dilapidated infrastructure, leading to increased poverty. Apart from degradation and loss of farms, oil spills have led to extensive deforestation with no adequate replanting practices. Mmom and Arokoyu (2009) submitted that mangrove forest deforestation is a product of the interaction of the many environmental, economic, social and political forces the Niger Delta region. As a result of deforestation, there are great concerns about rapid loss or decimation of biodiversity.

Persistent deforestation has resulted in shortened fallow periods, and use degradation and loss of soil fertility and consequently erosion of the topsoil. The slash and burn agriculture traditionally practised by shifting cultivators in the area is based on ecologically sound principles and minimises threats to the forest by leaving land fallow over periods of time long enough for regeneration. But landless peasants who have been forced from their own lands, increase the number of people pursuing such a subsistence lifestyle. This contributes to deforestation through further encroachment on forestland and reductions in fallow times (Uyigwe and Agho, 2007; World Bank, 2006).

The out-migration of displaced rural farmers in some communities as a result of environmental degradation caused by oil extraction in the region has led to a significant percentage of the local inhabitants to remain in cyclical poverty and penury. This has meant greater environmental degradation as a result of the intensive exploitation of the few remaining fertile land in the region by the residents. The oil producing communities have basically remained dependent and underdeveloped, economically marginalized and psychologically alienated. The wealth derived from oil resource exploitation and exports benefit directly only the operators of the oil industry and the bureaucrats in government and no one seems to be concerned with the low status masses in the area.

This study would provide answers to the following questions in the end: What are the features that farmers use to recognize a degraded land in Niger Delta? What are the causes of deforestation in Niger Delta? In the remaining parts of the paper, the materials and methods, results and discussions and recommendations are presented. The working hypothesis is that increasing population pressure is not significantly influencing farm-level deforestation.

Materials and Methods

The Study Area

The Nigerian Niger Delta is bordered by the Atlantic Ocean to the south and Cameroon to the east. The land surface is estimated at 112, 160 square kilometers or about 12% of the Nigeria's total land surface. The population is estimated at 35 million

housed in a state of Abia, Akwa Ibom, Bayelsa, Cross-River, Delta, Edo, Imo, Ondo and Rivers State .The region spans over 20,000 square kilometers hosting about 25% of the Nigerian population (from 2006 census, the total population of Nigeria is about 140 million people). About 2,370 square kilometers of the Niger Delta area consist of rivers, creeks and estuaries while stagnant swamp covers about 8600 square kilometers. The region falls within the tropical rain forest zone. The ecosystem of the area is highly diverse and supportive of numerous species of terrestrial and aquatic flora and fauna and human life. As opined by Iyayi (2004), it is richest wetland in the world. The region is divided into four ecological zones namely coastal inland zone, mangrove swamp zone, freshwater zone and lowland rain forest zone.

Method of Data Collection

Because the Niger Delta produces a unique homogeneity in climate and cultural values, the random sampling approach was used to select three states where data were collected. The 3 selected states were divided into local government areas (LGAs). Three local government areas were selected from each of the states. A total of 150 questionnaires were administered in each of the 3 states. At the end, due to insufficient information, only 428 were considered good to be included in the analysis. In all, 147 questionnaires were administered in Abia state, 146 in Akwa Ibom state and 135 in Rivers state. Also secondary data on population of the states were collected based on national population census of 2006. The total land area and forest land area were also collected from same publications of the National Bureau of Statistics (NBS).

Method of Data Analysis

The data collected for this study were analyzed with the aid of descriptive statistics and tobit regression analysis. Descriptive methods were employed to summarize the socioeconomic characteristics of the respondents. The analytical techniques used for descriptive analysis are frequency distribution tables, percentages. The characteristics highlighted include age, household size, sex, marital status, educational status etc.

Tobit Regression Analysis

This is the statistical tool that measures the stochastic relationship between independent variables (regressors) and dependent variables (regressand). In Tobit analysis, the regressand can assume a value of zero. For the data that were used, some farmers did not deforest any forestland, thus compelling application of Tobit regression. The importance of the regression technique is that it establishes the proportion of the variation in the dependent variable that can be explained by independent variables (Gujarati, 2007). In this study, the endogenous variable is the forestland that was cleared in the past five years. The estimated model is presented as:

$$D_i = \alpha + \sum_{j=i}^{19} \alpha_j X_j + v_i \quad .1$$

Where D_i is the deforested land areas (ha), X_j are the independent variables including population density, population per forest land, age of house heads (years), house head sex (male = 1, 0 otherwise), farming experience (years), marital status (married 1, 0 otherwise), number of people contributing to finances, education (years of education), market distance (km), inherited land (ha), purchased land (ha), borrowed land (ha), rented land (ha), community land (ha), problem getting land (yes = 1, 0 =otherwise), land conflict (yes = 1, 0 =otherwise), sold land (yes =1, 0 =otherwise), fertile land (yes = 1, 0 = otherwise and jointly owned community land (yes = 1, 0 = otherwise). v_i is the stochastic error term.

Results and Discussions

Socioeconomic characteristics of the respondents

The socioeconomic characteristics of the respondents are presented in tables 1 and 2. Table 1 shows the distribution of respondents according to sex. The table shows that 54.4% of the respondents are males, while 45.6% of the respondents are females. Proportion of females engaged in farming activities is high in the east when compared to the south-west where female farmers are engaged in processing activities. The table also shows the distribution of respondents according to marital status. This reveals that 87.1% of the farmers are married. Also according to the table, 5.8% are single, 1.4% are divorced. This implies that majority of them have reached marriageable age. According to table 4.7, 1.2% of the respondents did not respond. Also, 35.7% of the respondents have secondary education, 4.7% of respondents have university education, 17.3% of the respondents are not educated. The mean is 8.077103 while the standard deviation is 4.87801.

Table 1: Socio-economic characteristics of the farmers

Socio-economic characteristics	Frequency	%
Gender		
Male	233	54.4
Female	195	45.6
Marital status		
Single	25	5.8
Married	373	87.1
Divorced	6	1.4

Others	24	5.6
Education		
Primary	141	32.9
Secondary	153	35.7
NCE	13	3.0
OND	12	2.8
HND	10	2.3
University	20	4.7
None	74	17.3
Age		
< 30	23	5.37
30 < 40	69	16.12
40 < 50	91	21.26
50 < 60	106	24.77
60 < 70	89	20.79
≥70	50	11.68
Total	428	100.00

Source: Survey data, 2006

The table also shows the age distribution of the farmers. It reveals that only 5.37 percent of the respondents is less than 30 years old. Also, the highest proportion (24.77 percent) of the farmers belongs to age group 50<60 years. Similarly, average age for all the farmers is 51.55 years with standard deviation of 15.04. This shows that the farming households are ageing.

Indigenous knowledge for identifying degraded land

Table 2: Features that farmer used to recognize a degraded land

Land features	Frequency	%
Colour of soil	89	20.79
Depth of soil	19	4.44
Ease of tillage	38	8.88
Intensity of weed growth	71	16.59
Type of weed most common	116	27.10
Performance of maize crops	307	71.73
Performance of root and tuber crops	161	37.62
Performance of cash crops	68	15.89
Texture of the soil	42	9.81
Water drainage	99	23.13
Type of soil	165	38.55

Source : Survey data, 2006

Table 2 shows how farmers recognized a degraded land. 20.79% of the respondents recognized a degraded land through the colour of soil .4.4% of the respondents recognized a degraded land through the depth of the soil. 71.73% of the respondents recognized a degraded land through the performance of maize crops. This indicates that majority of the farmers recognized a degraded land through the performance of maize crops as a result of decrease in yield because maize does well in a fertile soil.

Determinants of deforestation

Average forest land cleared is 0.891355 ha. The kernel density graph of the deforested land is shown in figure 1. This reveals that land areas cleared by farmers are positively skewed.

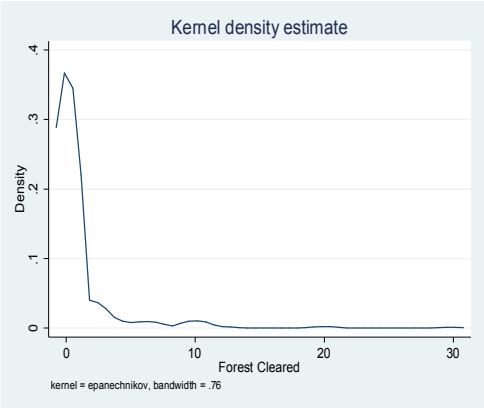

Figure 1: Kernel density estimate of deforested land

Table 3: Tobit regression results of the determinants of deforestation

Forest clear	Coefficient	Std. Err.
Population density	14.84385***	3.614335
Population per forest land	. 0103274*	.0055817
House head age	. 0189594	. 0475595
House head sex	3.148989**	1 .27783
Farming experience	. 016667	.0478119
Marital status	1.330737	1 .761825
Number of people contributing to finances	.2196888	. 3577706
Education	.2439454	. 1551384
Market distance	.0719507**	.0357592
Inherited land	.0750085	. 082371
Purchased land	.2974703*	.153391
Borrowed land	.730404	.488538
Rented land	.5273266	.4078519
Community owned land	. 1149553	. 1492038
Problem getting land	. 0948668	1. 419265
Land conflict	.3540434	1 .324347
Sold land	3.104828*	1 .777894
Fertile land	4 .232547*	2.508531
Jointly owned community land	-1. 979196	1 .31834
Constant	-103.5917	20.96902
sigma	7.316039***	.649775

Significant at 1% = ***, Significant at 5% = **, Significant at 10%=*

The sigma parameter is statistically significant (p<0.01). This means that the model properly fits the data. Population density is statistically significant at 1%. Variables that are statistically significant at 5% include house head sex, market distance. Other variables that are statistically significant at 10% include population per forest land, purchased land, sold land and fertile land. Population density has a positive relationship with the level of deforestation. This implies that an increase in population density will lead to an increase in the level of deforestation. Therefore, the null hypothesis should be rejected. This was also documented by Palo *et al* (1987) who identified a strong positive link between tropical deforestation and population growth for 72 tropical countries.

The coefficient of sex shows that male sex increases deforestation by 3.14898. If market distance increases by one unit, the level of deforestation will increase by 0.0719507. If population per forest land increases by one unit, deforested land will increase by 0.0103274. Purchased land has a positive relationship with deforested land. This implies that an increase in purchased land will lead to increased deforestation. If sold land increases by one unit, deforested land will increase by 3.104828. If fertile land increases by one unit, deforested land will increase by 4.232547

Conclusion

In this study, an attempt has been made to provide empirical analyses of deforestation and degradation in Niger delta. The findings will assist Nigerian policy makers to know the appropriate direction for policy formulation in order to ensure drastic reduction in the rate of deforestation and degradation in Niger delta. Land use policy in which ministry of environment and forest can take coordination role for integrating land sectoral policies in association with ministry of land is necessary. Also, policy and policy level integration in which department of environment under the ministry of environment and forest can take the lead into integrating environment and climate change issues into sectoral policies. In order to reduce the rate of deforestation and degradation in the Niger delta, policies should also encourage forest conservation bringing an end to unsustainable extraction and population contro

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Benefit Incidence Analysis of Public Health Expenditures and Households' Caregiver Preferences in Ogun State, Nigeria

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Abstract: The study seeks to determine the factors that influence public health care seeking behaviour based on the poverty status of households in rural areas. The primary data were collected with well-structured questionnaire while the secondary data were obtained from the local government health records. Descriptive analytical methods, benefit incidence analysis and Multinomial logit regression were employed for data analysis. The results show that average spending on health by households is ₦456.40, while the health subsidy accruing to household from government is ₦687.98. Benefit incidence analysis shows majority of government spending accrue to the poorest segment of the population. Also, the factors that significantly influence ($p<0.05$) households patronage of private clinics are years of education, presence of hygiene facilities and registration cost. Furthermore, age of household head, family size, presence of hygiene facilities, communication access (GSM), drug and registration cost will make household patronise chemist. Years of education of household head, registration cost and hygiene facilities will make household patronise self-care. Adequate education to alert households of the risks involved in inadequate decision regarding health problems and adequate funding of government hospitals were recommended, among others.

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Keywords: public health, poverty, benefit incidence analysis, Ogun state

Introduction

The World Health Organization (WHO) defined health as a state of complete physical, mental and social well being, not merely the absence of disease or infirmity. In 1986, this definition was reformulated and health was defined as a resource for everyday life, not the objective of living. Individual health is a positive concept that emphasizes social and physical resources as well as physical and mental capacity (WHO, 1986). That is why people often say that "health is wealth" (FMOH, 2009).

Health care in much of developing world has developed into two tier systems comprising a sophisticated and expensive hospital care system in urban areas, and a network of primary health care (PHC) clinics that complement the hospital system and offer basic preventive services to low income families in both urban and rural areas. The latter concept gained widespread support following the Alma Ata Declaration of 1977 where serious emphasis was placed on disease prevention. After approval of this approach, PHC system proliferated across developing world. All over the world, health promotion programmes are gradually focusing on the idea that providing knowledge about causes of ill health and choices availability will go a long way towards promoting a change in individual and household behaviour toward more beneficial health seeking behaviour (Ichoku and Fonta, 2006; FMOH, 2009).

A range of factors could influence people's health. Some of these may be fixed, while many are informed by socio-economic circumstances. There is also a growing acceptance that a wide range of social, economic, cultural and environmental factors, including poverty affect health. These may relate to living and working conditions and include experience of unemployment, quality of accommodation, level of education, social and community network and supports, the built environment and work environment as well as access to health care services (Onwujekwe *et al*., 2011).

Million of people are trapped in a vicious circle of ill health and poverty. Over the past one and a half decades, the declining quality of life in Nigeria has received considerable attention in literature. Such studies have examined the incidence and dimension of poverty (NBS, 2005). The major conclusion

from these studies is that poverty is intense and widespread in the country. Thus, the way in which sick individuals or their caretakers in the home perceive their illness could determine what type of health care they will seek and how much money and household member' time is committed for seeking treatment. Also, while seeking care in the health sector, the sick face choices that vary from government hospitals and health centres or clinics to mission institutions among others (Ogunbekun, 2004).

In Nigeria where less than 6% of the population have access to modern health care services (Okojie, 1994), it suffice to say that health care delivery system is in shame and much serious effort needs to be exhibited by everyone involved in the health sector because it has taken many years of gross neglect. Inadequate funding, poor management of limited facilities and resources, social depreciation and economic depression of the people who have become underprivileged and deprived of their due share to enjoy good health are notable among the key contributing factors. Therefore, understanding the main determinants of health care demand behaviour can be vital in furthering knowledge of how changes in government policy will impact on individuals and their demand of health care services (FMOH, 2009; Okeke and Okeibunor, 2010).

The broad objectives of this study is to determine the factors that influence public health care seeking behaviour based on the poverty status of households. The specific objectives are to describe household access to public health services, compute the poverty status of the household in the study area, analyze the benefit incidence of government expenditure on primary health care, determine the factors that affect public health care seeking behaviour.

Materials and Methods

Study area and sampling

The area of study is Ijebu North East Local Government. The local government came into being on the 13th of December 1996 having been carved out of Ijebu Ode Local Government. The local government area is made up of 10 political wards. The predominant occupation of the people of the local government can be classified as farming, trading and civil service. The local government has 26 health care centres.

The study involved the use of primary data with the aid of a well structured questionnaire. The secondary data were collected from the health department and finance department of the local government area to know government expenditure on health services. A simple random sampling technique was be used to sample 10 households each in all the 10 wards of the local government. Secondary data were sourced from the Health Department of the local government.

Analytical methods

The benefit incidence equation was used in the analysis of government expenditure on health. Multinomial logit regression model demand for health care from providers (self care, private clinics, government clinics, home nurses, religious centres, chemist, drug hawkers, traditional healers, quacks and herb sellers) in which the model assumes that household chooses the provider that gives highest level of utility was used to determine the factors that influence household behaviour in seeking health care.

Poverty line computation

This was embarked upon to categories households into different expenditure groups. The poverty status was used based on the headcount index using Foster, Greer, Thordecke (1984) poverty measure. The FGT is given by;

$$P\alpha = n^{-1}\sum^{q}\left[\frac{Z - Y_1}{Z}\right]$$

P = poverty status of respondents
Z = poverty line
Y_1=per capital expenditure of each poor household
n = sample size
q = number of household below poverty line.

The FGT measure is calculated by taking the proportional shortfalls in expenditure for each poor person, raising the shortfalls to a power to reflect the concern for the depth of poverty, taking the sum of these for all poor individuals and normalizing the sum by the population size. The poverty line is an arbitrary divider of the poor and non-poor. The poverty analysis requires establishing a poverty line that was used in combination with welfare indicators. The poverty line is based on income or consumption/expenditure data. The proportion of the population below the poverty line provides a quick indicator of the scope of the poverty problem. Thus, from the analysis, households spending less than one-third of the mean per capital household expenditure (MHHE) are considered core poor, while moderately poor spend less than two-third of MHHE and the noon poor are those with equal or greater than MHHE.

Benefit incidence analysis

The group specific benefit incidence of government spending on primary health care is given as;

$$X_j = \frac{H_{ij}S_i}{H_i}$$

Where, Xj = value of total health subsidy charged to group i.e. expenditure groups
Si = Government net spending on health
i = primary health care
Hi = total number of registered patients
Hij = Number of registered patients of group j
Si = Unit subsidy of providing health centre
Hi
j = groups (poor and non poor)

Household per capital expenditure = Total household expenditure

Household size
The total household per capital expenditure is calculated by finding the summation of the entire household's per capital expenditure for the sample household studied.
The mean per capital expenditure is calculated by dividing the total expenditure by the total number of household surveyed.
Mean per capital household expenditure = Total household expenditure

Household Surveyed

Multinomial Logit regression

The household's choice of medical providers is a discrete decision, which is consistent with qualitative choice models. In this qualitative choice situation, we presume that an individual/household can choose several alternatives: to seek self care treatment, private clinics treatment, government clinics, home nurses, religious centres, chemist, drug hawkers, traditional healers, quacks and herb sellers. In choosing to obtain medical services from whom, individuals and households consider a variety of characteristics of the alternative providers, such as proximity and quality. The decision is also affected by the characteristics of individual's health status in the households, education, age, gender and so on. This can be elaborated upon with general descriptive with concepts from the standard micro economics theory of utility maximization. Utility in this instance, therefore depends upon the attributes of health care choice j which varies with both the choices and characteristics of the individual.
An individual or household chooses among alternatives based on the utility of each alternative. More specifically, based on McFadden and Train (2000) we can posit that the utility of choice option j to individual or household I, Uij is:

$$U_{ij} = V_{ij}\left(M_j H_i\right) + \varepsilon ij \qquad (1)$$

$V(M_jH_i)$ represents utility determined by observed data.
M is a vector of provider characteristics.
H is a vector of individual economic and health status.
ε is a vector of unobserved components.
Where j denotes provider choice alternatives and ε which will be treated as a random variable. Utility-maximizing behaviour implies that an individual/household I will only choose a particular alternative j if $U_{ij} > U_{jk}$ is also random. The probability of any given alternative j being chosen by an individual/household can be expressed as:

$$P = P\left(U_{ij} > U_{ik}\right) \text{ for all k <> j} \qquad (2)$$

By substitution of (9)
P =P(Vij + ε ij>Vik + ε ij, for all k <>j) (3)
Rearranging,
P =P(ε ij- ε ij) >(Vij –Vik), for all k<>j)
By knowing the distribution of the random ε 's the distribution of each difference ε ij-ε ik for j, J<> k, and by using equation (3) calculate the probability that the individual/household will choose alternative j.
Letting Xijj = (M_j H_i) and assuming V to be a linear function of components of X, we operationalize equation 3 as;

$$U_{ij} = \beta_j X_{ij} + \varepsilon ij \qquad (4)$$

Where β_j is a vector of coefficient values indicating the effect of the various X_{ij}'s on individual i's utility for option j.
Assuming that each β_{ij} for all alternative j is distributed independently, identically in accordance with the extreme value distribution and given this distribution for the unobserved components of utility, the probability that the household will choose alternative j is

$$\text{Prob (option}_j/X_{ij}) = \frac{Exp\left(\beta_j X_{ij}\right)}{Exp\left(\beta_{jk} X_{ijk}\right)} \qquad (5)$$

where k=1. The parameters of this model can be estimated straightforward using maximum likelihood methods.

Results and Discussions

Socio-economic characteristics of respondents

Distribution of some socio-economic characteristics of the respondents is in table 1. This shows that 82% of the household heads that were sampled were male while only 18% were female. It was observed that there was a dominance of male over female household head, in cases where there were female household heads, it was due to the fact that they were either divorced or widowed or single individual.

The table shows that 78% of the household head sampled were married while only 22% of the household heads were not married. This shows that majority of the households survey are married. Table shows that 24% of the total household sampled have their household head less than or equal to 30 years in age, while 41% have their household head between the ages of 31-40 yrs. Also 24% of the household heads are between 40 and 59 years while only 11% have their household heads above 60years of age. This shows that majority of the household heads in the study area are middle age individuals who are actually engaged in one activity or the other because the average age of household head is 40 years.

The table further shows that 19% of the total household head samples only had primary education, 34% had up to secondary school education, 29% had tertiary education and 18% of the household head had no education formal education. Majority (63%) of the sampled household heads in the study area are educated. Also, 21% of the household head are traders who are involved in selling 27% are Artisans 25% are civil servants who work with the government, 20% are farmers while 3% work with private establishments .The table shows that majority of the respondents are artisans and civil servants

Table 1: Frequency and percentage distributions of household heads' socio-economic characteristics

Socio-economic variables	Frequency	Percentage Distribution (%)
Sex		
Male	82	82
Female	18	18
Marital status		
Married	78	78
Not Married	22	22
≤ 30years	24	24
31 – 40	41	41
40 – 59	24	24
≥ 60	11	11
Primary	19	19
Secondary	34	34
Tertiary	29	29
None	18	18
Trading	21	21
Artisan	27	27
Civil Service	25	25
Farming	20	20
Others	4	4
Private Establishment	3	3

Table 2. Distribution of treatment venue among households

	Frequency	Percentage Distribution (%)	Cumulative Percentage
Government	51	51	51
Self care	10	10	61
Private	15	15	76
Chemist	24	24	100
Total	100	100	

Table shows that 51% of the household patronise government hospitals, 15% private hospitals and 24% visits chemist and over the counter stores for treatment when they are ill. This shows that households prefer to go to government hospital when in need of treatment. However it is worth noting that a considerable visit of households to chemist gives room for concern.

Table 3: Distribution of ailments that affects households

Ailment	Frequency	Cumulative
Malaria	70	70
Others	30	100
Totals	100	

Table 3 shows that 70% of the ailment that affects household is malaria while 30% constitute other ailments such as tuberculosis, dysentery etc. this shows that households in the sample area are more prone to malaria.

Table 4: Distribution of preferred treatment venues across marital status of household head

	Marital Status		Total
	Single	Married	
Government Hospital	4	47	51
Self care	5	5	10
Private hospital	6	9	15
Chemist	7	17	24
Total	22	78	100

Table 4 shows that 47 of married household heads patronise government hospital while 17% of married household heads prefer patronising chemists, 7 household heads that are single patronise chemist. This implies that married household have more confidence in

government hospitals and chemist shops in times of ill health of any member of the household.

Poverty status of respondents

Poverty status of respondents was based on the head count index using Foster, Greer, Thordecke (1984) poverty measure. This was done by taking the proportional shortfall in expenditure for each poor household, raising the shortfall to a power to reflect the concern for the depth of poverty, taking the sum of these for all poor individuals and normalising the sum by the population size. The degree of concern for the poverty was fixed at α equals zero. This gave the headcount index the respondents were categories into core poor, moderately poor and non-poor based on the mean per capita household expenditure on basic needs. The relative poverty measure was used. The categories are

1. Those that spend less than $1/3$ of the mean household per capita expenditure are referred to as core poor.
2. Those that spent more than $1/3$ of the mean household per capita but not more than $2/3$ of it are known as moderate poor group
3. Those that spend more than $2/3$ of mean per capita household expenditure are called non poor.

From the survey, the mean per capita household expenditure is ₦7,003/month because there are average of 4 persons per household. However, two-third of that is N4668.7 which is the poverty line.

Table 5: Poverty status category of the respondents

Group	Amount (N)	Percentage Distribution (%)
Core poor	<2,334.37	20
Moderate poor	2334.37 – 466878	40
Non poor	>4668	40

The table shows that 40% of the households belong to the non poor group while 20% are in the core poor group. Also, 40% of the respondents are moderately poor. This implies that about 60% of the total surveyed area are poor and do not enjoy better quality of the basic requirements. With almost more than half of the household survey being poor, it confirms the growing concern of the increase in the number of poor.

Table 5: Distribution of Treatment venue Across Poverty Status

	Poverty status			Total
	Non Poor	Moderate Poor	Poor	
Government	11	27	13	51
Self care	16	8	7	31
Private	11	4	-	15
Chemist	2	1	-	3
Total	40	40	20	100

Table 5 shows that 11% of households who patronise government hospitals are non-poor, 16% who prefer self-care are non-poor, 11% who prefer private clinics are non-poor. It also shows that 40% of poor households patronise government hospital most, while only 15% prefer self-care treatment. This shows that majority of poor households prefer to go to government hospital because of the low cost of health services.

Distribution of Government Subsidy in the Provision of Health Care

In order to determine government subsidy in the provision of health care, government expenditure account was used in estimating unit subsidies. Unit subsidy is based on actual expenditures by government. Thus, government unit subsidy represents the total amount of government spending per patient.

It was calculated using the $\chi_{(poor)} = H_{(poor)} \times \frac{S_i}{H_i}$ formula where

s_i = Government spending in the local government

H_i = Total number of required patients in the local government

From the data obtained from the local government health authority in Ijebu North East Area,

Total number of registered patient = 11,6144

Total expenditure on health per annum = ₦7,990,142. 80

Therefore, using $\frac{Si}{Hi}$ to calculate government unit subsidy

Unit subsidy = $\frac{7,990,142.80}{11,614} = ₦687.98$

Average amount spent by household on health/month = ₦456.40

Total spending on health = N 687.98 + N456.40 = ₦1144.38

Table 6: Household and government spending on the health care

Health spending	Amount (N)	% Distribution
Average household spending	456.40	39.9
Government unit subsidy	687.98	60.1
Total	1144.38	100.0

From the table, it shows that government health care spending is higher than household health spending in the local government area. This implies that for every ₦1 of government unit subsidy for proving health care to households, the household spend 0.60k in gaining access to the health care provided by the government.

Specific benefit incidence of government spending on health case according to group.

Benefit incidence of government expenditure is given by $X_j = H_{ij} \frac{S_i}{H_i}$

Where Xj = value of health subsidy charged to group

H_{ij} = number of patients registered of group g at the group level.

government subsidy = ₦ 687.98

total number of patient = 11,614

total health subsidy = 687.98 x 11,614

=₦7,990,199.72

Hi = total number of patients (poor group)

= 11,614 x 0.60

= 6,968.4 ≈ 6,968 patients

Hi = total number of patients (non-poor group)

= 11,614 x 0.4

= 4,645.6 ≈ 4,646 patients

The benefit incidence of government spending on health care to the moderately poor group

$$\chi_{(poor)} = H_{(poor)} \times \frac{S_i}{H_i}$$

Where $X_{(poor)}$ = value of total health subsidy changed to the poor

$H_{(poor)}$ = Number of registered patients of the poor group

:- $X_{(poor)}$ = 6968 x 687.98

=₦ 4,793,844.64

Benefit incidence of government spending on health care to the non poor

Where: $X_{(non\ poor)}$ = value of total health subsidy changed to the non poor

$H_{(non\ poor)}$ = Number of registered patients of the non poor group

:- $X_{(non\ poor)}$ = 4,646 x 687.98

₦3,223,874.28

Table 7: Benefit incidence of health spending by group

Group	Benefit incidence	Percentage Distribution
Poor	4,793,844.64	59.8
Non poor	3,223,874.28	40.2
Total	8,017718.92	100.0

From the table, it is observed that the higher percentage of government spending accrues to the poor group. This is so because this group utilizes the services provided by the government most since they have the highest number of patients. This implies that the more the use of government provided facilities, the greater the benefit incidence of government unit subsidies accruing to the poor people in the society.

Factors Explaining Households' Health Facility Preferences

From the Multinomial Logit regression analysis that was carried out to determine the preferred health care alternatives i.e. (government, self care, private clinic and chemist) in which government health centres stands as the reference, the following observations were inferred. It was observed that factors that make households to prefer private clinics (heath centres owned by individuals ,groups and specialists) are number of years of education of the household head, ownership of means of transport e.g. car, presence of hygiene facilities(flush toilet and piped water) and ability to afford transport cost.

It was observed that the total number of years of education of the household head exhibited a positive relationship with the patronage of private clinics in that the higher the level of education (access to further education from elementary to tertiary level) the higher the preference for private clinics to government owned hospitals. This can be attributed to increase in tastes, exposure and knowledge due to education.

It was observed that the ownership of means of transport by household makes them patronize private clinics because distance to their choice of clinics that gives them the utility they want is not a barrier. Though the presence of hygienic facilities were significant, they exhibited a negative relationship with private clinics patronage i.e. the lower and lesser in number of hygiene facilities, the higher the tendency to patronize private clinics for proper check up.

It was observed that the significant factors that will make household to patronize chemist rather than government hospitals were age of household head, household size, presence of communication facilities, availability of hygiene facilities in the house and cost of transportation and drugs. The result showed that the younger household head will patronize chemist because of little experience in family management and may not see the need for proper medical attention due to lack of experience and the rush for career development for successful living. Household with small number of members will also patronize chemist because of cost effectiveness. The presence of hygiene facilities such as fridge, flush toilets and piped water makes the hygiene status of household higher and this makes household not to patronize government hospitals but rather chemists in times of mild ailments.

The higher the cost of drugs, the less likelihood of the patronage of government hospital and the higher the tendency to go to chemists for dispensing because it will be cheaper. It was observed that the factors that will make households patronize self-care (quacks, religious centres, traditional healers, hawkers, home nurses, and herb sellers) were the number of years of education of household head, presence of hygiene facilities and cost of registration for health care needs.

The result showed that the lower the level of education of the household head, the more likely of the patronage of self-care. This is due to the low level of exposure and knowledge of the decision maker in the house. Many other households visit self-care medication due to the diabolical and mystical nature of their illness. High cost of registration will also make the patronage of self-care higher due to the inability of households to afford the cost.

Table 8: Multinomal logit regression of a health care provider

	Private	Chemist	Self care
Constant	-7.2828	6.6094	-0.7707
Household head age	-0.0648	-0.0898**	-0.0392
Years of Education	0.8921**	0.0584	-0.4084**
Household size	-0.0199	-0.2579**	0.1169
Fridge	4.3983	4.3833	-2.4433
Car	3.2739**	1.9678	1.2812
GSM	-1.8527	-2.7144**	0.8197
Flush Toilet	-5.4909**	-4.8699**	8.9537**
Piped Water	-3.4273	-2.2461	-0.9480
Transport Cost	0.0220	0.0347**	0.0103
Registration cost	0.0040	-0.0030	-0.0408**
Drug	-0.0046	0.0098**	0.0031
Waiting time	-0.0811	-0.0042	0.0285
Severity of illness	0.1513	0.2579	0.5473

Significant at 5%**

Conclusion and Recommendation

This paper shows that more households were using health care facilities provided by government. The study revealed that government subsidy is adequate in the provision of health care services and that poor households cannot conveniently access and utilize health care services provided by private owners because of high cost. It is recommended that there should be a better way of educating the public on their health needs and the way they should go about seeking treatments. This is very paramount because our results show that self care was used by many households which portends some risks. Also it was found that education reduces the tendency of indulging in self care by the households. Also, there is the need for better funding of public health care services in Nigeria because our findings show that the poor were benefiting more than the rich from such expenditures. Also, due to its expensiveness, private health centres were least patronized. The government needs to consider a workable health insurance that can stimulate demand for private health services in Nigeria.

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Environmental Impact Assessment of Large Recreational, Sports, and Cultural Complexes On Urban Spaces Case Study: Hezar O Yek Shahr Recreational, Sports, and Cultural Complex District 22 of Tehran Municipality

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Abstract: Result of studies conducted and predictions made by World Bank and other international organizations indicate that two third of world population will live in the urban areas in the third millennium. Urban living in Iran has experienced a rapid growth during past 4 decades. Increased numbers of cities, wide spread migration from rural areas, and higher birth rate have significantly increased urban population. Higher disposable income and reduced work hours have improved social welfare. These changes have produced complicated and encompassing consequences in urban areas at national and local levels, including air and environmental pollution, densely populated urban areas, plus difficulties and inequalities in city and social services. Providing for social needs of various age groups in such circumstances and under heavy influence from world developments require extensive planning. A part of this planning includes provision of additional public recreational spaces. The complicated nature of urban living demands special focus on projects which may contribute to social welfare by increasing the available public areas including recreational parks. One such project is *Hezar O Yek Shahr* Recreational, Sports, and Cultural Complex in District 22 of Tehran Municipality. This project is being implementing on a 150 hectare divided into seven zones assigned for recreation, tourism, sports, education, cultural activities, and public services. This project is one of a kind in Iran and Middle East. *Hezar O Yek Shahr* project is already under development at a total estimated cost of about $2 billion. As an indication of the size of this undertaking, this project will require 993,000 cubic meters of earth removal and 404,000 cubic meters of filling. This complex will have various subdivisions for hotels, shopping centers, recreation area, water-park, and amusement center. The amusement park alone will have 83 attractions. It is estimated that 141961 visitors will visit this complex per day. Article 50 of Iran Civil Laws and article 172 of The 5th Iran National Development Plan require environmental impact assessment of any project to be undertaken by any large production, industrial, service, and infrastructural unit. In compliance with the legal requirements, the environmental impact of this large and significant project was assessment in this study by using Pastakia Matrix. This study examined the impact of 18 civil and infrastructural activities on environmental parameters in two phases of project construction and operations. The results of this study showed that the project implementation option had higher number of positive impacts. Consequently, the implementation of this project is preferable over non-implementation option. However, project implementation is supported as the final decision subject to implementation of certain management measurements.

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Introduction

Tourism has turned into an industry in many countries because of its contribution to national economy and national growth product. Income and profit from tourism is so high in some countries that rival petroleum income in Iran.

Tourism now ranks third in employment after petroleum and auto industries. Governments, authorities, and economic experts show keen interest in tourism development aiming for a larger share of international tourism market.

Tourism has turned into an important segment of economy during past several decades. World

Tourism Organization has estimated that international tourism will reach 1.6 billion arrivals by 2020. Tourism industry is expected to take a significant part of international gross products because of its global reach.

Recreation and entertainment centers play the same role industrial units had in nineteenth century. Recreation and amusement parks have cultural, economic, and social dimensions. They provide backward and forward linkage to city services and industries including recreational equipment producers, construction and architectural companies, transportation industry, and service industries such as banks and insurance companies. Large scale amusement parks have turned into *global villages* acting like crossroads for bringing people from different countries together.

Iran has experienced rapid urbanization in recent decades. Insufficient employment opportunities to attract the increasing flow of workers migrating from rural areas, petroleum based economy, and inability of active industries to compete in international markets as a base for increased production and further employment, has expanded the non-producing segment of economy to the detriment of national economy. The non-producing segment engages in the risky and speculative business of buying and reselling properties to push prices higher for personal gains.

The demand pull from this segment of economy has fashioned urban spaces in such a way that cannot provide the necessary products and services required by modern urban living. The outcome of this shortcoming is wider inequality and disregard for citizens' rights. The present circumstances require thoughtful space management to allocate urban spaces based on needs of society. Proper and efficient management of recreational spaces together with place marketing may improve living quality in urban areas and help expand urban economies.

Planning experience in Iran shows that urban planners have always lagged in urban development for several reasons including lack of integrated management approach, segment oriented view instead of space-place view, high population growth rate, high ratio of young population, elevated demands and expectations of citizens for city services, and economic changes.

Living quality and life satisfaction now have new meanings. Recreation centers and city tourism in metropolitan areas have to address residences' new multi dimensional demands. These demands stem from different definitions people have about recreation and entertainment. Understanding and paying attention to these differences can help urban planners in providing the required cultural and recreational spaces.

Tehran Municipality has undertaken *Hezar o Yek Shahr* project to create the largest recreational and tourism complex in the Middle East. This project is intended to address the extended culture changes that have occurred in Tehran in recent years. It will address the needs of young generation and shortage of recreational spaces. This project will meet a part of recreational needs of residence, improve economic conditions in Tehran, and create a sustainable source of income for City.

Hezar o Yek Shahr recreational and tourism projects will provide an amusement park in the Capital of Iran with extensive and diverse recreational and entertainment services that cannot be found anywhere in the Middle East. This huge undertaking is made possible with the initiative and participation of Tehran Municipality. It is intended to fulfill the cultural and recreational needs of Iranian people when visiting Tehran metropolitan. This project will be implemented in compliance with Islamic and Iranian values and principles. The main objectives of this project are to attract domestic investment funds, create employment, and provide recreational and entertainment spaces.

Three effective groups of factor should be considered in fulfilling the objectives of this project:

- Effective factors in project design
- Effective factors in project management
- Effective factors in project implementation

These three groups of factors should consider the following issues.

- Residences demand and their living style
- Investment potential of private sector in tourism development
- Required legal support from public sector and city management.

The following environmental factors should be considered in space relations of the project:

- The extent of using environment without harming natural resources and creating social, cultural, and economic problems
- The balance between development and prosperity of a district (for example, directing private and public investment funds toward city tourism industry and the related producing services for attracting international and regional investments, away from non-producing services in the intermediary and speculative business or irregular construction or industries that cannot compete in international market.)
- Potential employment and income opportunities, urban development and

prosperity, infrastructure and public service development

- Converting west entrance of Tehran into the most attractive recreational-commercial space

Implementation of this project will produce a cultural space with positive and/or negative impacts such as:

- environmental pollution
- qualitative and quantitative changes in city infrastructures
- traffic increase in the area and increased intercity traveling
- changes in land and property values
- zoning changes in district 22 and neighboring districts
- population change in the area
- life-satisfaction
- quality change in city management services, and
- changes in city revenues

This study takes an analytical and future oriented approach for evaluation of the potential environmental impact of this project as one of the most important consequences of its implementation.

Research Objective

The most important objective in evaluation of the environmental impact of this project was gaining assurance about compliance of planned policies, objectives, and activities of this project with environmental terms, criteria, laws and regulations set by Government.

An effective evaluation shall consider every critical and significant issue that may impact environment. The evaluation report should present various rational and acceptable options that have the lowest negative environmental impact, consider every condition that may improve the quality of environment, and create the highest level of confidence and assurance among decision makers and the public.

The environmental impact of this project is assessed by considering the following issues:

- Removing or recovering from potential environmental damages
- Increased public awareness
- Using public opinions in decision making
- Awareness about potentially dangerous environmental problems
- Predicting critical and permanent environmental impacts
- Striking a balance between long-term developmental objectives and the requirement to make resources available to the majority of people
- Propose developmental program in line with environmental protection
- Increased cooperation and coordination between public and private sectors
- Complying with and inclusion of environmental criteria in national development plans
- Identification of governmental responsibilities for environmental protection
- Striking a balance between population growth and environmental resources
- Maintaining the quality of renewable resources for maximum productivity with consideration given to keeping a proper life cycle
- Provision of healthy and active life for society
- Identification of correct methods of using environment
- Understanding critical environmental problems that need further studies, reviews, controls, and cares.

Environmental Impact Assessment

Environmental impact assessment (EIA) is the process of formal reviews and studies to predict the potential impacts on environment, human health and social well-fare resulting from activities and performances of a project. It is a systematic identification and assessment process to measure the consequences of carrying out a project, program, or plan on physical, chemical, biological, cultural, economical, and social aspects of the environment. Environmental impact assessment as a planning tool determines positive and/or negative impacts of a project on the environment.

Environmental impact assessment develops and implements an environmental monitoring system by the help of suitable environmental criteria in order to provide enough control over implementation and operations of a project to minimize its potential damaging impacts.

Tourism industry, like many other industries, may be harmful to environment if expanded without proper and logical planning. Economic losses may be suffered because of land price increases, workforce changes, overloading infrastructure, inflationary pressures, and economic gap between different parts of a country. Tourism expansion without proper plan may produce damaging consequences to the environment and historic heritage by producing negative cultural and social impacts, introducing changes in values, and promotion of social indecencies.

Negative tourism impacts are not limited to foreign tourism. Domestic tourism can also produce similar damaging impact if pursued without proper planning.

Tourism planning can decrease its negative impacts and create an opportunity for its sustainable development.

Sustainable tourism development is defined as tourism industry growth by attracting additional tourists through the available resources in such a way that it addresses the economic, social, and cultural needs of a society while observing the related laws and regulations, fulfilling tourists' expectations, and supporting social unity, cultural identification, environmental safety, economical growth, social welfare, and tourists enjoyment (Mansoori, 2002, p. 73).

A sustainable tourism program should be always flexible and support changes. New work procedures and approaches should never stand in the way of innovation, creativity, and experimentation. Such tourism program should adapt itself to environmental changes and fulfill tourists' new expectations and needs (Alvani, 1994, p.271).

The main objective of a sustainable tourism program should be the provision of rational approaches for utilization of natural and human resources. It means that such a program should prevent irrational use of resources. Sustainable tourism development should protect environment and natural resources as well as historical and cultural heritage of a society. It requires a well-defined set of policies to help the development of a tourism program that contributes to the overall development of the country (Mansoori, 2002, p. 73).

Key environmental variables of the tourism industry can be studies and analyzed from four points of view (Iran Tourism Organization, 2002, p. 4):

1) Physical impact on environment including the effects on soil, water, ecology, sound, and raining;
2) Natural impact on environment including the effects on plants, animals, and natural habitats;
3) Social and cultural impact including the effects on public health, employment, housing, and culture; and
4) Impact on national development programs including the effects on agricultural, industrial, and service development, plus the effects on land preservation and use

The following principles should be considered in environmental impact assessment in order to obtain a desirable outcome and provide for a controlled development of tourist recreational centers (Poorokhshoori, 2001, pp. 46-56 and Majnoonian, 1997, p. 4):

- Exclude geologically unstable areas (i.e. areas with high land erosion);
- Identify candidate areas for development;
- Minimize wind impact by considering height, shape, and position of buildings within the surrounding vegetation;
- Design facilities and services with the lowest possible impact on environment;
- Select environmental friendly materials and construction methods;
- Exercise total control over proper garbage and waste disposal; and
- Supervise sewage disposal to minimize environmental impact
- Provide utilities to lowland areas wherever possible.
- Position buildings, roads, and parking lots where there is the highest harmony with the surrounding area.
- Select the height and position in such way that they do not stand out to obstruct the natural view.
- Select construction material that conforms to local ecology.
- Design buildings that fit the local habitat.
- Procure construction material from outside the area. .

Tourism development without proper planning can damage social and environmental setting of the area in spite of all its benefits and advantages. Environmental impact assessment of a tourism project can prevent serious social and environmental consequences. A project shall not be approved unless it is changed to satisfy certain requirements. Environmental impact assessment should consider all environmental, economic, social, and cultural impacts. A highly profitable project may be unacceptable because of the problems it creates for environment and society. Such projects shall not be approved without revisions (Abdollah Zadeh, 2000, p. 95).

District 22 as Project Location

District 22 of Tehran Municipality is located northwest of Tehran at the downstream of Kan and Vardige Rivers. The area is surrounded by central Alborz Mountain on the north, Kan River on the east, Tehran-Karaj Highway on the south, and Vard Avard manmade forest on the west. District 22 neighbors districts 5 and 21 of Tehran Municipality.

Alborz Mountain embraces Tehran like an arc. It has been subject to urban development during past 30 years. Tehran has expanded throughout the foothills of ALborz up to 1800-meter elevation line. High slops and raggedness of the area above this line has prevented further city expansion above this elevation. The northern part of district 22 extends up to 1800-meter elevation line of southern foothills of

Alborz Mountain making it a suitable area for vegetation, plantation, recreation, and tourism.

District 22 is the largest Tehran municipality district. It was the first district with an approved plan with zoning for recreation, entertainment, and tourism before Tehran Comprehensive Plan was prepared. It had been decided that projects for this area should be limited to projects for recreation and tourism attraction. The first comprehensive plan devised in 1999 kept the original plan for this district 22 intact. The revised comprehensive plan of 2007 reconfirmed the same original plan for district 22 with an additional plan to reduce population from 675,000 to 400,000 (District 22 Comprehensive Plan, Bavand Consulting Engineers 1998).

Various projects have been completed in the area since 1999 including access roads to green land areas of Technology and Chitgar Parks, man-made lake at Azadi Stadium, Kan and Vard Avar Rivers, Khargoosh Darreh Park, and southern foothills of Alborz Mountain. The latter area was subject to residential developments before this plan. The present municipality management has been careful not to issue permits for residential development in the area leaving it strictly for public, recreational, and entertainment land use.

Figure 1: *Hezar O Yek Shahr* Project Position within district 22, Tehran Province, north central part of Iran and Iran.

Source: Google Earth

The proposed land for *Hezar O Yek Shahr Park* is located in west and northwest of Tehran stretching from 35.75 to 35.77 latitudes and from 51.17 to 51.20 longitudes. Tehran Park Project is located at North of Hemmat Highway, northwest of district 22 after Shahid Bagheri Residential Complex. The size of the project can be measured by the volume of earth removal and filling which are estimated to be 993,000 and 404.000 m^3 respectively. This park will have an estimated 141,961 visitors per day.

An important point of this project is its positioning. According to the studies conducted on Land Preservation Plans in the district, District 22 Comprehensive Plan, plus new Development Plans for Tehran, about 24 percent of land in Tehran are suitable for green zone (G). The land selected for this project is located in zone G with the potential for recreation, tourism, entertainment, and vegetation (Research Center for Environmental and Energy Studies, 1381/2002).

Hezar O Yek Shahr Project will include various subdivisions as listed below.

- Amusement Park
- Commercial Center
- Covered Water Park
- Five Star Hotel
- Four Star Hotel
- Three star hotel

The project design is fashioned after Islamic and Iranian architectural. The construction of this project will take 5 years with the first project to be the amusement park. Amusement Park has been organized into seven Diar (meaning land) and is expected to be completed within 2 years. The number seven was chosen because of its importance in Islamic teaching. The names of these diars were selected based on their usage and significance in Iranian literature. Next three tables provides additional information about this project.

Table 1: Project Subdivision

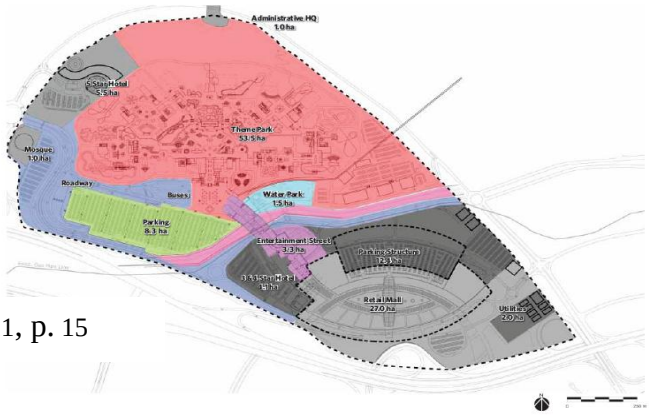
Subdivisions	Square Area (Hectare)	Capacity	Employment
Amusement Park	52.941	47941 Visitors	5000
Commercial and Shopping Centers	95.802	89815 Stores	5988
Water Park	6.851	6201 Visitors	650
Parking Lots		10000 Cars	

Table 2: Detailed Information of Hotels

Hotels	Rooms	Number of Guests	Number of Employees	Square Area
Three star	600	1200	600	1800
Four star	400	800	400	1200
Five star	300	600	300	900
Total	1300	2600	1300	3900

Table 3: Name and Description of Diars in Amusement Park

Diar	**Name**	**Description**
1	Afarinesh	The tail of earth and human creation based on Islamic and Iranian interpretation in attractive and different forms.
2	Afsaneh va Qesseh	A selection of Iranian myth and tails (such as Jamshid, Rostam, and Arash) and Islamic stories (such as The Prophet Nooh, peace be upon him)
3	Tarikh	An exploration into world and Iranian history
4	Asre No	A selection of materialistic and spiritual achievements in the new era.
5	Ekteshafat	Prediction of future and what can possibly happen
6	Majaraha	A variety of exciting and dangerous games with the help of new technologies based on myths.
7	Jaddeh Abrisham	A selection of the most important aspects and symbols of ancient civilizations enroute Abrisham Road.



Source: Forrec, 2011, p. 15

Figure 2: *Hezar O Yek Shahr* Project Site

Environmental Impact Analysis

Impact analysis is a versatile scientific tool for identification, collection, and organization of information about environmental impact of developmental plans. Environmental impact assessment of *Hezar O Yek Shahr* and option evaluation are the main part of this study. The data related to the current location and the its predicted impacts on different environments are analyzed. Qualitative-quantitative information and environmental impact predictions are presented in the same scale. The evaluation of different options will lead to selection of the best option.

Assessment Methodology

Developmental projects have different nature and therefore have different impact. The best approach for identification and evaluation of environmental impacts of developmental projects is using complex methods such as checklists, matrixes, and other similar methods. The evaluation team proposed different assessment techniques for this project based on various processes including decision making, impact identification, impact measurement, and impact interpretation. These processes provide easier, certain, definite, and realistic access to the results of this project. Evaluation team proposed a checklist for the whole project to be used with matrix method in consideration of different sub-projects and within the scope of the study.

The evaluation process was divided into four parts conforming to Warner and Preston study (1973) in order to prepare an objective oriented checklist for this project,.

1. Identification
2. Measurement
3. Interpretation
4. Conclusion

Objective-oriented Checklist with Patakia Matrix was selected for this study. It was chosen because of its versatility, independence, specialization, objective orientation, classification, flexibility, and reliability in its prediction of environmental impacts and how it compares the possible outcome of those impacts.

The following key questions were considered in prediction of possible environmental impacts of the project and its subprojects in order to obtain objective oriented and applicable results:

1- Does project implementation involve earth removal and filling?
2- Does project require service covering that may interfere with soil permeability?
3- Does project involve building construction?
4- Does project increase traffic and cause air pollution?
5- Does project consume water, uses insecticide, and produces sewage and waste?
6- Does project compete with vegetation growth and local ecology?
7- Does project cause biota increase or decrease?
8- Does project produce light and sound?
9- Does project pollute surface and/or in ground water sources?
10- Does project threaten human health?
11- Does project endanger human safety and/or produce human hazard?
12- Does project effect health, welfare, and security in local community?

The potential impact from different phases of construction and operation were classified. The environmental impacts of each class were grouped as negative, cumulative negative, non-recoverable negative or the like based on the negative impacts that they might have on environmental parameters. After analyzing and summarizing evaluation output,

the factors that may have the most critical impacts on environment were identified.

Pastakia Matrix method analyzes activity matrix against environmental parameters. This method was first proposed by Pastakia (1998) and uses certain standards as critical evaluation criteria.

This method identifies various activities of the proposed project and assesses their impacts on each environmental parameter including physical, chemical, biological, ecological, social, cultural, economical, and technical parameters.

Each environmental element was graded based on defined criteria and according to the prepared objective oriented checklists and prioritization of the identified impacts for the best use of this method. After assessment and mathematical calculation by using the related software, the range of environmental impacts were divided from highly positive to highly negative. Finally, a management and monitoring plan, plus approaches for reducing the undesirable impacts were prepared based on the tables and diagrams of environmental elements and predicted impacts.

Table 4: Pastakia Criteria

Criteria	Score	Description
A_1 - Impact Significance	4	National and/or international significance
	3	National or regional significance
	2	Regional significance but not within local terms
	1	Significant only for local conditions
	0	No significance
A_2 - Impact Range	+3	Impact with highly beneficial and positive changes
	+2	Certain local improvement
	+1	Local improvement
	0	No local impact
	-1	Negative local impact
	-2	Highly damaging impact
B_1 - Impact Period	1	No changes
	2	Temporary
	3	Permanent
B_2 - Recoverability	1	No change
	2	Recoverable
	3	Non-recoverable
B_3 - Cumulative Impact	1	No change - Impossible
	2	No cumulative impact
	3	With cumulative impact

Environmental Impact Checklist

Evaluation team identified and classified the most important construction and operation activities of *Hezar O Yek Shahr* Project based on the applicable guidelines and using checklist and matrix methods. This team identified the critical environmental factors that may be impacted by the project construction. Next, the environmental impact of those activities were predicted and studied. Table 6 provides a checklist of the identified and classified impacts of the proposed project on different environmental factors.

Table 5: Impact Range Classifications

Description	Range	Scores
High beneficial and positive impact and changes	+E	+72 to +108
High certain positive impact and changes	+D	+36 to +71
Medium positive impact and changes	+C	+19 to +35
Positive impact and changes	+B	+10 to +18
Minuscule positive impact and changes	+A	+1 to +9
No local impact and changes and/or Impossible	N	0
Minuscule negative impact and changes	-A	-9 to -1
Negative impact and changes	-B	-18 to -10
Medium negative impact and changes	-C	-35 to -19
Certain negative impact and changes	-D	-71 to -36
High negative impact and changes	-E	-108 to -72

The evaluation team discussed the resulting checklist, reviewed the predicted impacts, and classified projects based on their potential impacts during construction and operations. The classification of the subprojects was based on the nature of the impacts, the extent of the impacts, the application of project, and its intended audience.

Table 6 - Environmental Impact Checklist - Construction

Environmental parameters		Project activities									
		Equipping	removal &	roads &	construction	design &	Transport	& Co	& Sewa	& debris	employment
Physical Environment	Microclimate										
	Land form		•	•	•	•					
	Soil quality		•	•	•		•	•	•		
	Surface water volume					•			•		
	Surface water quality										
	Underground water volume										
	Underground water quality							•	•		
	Air quality		•	•	•		•	•			
	Sound pollution		•	•			•				
Biological Environment	Vegetation type		•	•	•						
	Animal species		•	•							
	Areas under management of Environmental Protection Agency										
Social-cultural Environment	Population										
	Literacy & specialization										
	Social culture improvement										
	Views and perspectives	•	•	•	•						
	Security, health, and hygiene						•	•	•	•	
	Conveniences										
	Tourism and recreation										
	Historical-cultural and religious heritage										
	Living standard										
	Local-cultural identification					•					
	Police protection and social security			•		•	•				
Economical-technical Environment	Realization of Comprehensive Plan objectives					•					•
	Employment										•
	Property value			•	•						•
	Income and expenses					•					
	Services					•					•
	infrastructures					•					
	Regional economy					•					•

Number of impacts on each project	1	7	9	6	9	5	4	4	1	5

Table 7 - Environmental Impact Checklist - Operations

Environmental parameters		Project activities									
		Transport	Water consumption	Energy consumption	Employment	Sewage disposal	Garbage disposal	operation (water park, cultural center, hotels, & commercial	Tourists visit and activities (amusement Park)	Garbage & debris disposal
Physical Environment	Microclimate							•		•
	Land form									
	Soil quality						•			•
	Surface water volume		•							•
	Surface water quality									
	Underground water volume		•							•
	Underground water quality					•				•
	Air quality	•		•						•
	Sound pollution	•							•	•
Biological Environment	Vegetation type									•
	Animal species									•
	Areas under management of Environmental Protection Agency									
	Disease carriers							•		
Social-cultural Environment	Population				•				•	
	Literacy & specialization									
	Security, health, and hygiene improvement							•	•	•
	Conveniences				•			•	•	•
	Social culture improvement				•				•	
	Public participation							•	•	•
	Views and perspectives							•		•
	Tourism and recreation							•	•	•
	Living standards							•		
	Historical, cultural, and religious heritage									
	Local-cultural identification							•	•	
	Police protection and social security							•	•	•
Economical-technical Environment	Employment				•			•	•	•
	Realization of Comprehensive Plan objectives							•	•	
	Green belt development		•			•				•
	Infrastructures							•	•	
	Property value				•			•		
	Income and expenses				•			•	•	
	Services				•				•	
Number of impacts on each project		2	3	1	7	2	1	15	14	17

Table 8 - Environmental Impact Classification - Construction

Environmental parameters	Impact type											
	inharmonious	Recoverable	Unrecoverable	Temporary	Permanent	Direct	Indirect	Without cumulative	With cumulative	Avoidable	Unavoidable	Beneficial
Microclimate												
Land form	•		•		•	•		•			•	
Soil quality	•		•		•	•		•			•	
Surface water quality	•	•		•				•			•	
Underground water quality	•		•	•			•	•		•		
Air quality		•		•		•		•			•	
Sound quality		•		•		•		•			•	
Vegetation type	•		•		•	•		•			•	
Animal species			•		•		•	•			•	
Habitats												
Disease carriers	•	•		•			•	•		•		
Population	•	•		•			•	•			•	
Literacy & specialization												•
Income and expenses												•
Security and hygiene	•		•	•		•		•		•		
Conveniences												
Property value			•		•		•	•			•	•
Tourism-industrial development			•		•	•						•
Commerce												
Services			•				•	•		•		•
Infrastructures												
Employment		•		•		•		•			•	•
Living standards												
Views and perspective		•		•		•		•			•	•
Social cultural improvement								•				
Public participation								•				
Local cultural identification												
Police Protection & Social Security		•			•	•		•				•
Conveniences												
Tourism and Recreation												
Total Impact	8	7	7	9	5	9	5	16		3	11	8

Table 9: Environmental Impact Classification - Operations

Environmental parameters	Impact type											
	inharmonious	Recoverable	Unrecoverable	Temporary	Permanent	Direct	Indirect	Without cumulative	With cumulative	Avoidable	Unavoidable	Beneficial
Microclimate			•		•	•		•			•	
Land form			•		•	•		•			•	
Soil quality			•		•	•		•		•		
Surface water volume		•			•	•		•			•	

Surface water quality												
Underground water		•			•	•		•		•		
Underground water quality			•		•		•		•	•		
Air quality			•		•	•		•			•	
Air pollution		•			•	•		•			•	
Sound quality			•		•	•		•			•	
Sound pollution	•	•		•		•			•		•	
Vegetation type												
Animal species	•		•		•		•	•			•	
Areas under management of Environmental Protection Agency												
Natural habitats												
Disease carriers	•	•		•			•	•		•		
Population	•	•		•		•		•			•	
Literacy & specialization												
Income and expenses			•		•		•					•
Security, health, & hygiene increase												
Conveniences			•		•	•		•			•	
Property value			•		•		•	•			•	
Tourism-industrial development			•		•		•					•
Commerce												
Services			•		•		•					•
Infrastructures			•		•	•		•			•	•
Employment			•		•	•		•			•	•
Living standards			•		•		•	•			•	•
Realization of comprehensive plan objectives			•		•	•		•			•	•
Green space			•		•	•		•			•	•
Tourism and recreation			•		•	•		•			•	•
Views and perspective			•		•	•		•			•	•
Social cultural improvement			•		•			•			•	•
Public participation			•		•			•			•	•
Total Impact	4	6	18	3	20	17	7	20	4	4	20	12

The evaluation team judged the impacts of water-park, hotels, cultural complex, and shopping centers on the surrounding environment to be close to each other and therefore put them into one group. The impact of this group as a special case was assumed to be the highest average of its members. Various parts (Diars) of amusement park were also grouped together. Maintenance activities and green space development were put into a separate group.

The resulting checklists were analyzed after identification and classification of environmental impacts. Table 10 provides the results of this analysis.

Table 10: Environmental Impact Analysis

Construction phase	Site Equipping	Earth removal & filling	Access roads	Building construction	Green space design & implementation	transport	Energy storage & usage	Water Usage & Sewage disposal	Garbage & debris disposal	employment	Average of impact ratio on environmental parameters
Impact score	1	7	9	6	9	5	4	4	1	5	32.6

Operation phase	Transport	Water consumption	Energy consumption	Employment	Sewage disposal	Garbage disposal	Total operation (water park, cultural center, hotels, & commercial	Tourist visit and activities (amusement Park)	Garbage & debris disposal	----	----
Impact score	2	3	1	7	2	1	15	14	17	---	32.11

According to this table, the total impact score of implementing this project on surrounding environmental parameters in construction period is 6 out of 32 and in operation period is 11 out of 32. About 50% impact increase represents increased impact on surrounding environment, which may include positive and negative impacts.

Table 11: Impact on Each Environment - Construction

Environmental parameters	Project activities										Total Project impact
	Site Equipping	Earth Removal & Filling	Access Roads	Building Construction	space Design & Implemen	Transport	Energy Storage & Consumption	Water Usage & Sewage Disposal	Garbage & Debris Disposal	Employment	
Physical environment		4	4	3	2	3	3	3	-	-	24
Biological environment	-	0	0	1	-	-	-	-	-	-	5
Social-cultural environment	1	1	2	1	2	2	1	1	1		13
Economic-technical environment	-	-	1	1	6					5	13

This table shows that the project activities during operations have the highest impact on social-cultural environment. Economic-technical and biology environments receive the next highest impact. Physical environment ranks third on impact received from the project during operations. Social-cultural and economic-technical environments show the highest positive impact. Green space development has the highest positive impact on physical environment.

Table 12: Impact Analysis of Classification in Different Phases

Project phase	Number of projects and activities	Negative impact on environmental parameters & elements	Cumulative impact on environmental parameters & elements	Non-recoverable impact on environmental parameters & elements	Indirect impact on environmental parameters & elements	Beneficial impact on environmental parameters & elements
Construction	10	30.8	30.0	30.7	30.5	30.8
Operation	9	34.4	34.4	34.18	34.7	34.12

This table shows that the negative project impact is higher during construction than during operations. The higher negative impact is attributed to earth removal, leveling, and site equipment. The beneficial project impact is significantly higher during operations versus construction period.

The evaluation team assessed the project and analyzed the checklists by considering the interactions and overlapping between various activities in different subprojects. The result of analysis showed that this project had long-term beneficial and harmonious impact to the surrounding social, economic, cultural and technical environments. These impacts are recoverable.

Impact Matrix and Evaluation Results

In order to understand the final result which is required for "go/no go" decision, the related scores were entered into a matrix with entries for impacts on physical, biological, social, economic, cultural,

environmental prolusion, and developmental plans. The numbers representing positive or negative impact on different options were summed up and multiplied by the related factors. The sum shows the project impact on each environmental factor. The total algebraic sum is used in the final decision making.

Special attention should be made to the negative numbers in the table in order to decide on what approaches to take to reduce the impact. These numbers shall be used in project management and monitoring. Project management shall improve positive impacts and reduce negative impacts obtained from the matrix.

Project Implementation Option

The next tables and bar diagrams show the result of the analysis performed on the impact of project activities on different environmental parameters during construction and operations for project implementation option.

Table 13: Impact on Physical-Chemical Environment (P/C) - Construction Project Implementation Option

Criteria							Activity impact on environmental parameters	Code	
A_1	A_2	B_1	B_2	B_3	ES	R			
2	-1	2	2	3	-14	-B	Transportation impact on air quality	P/C	1
2	-1	2	2	3	-14	-B	Transportation impact on sound quality	P/C	2
2	-1	2	2	3	-14	-B	Transportation impact on traffic	P/C	3
1	-1	3	3	1	-7	-A	Ground and concrete works impacts on water drainage	P/C	4
1	-1	3	3	1	-7	-A	Ground and concrete works impacts on land form	P/C	5
2	-1	2	2	3	-14	-B	Ground and concrete works impacts on air quality	P/C	6
2	-1	2	2	3	-14	-B	Ground and concrete works impacts on noise production	P/C	7
1	-1	3	3	1	-7	-A	Ground and concrete works impacts on surface water	P/C	8
0	0	1	1	1	0	N	Ground and concrete works impacts on soil characteristics	P/C	9
0	0	1	1	1	0	N	Access road impact on surface water	P/C	10
2	-1	2	2	3	-14	-B	Construction material unloading impact on noise production	P/C	11
0	0	1	1	1	0	N	Access road impact on soil characteristics	P/C	12
1	-1	3	3	1	-7	-A	Sewage disposal impact on soil characteristics	P/C	13
2	-1	2	2	3	-14	-B	Construction work impact on noise production	P/C	14
0	0	1	1	1	0	N	Construction work impact on air quality	P/C	15
1	-1	3	3	1	-7	-A	Construction work impact on land form	P/C	16
0	0	1	1	1	0	N	Construction work impact on surface water	P/C	17
0	0	1	1	1	0	N	Construction work impact on soil characteristics	P/C	18

Table 14: Impact on Biological-Ecological Environment (B/E) - Construction Project Implementation Option

Criteria							Activity impact on environmental parameters	Code	
A_1	A_2	B_1	B_2	B_3	ES	R			
1	-1	3	3	2	-8	-A	Deforestation impact on land ecosystem	B/E	1
0	0	1	1	1	0	N	Deforestation impact on water ecosystem	B/E	2
1	-1	3	3	2	-8	-A	Ground and concrete works impacts on vegetation habitat	B/E	3

1	-1	3	3	2	-8	-A	Ground and concrete works impacts on plant concentration	B/E	4
2	-1	3	3	2	-16	-B	Ground and concrete works impacts on animal behavioral pattern	B/E	5
0	0	1	1	1	0	N	Ground and concrete works impacts on animal habitat	B/E	6
0	0	1	1	1	0	N	Construction work impact on land ecosystem	B/E	7
0	0	1	1	1	0	N	Construction work impact on water ecosystem	B/E	8
1	-1	3	3	2	-8	-A	Transport impact on vegetation habitat	B/E	9
0	0	1	1	1	0	N	Transport impact on animal habitat	B/E	10

Table 15: Impact on Social-Cultural Environment (S/C) - Construction Project Implementation Option

Criteria							Activity impact on environmental parameters	Code	
A_1	A_2	B_1	B_2	B_3	ES	R			
2	-1	2	2	3	-14	-B	Impact of supply & transport scraps, construction materials, equipments, machineries, and personnel on local traffic	S/C	1
2	-1	2	2	3	-14	-B	Impact of noise prolusion on local community	S/C	2
2	+2	2	2	3	+28	+C	Impact of employment and construction on people and local community participation	S/C	3
2	+1	2	2	2	+14	+B	Impact of employment and construction on local population density	S/C	4
2	+1	2	2	2	+12	+B	Impact of project hiring on local employment	S/C	5

Table 16: Impact on Economical-Technical Environment (E/O) – Construction Project Implementation Option

Criteria							Activity impact on environmental parameters	Code	
A_1	A_2	B_1	B_2	B_3	ES	R			
3	+2	2	2	2	+36	+D	Impact of transportation and equipment on economy	E/O	1
2	+2	2	2	2	+24	+C	Impact of employment on income	E/O	2
1	0	1	1	1	0	N	Costs involved in changing land zoning	E/O	3
3	-2	2	2	2	-36	-D	Construction costs	E/O	4
2	-1	2	2	2	-12	-B	Impact of demand for energy	E/O	5
3	+2	2	2	2	+36	+D	Impact of material and equipment procurement on economy	E/O	6

Table 17: Impact on Physical-Chemical Environment (P/C) – Construction Project Implementation Option

Criteria							Activity impact on environmental parameters	Code	
A_1	A_2	B_1	B_2	B_3	ES	R			
2	-1	3	2	3	-16	-B	Impact of operation process on air quality	P/C	1
2	-2	3	2	3	-32	-C	Impact of operation process on sound quality	P/C	2
2	-1	2	3	3	-16	-B	Impact of operation process on surface water quality	P/C	3
1	-1	3	3	2	-8	-A	Impact of operation process on underground water quality	P/C	4
2	+1	3	2	2	+14	+B	Impact of green land on air and sound quality	P/C	5
2	-1	1	1	1	-6	-A	Impact of spillage and accidents on air and sound quality	P/C	6
2	-1	3	3	3	-18	-B	Impact of spillage and accidents on surface water	P/C	7
2	-1	3	3	3	-18	-B	Impact of spillage and accidents on soil characteristics	P/C	8
1	+1	3	2	2	+7	+A	Impact of green land on soil characteristics	P/C	9

Table 18: Impact on Biological-Ecological Environment (B/E) – Construction Project Implementation Option

Criteria	Activity impact on environmental parameters	Code

A_1	A_2	B_1	B_2	B_3	ES	R			
2	-1	3	3	2	-16	-B	Impact of operation process on land ecology	B/E	1
2	-1	2	2	3	-14	-B	Impact of operation process on water ecology	B/E	2
1	+1	3	2	1	+6	+A	Impact of green land on land ecology	B/E	3
0	0	1	1	1	0	N	Impact of green land on water ecology	B/E	4
1	+3	2	2	2	+18	+B	Impact of green land on land	B/E	5
2	-1	3	3	2	-16	-B	Impact of operation process on vegetation habitat	B/E	6
2	-1	3	3	2	-16	-B	Impact of operation process on animals	B/E	7
2	-1	2	3	2	-14	-B	Impact of spillage and accidents on vegetations	B/E	8
2	-1	2	2	2	-12	-B	Impact of spillage and accidents on animals	B/E	9
2	-1	2	3	3	-16	-B	Impact of spillage and accidents on water ecosystem	B/E	10
2	-1	2	2	3	-14	-B	Impact of spillage and accidents on land ecosystem	B/E	11

Table 19: Impact on Social-Cultural Environment (S/C) – Operations Project Implementation Option

Criteria							Activity impact on environmental parameters	Code	
A_1	A_2	B_1	B_2	B_3	ES	R			
2	+2	3	2	3	+32	+C	Impact on future development plans	S/C	1
2	+3	3	2	3	+48	+D	Impact on service provision	S/C	2
2	+2	3	2	3	+48	+D	Impact on communities	S/C	3
2	+1	3	2	3	+16	+B	Impact on hygiene indexes	S/C	4
2	+2	3	2	3	+32	+C	Impact on security and safety	S/C	5
3	+2	3	2	3	+48	+D	Impact of guest services on tourism growth	S/C	6
2	+1	3	2	3	+16	+B	Impact of shopping and convenience services on public welfare	S/C	7
3	+2	3	2	3	+48	+D	Impact of recreational services on public temperament/happiness	S/C	8

Table 20: Impact on Economic-Technical Environment (E/O) – Operations Project Implementation Option

Criteria							Activity impact on environmental parameters	Code	
A_1	A_2	B_1	B_2	B_3	ES	R			
3	+2	3	2	3	+48	+D	Impact on employment	E/O	1
2	+1	3	3	3	+18	+B	Impact on property and land prices	E/O	2
2	-1	3	2	2	-14	-B	Impact on community energy consumption	E/O	3
2	+2	3	3	3	+36	+D	Impact on different economic-technical activities	E/O	4
2	+2	3	2	3	+32	+C	Impact on shopping centers on local economy	E/O	5

Table 21: Number and Range of Impact – Construction Project Implementation Option

Environments Impact range	Economic-technical (E/O)	Social-cultural (S/C)	Biological-ecological (B/E)	Physical-chemical (P/C)	Total score
E	0	0	0	0	0
D	2	0	0	0	2
C	1	1	0	0	2
B	0	2	0	0	2
A	0	0	0	0	0
N	1	0	5	6	12
-A	0	0	4	5	9
-B	1	2	1	7	11
-C	0	0	0	0	0
-D	1	0	0	0	1
-E	0	0	0	0	0

Diagram 1: Impact Number and Range - Construction Phase Project Implementation Option

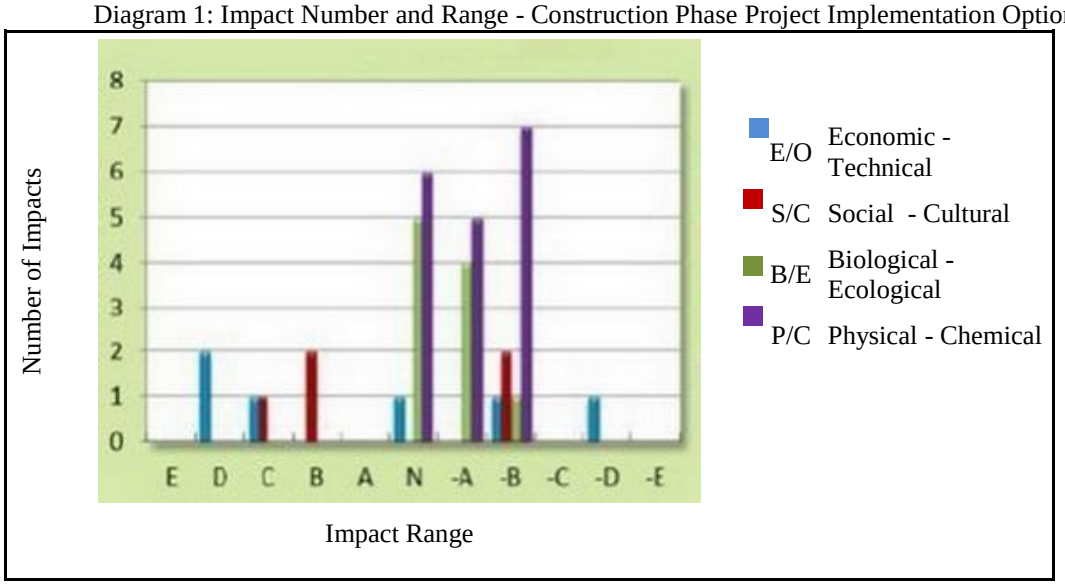
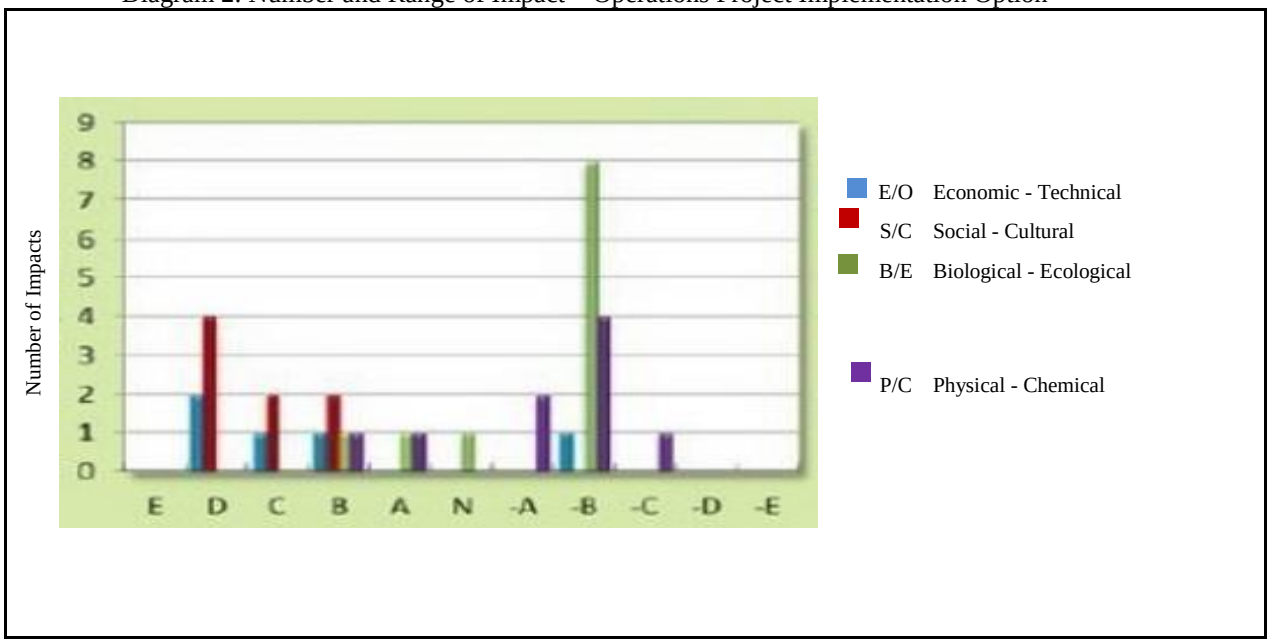


Table 22: Number and Range of Impact – Operations Project Implementation Option

Environments Impact range	Economic-technical (E/O)	Social-cultural (S/C)	Biological-ecological (B/E)	Physical-chemical (P/C)	Total score
E	0	0	0	0	0
D	2	4	0	0	6
C	1	2	0	0	3
B	1	2	1	1	5
A	0	0	1	1	2
N	0	0	1	0	1
-A	0	0	0	2	2
-B	1	0	8	4	12
-C	0	0	0	1	1
-D	0	0	0	0	0
-E	0	0	0	0	0

Diagram 2: Number and Range of Impact – Operations Project Implementation Option



Non Implementation Option

The future situation of this area is discussed in this section if project is not implemented. The following tables and diagrams show the result of environmental impact analysis for construction and operations phases in non implementation option.

Table 23: Impact on Physical-Chemical Environment (P/C) – Construction Non Implementation Option

Criteria							Activity impact on environmental parameters	Code	
A_1	A_2	B_1	B_2	B_3	ES	R			
0	0	1	1	1	0	N	Transportation impact on air quality	P/C	1
0	0	1	1	1	0	N	Transportation impact on sound quality	P/C	2
0	0	1	1	1	0	N	Transportation impact on traffic	P/C	3
0	0	1	1	1	0	N	Ground and concrete works impacts on water drainage	P/C	4
0	0	1	1	1	0	N	Ground and concrete works impacts on land form	P/C	5
0	0	1	1	1	0	N	Ground and concrete works impacts on air quality	P/C	6
0	0	1	1	1	0	N	Ground and concrete works impacts on noise production	P/C	7
0	0	1	1	1	0	N	Ground and concrete works impacts on surface water	P/C	8
0	0	1	1	1	0	N	Ground and concrete works impacts on soil characteristics	P/C	9
1	-1	3	3	1	-7	-A	Access road impact on surface water	P/C	10
0	0	1	1	1	0	N	Construction material unloading impact on noise production	P/C	11
0	0	1	1	1	0	N	Access road impact on soil characteristics	P/C	12
0	0	1	1	1	0	N	Sewage disposal impact on soil characteristics	P/C	13
0	0	1	1	1	0	N	Construction work impact on noise production	P/C	14

0	0	1	1	1	0	N	Construction work impact on air quality	P/C	15
0	0	1	1	1	0	N	Construction work impact on land form	P/C	16
0	0	1	1	1	0	N	Construction work impact on surface water	P/C	17
0	0	1	1	1	0	N	Construction work impact on soil characteristics	P/C	18

Table 24: Impact on Biological-Ecological Environment (B/E) – Construction Non Implementation Option

Criteria							Activity impact on environmental parameters	Code	
A_1	A_2	B_1	B_2	B_3	ES	R			
0	0	1	1	1	0	N	Deforestation impact on land ecosystem	B/E	1
0	0	1	1	1	0	N	Deforestation impact on water ecosystem	B/E	2
0	0	1	1	1	0	N	Ground and concrete works impacts on vegetation habitat	B/E	3
0	0	1	1	1	0	N	Ground and concrete works impacts on plant concentration	B/E	4
0	0	1	1	1	0	N	Ground and concrete works impacts on animal behavioral pattern	B/E	5
0	0	1	1	1	0	N	Ground and concrete works impacts on animal habitat	B/E	6
0	0	1	1	1	0	N	Construction work impact on land ecosystem	B/E	7
0	0	1	1	1	0	N	Construction work impact on water ecosystem	B/E	8
0	0	1	1	1	0	N	Transport impact on vegetation habitat	B/E	9
0	0	1	1	1	0	N	Transport impact on animal habitat	B/E	10

Table 25: Impact on Social-Cultural Environmental (S/C) – Construction Non Implementation Option

Criteria							Activity impact on environmental parameters	Code	
A_1	A_2	B_1	B_2	B_3	ES	R			
0	0	1	1	1	0	N	Impact of supply & transport scraps, construction materials, equipments, machineries, and personnel on local traffic	S/C	1
0	0	1	1	1	0	N	Impact of noise prolusion on local community	S/C	2
2	-1	2	2	2	-12	-B	Impact of employment and construction on people and local community participation	S/C	3
2	-1	2	2	2	-12	-B	Impact of employment and construction on local population density	S/C	4
2	-2	2	2	2	-24	-C	Impact of project hiring on local employment	S/C	5

Table 26: Impact on Economical-Technical Environment (E/O) – Construction Non Implementation Option

Criteria							Activity impact on environmental parameters	Code	
A_1	A_2	B_1	B_2	B_3	ES	R			
1	-1	2	2	2	-6	-A	Impact of transportation and equipment on economy	E/O	1
2	-2	2	2	2	-24	-C	Impact of employment on income	E/O	2
0	0	1	1	1	0	N	Costs involved in changing land zoning	E/O	3
0	0	1	1	1	0	N	Construction costs	E/O	4
2	1	2	3	2	+14	+B	Impact of demand for energy	E/O	5
3	-1	2	2	2	-18	-B	Impact of material and equipment procurement on economy	E/O	6

Table 27: Impact on Physical-Chemical Environment (P/C) – Construction Non Implementation Option

Criteria							Activity impact on environmental parameters	Code	
A_1	A_2	B_1	B_2	B_3	ES	RV			
0	0	1	1	1	0	N	Impact of operation process on air quality	P/C	1
0	0	1	1	1	0	N	Impact of operation process on sound quality	P/C	2
0	0	1	1	1	0	N	Impact of operation process on surface water quality	P/C	3
0	0	1	1	1	0	N	Impact of operation process on underground water quality	P/C	4
2	-1	2	2	2	-12	-B	Impact of green land on air and sound quality	P/C	5
0	0	1	1	1	0	N	Impact of spillage and accidents on air and sound quality	P/C	6
0	0	1	1	1	0	N	Impact of spillage and accidents on surface water	P/C	7
0	0	1	1	1	0	N	Impact of spillage and accidents on soil characteristics	P/C	8
1	-1	2	2	2	-6	-A	Impact of green land on soil characteristics	P/C	9

Table 28: Impact on Biological-Ecological Environment (B/E) – Operations Non Implementation Option

Criteria							Activity impact on environmental parameters	Code	
A_1	A_2	B_1	B_2	B_3	ES	RV			
0	0	1	1	1	0	N	Impact of operation process on land ecology	B/E	1
0	0	1	1	1	0	N	Impact of operation process on water ecology	B/E	2
1	-1	3	1	1	-5	-A	Impact of green land on land ecology	B/E	3
1	1	3	1	1	5	+A	Impact of green land on water ecology	B/E	4
1	-2	3	1	1	-10	-B	Impact of vegetation on land	B/E	5
0	0	1	1	1	0	N	Impact of operation process on vegetation habitat	B/E	6
0	0	1	1	1	0	N	Impact of operation process on animals	B/E	7
0	0	1	1	1	0	N	Impact of spillage and accidents on vegetations	B/E	8
0	0	1	1	1	0	N	Impact of spillage and accidents on animals	B/E	9
0	0	1	1	1	0	N	Impact of spillage and accidents on water ecosystem	B/E	10
0	0	1	1	1	0	N	Impact of spillage and accidents on land ecosystem	B/E	11

Table 29: Impact on Economic-technical Environment (E/O) – Operations Non Implementation Option

Criteria							Activity impact on environmental parameters	Code	
A_1	A_2	B_1	B_2	B_3	ES	R			
2	-2	2	2	1	-20	-C	Impact on employment	E/O	1
2	-1	2	2	1	-10	-B	Impact on property and land prices	E/O	2
0	0	1	1	1	0	N	Impact on community energy consumption	E/O	3
2	-1	2	2	1	-10	-B	Impact on different economic-technical activities	E/O	4
2	-1	2	2	1	-10	-B	Impact on shopping centers on local economy	E/O	5

Table 30: Total Number and Range of Impacts – Construction Non Implementation Option

Environments Impact range	Economic-technical (E/O)	Social-cultural (S/C)	Biological-ecological (B/E)	Physical-chemical (P/C)	Total score
E	0	0	0	0	0
D	0	0	0	0	0
C	0	0	0	0	0
B	1	0	0	0	1
A	0	0	0	0	0
N	2	2	17	10	31
-A	1	0	0	1	2
-B	1	2	0	0	3
-C	1	1	0	0	2
-D	0	0	0	0	0
-E	0	0	0	0	0

Diagram 1: Total Number and Range of Impacts - Construction Non Implementation Option

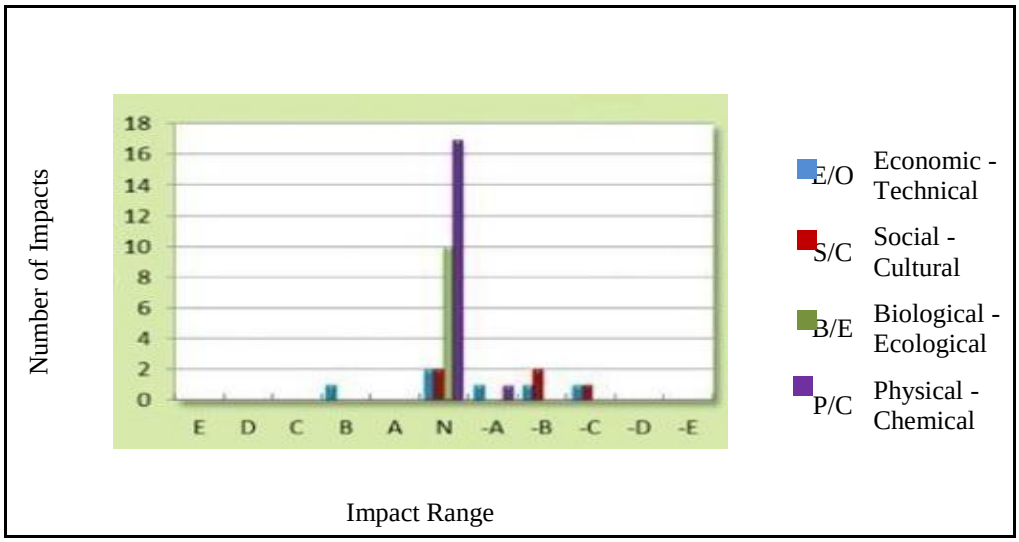
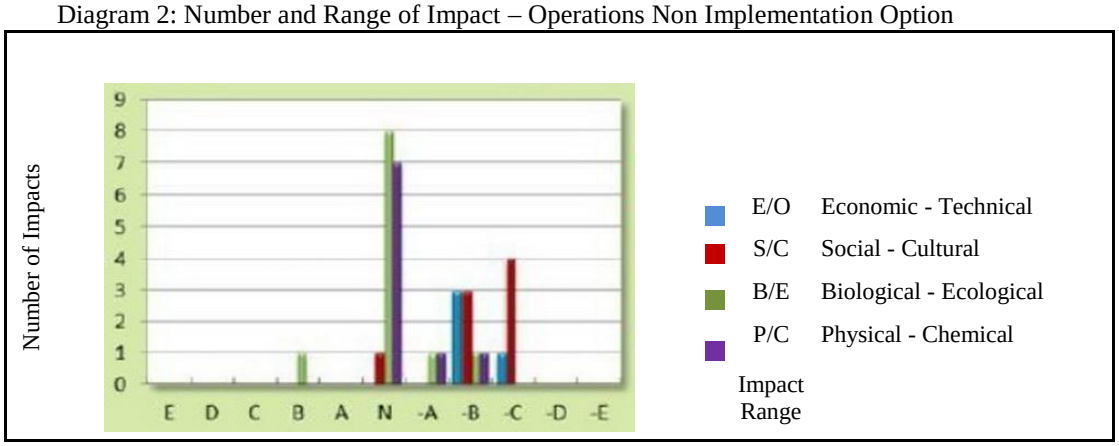


Table 31: Total Number and Range of Impacts – Operations Non Implementation Option

Environments Impact range	Economic-technical (E/O)	Social-cultural (S/C)	Biological-ecological (B/E)	Physical-chemical (P/C)	Total score
E	0	0	0	0	0
D	0	0	0	0	0
C	0	0	0	0	0
B	0	0	1	0	1
A	0	0	0	0	0
N	0	1	8	7	16
-A	0	0	1	1	2

-B	3	3	1	1	8
-C	1	4	0	0	5
-D	0	0	0	0	0
-E	0	0	0	0	0

Diagram 2: Number and Range of Impact – Operations Non Implementation Option



Conclusion and Final Selection

The comparison between the number and range of impacts from construction and operations activities for implementation and non-implementation options shows that:

1- Implementation option has 9 minuscule negative impacts, 11 ordinary negative impacts, and 1 specific negative impact during construction.
2- Non implementation option has 2 minuscule negative impacts, 3 ordinary negative impacts, and 2 medium negative impacts on region.
3- Implementation option has 2 minuscule positive impacts, 2 ordinary positive impacts, and 2 specific positive impacts during construction if environment protection provisions and regulations are observed.
4- Non implementation option has only one minuscule positive impact.
5- Non implementation option has 1 minuscule positive impact during operation on region.
6- Implementation option has 2 minuscule positive impacts, 5 ordinary positive impacts, and 3 medium positive impacts, and 6 specific positive impacts during operations on region.
7- Implementation option has 2 minuscule negative impacts, 13 ordinary negative impacts, and 1 medium negative impact during operations.
8- Non implementation option has 2 minuscule negative impacts, 8 ordinary negative impacts, and 5 medium negative impacts.

The total sum of positive and negative impacts shows that positive impacts from implementation option are higher than the negative impacts. Because this project is considered important as national and regional project, therefore, its implementation is prepared. The evaluation team recommended the implementation of the project after making specific changes in the plan and provisions for management and monitoring systems.

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