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Assessment of interleukin -10 and interferon-gamma with oral squamous cell carcinoma patients compared with healthy subjects as a prediction for early detection and monitor treatment response

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Abstract

Background: In the worldwide oral squamous cell carcinoma (OSCC) is considered to be one of the most deadly diseases, due to it is late in diagnosis and absence of availability of established reliable biomarkers. The aim of current study was designed to detect cytokines in saliva of patient with oral squamous cell carcinoma and compared their levels with the healthy subjects and evaluate their validity as diagnosis and investigated changes of cytokines level in saliva pre-and post- surgical treatment to provide knowledge for exploring the use of saliva to monitor treatment response.

Material and method: whole unstimulated saliva was collected from each individual (25 OSCC patients Pre- and post- surgical removal of tumor and 25 healthy individuals matching with age and gender as control group. saliva was evaluated both cytokines IL 10 and interferon gamma by ELISA.

Result: IL-10 level is increased significantly in group of OSCC patients compared to healthy subjects (control group). Ten days after surgical removal of tumor, salivary level IL10 was decreased significantly in comparison to its level pre-operation. In OSCC patients salivary level interferon gamma was decreased significantly than its level in healthy subjects, ten days after surgical removal of tumor salivary level interferon gamma was increased significantly in group of OSCC patients in comparison to their level before surgical removal of tumor.

Conclusion: salivary levels of both cytokines IL-10, and interferon gamma could prove to be potential biomarkers of OSCC.

Key words: Oral squamous cell carcinoma (OSCC), Interleukin 10 (IL10) and interferon gamma (INF- γ).

Introduction

OSCC is the one of most frequent, aggressive malignant tumor of the oral cavity. In Iraq, OSCC Squamous cell carcinoma (SCC) was the most

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common oral malignancy accounting 90.92% [1]. Oral squamous cell carcinoma is a multi-factorial disease in origin, mostly it is linked with life style of person it may be modify by many extrinsic agents, various form of tobacco smoking and chewing (e.g., pipes, cigars and cigarettes), Smokeless tobacco such as oral snuff (wet or moist snuff) and Betel quid chewing has become common over the world are considered as the most important cancer risk factors and as responsible for millions of cancer deaths annually [2]. Alcohol both independently as well as synergistically with smoking acting as the major risk factors for oral squamous cell carcinoma. Long-term and heavy smoking and alcohol drinking habit might be increase the risk of triggering oral squamous cell carcinoma [3]. Surgery, with or without radiotherapy for patients with locally advanced OSCC has been widely accepted as the typical treatment and is supposed to be the most curative therapy, but although improvement in treatment the disease still has a low overall survival rate, may be due to lack of improvement in diagnosis of OSCC or not diagnosed until reach to advance stage and invasive deeply to surrounding tissue [4]. Saliva has been used as a diagnostic fluid because salivary composition reflects disease or physiologic conditions. [5] Saliva has many advantages, containing non-invasive collection method, so does not require specialized training. In addition, whole saliva contains proteins resulting from desquamated epithelial cells, leukocytes, gingival sulcular fluid, and bronchial and nasal secretions. [6] Therefore, saliva may be more sensitive than serum for detection of disease [7&8]. In saliva cytokines, especially inflammatory and angiogenic cytokines, have been investigated as potential protein biomarkers of head and neck squamous cell carcinoma [9]. In OSCC cytokines sources may be tumor cells and the immune response to the tumor [10]. IL-10 is anti-inflammatory cytokines, a 35 kD cytokine identified in 1989, and is produced by activated macrophages, B cells, and T cells [11]. IL10 suppresses MHC-II expression in activated macrophages and is thus a potent inhibitor of antigen presentation [12 &13]. The particular interest is that IL-10 inhibits the production of IFN-γ by Th1 and NK cells, and induces the growth, differentiation, and secretion of IgGs by B cells and macrophages themselves are affected by IL-10 in that exposure to this cytokine lowers their microbicidal activity and diminishes their capacity to respond to IFN-γ [13].

Interferon gamma (IFN γ) is one of the dominant cytokines whose biological activity is conventionally associated with cytostatic/cytotoxic and antitumor mechanisms during cell-mediated adaptive immune response [14]. Beside its ability to trigger cell cycle arrest and apoptosis, IFN-γ can illustrate its anti-tumor activity in patients with progressive head and neck squamous cell carcinoma. Type II immune IFN- γ are considered as more potent cytokines actually they are cyto-protective against tumor genesis &promotes anticancer activities by attenuating cancer cell growth, in humans reduced of IFN-γ levels and/or the generation of genetic defects in IFN-γ signaling factors, including IFN-γ single nucleotide polymorphisms, IFN-regulating factors (IRFs), & IFN receptor are danger factors for tumor genesis process [15]. There is significant lack of information regarding role of these potential markers in oral cancer pathophysiology, also, it needs to be explored whether these immune-suppressive cytokines could be exploited as diagnostic and prognostic markers in OSCC patients. Salivary cytokine levels estimation may be

afforded most important indicators for early diagnosis and represent the immunosuppressive cytokines, as prospective salivary biomarkers in high-risk populations to OSCC [10].

Materials and methods

The present study was conducted at different hospitals in Baghdad \ Iraq at department of maxillofacial surgery, the samples included in the study 25 patients, histo-pathologically confirmed OSCC of both genders within age (41-77) years old. The OSCC patients recruited in the study did not have any oral or systemic illness, salivary sample taken from OSCC patient two days before surgical treatment and 10 days after surgical treatment. Control group enrolled in this study, included 25 healthy subject matching in age and gender to the study group with no history of OSCC or any other types of tumor. (Table 1). Whole unstimulated saliva was collected between 8 am and 11 am was done in a sterile graduated test tube, then these tubes were subjected for centrifugation at 4000 rpm for 10 minutes. 100 µl of supernatant was stored in tubes under standardization conditions following Navazesh and Kumer (2008) [16] instruction for analysis salivary Interleukin 10 and interferon gamma.

Measurement of these proteins was determined using salivary IL 10 and Interferon gamma ELIZA kit by Enzyme Linked Immuno-Assay machine. However, reagent preparation principle, assay of procedure and results of calculation were all performed following to the instructions of Shanghai Yehua Biological Technology\ China manufacturer's procedure.

Finally, calculation by Make concentration of standards the abscissa and absorbance (OD) value the ordinate. Draw the standard curve on the graph paper. According to the absorbance

(OD) value of the sample, locate its corresponding concentration (which is the concentration of the sample); or calculate the linear regression equation of the standard curve according to the standard concentration and the absorbance (OD) value. Then substitute with the OD value of the sample to calculate its concentration.

The demographic variables were presented as simple descriptive statistics calculating mean and standard deviation (SD) of numerical data-like age, the salivary concentrations of studied cytokines were presented as mean and standard error of mean (SEM). Independent samples t-test was used to compare the salivary cytokine levels in studied groups. Pearson's correlation coefficient was calculated to evaluate the correlation between two studied cytokines value <.01 was considered as statistically. All statistical analyses were conducted using the SPSS statistical software program.

Results

In the current study salivary cytokine levels of IL-10, and interferon gamma of 25 patient with OSCC (study group) compared with 25 healthy individuals (control group) were estimated. In the current study, the age of OSCC patients ranged between (41-77) years with a mean age of (56.7 ± 10.4) years. However, the majority of patients are in the age group of (50-59) years. Regarding gender there is a significant male's predominance (68.0%) among study group, no statistically significant differences has been recorded in age or gender existed between patients and controls, as shown in table 1.

Regarding salivary IL-10 levels and before surgical treatment of tumor, table 2 illustrated that a high mean value was recorded among study group with statistically significant difference compared with healthy group.

Ten days after surgical removal, table 3 shows that a significant decrease in the level ofIL-10 among study group compared with the level before surgical treatment. IL-10 have an excellent ability to discriminate between OSCC patients and healthy subjects with 100% SN, 96 SP, 98% accuracy, 96.2% PPV and 100% NPV. The optimal cut point to discriminate OSCC patients from control was salivary level above >4.48335, as shown in table 4 and figure 1.

concerning salivary interferon gamma levels and before surgical treatment of tumor, table 5 illustrated that a low mean value was recorded among study group with statistically significant difference compared with healthy group. Since data did not follow normal distribution Mann Whitney U test used and median and the interquartile range used to represent the data.

Ten days after surgical removal, table 6 shows that a significant increase in the level of interferon gamma among study group compared with the level before surgical treatment. Since data did not follow normal distribution Wilcoxon signed rank test used and median and the interquartile range used to represent the data. Salivary interferon gamma has excellent ability to discriminate between OSCC and control with 100% SN, 100% SP, 100% accuracy, 100% PPV and 100% NPV. The optimal cut point to discriminate OSCC from control was serum level below or equal 176.212. As illustrated in table 7 and figure 4.

Discussion

Among patients with OSCC analysis of selected salivary biomarkers appears to be a major important and the salivary biomarkers are used since saliva includes a wide range of compounds [17]. Relative levels of cytokines in saliva between head and neck SCC patients and healthy individuals focused many studies [18,19,20,21,22], but changes of salivary levels of cytokines between pre-and post treatment have not been extensively explored, may be due to difficulties to follow up the patient after treatment or due to changes in salivary physicochemical properties like subsequent xerostomia with conventional radiation [5, 23 and 12]. In the current investigation, none invasive collection approach could dramatically reduce anxiety and discomfort and increase patient's willingness to undergo health checkup routinely that will greatly increase the opportunity to monitor their general health over time and to diagnose morbidities [25].

In this study, mean value of salivary-IL 10 was significantly elevated with OSCC patients before surgical removal of tumor (7.8049 ± 0.9176p pg/ml) in comparison to healthy subjects (3.3420 ± 0.8058 pg/ml). The IL 10 level in normal health individual was similar to finding by other studies [26,27and 28]. The level was significantly decrease ten days after surgical removal of tumor, these finding of the current investigations may be due to anti-inflammatory IL-10 negatively regulate the anti-tumor immune responses and counteract the proliferative potential of their pro-inflammatory counterparts [28], a IL-10 is a pleiotropic cytokine which is produced by macrophages, T-helper and B lymphocytes and has the ability to either stimulate or suppress the immune system [29]. IL-10 has dual effects on solid tumors which appear as

anti-tumor effects or tumor cells evasion of immune system. By preventing tumor antigen from reaching antigen-producing cells, IL-10 causes tumors to escape T-cells [30]. Antitumor mechanism of IL-10 is not quite well known yet, may be cause anti-tumor and anti-metastatic effects by inhibition of angiogenesis. IL-10 can have anti-angiogenesis effects via reducing the production of angiogenic factors, such as TNF-α, IL-6 and, by macrophages associated with tumor. IL-10 is also directly effective on secretion of angiogenic factors from tumor cells [31]. The pro inflammatory cytokines on one hand are responsible for growth and proliferation of immune as well as tumor cells, while on the other hand, enhance tumor immune surveillance program, also anti- inflammatory cytokine are active immunosuppressive agents [32]. The immunosuppressive role of IL10, mediate a shift of differentiation from T-helper 1 cells to T-helper 2 cells, thus down-regulating the anti-tumor immunity [22], thus preventing tumor rejection [32,33 and 34].

In the tumor, micro environment IFN- gamma is secreted by various leukocytes and modulate immune function, IFN- gamma has been shown to promote host anti-tumor immunity, deficiency in IFN-gamma signaling proved susceptible to carcinogen-induced tumor formation [35]. In this study, mean value salivary interferon gamma in health individual 244.5 (206.1 – 251.4) pg/ml, which is similar finding to other studies [13and 36]. Tumor arises as a result of cellular changes that disturb normal balances between growth of cell and cell death, these changes could be caused by an altered expression of many factors including IFN γ [37]. In this study salivary low level of interferon gamma in the OSCC 120.2(110.6 –143.1) pg/ml in comparison to normal subject, IFN-γ considered as potent cytokine which is cyto-protective against tumorigenesis [38]. In humans decreased levels of IFN-γ is risk factors for tumorigenesis [39]. IFN-γ exhibits a variety of important biological activities: not only does IFN-γ confer antimicrobial and immunomodulatory effects—inducing major histocompatibility complex (MHC)-mediated antigen presentation pathways, developing type 1 T helper cell (Th1) responses, causing anti-microbe effects, regulating leukocyte trafficking, and facilitating Toll-like receptor signaling—but it also promotes anticancer activities [40]. The loss of major histocompatibility complex (MHC) and cytotoxic priming proteins may result from cellular hypo- responsiveness to IFN-γ [41]. According to this hypothesis, changes in the activation of IFN-γ signaling should be considered as an alternative escape pathway from IFN-γ-dependent immune surveillance in tumor genesis.

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Table 1: Distribution of sample study and control groups by age and gender.

	Control	OSCC	P value
Number	25	25	-
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%)	
Gender			0.556 [b]
Female	10 (40.0%)	8 (32.0%)	
Male	15 (60.0%)	17 (68.0%)	
[a] Independent t test, [b] chi square test			

Table 2: salivary levels of IL-10 mean and standard deviation between study and control groups

Control	OSCC	P -value
3.342 ± 0.805 pg/ml	7.804 ± 0.917p pg/ml	<0.001
Independent test		

Table 3: Salivary level of IL-10 mean and standard deviation in oral squamous cell carcinoma patients 10 days post-op

Baseline	10 days post-op	P value
7.804 ± 0.917 pg/ml	4.549 ± 1.302 pg/ml	<0.001
Paired test		

Table 4: Validity for IL-10 for predicting oral squamous cell carcinoma patients

Markers	AUC	Cut point	SN	SP	Accuracy	PPV	NPV
IL 10	0.998	>4.48335	100%	96%	98.0%	96.2%	100%
SN: sensitivity, SP: specificity, PPV: positive predictive value, NPV: negative predictive value							

Table 5: salivary levels of Interferon-γ mean and standard deviation between control and study group

	Control	OSCC	P value
Interferon-γ	244.5 (206.1 – 251.4)	120.2 (110.6 – 143.1)	<0.001
Mann Whitney U test			

Table 6: salivary level of Interferon-γ in oral squamous cell carcinoma patients 10 days post-op

	Baseline	10 days post-op	P value
Number	25	25	-
Interferon-γ	120.2 (110.6 – 143.1)	194.5 (190.6 – 197.0)	<0.001
Wilcoxon signed rank test			

Table 7: Validity for Interferon-γ for predicting oral squamous cell carcinoma patients

Markers	AUC	Cut point	SN	SP	Accuracy	PPV	NPV
Interferon-γ	0.960	≤176.212	100%	96%	98.0%	96.2%	100%

SN: sensitivity, SP: specificity, PPV: positive predictive value, NPV: negative predictive value

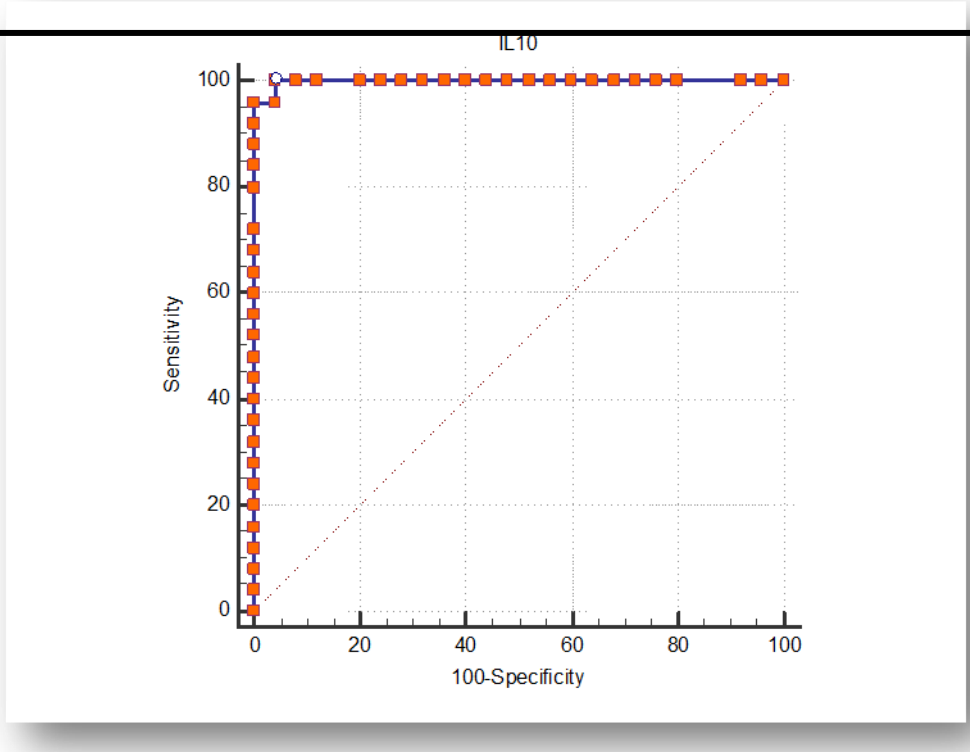

Figure 1: Receiver operator curve (ROC) of IL-10 as discriminator of oral squamous cell carcinoma from control

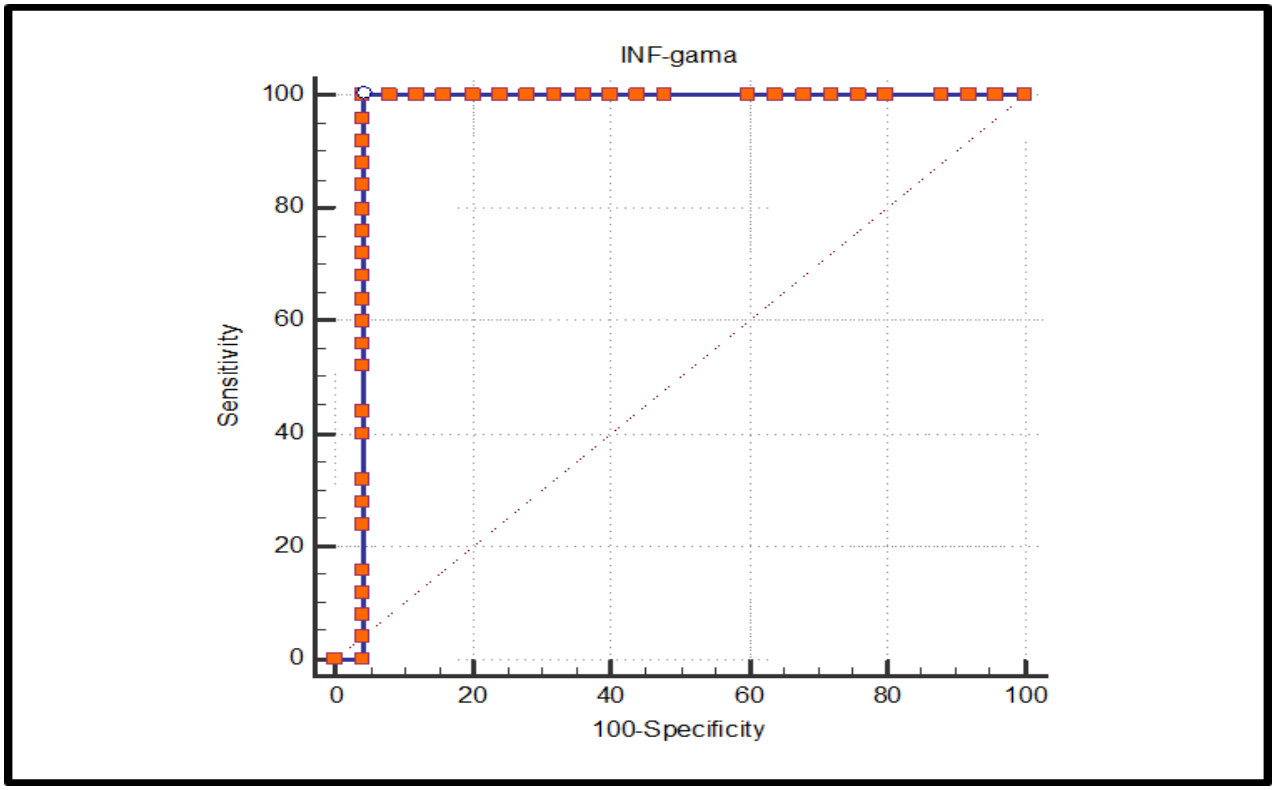

Figure 2: Receiver operator curve (ROC) of interferon gamma as discriminator of OSCC from control.