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)

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Abstract. The first part of the digestive tract is the oral mucosa, which is 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 wherefore, 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

1. 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. Therefore, Oral malignancy considers as a large public health problem and one of the few life debilitating oral illness worldwide. It is considered as a highly fatal and deforming disease. More than 90% of tumors in the head and neck are squamous cell carcinoma, 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 Sri Lanka it's about 40% or more. Females have a lower incidence than males at all age levels. 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).

2. Objectives
In Iraq, oral cancer represents 4.5% 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 interleukins (IL2, IL6, IL8) levels between patients and healthy control groups and the role of these interleukins in the diagnosis, development and pathogenesis of oral and oropharyngeal squamous cell carcinoma.

3. 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.

4. 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 interleukins levels in the serum were measured by using the following Elisa kits:
1-Human interleukin(6) Elisa kit, Mybiosource – (USA) Cat.No: MBS021993
2- Human interleukin(8) Elisa kit, Mybiosource – (USA) Cat.No: MBS013419
3-Human interleukin(2) Elisa kit, Mybiosource – (USA) Cat.No: MBS033567
5. 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 highly significant difference between the level of these serum interlukins between the two groups (p>0.01). Table(3)

6. 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 chromosome may lead to genetic anomalies which linked with alterations in DNA repair genes. (18) Other factors that may be 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 increased exposure to risk factors 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 is 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 buccal 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 importance 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 |
Figure (1) : Comparision between patients and healthy control group regarding socioeconomic status. Chi-square =10.224 , p<0.05

Figure (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 No | %  | Female No | %  | Total No | %  |
|---------------|--------|----|-----------|----|----------|----|
| palat         | 2      | 10 | 5         | 3  | 15       | 3  |
| Tongue        | 15     | 3  | 10        | 1  | 25       | 5  |
| cheek         | 5      | 1  | 5         | 1  | 10       | 2  |
| oropharynx    | 10     | 2  | 5         | 2  | 20       | 4  |
| maxilla       | 5      | 1  | 5         | 1  | 10       | 2  |
Table (3) Comparison of interleukins level (IL2, IL6, IL8) between patients and healthy control groups. p< 0.01

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

7. References

[1] Petersen PE. Strengthening the prevention of oral cancer: the 10
[2] Federation DentaireInternationale– FAD statement (1999) , Oral cancer. FDA World, 8:24.
[3] Sanderson, R.J. and Ironside, J.A.D.(2002) squamous cell carcinoma of the head and neck, clinical review.B.M.J., 325:822-827
[4] Zakrzewska, JM.(1999): Oral cancer:Fortnightly review. BMJ,318:1051-1054.
[5] Blot WJ, McLaughlin JK, Winn DM, et al:Smoking and drinking in relation to oral and pharyngeal cancer. Cancer Res 48:3282-3287, 1988
[6] Soames, JV. And Southam, JC.(1998):Oral epithelial tumours. In: Oral pathology,3rd Ed., Oxford University press, pp: 159 - 179 .
[7] Beehken, SW.; Kronkiras, H.; Moddox, WA.; Peters, GE.; Soong, and Urist, M.(1999): T1 and T2 squamous cell carcinoma of oral and tongue. Prognostic factors and the role of elective lymph node dissection. Head and Neck, 21: 124 – 130
[8] R. Virchow, Die Krankhaften Geschw¨ulste, Berlin, Germany,1863.
[9] F. Balkwill and A. Mantovani, “Inflammation and cancer: back to Virchow?” The Lancet, vol. 357, no. 9255, pp. 539–545, 2001.
[10] S. P. Hussain and C. C. Harris, “Inflammation and cancer: an ancient link with novel potentials,” International Journal of Cancer, vol. 121, no. 11, pp. 2373–2380, 2007
[11] B. F. Zamaron and W. Chen, “Dual roles of immune cells and their factors in cancer development and progression,”International Journal of Biological Sciences, vol. 7, no. 5, pp. 651–658, 2011.
[12] Talabani,N Gh and Al- Rawi, NH. (2002): Squamous cell carcinoma of oral cavity: A clinico- pathological analysis of 1425 cases from Iraq. Al- Rafidan Dent. J, 1:1-6.
[13] V T Beena, S S Binisree, T Ayswarya, Ismayil Pakkadan, S K Padmakumar, R Sivakumar. Oral Squamous Cell Carcinoma in Patients Younger than 40 Years: A 10 Year Retrospective Study International Journal of Scientific Study , vol.4, no.4 ,pp 150-153,2016
[14] Hellen-Bandeira-de-Pontes Santos 1, Thayana-Karla-Guerra dos Santos 2, Alexandre-Rolim Paz 3, Yuri-Wanderley Cavalcanti 4, Cassiano-Francisco-Weege Nonaka 5, Gustavo-Pina Godoy 5, Pollianna-Muniz Alves: . Clinical findings and risk factors to oral squamous cell carcinoma in young patients: A 12-year retrospective analysis , Med Oral Patol Oral Cir Bucal. Vol. 1, no.21 ,pp 151-156,2016
[15] Gilroy JS, Morris CG, Amdur RJ, Mendenhall WM. Impact of young age on prognosis for head and neck cancer: a matched-pair analysis. *Head Neck* 2005; 27: 269-73.

[16] Goldenberg D, Brooksbay C, Hollenbeak CS. Age as a determinant of outcomes for patients with oral cancer. *Oncol 2009; 45: e57-61.

[17] Hafkamp HC, Manni JJ, Speel EJ. Role of human papillomavirus in the development of head and neck squamous cell carcinomas. *Acta Otolaryngol* 2004; 124: 520-6.

[18] Kostrzewska-Poczekaj M, Gąwcki W, Illmer J, Rydzanicz M, Gajecka M, Szyfter W, et al. Polymorphisms of DNA repair genes and risk of squamous cell carcinoma of the head and neck in young adults. *Eur Arch Otorhinolaryngol* 2013; 270: 271-6.

[19] Llewellyn CD, Linklater K, Bell J, Johnson NW, Warnakulasuriya KA. Squamous cell carcinoma of the oral cavity in patients aged 45 years and under: a descriptive analysis of 116 cases diagnosed in the South East of England from 1990 to 1997. *Oncol 2003; 39: 106-14.

[20] Warnakulasuriya S. Global epidemiology of oral and oropharyngeal cancer. *Oncol 2009; 45: 309-16.

[21] Jacops, C. D. (1990) Etiologic consideration for head and neck squamous cancer. In: *Carcinoma of the head and neck*. Jacobs, C. ed. Kluwer Academic. 265 -282.

[22] Winn, D.M. (1995) Diet and nutrition in the etiology of oral cancer. *A.M.J. Clin. Nutr.* 61:4375 - 4455.

[23] Sapp, J.P.; Eversole, L. R.; ysocki, G.(1997). Epithelial disorders. In: *Contemporary oral and maxillofacial pathology*. Mosby. NewYork. 156 – 95

[24] Rugezi, J.A. ; Sciubba, J.J. and Jordan, R.C.K. (2003), *Oral pathology, clinical pathologic correlations*. 4th ed.Elsevier Science , USA. 52-74.

[25] Omar. m and Wasan. Y Oral cancer trends in Iraq from 2000 to 2008. The Saudi Journal for Dental Research; (2014) 5, 41–47

[26] Warnakulasuriya S. Global epidemiology of oral and oropharyngeal cancer. *Oncol 2009;45:309–16. Uehara M, Sano K, Ikeda H, Sekine J, Irie A, Yokota T, Tobita T, Ohba S and Inkuchi T. Expression of vascular endothelial growth factor and prognosis of oral squamous cell carcinoma. Oral Oncology. (2004); 40(3), 321-325

[27] Hashibe M, Jacob BJ, Thomas G, et al. Socio-economic status, lifestyle factors and oral premalignant lesions. *Oncol. 2003;39:664-671

[28] Rucha Bapat, Supriya Kheur1, Mohit Kheur, Tanya Sethi, and Archana A Gupta. RJPBCS 2016 ;7(2) :: 436-442

[29] Jinno T, Kawano S, Maruse Y, et al. Increased expression of interleukin-6 predicts poor response to chemoradiotherapy and unfavorable prognosis in oral squamous cell carcinoma. Oncol Rep. 2015;33:2161e2168

[30] Nazar G. Al Talabani ,Shanaz M. Gaphor ,Abdul-Wahab R. Hamad . Serum and salivary levels of proinflammatory cytokines as potential biomarkers in the diagnosis of oral .squamous cell Carcinoma. J Bagh College Dentistry. 2009; Vol. 21(1):60-65

[31] Thomas K. Hoffmann, Eniko Sonkoly, Bernhard Homey, Katrin Scheckenbach, Christian Gwosdz, I Murat Bas, Adam Chaker, Kerstin Schirau, Theresa L. Whiteside. Aberrant cytokine expression in serum of patients with adenosid cystic carcinoma and squamous cell carcinoma of the head and neck.2007 HEAD & NECK—DOI 10.1002/hed :427-478

[32] Biancotto A, Feng X, Langweiler M, Young NS, Philip McCoy J (2012) Effect of anticoagulants on multiplexed measurement of cytokine/chemokines in healthy subjects. Cytokine 60: 438–446

[33] Chen Z,Malhotra PS, Thomas GR, et al. Expression of proinflammatory and proangiogenic cytokines in patients with head and neck cancer. *Clin Cancer Res. 1999;5:1369-1379

[34] Lathers DM, Achille NJ, Young MR. Incomplete Th2 skewing of cytokines in plasma of patients with squamous cell carcinoma of the head and neck. Hum Immunol. 2003:64:1160e1166
[35] Tartour E, Deneux L, Mosseri V, et al. Soluble interleukin-2receptor serum level as a predictor of locoregional control and survival for patients with head and neck carcinoma: results of a multivariate prospective study. Cancer. 1997;79: 1401-1408

[36] Yohei Fujita, Masato Okamoto, Hiroyuki Goda, Tomoyuki Tano, Koh-ichi Nakashiro, Atsuro Sugita, Tomonobu Fujita, Shigeo Koido, Sadamu Homma, Yutaka Kawakami, Hiroyuki Hamakawa. Prognostic significance of Interlukin-8 and CD163-positive cell infiltration in tumor tissues in patients with oral squamous cell carcinoma. Plos one journal. 2014:1-17