

# Measurement of Serum and Salivary TNF- $\alpha$ in Oral Lichen Planus Patients

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## ABSTRACT

**Background:** Lichen planus is an inflammatory skin disorder with unclear etiology. TNF- $\alpha$  is a multifunctional cytokine which plays a major role in controlling cell proliferation, differentiation and apoptosis along with immunity and inflammation.

**Aim:** To determine the serum and salivary level of TNF- $\alpha$  in patients with oral lichen planus in comparison with the healthy participants.

**Materials and method:** A cross-sectional study was conducted on 30 subjects, including normal oral mucosa (15 subjects) and oral lichen planus (15 subjects). The levels of serum and salivary TNF- $\alpha$  were evaluated using TNF- $\alpha$  ELISA test.

**Results:** The serum and salivary TNF- $\alpha$  level was significantly higher in lichen planus patients than in patients with normal oral mucosa.

**Conclusion:** The findings in this study suggest that TNF- $\alpha$  plays a major role in the pathogenesis of oral lichen planus.

**Keywords:** Lichen planus, tumor necrosis factor- alpha, cytokine, saliva, serum

## INTRODUCTION

**Lichen Planus** was derived from the Greek leikhēn, meaning "a tree moss" and the Latin planus, meaning "flat".<sup>[1]</sup> Oral lichen planus is a common mucocutaneous disease with multifactorial etiology. It was first described by British physician Erasmus Wilson in 1869 and it is thought to affect 0.5-1 per cent of the world's population. The condition can affect either the skin or mucosa or both. It is usually present as a symmetrical, bilateral white striations, papules or plaques on the buccal mucosa, tongue and gingiva. Erythema, erosions and blisters may or may not be present.<sup>[2, 3]</sup> The cause is not clearly known but cell-mediated immune response plays an important role in the pathogenesis of oral lichen planus. The

immunological process results in vacuolar degeneration, lysis and finally, liquefaction of basal cells. The affected keratinocytes and associated inflammatory cells produce large amount of cytokines which play a key role in the recruitment of T-lymphocytes. T cells predominantly infiltrate in the subepithelial region and characterize oral lichen planus which further induces release of chemokines and cytokines belonging to either Th1 or Th2 groups.<sup>[4]</sup>

TNF- $\alpha$  is a molecule having 17Kd molecular weight, a polypeptide structure with a multifunctional proinflammatory cytokines that plays a major role in immune and host defense responses to infection,<sup>[5]</sup> stimulate angiogenesis<sup>[6]</sup> and influences tissue remodeling,<sup>[7]</sup> and takes part in the

regulation of cell proliferation and differentiation. It is a prototype molecule of a family belongs to the central mediator of acute inflammation. TNF- $\alpha$  is primarily released from stimulated monocytes and macrophages but Langerhans cells and active keratinocytes are also other cellular sources of TNF- $\alpha$ . [8]

TNF- $\alpha$  plays a pivotal role in innate inflammatory responses, [9] and it has been implicated in the pathogenesis of several chronic inflammatory disorders with an autoimmune component, such as psoriasis [10, 11] and systemic lupus erythematosus. [12]

In some of these diseases, serum and salivary TNF- $\alpha$  concentration correlated with the activity and intensity of the disease and may be used as a prognostic factor. The aim of the present study was to determine the serum and salivary level of TNF- $\alpha$  in patients with oral lichen planus in comparison with healthy participants.

## MATERIALS AND METHODS

A cross-sectional study was conducted on 30 subjects who were divided into two groups:

- Group A- 15 subjects of normal oral mucosa
- Group B- 15 subjects of oral lichen planus

The histopathological confirmation was done on all cases of oral lichen planus and normal oral mucosa. The study was approved by the ethical committee of the institution and an informed consent was taken from the study subjects. Patient with autoimmune disease, malignancy and patients who have medication were excluded from the study.

Sample collection was done in the morning from the subjects so as to prevent diurnal variation. Blood samples were taken from cubital vein and left to clot at 4<sup>o</sup>C in a sterile, clean, dry tube and kept inside the refrigerator. Serum were collected by centrifuge the blood at 3,000rpm for 5 to 10 minutes in 10cc sterile tubes and stored at -20<sup>o</sup>C until the time of analysis.

From each subject, 10 ml of unstimulated whole saliva were collected into a sterile centrifuge tube. After centrifugation, the separated clear salivary fluid was stored in disposable storage vials at -80<sup>o</sup>C until the test day.

Salivary and serum levels of TNF- $\alpha$  were determined using TNF- $\alpha$  Enzyme linked immunosorbent assay (ELISA) test (Ray Bio Human TNF- $\alpha$  enzyme immunoassay). The TNF- $\alpha$  concentration of salivary and serum samples were obtained using the optical density and concentration of the standard. The result was expressed as pg/ml.

The data was analysed by using statistical software (SPSS version 19.0). Mean and standard deviation were calculated for each individual group. The significance of the mean values of TNF- $\alpha$  in serum and saliva between the control and the patients were done using Student's independent *t*-test. A probability value (*p*) of  $\leq 0.05$  was considered to be statistically significance.

## RESULTS

In this study, Group A included 9 males (60%) and 6 females (40%). Group B comprised of 5 males (33.3%) and 10 females (66.67%) [Table 1].

The mean age of the patients in Group A and Group B was 26.75 years and 36.50 years respectively [Table 2].

The mean $\pm$ standard deviation (SD) of serum TNF- $\alpha$  in Group A and Group B was 33.65  $\pm$  11.12 pg/ml and 64.44  $\pm$  19.88 pg/ml respectively. The mean serum TNF- $\alpha$  level of patients with normal oral mucosa was significantly lower than that of oral lichen planus patients and the difference was statistically significant ( $<0.005$ ).

The mean  $\pm$  SD of salivary TNF- $\alpha$  in Group A and Group B was 15.32  $\pm$  5.6 pg/ml and 67.34 $\pm$ 2.78 pg/ml respectively. The mean salivary TNF- $\alpha$  level of patients with oral lichen planus was significantly higher than that of normal oral mucosa and the difference was statistically significant ( $<0.001$ ).

**Table 1: Gender wise distribution in normal oral mucosa and oral lichen planus**

Group	No. of cases	Gender	
		Male	Female
A	15	09(60%)	06 (40 %)
B	15	5 (33.33%)	10 (66.67%)

Group A- Normal oral mucosa, Group B-Oral lichen planus

**Table 2: Age wise distribution in normal oral mucosa and oral lichen planus**

Group	No. of cases	Mean $\pm$ Standard deviation
A	15	26.75 $\pm$ 5.09
B	15	36.50 $\pm$ 8.33

Group A- Normal oral mucosa, Group B-Oral lichen planus

**Table 3: SerumTNF- $\alpha$ in normal oral mucosa and oral lichen planus**

Parameter	Group	No. of cases	Mean $\pm$ Standard deviation	P-value
Serum TNF- $\alpha$ (pg/ml)	A	15	33.65 $\pm$ 11.12	<0.005
	B	15	64.44 $\pm$ 19.88	

Group A- Normal oral mucosa, Group B-Oral lichen planus

**Table 4: Salivary TNF- $\alpha$ in normal oral mucosa and oral lichen planus**

Parameter	Group	No. of cases	Mean $\pm$ Standard deviation	P-value
Salivary TNF- $\alpha$ (pg/ml)	A	15	15.32 $\pm$ 5.6	<0.001
	B	15	67.34 $\pm$ 2.78	

Group A- Normal oral mucosa, Group B-Oral lichen planus

## DISCUSSION

Oral lichen planus is an inflammatory keratotic disorder of the oral mucosa with unknown etiology that can affect all parts of the oral cavity, including buccal mucosa, tongue and gingiva. Various studies have suggested that immunological mechanism play an important role in the pathogenesis of oral lichen planus but the exact cause remain unclear. [13, 14] The immunological mechanism results in the vacuolar degeneration of the basal cells but histochemical studies have identified T lymphocytes as a predominant cell in this lesion. The major cells responsible for the damage of basal keratinocytes are CD8<sup>+</sup> T cells. [15] The major cytokines i.e. TNF- $\alpha$  and IFN- $\gamma$  released from activated T cells accumulate inflammatory cells in the region, and this leads to cell-mediated cytotoxicity and keratinocyte destruction. These two cytokines, along with other cytokines act locally and systemically, results in liquefaction degeneration in keratinocytes. In epithelial cells, TNF- $\alpha$  is cytotoxic and antiproliferative at high and low concentrations respectively. TNF- $\alpha$  has a vital regulatory effect in the onset and progression of lichen planus. [16, 17]

In this study, the mean serum and salivary level of TNF- $\alpha$  in oral lichen planus was significantly higher than that of normal oral mucosa and this was in agreement with the previous studies. [18-22] This indicates that TNF- $\alpha$  plays a major role in the pathogenesis of OLP and also considered as

an important marker in determining the disease activity. In this study, the lower levels of TNF- $\alpha$  in serum compared with those in saliva suggest that saliva have some advantages over serum because it can be collected non-invasively by subjects with modest training, and analysis of saliva has established values of numerous biochemical and immunologic parameters than those of serum.

## CONCLUSION

This study indicates that the serum and salivary TNF- $\alpha$  level was significantly higher in oral lichen planus patients when compared to patients with normal oral mucosa. These findings signifies that TNF- $\alpha$ , a proinflammatory cytokine plays an important role in the pathogenesis of oral lichen planus. Saliva can be used as a good substitute to serum to evaluate TNF- $\alpha$  in oral lichen planus patients.

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