

# T-helper Type 1 and 2 Cytokine Levels in Patients with Benign and Malignant Salivary Gland Tumors

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## ABSTRACT

**Background:** Salivary gland tumors are among malignancies that have high recurrence rate. Immune responses in salivary gland tumors have not been well elucidated. T helper type 1 (Th1) and Th2 cytokines have been reported to play a role in the outcome of head and neck cancers. **Objective:** To evaluate the serum levels of interferon gamma (IFN- $\gamma$ ), as the hallmark of Th1 cytokines, and interleukin-4 (IL-4), as the hallmark of Th2 cytokines, in patients with benign and malignant salivary gland tumors in comparison with healthy controls. **Methods:** Fifty patients with benign and 14 patients with malignant salivary gland tumors, as well as 23 healthy individuals were recruited. Serum levels of IFN- $\gamma$  and IL-4 were measured using ELISA method. Nonparametric tests were used for data analysis. **Results:** Serum levels of IFN- $\gamma$  and IL-4 were found not to be significantly different in patients compared to the control group ( $0.68 \pm 0.29$  vs.  $1.03 \pm 0.57$  pg/ml,  $p=0.58$  for IFN- $\gamma$ ,  $4.57 \pm 1.57$  vs.  $4.41 \pm 1.31$  pg/ml,  $p=0.28$  for IL-4). IFN- $\gamma$  and IL-4 serum levels were also not significantly different between patients with benign and malignant salivary gland tumors ( $p=0.54$  and  $p=0.86$ , respectively). **Conclusion:** The systemic levels of IL-4 and IFN- $\gamma$  seem not to be associated with salivary gland tumor in our study. Investigation of other cytokines produced by Th1 and Th2 cells are warranted.

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**Keywords:** IFN- $\gamma$ , IL-4, Salivary Gland Tumors, Serum

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## INTRODUCTION

Salivary gland tumors are a heterogeneous group of tumors with diverse clinicopathological and histological presentations that make their diagnosis and treatment complex (1). These tumors comprise 3-6% of all head and neck tumors. The average incidence rate per 100,000 populations is 4.7 for benign tumors and 0.9 for malignant ones per year. They can occur at any age but are more common in individuals older than 50 years. Approximately 80% of these tumors arise from parotid glands, but other major and minor glands can be affected with lower frequency (1,2).

The antitumor activity of the immune system is often promoted by T-helper (Th1) lymphocytes. Interferon gamma (IFN- $\gamma$ ) is one of the most important cytokines secreted by Th1 cells which activates cellular immunity in the tumor environment. It exerts its antitumor effects in many different ways, such as preventing the spread of tumor through inhibition of angiogenesis, increasing phagocytosis of cancer cells via activation of macrophages, and induction of apoptosis. IFN- $\gamma$ , by affecting the cells, can also inhibit their differentiation into Th2 cells (3). In salivary gland cells, it has been shown that IFN- $\gamma$  induces apoptosis and results in the activation of caspase 8 as well as the "death receptor" pathway (4).

Likewise, Interleukin-4 (IL-4) is a cytokine with pleiotropic activity. IL-4, by affecting Th0 cells, stimulates their differentiation into Th2 cells and suppresses IFN- $\gamma$  producing Th1 cells; therefore, antitumor immunity will be weakened (5). This cytokine increases the expression of MHC class II on the surface of B-lymphocytes. It also plays an important role in the Immunoglobulin class switching, and enhancing humeral immunity (6). In addition to the effects of IL-4 on the immune system, IL-4 receptor is detected on the surface of many solid tumors, such as breast cancer, melanoma, ovarian carcinoma, and carcinoma of the head and neck (7,8).

Salivary gland tumors, among other types of head and neck tumors, have more diversity, complexity of treatment and relapse. Imbalances in T-cell subsets as well as their cytokine profiles have been reported in head and neck tumors (9,10). Our previous study demonstrated that the disproportion in the Th17/Treg ratio may contribute to the progression of salivary gland tumors (11). In addition, changes in Th1 and Th2 cytokine profiles can be seen in various types of cancers, including lung, breast, kidney, liver, prostate and head and neck tumors, which is generally associated with an increase in Th2 cytokines including IL-4, and decrease in Th1 cytokines especially IFN- $\gamma$  (4-6,12). However, expression patterns of these two main cytokines have not been investigated in salivary gland tumors, yet. Considering the role that the immune system plays in tumor formation or regression, we aimed to study and compare the serum levels of IL-4, and IFN- $\gamma$  among patients with a benign and a malignant salivary gland tumor and normal population, and then checking out the mean and statistical issues in each group.

## MATERIALS AND METHODS

Sixty-four patients with salivary gland tumors who referred to the Khalili hospital, Shiraz, Iran were included in this study after getting the definite detection, checking out pathology, and detecting the tumor type. Patients were divided into two groups: 50 patients with benign salivary gland tumors, and 14 patients with malignant ones. The

details of clinicopathological characteristics of the patients are depicted in the Table 1. Twenty-three healthy individuals comprised the control group.

**Table 1. The clinicopathological characteristics of the patients with malignant and benign salivary gland tumors.**

	Characteristics	Classification	Frequency (%)
Malignant Tumors (N=14)	Histological tumor type	Mucoepidermoid carcinoma	5(35.7%)
		Adenoid cystic carcinoma	2(14.3%)
		Squamous cell carcinoma	4(28.6%)
		Metastatic melanoma	1(7.1%)
		Epthelialmyoepithelial carcinoma	2(14.3%)
	TNM stage	Stage I	1(7.2%)
		Stage II	3 (21.4%)
		Stage III	4(28.6%)
		Stage IV	3(21.4%)
		unknown	3(21.4%)
Metastasis	Yes	3(21%)	
	No	11(79%)	
Benign Tumors (N=50)	Histological tumor type	Pleomorphic adenoma	42(84%)
		Warth in tumor	7(14%)
		Basal cell adenoma	1(2%)

Blood samples were taken with consent of the candidates. The volume of blood was about 5 ml, which was drawn in 15 ml disposable endotoxin free tubes. Serum was separated by centrifuge and then was stored at -20°C until testing. Serum levels of IL-4 and IFN- $\gamma$  were measured using sandwich ELISA technique. Measurement method was in a manner that samples in both controls and case group were incubated with the monoclonal antibody against each cytokine. After rinsing, substrate was added to each sample and the enzymatic reaction was performed; which, in the presence of antigen, resulted in the appearance of blue color. After the time required reaching the equilibrium in reaction, the reaction was stopped by adding stop solution. The intensity of the color was proportional to the amount of each cytokine in the sample. For IFN- $\gamma$ , the ELISA kit (bioscience, Austria) with measurement sensitivity of 0.99 pg/ml, and for IL-4, the ELISA kit (bioscience, Austria) with measurement sensitivity of 0.66 pg/ml was used. Standard curve for each assay was drawn by showing the amount of absorption ratio to the amount of concentration on the curve based on the standard samples absorption. The concentration of each cytokine in samples was determined and recorded based on the standard curve.

**Statistical Analysis.** Kolmogorov-Smirnov test showed lack of normal distribution of variables; therefore, non-parametric analysis Kruskal-Wallis U-test using SPSS-24 statistical software package was used.

## RESULTS

The mean age of the case group was  $44.0 \pm 15.3$  (ranging from 21 to 80) and the mean age of the control group was  $44.3 \pm 16.5$  (ranging from 20 to 75). In the case group, 45.3% of patients were men and 55.7% of them were women. These numbers in the controls were 47.8% and 52.2 %, respectively.

The serum levels of IFN- $\gamma$  and IL-4 are shown in the Table 2. There was no significant difference between the serum level of IFN- $\gamma$  ( $0.68 \pm 0.29$  in the whole group patients and  $1.03 \pm 0.57$  pg/ml in controls;  $p=0.58$ ) and IL- 4 ( $4.57 \pm 1.57$  in patients vs.  $4.41 \pm 1.31$  pg/ml in control;  $p=0.28$ ).

The comparisons of serum levels of IL-4 and IFN- $\gamma$  are also shown separately in both patients with benign or malignant tumors in Table 2. No significant difference was observed between serum levels of IFN- $\gamma$  and IL-4 and in either patients with benign tumor or malignant tumor ( $p>0.05$ ).

The results of the present study showed that serum levels of IL-4 did not differ in both case group ( $p=0.39$ ), and the controls ( $p=0.88$ ) by the mean age. There was also no significant difference between the mean age and serum levels of IFN- $\gamma$  in both case group ( $p=0.33$ ), and the controls ( $p=0.51$ ). Moreover, regarding the gender, this parameter was not associated with serum levels of IL-4 in both case groups ( $p=0.32$ ), and the controls ( $p=0.69$ ). There was also no significant difference between the sex and serum level of IFN- $\gamma$  in both case group ( $p=0.80$ ), and the controls ( $p=0.56$ ). Therefore, patient's gender or aging with ranging studied here did not increase or decrease the cytokines in their serum.

## DISCUSSION

According to the results of the present study, mean serum level of IL-4 in patients and the controls were not statistically significant. No considerable differences in the levels of IFN-  $\gamma$  were observed, too.

To the best of our knowledge, our results are the first publication on the serum levels of IL-4 and IFN- $\gamma$  on salivary gland tumors. In other types of cancer, more data are available. In head and neck cancer and in the same ethnic background, it was found that serum IL-4 levels were increased in cancer patients compared to healthy individuals, but were not significantly associated with tumors stage (12). In a research done by Almatroodiet *al.* on serum levels of IL-4 in patients with large cell carcinoma of lung, there was a significant increase in serum level of IL-4 in comparison to the other *non-small cell lung cancer* subjects and controls (13). In a study by Golstein *et al.* on prostate cancer, results showed that serum levels of IL-4 was higher in refractory patients (14).

**Table 2. Serum concentrations of IFN- $\gamma$  and IL-4 in the patients with malignant and benign salivary gland tumors and control group.**

Cytokine	Concentration pg/ml Ranges, Mean $\pm$ SEM			P value (two-tailed)			
	Patients with malignant tumors (n=14)	Patients with benign tumors (n=50)	Healthy control subjects (n=23)	Global P value*	Comparison between Malignant and benign**	Comparison between Malignant and control**	Comparison between Benign and control**
IFN- $\gamma$	0.00-6.21 (0.64 $\pm$ 0.47)	0.00-11.57 (0.70 $\pm$ 0.35)	0.00-8.80 (1.04 $\pm$ 0.53)	0.74	0.54	0.99	0.50
IL-4	0.00-16.16 (2.7 $\pm$ 1.46)	0.00-80.17 (5.10 $\pm$ 1.97)	0.00-15.88 (4.41 $\pm$ 1.31)	0.54	0.86	0.47	0.34

\*Global P value; Kruskal-wallis H-test results comparing three groups

\*\* Mann-Whitney U-test results comparing two group

However, Kcvoacs *et al.* in patients with different types of cancer found that there was no significant difference between serum level of IL-4 in patients and the controls, which is similar to the result of our study on salivary gland tumors (15). Regarding IFN- $\gamma$ , Lee *et al.* have detected a significant association between serum levels of IFN- $\gamma$  and tumor size and stage in patients with Hepatocellular carcinoma (16). Similar to our results, lack of association between IFN- $\gamma$  and disease status (paired *t-test*,  $p < 0.05$ ) in breast cancer has been reported (17). Decrease in IFN- $\gamma$  serum levels have also been observed in patients with non-small cell lung cancer, making intervention of systemic level of IFN- $\gamma$  in cancer more complex (18).

In other body fluids of human body like pleural effusions, results are controversial, as well. For example, in a study by Ghayyumi *et al.* a wide variation in IL-4, and IFN- $\gamma$  levels in malignant pleural effusions was shown, and no significant difference was observed between levels of these cytokines compared to congestive heart failure case (19).

According to the above publications, controversies exist on serum levels of IFN- $\gamma$  and IL-4 as a prognostic factor and/or an index in determining the clinical disease process in most solid tumors. In our study, there was no significant association between the serum levels of IFN- $\gamma$  and IL-4 and clinic-pathologic factors of the salivary gland tumors. The discrepancies can be explained by the differences in the nature of tumors, in ethnic background, and in the number of patients' samples.

In conclusion, we found that there is no significant association between salivary gland tumors and serum levels of IFN- $\gamma$  and IL-4, the two main cytokines of Th1 and Th2 responses. However, no definite conclusion on the role of these two cytokines in antitumor immunology of salivary gland can be made only based on the current results. Although systemic levels of cytokines are footprint of cytokine levels in the body, the production of these cytokines in tumor cells are shown to play an important role in anti-tumor immunity. On the other hand, other cytokines produced by Th1 and Th2 cells such as IL-13 may be involved in anti-tumor immunity of salivary gland tumors.

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