

## Comparative Evaluation of Serum Tumor Necrosis Factor Alpha in Healthy Smokers and Healthy Non Smoker Subjects – A Case Control Study

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**Abstract:** Tumor Necrosis Factor- $\alpha$  (TNF- $\alpha$ ), a "major inflammatory cytokine" not only plays an important role in periodontal destruction, but also is extremely toxic to the host. Till date, there are not many studies comparing the levels of TNF- $\alpha$  in serum and its relationship with the smoking status. Our study aimed to assess the effect of smoking on serum values of Tumor Necrosis Factor alpha, and to compare the serum TNF alpha among the 2 study groups and establish a correlation between serum TNF alpha and smoking status. Hence, an attempt is made to estimate the level of TNF- $\alpha$  in serum, its relationship to smoking status in periodontally healthy individuals. 40 subjects participated in the study and were grouped into 2 groups Group A: 20 non smokers who were systemically and periodontally healthy subjects. Group B: 20 smokers who were systemically and periodontally healthy subjects. The serum samples were assayed for TNF- $\alpha$  level by Enzyme Linked Immunosorbent Assay (ELISA) method. The mean serum TNF- $\alpha$  Cytokines for group A (Non-smokers+ healthy) was  $0.867 \pm 0.865$ , and for group B (smoker healthy) was  $0.832 \pm 0.771$ . The range of serum TNF alpha was from (0.11 to 2.87). There was statistically no significant difference in Serum TNF- $\alpha$  Cytokines among Smokers & Non-Smokers Healthy subjects ( $P=0.892$ ). These observations suggest that there does not exist any association between serum Tumor necrosis factor alpha and smoking status. Within the limitations of this study, it is clear that additional studies with a larger sample size would be needed to draw any final conclusions regarding the effect of smoking on serum TNF alpha. It can be concluded that there is a prospect of using the estimation of TNF- $\alpha$  in serum as a "marker" of periodontal disease in future. However It remains a possibility that the absence or low levels of TNF- $\alpha$  in serum might indicate a stable lesion and elevated levels might indicate an active site but only longitudinal studies taking into account, the disease "activity" and "inactivity" could suggest the possibility of using TNF- $\alpha$  in serum as an "Indicator" of periodontal disease.

**Keywords:** Tumor necrosis Factor Alpha, Smoking.

### INTRODUCTION

Oral health is indispensable to overall healthy being. Man has been suffering from ailments of oral cavity since time immemorial. Oral diseases especially caries and periodontitis are known for their high prevalence and rapid morbidity. Periodontal diseases are a group of chronic, progressive bacterial infections resulting in inflammation and destruction of tooth supporting tissues [1]. The periodontal disease is known to have a complex pathogenesis with both bacterial and host factors contributing to the destruction of periodontium. Role of host immune response is most

important factor in periodontitis as it determines both disease progression and severity [2].

In smokers too, a suppressed immune response favour increased periodontal destruction [3,4]. Hence, immune function in periodontitis is a specialized function where every single molecule can have ramifications on the resultant tissue protective and destructive response.

Difficulty in determining active disease and ongoing destruction in periodontal tissue by traditional

diagnostic aids like probing depth and attachment loss has proved them to be inadequate in the modern era of periodontal therapeutics [5]. Search for a biomarker for periodontitis has resulted in researchers trying out and finding new molecules that can guide a clinician in many a decision regarding the patient's condition.

Tumor necrosis factor-alpha (TNF- $\alpha$ ) is a pro-inflammatory cytokine released by macrophages which is known for its substantial role in periodontitis mediated bone loss [6]. This can be detected in saliva, gingival crevicular fluid (GCF) and serum in both health and periodontitis [7]. Increased concentration observed in periodontitis correlate closely with the tissue destruction and immune response [8,9].

Enhanced expressions of serum TNF- $\alpha$  have been observed in smokers in rheumatoid arthritis and chronic obstructive pulmonary disease (COPD). Also, up-regulation of its expression in keratinocytes in chronic inflammatory skin diseases like psoriasis has also been observed [10]. This clearly indicates the role of TNF- $\alpha$  in smokers and chronic inflammation. Also, serum cytokines are increasingly being correlated with periodontal status and oral inflammatory burden in recent times [8,11].

In general, pathogenic species and their products are able to stimulate the production of a number of pro-inflammatory cytokines, including interleukin (IL)-1b and IL-6 and tumor necrosis factor-alpha (TNF- $\alpha$ ), which coordinate a local inflammatory response [12,13-25].

The role of TNF- $\alpha$  in the host immune response to local infection has been well documented in the literature. This cytokine triggers the production of adhesion molecules, pro-inflammatory cytokines, and chemokines, such as IL-1a, IL-1b, IL-6, and IL-8 and matrix metalloproteinases [14, 26-35].

In addition, TNF- $\alpha$  may significantly stimulate local bone resorption by inducing osteoclastogenesis and influencing the production of the essential osteoclast differentiation factors, such as receptor activator of nuclear factor-kappa B ligand and its soluble decoy receptor, osteoprotegerin [15, 36-45].

So the present study was carried out to evaluate the effect of smoking on levels of serum TNF- $\alpha$ .

## MATERIALS AND METHODS

A total of 40 patients were selected from the Out Patient Department of Periodontology, People's College of Dental Sciences and Research Centre, Bhanpur, Bhopal. They were divided into 2 groups of patients aged between 24-60 years.

The total study population was divided into 2 groups:

Group A: 20 non smoker subjects, who were systemically and periodontally healthy.

Group B: 20 smoker subjects who were systemically and periodontally healthy.

### Inclusion criteria

Patients should have at least 20 permanent teeth.

For Smokers: patients who smoked >10 cigarettes/day for more than 3 years

For Non Smokers: patients who have no history of smoking.

For Healthy Periodontium: periodontal probing depth as well as clinical attachment level  $\leq$  3mm.

### Exclusion criteria

Patients suffering from chronic systemic diseases.

Pregnant and lactating females.

Patients taking any medication 6 months prior to study other than vitamins or occasional analgesics.

Patients undergoing radiotherapy to head and neck region.

The nature and purpose of the study was explained to the patients and an informed consent was obtained from every patient. A detailed case history was recorded in a prepared proforma which included information regarding the patient's age, gender, past medical history and dental history including smoking history and various clinical parameters.

### COLLECTION OF BLOOD

From the selected patients, 5ml. of blood was withdrawn from the antecubital vein to evaluate the levels of Tumor Necrosis Factor alpha (TNF alpha) in serum through Human Serum ELISA detection.

### BIOCHEMICAL ANALYSIS

Biochemical analysis was carried out at Centre for Scientific Research and Development (CSR), People's University, Bhopal.

### ELISA KITS

- Tumor Necrosis Factor- $\alpha$  (TNF-  $\alpha$ ) is a potent lymphoid factor which exerts cytotoxic effects on a wide range of tumor cells and certain other target cells. Human TNF-  $\alpha$  is a 17.4 kD protein containing 157 amino acid residues.

### Intended Use

Human ELISA Kits are specifically designed for the accurate quantification of human TNF- $\alpha$ , from cell culture supernatant, serum, plasma or other bodily fluids. It is ready-to-use, accurate, and sensitive.

### STATISTICAL ANALYSIS

The data obtained was subjected to statistical analysis with the consult of a statistician. The data so obtained was compiled systematically. A master table was prepared and the total data was subdivided and distributed meaningfully and presented as individual

tables along with graph

Statistical procedures were carried out in 2 steps:

- Data compilation and presentation
- Statistical analysis

Statistical analysis was done using Statistical Package of Social Science (SPSS Version 20; Chicago Inc., USA). Data comparison was done by applying specific statistical tests to find out the statistical significance of the comparisons. Quantitative variables were compared using mean values and qualitative variables using proportions.

Significance level was fixed at  $P < 0.05$ .

## DISCUSSION

Cytokines are key modulators of inflammation. They participate in acute and chronic inflammation in a complex network of interactions. Several cytokines exhibit some redundancy in function and share overlapping properties as well as subunits of their cell surface receptors [60].

Periodontal diseases are characterized by the classic hallmarks of the inflammatory response, including erythema, and edema. Late sequelae of periodontal diseases include the loss of alveolar bone, periodontal ligament attachment, and ultimately, teeth. Therefore, periodontal disease can be viewed as a chronic inflammatory process in which bacteria-induced localized gingival inflammation results in destruction of bone and the attachment apparatus of the teeth [61]. It has also been considered a risk for a variety of systemic conditions, including cardiovascular disease, diabetes mellitus, rheumatoid arthritis, and respiratory disorders [62]. When the relationship between periodontitis and Rheumatoid Arthritis (RA) was examined, the findings suggested that circulating TNF- $\alpha$  is related to periodontal inflammation with regard to tissue destruction and vascular reaction in patients with RA [61].

## ROLE OF TNF ALPHA IN PATHOGENESIS OF PERIODONTITIS

Periodontitis is initiated by specific bacteria and the local host response to these bacteria includes the recruitment of leukocytes and the subsequent release of inflammatory mediators and cytokines such as interleukin (IL)-1, IL-6, IL-8, IL-10, IL-12 and TNF- $\alpha$ , which are thought to play an important role in the pathogenesis of the disease. These increased levels of several cytokines are involved in periodontal tissue destruction [21].

TNF- $\alpha$  is also a monocyte-derived protein that has a wide range of proinflammatory and immunomodulatory effects on a number of different cell populations. TNF- $\alpha$  can stimulate fibroblasts, including gingival fibroblasts, to produce collagenase [21], an enzyme implicated in the tissue destruction of

periodontal disease, and to stimulate bone resorption. TNF- $\alpha$  activates monocytes and stimulates the production of IL-1 $\beta$ , platelet activating factor and prostaglandins. Monocyte stimulation by lipopolysaccharide enhances the production of TNF- $\alpha$ , which has also been shown to induce collagenase release and bone resorption in vivo [21].

Pro-inflammatory cytokines (tumor necrosis factor [TNF]- $\alpha$ , interleukin [IL]-1 $\alpha$  and IL-1 $\beta$ ) are necessary for initiating an effective inflammatory process against infection. TNF- $\alpha$  also activates osteoclasts and thus induces bone resorption and has synergistic effects with the bone-resorptive actions of IL-1 $\beta$  [61].

Studies have shown a positive correlation between IL-1 $\beta$  and TNF- $\alpha$  in chronic periodontitis patients [63].

Reddy N.R. *et al.* [64] have shown a positive correlation of TNF- $\alpha$  concentration with the extent of periodontal destruction [64]. Level of circulating TNF- $\alpha$  in serum has been seen to have decreased following periodontal therapy [65, 66]. The concentration of IL-1 $\beta$  was reported to be higher in gingival crevicular fluid in cases of chronic periodontitis patients than in gingivitis and control group.

## TNF ALPHA AND ITS ASSOCIATION WITH SYSTEMIC DISEASES:

The levels of these cytokines were found to be positively correlated with systemic disease. The levels of IL-1 $\beta$  and TNF- $\alpha$  were higher in serum of diabetic patients with periodontal disease [6]. IL-6 and TNF- $\alpha$  concentrations were little higher in serum of patients with type-2 diabetes mellitus than that of control group [69].

The level of TNF- $\alpha$  level was significantly higher in patients with osteoporosis. It was concluded that osteoporosis patients are prone to overproduce TNF- $\alpha$ , which also activates the B-cells and promotes the B-cell activity in the periodontal inflammatory sites, aggravating the periodontal disease [67].

1. TNF- $\alpha$  level in serum act as diagnostic marker of periodontal disease in patients with Alzheimer disease [68].

## SMOKING AND TNF ALPHA

Smoking is one of the major environmental risk factors for periodontal diseases [21].

Active inflammatory cells produce various inflammatory mediators in response to smoking, and inflammatory cytokines are the important of them. Importance of smoking in increasing inflammatory cytokines has been reported in some previous studies [59].

Smoking does not seem to influence the subgingival colonization of some important periodontal pathogens as most previous studies suggest that smoking and non-smoking patients largely exhibit the same microflora [21].

This suggests as a possible alternative that smoking interferes with the immune response of the host. Tobacco components may also modify the production of cytokines or inflammatory mediators. Smokers have been reported to have increased gingival crevicular fluid (GCF) levels of TNF- $\alpha$ . Such alterations in host response may affect the reparative and regenerative potential of the periodontium in tobacco smokers [21, 46-50].

Some other studies also report increased production of TNF- $\alpha$  and serum or plasma concentrations in smokers than non-smokers [73, 74] and although similar to the findings of this study, some studies report no significant difference in the inflammatory cytokine between smokers and non smokers [70-72].

#### INTERPRETATION OF THE RESULTS

On comparing the results of mean values of serum TNF alpha between smokers+healthy and non smoker+healthy subjects, there was statistically no significant difference in Serum TNF- $\alpha$  Cytokine among Smokers & Non-Smokers Healthy subjects. (P=0.892).

These results led us to surmise that there is no relation between smoking status and serum values of TNF alpha. There are various other studies to support this hypothesis.

Our results are in accordance with a similar study conducted by Erdemir EO *et al.* [21, 51-55] which demonstrated that cigarette smoking increases the amount of dental plaque over time in smokers and does not influence GCF contents of IL-6 and TNF-alpha.

These data do not support the inflammatory property of smoking. Further studies are needed to clarify possible mechanisms by which smoking affects systemic inflammation by TNF- $\alpha$  or other cytokines.

Periodontitis is a chronic bacterial infection characterized by persistent inflammation, connective tissue breakdown and alveolar bone destruction mediated by pro-inflammatory mediators. Tumor necrosis factor-alpha (TNF- $\alpha$ ) is a pro-inflammatory cytokine released by macrophages which is known for its substantial role in periodontitis mediated bone loss [6]. This can be detected in saliva, gingival crevicular fluid (GCF) and serum in both health and periodontitis [7]. Increased concentration observed in periodontitis correlate closely with the tissue destruction and immune response [8,9].

Increased level of TNF- $\alpha$  in serum is related with an inflammatory state. High numbers of inflammatory cells in the connective tissue and gingival crevice can lead to the release of TNF- $\alpha$  upon stimulation by the bacterial products [69].

TNF- $\alpha$  may be synthesized and secreted by the local periodontal connective tissue cells, such as fibroblasts and endothelial cells or by infiltrating leukocytes i.e. mononuclear cells, macrophages and neutrophils.

#### MEAN GINGIVAL INDEX SCORE

Mean Gingival index in smokers+healthy was (1.26 $\pm$ 0.38) and among Non-Smokers+healthy was (0.032 $\pm$ 0.011) subjects. There was statistically highly significant difference in mean Gingival Index among Smokers & Non-Smokers Healthy (P=0.001).

These results are in accordance with the study conducted by Kanakdande V *et al.* [68] in 2015, where the mean gingival scores were higher in smokers than in non smokers, with statistically significant difference of values in both the group.

#### MEAN PLAQUE INDEX SCORE-

It was (2.12 $\pm$ 0.50) among Smokers+Healthy and (0.029 $\pm$ 0.013) among Non-Smokers+Healthy subjects. There was statistically highly significant difference in mean Plaque Index among Smokers & Non-Smokers Healthy (P=0.001).

A similar study by Kanakdande V *et al.* [68] also found that the mean plaque scores were higher in smokers than in non smokers, which was statistically significant.

#### MEAN PROBING POCKET DEPTH-

The Mean Probing Pocket Depth were (1.83 $\pm$ 0.37) among Smokers+Healthy and 1.49 $\pm$ 0.18 among Non-Smokers+Healthy subjects. There was statistically highly significant difference in mean Probing Pocket Depth among Smokers & Non-Smokers+Healthy (P=0.001).

A similar study by Kanakdande V *et al.* [68] also found that the probing depth were higher in smokers than in non smokers, with the differences being statistically significant.

#### MEAN CLINICAL ATTACHMENT LOSS-

The Mean Clinical Attachment Loss was 1.88 $\pm$ 0.39 among Smokers+Healthy and 1.49 $\pm$ 0.18 among Non-Smokers+Healthy subjects. There was statistically highly significant difference in mean Clinical Attachment Loss among Smokers & Non-Smokers Healthy (P=0.001).

These results are in accordance with the study by Kanakdande V *et al.* [68], which showed that the

values of clinical attachment loss were higher in smokers than in non smokers, with the differences being statistically significant.

As per the results and observations of our study, there was no statistically significant difference in mean values of serum TNF alpha between smokers and non smoker groups. This leads us to conclude that smoking status has no significant impact on serum TNF alpha levels.

Based on these findings, we can conclude that smoking deteriorates the periodontal health, which is depicted by the increased values of various clinical parameters like probing pocket depth, gingival index scores, plaque index scores and mean clinical attachment levels.

### LIMITATIONS

Although bacteria are the primary etiologic factors in periodontal disease, the patient's host response is a determinant of disease susceptibility, and smoking also interferes with the immune response of the host. The presence of excessive amount of subgingival and supragingival plaque makes the evaluation of the effect of smoking on periodontal health extremely difficult. Therefore, in the studies of smoking and periodontal health, the effect of oral hygiene must be controlled and higher oral hygiene must be provided [21].

Within the limitations of this study, it is clear that additional studies with a larger sample size would be needed to draw any final conclusions regarding the effect of smoking on serum TNF alpha.

Difficulty in determining active disease and ongoing destruction in periodontal tissue by traditional diagnostic aids like probing depth and attachment loss has proved them to be inadequate in the modern era of periodontal therapeutics [5]. Search for a biomarker for periodontitis has resulted in researchers trying out and finding new molecules that can guide a clinician in many a decision regarding the patient's condition.

### CONCLUSION

These observations suggest a positive association between periodontal disease and increased levels of TNF-a in serum. It can be concluded that there is a prospect of using the estimation of TNF-a in serum as a "marker" of periodontal disease in future.

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