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## IL-4 mRNA Expression in Laryngeal Squamous Cell Carcinoma Patients with or without Lymph Node Involvement

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### Abstract

**Background:** Interleukin (IL)-4 is a member of T helper 2 (Th2) axis produced by T-lymphocytes and mast cells. It has been shown that IL-4 expression changes in tumor tissues. The main objective of this study is to investigate the expression of IL-4 mRNA in patients with Laryngeal Squamous Cell Carcinoma (LSCC) with or without lymph node involvement. **Materials and Methods:** mRNA expression of IL-4 in LSCC tissues were detected by quantitative Real-Time PCR (qRT-PCR). Expression of IL-4 gene was compared between lymph node positive and negative patients with Squamous Cell Carcinoma of Larynx. **Results:** No statistically significant association was found in expression of IL-4 between lymph node positive and negative patients. **Conclusion:** It seems that IL-4 has no important effect on the involvement of lymph node in LSCC. However, to achieve a definite conclusion more investigations are certainly required. [GMJ. 2014;3(1):20-23]

**Keywords:** Cancer; Interleukin-4; Lymph Node Metastasis; Larynx; T helper 2

### Introduction

Laryngeal cancer consists of some types of Squamous cell carcinoma (SCC). Sarcoma and laryngeal lymphoma are other malignancies of larynx which are rare [1]. Laryngeal cancer is responsible for one fourth of all head and neck cancers, and is 4 times more common in males than in females [2]. Almost 10-15% of patients with laryngeal cancer have metastases to lung, liver and skeletal system when they are referred [3]. Thus, novel ways of detection and treatment are absolutely required and should be investigated [4].

Cytokines would change in different strategic sites such as peripheral blood, lymph nodes (LN) and tissues of patients with cancer. Interleukin (IL)-4 is one of T helper 2 (Th2) type cytokines with major roles in development of different types of cancer such as colon cancer, breast cancer, glioma, and melanoma [5]. IL-4 has a main role in differentiation of native T lymphocytes and causes the production of IL-4, IL-5, IL-10, and IL-13. These cytokines strongly inhibit the differentiation of interferon gamma (IFN $\gamma$ ) producing cells [6]. IL-4 inhibits IFN $\gamma$  to stimulate macrophages; therefore, it inhibits cellular immunity [7].

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Li *et al.* showed that endogenous IL-4 upregulates antiapoptotic genes and stimulates tumor growth, since using monoclonal antibody to neutralize IL-4 caused reduction of tumor growth [8]. Kanai *et al.* revealed that IL-4 decreases chorioembryonic antigen (CEA) and E-cadherin which may lead to lower attachment between colon tumor cells [9]. IL-4 has the ability to enhance lung metastases in rats with melanoma and injection of IL-4 has been shown to increase these metastases which would be ceased by using neutralizing IL-4 monoclonal antibodies [10]. Production of large amounts of IL-4 in tumor microenvironment compared to normal tissues can probably protect tumor cells against immune system [11]. In a study by Stassi and colleagues IL-4 was introduced as a reason for resistance of thyroid cancer cells to chemotherapy [12]. As IL-4 is one of the important cytokines known with cancer promoting characteristics such as metastasis to different organs, we aimed to investigate IL-4 gene in tumor tissues of laryngeal cancer patients with and without LN involvement. Results of this study would open new insights for LSCC tumor progression.

## Materials and Methods

### *Sample Size and Sampling Method*

This study was performed on tumor tissues from 58 patients with laryngeal cancer, among which 7 patients had LN involvement and 47 patients had no LN involvement. Patients underwent surgeries in Khalili hospital (Shiraz University of Medical Sciences, Shiraz, Iran) and 0.5 × 0.5 cm of tumour tissues were sent to the laboratory on ice. Those patients with a pathology diagnosis other than squamous cell carcinoma or with previous radiotherapy or chemotherapy were excluded from the study. After pathologist's approval for squamous cell carcinoma, the samples were held at -70 oC till the lab studies were started.

RNA extraction and complementary DNA (cDNA) synthesis

A 500 mg frozen sample was powdered with liquid nitrogen and was then transferred to a 1.5 ml tube while RNA was extracted by using RNA extraction kit (Roche™, USA) based on

the manufacturer's instructions.

Finally optic density (OD) of extracted RNAs was determined using spectrophotometer. cDNA was produced from the extracted RNAs using the cDNA synthesis kit based on the manuscript (Fermentas™, Canada).

Quantitative Real Time Polymerase Chain Reaction (Q-PCR)

Q-PCR method was performed using an Applied Biosystem thermal cycler. Approximately 2 µl cDNA was amplified in each 25 µl PCR reaction containing 12.5 µl of 2x SYBR Green Master Mix (Fermentas™, Canada), 0.3 µl of each 10 pmol forward and reverse primers (designed in primer 3 software) and 9.9 µl DEPC water. PCR amplification was done in 50 cycles using the following program: 95°C for 10 min, 95°C for 15 s, 57 °C for 30 s and 60 °C for 34 s. Expression of 18S rRNA was used as reference gene.

### *Statistical Analysis*

Statistical analyses were done using the SPSS software for windows version 15 (SPSS, Chicago, IL, USA). Non-parametric Mann-Whitney U test was used to compare the rate of gene expression of IL-4 between patients with and without LN involvement. The relative amounts of IL-4 transcripts were determined from  $\Delta C_t$  and  $2^{-\Delta C_t}$  formulas. Significant difference was accepted if P value was less than 0.05.

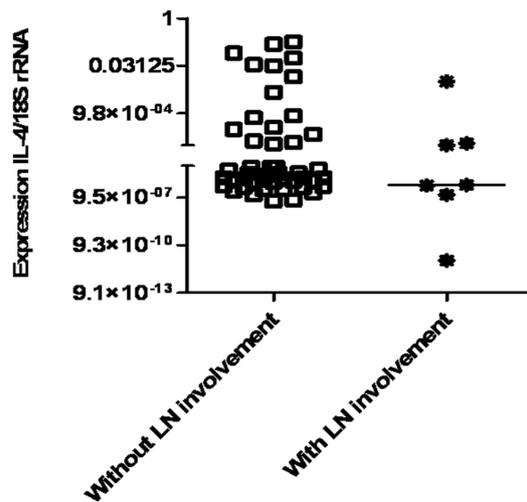
## Results

### *Clinicopathological Characteristics of Patients*

All 58 patients were male with a minimum age of 38 years old and maximum of 84 years old (mean age was 53± 12 years). 87% of the patients had LN involvement whereas 13% had no involvement.

### *IL-4 Transcripts and Lymph Node Involvement*

As shown in Figure-1 the median of  $2^{-\Delta C_t}$  of IL-4 expression in tumor tissues of patients without LN involvement was slightly higher than those with LN involvement. However, this difference was not statistically significant (P value>0.05).



**Figure 1.** Expression of IL-4 mRNA in tumor tissues of laryngeal squamous cell carcinoma patients with and without lymph node involvement. Horizontal lines showed the median of  $2^{-\Delta CT}$ .

## Discussion

Expression of cytokines in tumor tissues acts as one of the prominent factors mediating tumor growth and metastasis. Th2 type cytokines such as IL-4 favor tumor growth by stimulating cell proliferation and inhibiting cell apoptosis. Some studies showed a higher expression of Th2 cytokines in the serum of patients with cancer. Mojtahedi and colleagues studied serum level of IL-4 and IL-10 in head and neck squamous cell carcinoma (HNSCC) patients and in a group of normal individuals. They showed that serum IL-4 is significantly higher in patients than controls [13]. Similarly, Cheng *et al.* detected much higher expression of IL-4 in the serum of patients with nasopharyngeal cancer [14]. Peripheral blood mononuclear cells (PBMC) of laryngeal squamous cell carcinoma patients also showed higher level of IL-4 mRNA in comparison with control subjects [15]. Interestingly, Manchanda studied tobacco related SCC of oral cavity and revealed that T-lymphocytes of these patients had significantly higher expression of IL-4 than control group

[16]. Recently, Seto *et al.* designed a peptide against IL-4 receptor and found out that this peptide has the ability to decrease the rate of tumor growth in mice with HNSCC [17]. In contrast, Myers and co-workers showed much more increase in tumor growth in 6 cell lines of HNSCC in response to IL-4 [18].

In this study we aimed to evaluate the expression of IL-4 mRNA in tumor tissues of patients with LSCC and its relationship with LN involvement in these patients. Based on the results of our study, no relationship was found between IL-4 gene expression in tumor tissues of LSCC patients and LN involvement. Consistently, Oliveira *et al.* reported no significant relation between clinicopathological characteristics of patients with invasive oral SCC and IL-4 expression [19]. Similarly, it has been determined that the immune system of patients with HNSCC has a shift toward Th2 type responses with increased level of IL-4, IL-6 and IL-10 but without relationship with important tumor prognostic factors [20].

## Conclusion

In conclusion, it would be reasonable to say that IL-4 may have no effect on LN involvement in LSCC patients and it could not be used as a biomarker for prognosis in these patients. However, there were some limitations such as type of technique and sample size in our study which may have important effects on this result. Thus, using more accurate techniques such as western blot compared to real time PCR and study on a larger sample size contribute to the better elucidation.

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## Declaration of interest

The authors report no conflicts of interest.

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