THE EXPRESSION OF INTERLUKIN 2(IL- 2), INTERLUKIN 8(IL-8) AND INTERLUKIN 6(IL-6) IN PATIENTS WITH ORAL AND OROPHARYNGEAL SQUAMOUS CELL CARCINOMA IN BASRAH CITY (A CASE CONTROL STUDY)

Maha M. Al-mahfoud[1], Ihsan E. AlSaimary[1] and Ali. A. Al shawi[2]

[1]Department of Microbiology/Collage of Medicine/ University of Basrah / Basrah.
[2]Collage of Dentistry / University of Basrah / Basrah.

ABSTRACT: *The first part of the digestive tract is the oral mucosa, which exposed to different exogenous toxins ,long period of exposure could lead to malignant changes /tumors. One of the prevalent cancers of the body is Oral squamous cell carcinoma, oral cancer sometimes may be resemble benign lesions in the mouth therefor, the diagnosis may be not easy clinically. There appear the importance of serum cytokines in distinguishing different pathologies. Aims :The aim of this study was to identify the role of serum Interlukins (IL2 ,IL6, IL8) in the early detection and pathogenesis of oral and oropharyngeal squamous cell carcinoma. Patients and Methods: This study included 20 patients with oral and oropharyngeal squamous cell carcinoma were clinically diagnosed and then confirmed by histopathological examination and 26 healthy control . Age, gender, as well as the level of interlukins in the serum of patients and healthy control were measured,in addition clinical signs and the site of lesions were recorded for patients group. Results: There were 20 new cases of oral   and oropharyngeal squamous cell carcinoma in Basrah from September 2015 to December 2016 , 12 in men and 8 in women . 26 healthy control individuals 17 men and 9 female were included in this study . Cancer at all oral sites affected men more than women. The Tongue is the most frequent site .The level of serum Interlukins (IL2, IL6, IL8) in patients group were much more than those in healthy control group.*

KEYWORDS: SCC, Oral, Oropharyngeal Squamous Cell Carcinoma, Inflammation, Interlukins

INTRODUCTION

In general, the term oral cancer refers to cancers of the oral cavity and the oropharynx. there are 220 000 new cases per year in men (5% of all cancers and 90 000 in women (2% of all cancers) recorded globally. (1)

Therefore, Oral malignancy considers as a large public health problems and one of the few life debilitating oral illness worldwide. It is considered as a highlyfatal and deforming disease (2).

More than 90% of tumors in the head and neck are squamous cell carcinoma (3).,therefore it is the commonest malignant tumor of oral mucosa .In the UK and USA Oral cancer, represents about 2% of all malignant tumors, while in India and Srilanka it's about 40% or more Females have a lower incidence than males at all age levels (4). The most important risk factors for OSCC are tobacco and alcohol uptake(5) more than 90% happened in patients over 40 years, for that reason, the incidence of oral cancer is a linear increase with age(6).

Age and sex variables have important prognostic factors (7), as well as Environmental carcinogens for example chemicals, radiation and viruses are critical enhancing factors in the advancement of oral cancer (6).

The role of inflammation in the development of cancer was described as early as 1863, by Rudolf Virchow. His observations that inflammatory cells infiltrate tumors led him to hypothesize that cancer arises from inflammatory sites ("lymphoreticular infiltration") (8,9). In the last decades, Virchow's postulation has been supported by abundant evidence that various cancers are triggered by infection and chronic inflammatory disease (10)

Cytokines are low-molecular-weight proteins that mediate cell-to-cell communication. Immune and stromal cells, such as fibroblasts and endothelial cells, synthesize them and they regulate proliferation, cell survival, differentiation, immune cell activation, cell migration, and death. Depending on the tumor . Microenvironment, cytokines can modulate an antitumoral response, but during chronic inflammation, they can also induce cell transformation and malignancy, conditional on the balance of pro- and anti-inflammatory cytokines, their relative concentrations, cytokine receptor expression content, and the activation state of surrounding cells (11)

Objectives:

In Iraq, oral cancer represents4.5% of all malignant cases, as recorded by Iraqi cancer registry (12)

The aim of this study was to:

1- Identify differences in oral and oropharyngeal squamous cell carcinoma patients and healthy control group according to gender, age groups and site of the tumor in Basrah

2- Estimate the difference in interlukins (IL2,IL6,IL8) levels between patients and healthy control groups and the role of these interlukins in the diagnosis ,development and pathogenesis of oral and oropharyngeal squamous cell carcinoma

Patients and methods

This is a case control study involved twenty blood samples were taken from patients with Head and neck squamous cell carcinoma , All patients were examined before receiving anti-cancer therapy and diagnosed by specialist physician, the samples were collected from maxillofacial and ENT unit in Basrah general Hospital , oncology department in Al-Sadder Hospital during the period from September 2015 to December 2016

The clinical features recorded as well as age ,gender, site of the lesion history of the illness . The clinical diagnosis of oral squamous cell carcinoma then confirmed by histopathological examination through staining with Hematoxyline and Eosin stain.

Blood samples were collected from 26 healthy control subjects only if they were not receiving any medications, did not have a history of any chronic or acute illnesses, and gave no smoking

Sample collection

Blood samples

Five ml of Blood specimen were collected from all patients and control group in a sterile tubes , and left to stand for an hour at room temperature then centrifuged at 1500 rpm for 10 minutes and the serum obtained was divided into several parts and kept at -20 C until needed to examination by Eliza , caution was taken to avoid repetitive freezing and thawing of the serum samples

The interlukins levels in the serum were measured by using the following Elisa kits:

 1-Human interlukin(6) Elisa kit, Mybiosource – (USA) Cat.No: MBS021993

2- Human interlukin(8) Elisa kit, Mybiosource – (USA) Cat.No: MBS013419

3- Human interlukin(2) Elisa kit, Mybiosource – (USA) Cat.No: MBS033567

Picture (1) Female patient with irregular ulcer on the left maxilla , size 4*3
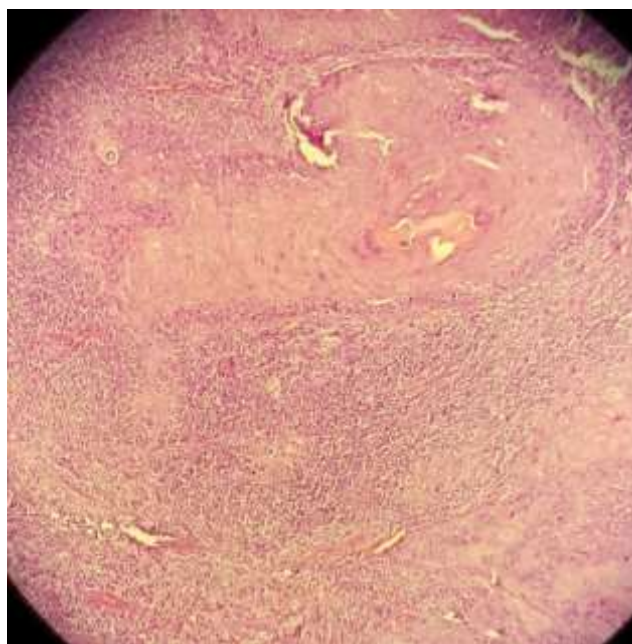
Picture (2) Oral squamous cell carcinoma (OSCC) of the lateral edge of the tongue, (stain, H&E; magnification, x50

RESULTS

The mean age range of twenty patients with oral and oropharyngeal squamous cell carcinoma were included in this study was (3.1 ± 0.91) year, with an age range was between 15 to 80 years, While the mean age for 26 individuals in healthy control group was (2.92 ± 0.935) with an age range was between 28 to 75 years. There is no statistical significant difference in age group between patients and healthy control groups. Table(1)

The results in figure (1) showed a significant difference $(p< 0.05)$ in socioeconomic status between patients (2.40 ± 0.75) and healthy control(1.88 ± 0.59)

The percentage of OSCC cases was reported in the center of Basrah (45%) , from other cities Thi-qar and Missan) was 30% ,and finally it was 25% from rural area .The association between oral and oropharyngeal squamous cell carcinoma and residency was not significant. $p>0.05$.Figure (2)

Regarding the tumor site involved by OSCC, the most common site encountered in this study was the tongue comprise (5 cases) 25%, followed oropharynx (4 cases) 20% , palat (3 cases) 15% ,then cheek and maxilla (2 cases) for each 10% , and finally post nasal space, supra glottic and jaw (one case) for each 5% , as shown in Table (2)

According to comparison between patients and healthy control groups in serum level of interlukins (IL2 ,IL6,IL8) ,statistically there is a highlly significant difference between the level of these serum interlukins between the two groups $(p>0.01)$. Table(3)

DISCUSSION

In this study, twenty oral and oropharyngeal squamous cell carcinoma surgical specimens were evaluated and analyzed, therefore the incidence and prevalence could not be expressed due to small sample size, yet there was a correlation between present and previous data regarding the incidence of OSCC in Iraq.

As shown in table (1) there is no significant difference in age group between patients and healthy control group, although among patients group there was an increase in the number of patients in the age group less than 60 years in comparison with those over 60 years of old. These results was in agreement with other recent studies which found that there is increasing incidence of OSCC in young patients in the recent years (13,14)

In the other hand, results of other studies, have not found any significant differences in outcomes between different age groups (15,16,17)

These results may be due to a genetic predisposition for the cancer development at a young age, especially in those patients with no known risk factors .in addition , an increase in chromosome fragility after exposure to mutagen , significantly increased in young patients than older individuals ,this weakness in chromosom may lead to genetic anomalies which linked with alterations in DNA repair genes. (18)

Other factor that may associated with increased incidence of tumor among young people was diet , as estimated by some researchers ,who found that diet low in fruits and vegetables and fish oils are generally correlated with a risk of oral cancer (19)

Oral carcinoma occurs less frequently in women than in men this may be due to increase exposure to risk factor by men(20) .in addition to other intrinsic factors like malnutrition or iron deficiency anemia that may present in males more than in females(21,22).Poor oral hygiene which consider as having a identical modifying effect may explain increase the incidence in male than in females patients (23,24)

Although the result was not significant , but the most frequent site of OSCC lesion was on the tongue both in male and female patients ,this result in agreement with other Iraqi study in 2013 (25),and with studies in European and the US populations, but the most common site among Asian populations is bucal mucosa which is attributed to betel quid/tobacco chewing habits.(26)

The percentage of OSCC cases was reported in the center of Basrah (45%) more than in rural area (25%), this result can be attributed to the presence of many hospitals and the location of the oncology center in Basrah which made the referral cases reach the institute easier

Most patients are with low socioeconomic status ,this may be due to involvement of these patients in habits that are less healthy, like tobacco and alcohol consumption, in addition eating fewer fruits and vegetables, than those of higher socioeconomic status.

Yet, studies on the correlation between socioeconomic factors and   the incidence of OSCC are disagreeing. (27)

The results showed a highly significant difference in serum interlukins level (IL2 , IL6, IL8) between patients and healthy control groups. This results were in agreement of other studies that found an increase level of (IL2 ,IL6,IL8) in patients with HNSCC (28 ,29,30, 31)

A study by Biancotto et al estimated that the   serum level of IL8   correlate negatively with favourable outcome in patient with OSCC ,after the examination of different cytokines including IL8 in the sera derived from those patients by using a multiplexed measurement system(32)

Other investigators showed   that IL-6 is detected at higher concentrations in the serum of patients with head and neck squamous cell carcinoma   when compared with age-matched control individuals (33)

These results indicate   the importans of these markers as a prognostic   signals   for patients with HNSCC,   , tumor proliferation , metastasis, and finally the therapeutic potential of targeting their pathway .

Increase level of IL2 and IL6 suggest the activation of the Th2 arm of the immune response in HNSCC patients (34). In addition a higher level of IL6 indicate higher tumor stage and positive lymph nodes (35)

In the other hand ,high level of IL8   suggest poor clinical outcomes of patients with OSCC through activation and generation of M2 macrophage (36)

Table (1) Comparison of   different age groups between   patients and   healthy control . Chi –square = 0.244   , p>0.05

		Patients	%	control	%
age	15-29	1	5	2	7.7
	30-44	4	20	6	23.1
	45-59	7	35	10	38.5

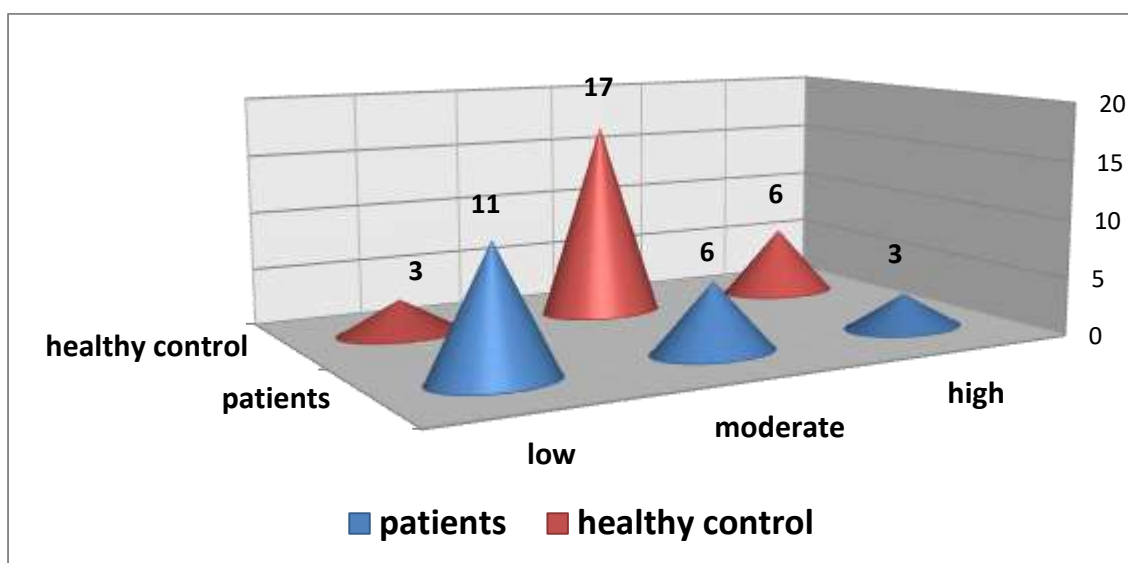| over 60	8	40	8	30.7
Total	20	100	100	100

Fig (1) : Comparision between patients and healthy control group regardind socioeconomic status . Chi-square =10.224 ,p<0.05
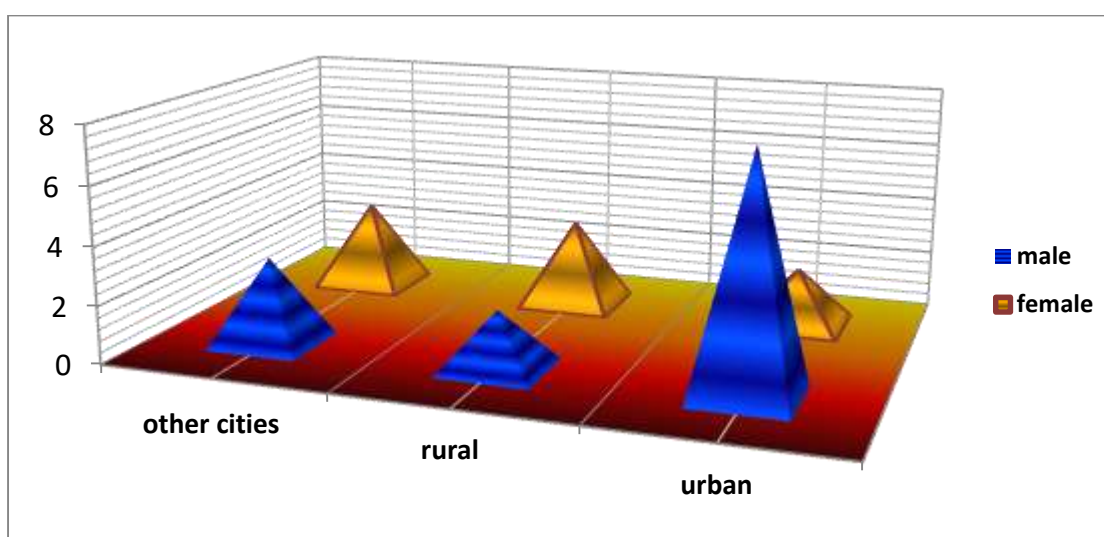

Fig. (2) Distribution of patients according to residency. Other cities (Thi-qar and Missan) . Chi- square =2.29 p>0.05

Table(2) Distribution of patients based on the site of the lesion and gender. Chi-square =1.81 df= 7 ,p>0.05

Sites of tumor	Male		Female		Total	
	No	%	No	%	No	%
palat	2	10	1	5	3	15
Tongue	3	15	2	10	5	25
cheek	1	5	1	5	2	10
oropharynx	2	10	2	5	4	20
maxilla	1	5	1	5	2	10
Post nasal space	1	5	0	0	1	5
Supra-glottic	1	5	1	5	2	10
Jaw	1	5	0	0	1	5
Total	12	60	8	40	20	100

Table(3) Comparision of interlukins level (IL2 ,IL6,IL8) between patients and healthy control groups . p< 0.01

	tumor	N	Mean pg/ml	T value	Sig(2 tailed)
IL2 serum	patients	20	204.3	2.539	0.015
	control	26	132.1		
IL6 serum	patients	20	248.7	10.670	0.00
	control	26	52.2		
IL8 serum	patients	20	168.1	2.978	0.005
	control	26	107.5		

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