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The role of salivary Interleukin 17 as a dependent positive predictive biomarker among Iraqi patients with oral squamous cell carcinoma

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Abstract

Background: Head and neck cancer is the sixth most common cancer in human. Over 90% of malignant neoplasms of the head and neck are diagnosed as squamous cell carcinomas of oral cavity. In addition, its diagnosis is often late due to unavailability of predictive reliable measurable biomarkers. Interleukin 17 is one of pro-inflammatory cytokines manufactured by T-helper cells. It usually binds to type I cell-surface receptor named interleukin. The purpose of the current study was to explore the predictive role of salivary interleukin 17 in the diagnosis of oral squamous cell carcinoma in comparison with healthy individuals.

Method: Patients with histopathologically-confirmed oral squamous cell carcinoma (25 patients) and 25 age- and gender-matched normal healthy subjects (controls) were involved in this study. Unstimulated saliva was collected from each individual (for oral squamous cell carcinoma patients, before and 10 days after surgical treatment). Salivary interleukin-17 levels were evaluated for both groups using Enzyme-Linked Immuno-Sorbent Assay (ELISA) technique.

Results: Mean valueS of IL-17 levels were significantly increased in oral squamous cell carcinoma patients as compared to controls and also were higher in oral squamous cell carcinoma patients before surgical removal of tumor than their levels, for the same patients, ten days after surgical removal of tumor.

Conclusion: Interleukin-17 has paticular advantage to be used as a positive predictive factor for prognosis and diagnosis of OSCC.

Key words: Oral squamous cell carcinoma, Interleukin-17, ELISA, Saliva, Cytokines.

INTRODUCTION

Oral squamous cell carcinoma (OSCC) is well-defined as a malignant neoplasm of the oral cavity which develops from uncontrolled division of squamous epithelial cells [1]. It is considered the most common cancer that affects humans worldwide. It has an incidence of 95%-96% of the other oral cancers, worldwide, with increasing incidence and prevalence of 50% [2,3&4]. Its incidence varies in different areas of the world and this difference is mostly attributed to exposure to risk factors specific to the geographical area [5]. It is frequently reported in the fifth to seventh decade of life with male preponderance.

In Iraq, one of the studies reported that in Baghdad, the capital of Iraq, people within the age group 51-60 years old were highly effected [6,7]. Saliva is one of the most important, complex and unique aqueous fluid institutes in the oral cavity.

At present time, a great deal of focus from researchers is on the use of saliva as an alternative sample for the diagnosis, prediction and progression of several diseases [8]. Biomarkers can be demarcated as a quantifiable and calculable biological parameters which can serve as indicators for health and physiology-related assessments such as pathological processes, environmental exposure, diagnosis and prognosis of disease or pharmacologic responses to a healing intervention [9].

Interleukin 17 (IL-17) is one of pro-inflammatory cytokines manufactured by T-helper cells [10]. After year 2000, the efforts of researchers were focused on understanding of the IL-17 family members and their corresponding receptors [11]. Six IL-17 family ligands [IL-17A, IL-17B, IL-17C, IL-17D, IL-17E (IL-25) and IL-17F] and five receptors (IL-17RA, IL-17RB/IL-25R, IL-17RC, IL-17RD/SEF and IL-17RE) were studied. Firstly, IL-17 was believed to be produced absolutely by T- cells [12], but recently it was found that it is secreted by a variety of innate cells including macrophages, dendritic cells (DC), natural killer, natural killer T and lymphoid tissue inducer [13]. A major development in this field occurred with the recognition that IL-17-producing $CD4^+$ T cells arise as a distinctive population from the classic T helper type 1 (Th1) and Th2 cells [14, 15]. Interleukin-17 is considered as a more potent mediator in delayed-type reactions as it can increase chemokines production in different tissues. At the site of inflammation, signaling from IL-17 results in recruitment of monocytes and [16]. Interlukine-17 is one of most important cytokines for protective immunity against extracellular pathogens in addition to clearance of intracellular pathogens [17]. Also plays a critical role in the pathogenesis of various autoimmune inflammatory diseases [18].

The pro-tumor and antitumor paradox of Interleukin -17 function

Interleukin -17 in the tumor microenvironment has many functions which may contribute to tumor progression. The main pro-tumor role of interleukin -17 is inflammation-related to tumor depending on its pro-angiogenic property of surrounding endothelial cells and fibroblasts. Also, Il-17 has a direct effect on multiplication and survival of cancer cells [19]. In the inflammatory process, IL-17 activity may be associated with destruction of tissues through matrix metalloproteinases (MMPs). It is important to know that IL-17 has been recognized to facilitate the spread of tumors either through MMPs activity or inducing angiogenesis of tumor. On the other hand, a number of reports have revealed tumor-inhibitory and tumor regression properties of interleukin -17 through T cell and IFN-Y activity by acting on tumor and/or stromal cells [20].There are Different mechanisms that link between interleukin -17 and the development of tumor-specific cytotoxic T lymphocytes. For example, IL-17 has been revealed to encourage the release of IL-6 from a variety of cells. Moreover, its stimulation can induce IL-12 production from macrophages [21]. IL-6 and IL-12, together, have been associated with the induction of tumor-specific cytotoxic T lymphocyte (CTL). Even though, it has been revealed that IL-17can stimulate tumor growth by inducing angiogenesis. As a result, IL-17-induced angiogenesis may also encourage antitumor immunity by creating a route for immune cells to reach and attack the inner mass of a solid tumor [20].

Therefore, the aim of current study was to evaluate the levels and diagnostic validity of cytokines in saliva of patients with oral squamous cell carcinoma in comparison with apparently healthy subjects. In addition, to investigate the changes in levels of these cytokines, measured before and after surgical intervention, to

draw a conclusion regarding the potential advantage of using saliva in monitoring patients' responses to treatment.

MATERIALS AND METHODS

The present study was conducted at department of maxillofacial surgery in different hospitals in Baghdad, Iraq. The study samples were obtained from 25 patients who had histopathologically-established untreated oral squamous cell carcinoma of both sexes. Their age ranged from 41 to 77 years. The patients were free from any other oral or systemic illness. Salivary samples have been taken from oral squamous cell carcinoma patients two days before surgical removal of tumor and ten days after removal of tumor surgically. The control group included twenty five apparently healthy subjects who were age- and gender-matched to patients in the study group. Unstimulated saliva was collected in the morning between 8 am. and 11 am [9]. Salivary samples were centrifuged at 4000 rpm for 10 minutes then 100 µl of supernatant was withdrawn and stored in sterile eppendorf tubes at (-20 ^{0}C) for analysis of IL-17.

Interleukin 17 production was measured using ELISA kits (IL-17 ELISA Kit) Shanghai Yehua Biological Technology/ China.

Data from current study were analysed using computer-based software, the Statistical Package for Social Sciences (SPSS) version 20.-0. Demographic variables of participants were presented as mean and standard deviation (SD) using simple descriptive statistics. The concentrations of studied salivary cytokines were presented as mean and standard error of mean (SEM). Unpaired *t*-test was used to compare the levels of salivary cytokines in patients and controls. Pearson's correlation coefficient was calculated to evaluate the correlation between two studied cytokines. P value < 0.05 was considered s statistically significant with a confidence interval of 95%.

RESULTS

Results of current study revealed that out of the 25 oral squamous cell carcinoma patients 17 (68.0%) were males and 8 (32.0%) were females with a mean age of 57.9 ± 10.4 years. There were no significant differences in age and gender distribution of patients and control subjects of current study (Table 1).

The results indicated that salivary levels of IL-17 were significantly higher in patients with OSCC (206.45 ± 47.76pg/ml) in comparison with their healty controls (59.17 ± 13.34 pg/ml) (P<0.001; Table 2 and Figure 1).

In addition, current study revealed that, in OSCC patients, the elevated salivary levels of IL-7 before surgical operation (206.45 ± 47.76 pg/ml) significantly decreased (125.40 ± 33.69 pg/ml) ten days of surgical removal of tumor (P<0.001; Table 3 and Figure 1).

Moreover, data of current study showed that salivary IL-17 have excellent ability to discriminate between OSCC and healthy controls with 100% SN, 100% SP, 100% accuracy, 100% PPV and 100% NPV. The optimal cut point for IL-17 to discriminate OSCC from control was serum level above 88.115pg/ml (Table 4 and Figure 2).

Table 1 Age and gender distributions of study participants

	Control n= 25	**OSCC patients n= 25**	**P value**
Age/years	56.7 ± 10.4	57.9 ± 10.4	0.685 [a]
40 – 49	4 (16%)	4 (16%)	
50 – 59	8 (32%)	9 (36%)	
60 – 69	8 (32%)	6 (24%)	
70 – 79	5 (20%)	6 (24%)	
[a] Independent –t- test			
Gender			0.556 [b]
Female	10 (40.0%)	8 (32.0%)	
Male	15 (60.0%)	17 (68.0%)	
[b] chi square test			

Table 2 Salivary levels of IL-17 in OSC patients and their normal controls

Variable	**C OSCC patients n= 25**	**ControlsControl n= 25**	**PP ** value**
Salivary level of IL-17 Mean±SD*(pg/ml)	206.45±47.76	59.17±13.34	<0.001

*Standard Deviation. ** For unpaired *t*-test.

Table 3 Salivary levels of IL-17 in patients with OSCC (n= 25) before surgery and 10 day post-operatively

Variable	**Before surgery**	**10 dTen days after surgeryays post-op**	**valueP****
Salivary level of IL-17 Mean±SD* (pg/ml)	206.45±47.76	125.40±33.69	<0.001

Standard Deviation. ** For paired *t*-test.

Table 4 Validity of serum level of IL-17 as a predictive biomarker for OSCC

Markers	**AUC**	**Cut point**	**SN**	**SP**	**Accuracy**	**PPV**	**NPV**
IL-17	1.0	>88.115	100%	100%	100%	100%	100%
SN: sensitivity, SP: specificity, PPV: positive predictive value, NPV: negative predictive value							

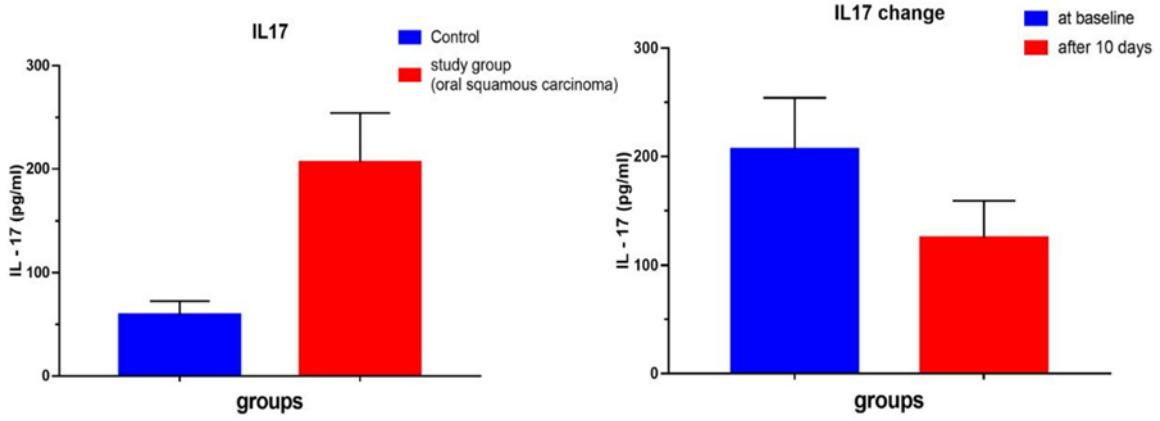

Figure 1 Salivary levels of interleukin-17(pg\ml) at baseline and 10 days post-operatively

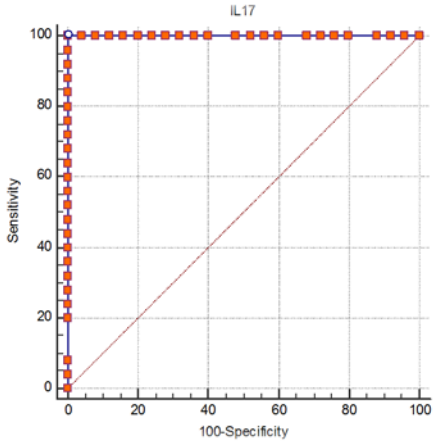

Figure 2 Receiver operator curve (ROC) of IL-17 as a discriminator of OSCC from control

DISCUSSION:

All over the world, oral cancer was appeared as a problem threatening public health accompanied with escalating incidence and mortality rates [22]. So that, there is a growing need for carrying out newer screening and early diagnostic approaches in order to diminish the morbidity and mortality owing to this disease. For this dreaded malignancy, sensitive and specific biomarkers are to be considered effective in respect to screening, diagnosis, staging and follow-up [23]. Saliva was looked upon as a true reflection of blood and its various elements and represents a true mirror for body's health. It has been used for detection of various diseases ranging from autoimmune diseases to infections and cancers [24, 25,26]. IL-17 is a relatively novel cytokine family and it plays an important role in connecting innate and adaptive immune responses. The mean value of salivary levels of IL-17A in normal healthly individuals reported in current study were similar to those reported in a previous study [27]. In addition, the mean values of salivary levels of IL-17A in saliva samples were significantly increased in OSCC patients in comparison to salivary levels of healthy control subjects and its level was decrease significantly ten days after surgical removal of tumor. These results are in agreement with those presented by [28], that T helper 17 cells and IL-17 levels are increased in patients with head and neck squamous cell carcinoma. The high salivary levels of IL-17 with OSCC patients may be due to the tumor microenvironment. It had been stated that the functions of IL-17 contribute mainly to progression of tumor as IL-17 directly affects reproduction and survival of tumor cells [19]. In addition, interlukine has two effects; pro-tumor and anti-tumor. Pro-tumor role of IL-17 is mainly via promoting tumor angiogenesis via activation of fibroblasts and endothelial cell [26]. IL-17 spurs the excretion of IL-8 that boosts the angiogenic signals of endothelial cells. That cytokine stimulates reproduction and survival of endothelial as well as tumor cells and of cells of tumor and whets the recruitment of neutrophiles at tumor site [29]. Anti-tumor effect of IL-17 is mediated via enhancing cytotoxic T lymphocyte (CTL) activity [30]. Various types of mechanisms have been proposed to illustrate the IL-17 boost of tumor-specific T lymphocytes cytotoxic activity (CTL). For example, it has been notably indicated that IL-17 spurred the excretion of the activating inflammatory cytokine, IL-6, from various cells at tumor site [31].

Ethical Clearance: It was obtained from the Scientific Research Committee at Department of Maxillofacial Surgery in the concerned hospitals in Baghdad, Iraq.

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Conflict of Interest: None to declare.

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