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ORIGINAL ARTICLE

Estimation of Salivary Cytokine TNF-α in Chronic and Aggressive Periodontitis: A Case Control Study

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ABSTRACT:

Introduction: Periodontitis is defined as an inflammatory disease of supporting tissues of teeth caused by specific microorganisms or groups of specific microorganisms, resulting in progressive destruction of the periodontal ligament and alveolar bone mediated by pro-inflammatory mediators. Tumor necrosis factor-alpha (TNF-α) is an important pro-inflammatory mediator that produced causes destruction of periodontal tissues. **Aim**: The aim of the study is to estimate the salivary TNF-α in chronic and aggressive periodontitis and control subjects and further correlate the levels with clinical parameter such as gingival index (GI), plaque index (PI), probing pocket depth (PPD) and clinical attachment loss. Materials and methods: The study consisted of 90 subjects divided into groups Groups 1 (control), 2 (generalized chronic periodontitis) and 3(aggressive periodontitis). Salivary samples from the participants were used to assess the TNF-α levels using enzyme-linked immunosorbent assay. Results: GI and PI were found to be significantly higher in chronic and aggressive periodontitis compared to the controls. The mean TNF-α value in chronic periodontitis patients (11.8±0.40pg/ml) and aggressive periodontitis patients (10.4±0.52) was significantly higher than in control subjects (3.6±0.25 pg/ml). Among periodontitis patients, aggressive periodontitis subjects exhibited a significant positive correlation between the salivary TNF-α and PPD. Conclusion: Salivary TNF-α levels are significantly higher in chronic periodontitis than in healthy subjects, but there was no significant correlation with the clinical parameters.

Key words: Chronic periodontitis, Aggressive periodontitis, tumor necrosis factor.

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INTRODUCTION:

Periodontitis is defined as an inflammatory disease of supporting tissues of teeth caused by specific microorganisms or groups of specific microorganisms, resulting in progressive destruction of the periodontal ligament and alveolar bone mediated by pro-inflammatory mediators.[1] Inflammatory mediators and tissue breakdown products have been frequently detected in gingival tissues, gingival crevicular fluid, serum, and saliva. Periodontal pathogens activate the host immunity- related responses against the bacterial antigens and lead to stimulation a series of cytokines that play an important role in the immune response of patients.[2]

Tumor necrosis factor-alpha (TNF-α) cachexin, or cachectin is an important pro-inflammatory mediator that produced causes destruction of periodontal tissues. It is a cell signaling protein (cytokine) and is one of the cytokines that make up the acute phase reaction. TNF is active in 2 forms: TNF-α and TNF-β. TNF-α is produced by the activated macrophages, neutrophils, keratinocytes, monocytes, and mast cells in response to lipopolysaccharides (LPSs). TNF-β is produced by TH1 subsets of CD4+ T cells that have been activated by antigens or mitogens.[3,4] The primary role of TNF is in the regulation of immune cells. TNF, being an endogenous pyrogen, is able to induce fever, inflammation and to inhibit tumorigenesis and viral replication.

Out of all the various biological media, saliva is widely studied because of its non invasive method of collection , readily availability and it contains both locally and systemically produced serum markers which are of significant diagnostic importance. It has long been used to assess periodontitis and its role as a diagnostic medium

has been a subject of considerable research activity. The various biomarkers in saliva of diseased person help in identifying susceptible patients and serve as surrogate endpoints for monitoring the end of therapy.[5]
Thus this study aimed to estimate and compare salivary TNF-α levels in patients with generalized chronic periodontitis, generalized aggressive periodontitis and healthy periodontium and also to assess the correlation of TNF-α with the clinical variables.

MATERIALS AND METHODS:

The study comprised 90 subjects (45 males and 45 females) which were divided into three major groups. Groups 1 included 30 subjects (15 males and 15 females) with healthy periodontium, group 2 included 30 subjects (15 males and 15 females) with chronic periodontitis and group 3 included rest 30 subjects (15 males and 15 females) with generalized aggressive periodontitis.
Inclusion criteria comprised of patients in the age range of 18–45 years with a minimum of 18 teeth. Group 1 patients had a healthy periodontium with no gingival inflammation (gingival index [GI] = 0; pocket depth ≤3 mm and clinical attachment loss = 0). Patients were categorized as generalized chronic or aggressive periodontitis based on the American Academy of Periodontology criteria. Only those participants who presented with deep pockets with a minimal subgingival plaque and healthy tissue response, free of inflammation were selected for aggressive periodontitis category. Exclusion criteria consisted of patients with systemic diseases, patients on medications, patients who gave a history of periodontal treatment in the last 3 months, Smokers and alcoholics. Plaque index (PI), GI, periodontal pocket depth and loss of attachment was measured by using a Williams periodontal probe after the salivary sample collection.
An informed consent was taken from the patients prior to the study. Patients were instructed to rinse their mouth with water followed which unstimulated whole expectorated salivary samples were collected into sterile containers and stored.
The subjects washed their mouth with water before sampling; then their unstimulated saliva was collected in 5 ml sterile tubes. The samples were immediately centrifuged at 10,000 rpm for 10 min, in order to remove cells and microparticulate material. The clear supernatant of the centrifuged and collected.The assay was then carried out with a commercially available enzyme-linked immunosorbent assay kit (human quantitative high sensitivity TNF-α assay by R&D system using ELISA).

STATISTICAL ANALYSIS

The overall comparison of mean values from the study groups was done by Kruskal–Wallis one-way ANOVA. Comparison between any two study groups was done by Mann–Whitney U-test. The correlation between TNF-α and the clinical variables in each group was done by Spearman's correlation test.

RESULTS:

As compared to group 1 (controls) significantly higher results ($P < 0.05$) of both gingival and periodontal index were seen in both group 2 (chronic periodontits) and 3 (aggressive periodontitis). Among group 2 and 3, Group 2 showed significantly higher results of PI. There were no statistically significant differences in mean periodontal pocket depth and clinical attachment level between Groups 2 and 3. (TABLE 1)
The TNF-α was detected in all saliva samples of the three groups and the average concentrations of TNF-α in the experimental groups were calculated as 3.6 (pg/ml) in the group 1 and 11.8 (pg/ml) in group 2 and 10.4 (pg/ml) in group 3. Thus the mean TNF-α value in Group 2 (chronic periodontitis) and group 3 (aggressive periodontitis) patients was significantly higher than in control subjects (Group 1).
However, there was no significant difference in TNF-α values between Group 2 and 3. (TABLE 2). When clinical parameters were compared with among all the three groups, it was found that only Group 3 (aggressive periodontitis) subjects had a significant positive correlation between the salivary TNF-α and periodontal pocket depth.

TABLE 1: Comparison of clinical variables (PI, GI, PD and CAL) between all three study groups

	PI	GI	PD	CAL
GROUP1	0.25±0.55	0.27±0.30	-	00±0.00
GROUP 2	1.35±0.35	1.85±0.25	7.23±0.32	7.1±0.30
GROUP 3	1.39±0.26	0.75±0.40	6.9±0.30	7.0±0.40

(PI: Periodontal index, GI: gingival index, PD: probing depth, CAL: clinical attatchment loss)

TABLE 2: Comparison of TNF-α among different study groups

GROUPS	MEAN TNF-α (pg/ml)
GROUP1	3.6±0.25
GROUP 2	11.8±0.40
GROUP 3	10.4±0.52

DISCUSSION:

In this study among the various biological media, saliva was chosen because of its non invasive method of collection, readily availability and it contains both locally and systemically produced serum markers which are of significant diagnostic importance. Unstimulated saliva (Whole saliva) was preferred and collected for use in the study, as the salivary composition is altered in stimulated saliva.[6,7]

The results of this study indicated a significant increase in salivary TNF-α levels in generalized chronic periodontitis patients (group 2) when compared to healthy controls (group 1). These findings were in accordance with a study by Geng *et al* who also stated that the levels of TNF-α and IL-6 cytokines were higher in patients with chronic periodontitis than the healthy individuals.[8] In another study conducted by Rai *et al*. it was found salivary levels of TNF-α were significantly higher in patients with periodontitis than the control group ($P < 0.001$).[9]

Salivary TNF-α levels of aggressive periodontitis (group 3) subjects were also found to be significantly higher when compared with the healthy controls (group 1).Though no significant difference in TNF-α values was seen when Group 2 and 3 were compared amongst each other. The results of this study were in accordance with the study of Gumus *et al*. Who found that salivary TNF-α levels were significantly higher in aggressive periodontitis group than healthy controls.[10] Where as in contrary Sun *et al*. reported that plasma TNF-α levels were not significantly different between aggressive periodontitis and healthy controls.[11]

Salivary TNF-α levels showed no correlation with most of the clinical parameters except the probing pocket depth (PPD) which showed significant positive correlation with salivary TNF-α in aggressive periodontitis group (Group 3).

CONCLUSION:

From this study we conclude that as Salivary TNF-α levels are significantly elevated in chronic periodontitis and aggressive periodontitis when compared to healthy subjects, It can be suggested that the salivary TNF-α analysis can be used as a useful diagnostic tool and as a prognostic biomarker to diagnose chronic and agressive periodontitis. Due to variable results seen in literature accounted by different authors,we hereby suggest more studies with a larger sample size to further evaluate the role of TNF-α in periodontitis patients so that this can be helpful in identifying susceptible patients and serve as surrogate endpoints for monitoring the end of therapy.

REFERENCES:

1. Newman MG, Carranza FA, Takei H, Klokkevold PR. Carranzas clinical Periodontology. 10th ed. Elsevier health sciences; 2006.
2. Benakanakere M, Kinane DF. Innate cellular responses to the periodontal biofilm. Front Oral Biol 2012;15:41-55.
3. Olszewski, et al. (2007). "TNF Trafficking to Human Mast Cell Granules: Mature Chain-Dependent Endocytosis". The Journal of Immunology. 178: 5701–5709.
4. Newman MG, Takei HH, Klokkevold PR, Carranza FA. Clinical Periodontology. 10th ed. Northwestern University, United States: Saunders Elsevier; 2006. Microbial interactions with the host in periodontal disease; p. 239
5. Varghese S, Thomas H, Jayakumar ND, Sankari M, and Lakshman R :Estimation of salivary tumor necrosis factor-alpha in chronic and aggressive periodontitis patients. Contemp Clin Dent. Sep; 6(Suppl 1): S152–S156.
6. Polland KE, Higgins F, Orchardson R. Salivary flow rate and pH during prolonged gum chewing in humans. J Oral Rehabil. 2003;30:861–5.
7. Nederfors T, Nauntofte B, Twetman S. Effects of furosemide andbendroflumethiazide on saliva flow rate and composition. Arch Oral Biol. 2004;49:507–13.
8. Geng W, Ying-Hui T, Sheng-Gen S. Expression of IL-6 and TNF-α activities in saliva of chronic periodontitic patients. Chin J Conserv Dent. 2009;5:11.
9. Rai B. Salivary levels of tumor necrosis factor-alpha in periodontitis. Adv Med Dent Sci. 2008;2:40–1.
10. Gumus P, Nizam N, Lappin DF, Buduneli N. Saliva and serum levels of B-cell activating factors and tumor necrosis factor – A in patients with periodontitis. J Periodontol. 2014;85:270–80.
11. Sun XJ, Meng HX, Chen ZB. Levels of interleukin-1beta and tumor necrosis factor-alpha in patients with aggressive periodontitis. Beijing Da Xue Xue Bao. 2008;40:24–7.

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