Original Article

Salivary Tumor Necrosis Factor-Alpha Levels In Nonsmokers And Smokers With Chronic Periodontitis Before And After Phase-I Periodontal Therapy: A Comparative Study

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Abstract

Introduction: The proinflammatory protein TNF-alpha (TNF- α) is one of the pathophysiologies that has been thought to be influenced by inflammatory cytokines. As tissue damage agents and a biomarker for periodontal disease development, TNF-α levels are higher in the oral fluids of smokers. Recent developments have made it possible to utilize saliva as a diagnostic fluid to identify the concentrations of these biomarkers.

Methods: Before and after phase-I therapy, salivary TNF- levels were measured in nonsmokers with chronic periodontitis (control group, n=15) and in smokers with chronic periodontitis (study group, n=15). The results were compared with clinical parameters like gingival bleeding index, plaque index, probing pocket depth, and clinical attachment level.

Results: At baseline, smokers had higher salivary TNF- α than nonsmokers. Both smokers and nonsmokers saw a clinical parameter and salivary TNF-α level decrease that was statistically significant (p=0.00) three months following Phase-I treatment. However, compared to smokers, nonsmokers had a substantially higher decrease in clinical parameters and salivary TNF-α levels.

Conclusion: The current data demonstrate that smoking is related to increased levels of salivary TNF-α and that nonsmokers respond better to phase-I treatment than smokers, as seen by a considerable drop in salivary TNF-α levels.

Keywords: Smoking, TNF-α, Phase-I Therapy, Chronic Periodontitis

Introduction:

A chronic bacterial infection called periodontitis is characterized by elongated inflammation, connective tissue deterioration, and alveolar bone loss. An inflammatory reaction in the body is brought on by microbial antigens found in the periodontal tissue. A protracted yet unprotected host response, which may be mediated by genetic, environmental, and systemic variables, is linked to tissue damage and disease development in periodontal disorders. Proinflammatory cytokines, kinins, and eicosanoids are released over a prolonged period due to this host response[1]. Proinflammatory cytokines play a crucial role in cell signaling, encourage systemic inflammation, and regulate inflammatory responses. Antiinflammatory cytokines fight back and slow down disease development, yet they are associated with significant tissue damage. One such proinflammatory cytokine is tumor necrosis factor-alpha (TNF-α), which has several effects, including facilitating leukocyte recruitment to the site of inflammation, changing vascular permeability by stimulating the expression of selectins and adhesins, inducing the production of interleukins and prostaglandins, and inducing vascular proliferation in the development of periodontal granulation tissue[2]. Excessive bone resorption comes from TNF- production because it promotes osteoclastogenesis by activating the RANK-RANKL coupling mechanism and by raising the levels of other proinflammatory cytokines such as IL-1, 6, and 8[(3]). Smoking has been discovered to significantly impact the immune system's defense mechanisms, causing periodontal damage to be more extensive and severe. However, in terms of the periopathogens, smokers and nonsmokers with the periodontal disease typically have a comparable micro flora [4]. Smoking changes vascular function, neutrophil/monocyte activity, adhesion molecule expression, antibody formation, and the release of cytokines and inflammatory mediators [5]. These alterations most likely lead to smoking's detrimental effects on the periodontium's capacity for repair and regeneration. Periodontal disease is treated with both non-surgical and surgical methods. Scaling and root planing are still the mainstays of non-surgical periodontal treatments for halting disease development. Gingivitis to periodontitis is a lengthy process leading to attachment loss. This also causes fast attachment loss in episodic bursts in short periods [6]. Rich vasculature exists in salivary glands, where saliva is filtered and processed. Salivary glands may be the only source of its constituent parts, or blood may be used to create saliva by passive diffusion or active transport. Levels of biochemical and immunological components evaluated in saliva may correspond to blood levels when such components are taken from the blood[7]. When compared to 39 healthy controls and 35 participants with moderate to severe chronic periodontitis, the salivary concentrations of TNF-, RANKL, and ICTP were examined by Frodge et al. (2008)[8]. According to the scientists, individuals with chronic periodontitis had considerably higher salivary TNF levels, which may make it easier to detect, diagnose, and treat periodontal illnesses.

Materials and Methods:

Patients who attended the outpatient Department of Periodontology, Tamilnadu Government Dental College and Hospital, Chennai, were randomly selected and enrolled in the study. A total of 30 subjects were selected. The subjects were divided into two groups based on the habit of smoking:

Group 1 – Control group (15 subjects)

Group 2 – Study group (15 subjects)

Inclusion criteria

· Age 30-60years.

· Gender – male.

· Systemically healthy individuals.

· Generalized Chronic Periodontitis - loss of attachment ³5mm and bone loss

in radiograph (involving more than 30% of the sites).

· Current smokers - ³10 cigarettes per day for more than one year (only cigarette

smokers).

Clinical parameter:

Following clinical parameters were estimated in Group-I and Group II subjects before and after phase-I therapy.

o Plaque index – Silness and Loe 1964[9]

o Gingival bleeding index – Ainamo and Bay 1975[10]

o Probing depth in mm (PD) – Carranza 10th ed[11]

o Clinical attachment level in mm (CAL) – Carranza 10th ed[11]

Saliva collection: Disposable syringe, Eppendorf's tube.

Before eating breakfast, the individuals' saliva was collected after they had rinsed their mouths with water. Afterward, participants were told to spit the collected saliva (2ml) into an Eppendorf tube. All individuals (group I and group II) had saliva samples obtained at baseline before treatment and three months after surgery. The samples were utilized for further ELISA analysis after being centrifuged at 2600 rpm and kept at -80° C. The Enzyme-Linked Immunosorbent Assay (ELISA) technique (Bioassay Technology Laboratory®) was used to measure salivary TNF-α quantitatively.

Statistical Analysis: The SPSS V20 statistical tool (Statistical Package for Social Science, Version 120; SPSS Inc.; Chicago IL, USA) was utilized.

Comparison between groups At baseline, the mean difference between groups I and II was 9.7301.330, which was statistically significant (p=0.000), and it was 10.1140.925 at three months post-op, which was statistically non-significant (p=0.000).

Results: At baseline, smokers had higher salivary TNF levels than nonsmokers. Both smokers and nonsmokers saw a clinical parameter and salivary TNF-level decrease that was statistically significant (p=0.00) three months following Phase-I treatment. However, nonsmokers had a substantially higher decrease in clinical parameters and salivary TNF-level compared to smokers.(Table 1-5)

Table 1: COMPARISON OF CLINICAL PARAMETERS AT BASELINE AND

3MONTHS AFTER PHASE-I THERAPY

GROUP –I

Variables	Paired Differenc es					t	df	Sig. (2tailed)
	Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval				
				Lower	Upper			
GBI BL - GBI 3M	67.168	2.772	.715	65.632	68.703	93.840	14	.000
PPD BL - PPD 3M	2.366	.829	.214	1.906	2.825	11.051	14	.000
CAL BL - CAL 3M	2.366	.825	.213	1.909	2.823	11.102	14	.000
PI BL - PI 3M	1.340	.293	.075	1.177	1.502	17.688	14	.000
TNF-α BL - TNF-α 3M	15.548	1.102	.284	14.937	16.158	54.601	14	.000

GROUP-II

Variables	Paired Differenc es					t	df	Sig. (2tailed)
	Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval				
				Lower	Upper			
GBI BL - GBI 3M	62.692	5.468	1.411	59.663	65.720	44.404	14	.000
PPD BL - PPD 3M	2.766	.479	.123	2.500	3.031	22.331	14	.000
CAL BL - CAL 3M	2.765	.474	.122	2.502	3.028	22.551	14	.000
PI BL - PI 3M	1.302	.242	.062	1.168	1.436	20.835	14	.000
TNF-α BL - TNF-α 3M	15.164	1.135	.293	14.535	15.793	51.702	14	.000

Table 2: COMPARISON OF CLINICAL PARAMETERS BETWEEN CONTROL AND STUDY GROUPS

BASELINE

Variables	Differences				t	df	Sig. (2tailed)
	Mean	Std. Error Mean	95% Confidence Interval				
			Lower	Upper			
G-I GBI BL – G-II GBI BL	3.150	2.350	1.665	7.965	1.339	28	0.095
G-I PPD BL - G-II PPD BL	.803	.389	.005	1.601	2.062	28	0.024
G-I CAL BL - G-IICAL BL	.802	.390	.001	1.602	2.053	28	0.024
G-I PI BL – G-II PI BL	.016	.143	.278	.311	0.115	28	0.454

3 MONTHS

Variables	Differences				t	df	Sig. (2tailed)
	Mean	Std. Error Mean	95% Confidence Interval				
			Lower	Upper			
G-I GBI 3M - G-II GBI 3M	1.326	2.092	2.960	5.612	0.633	28	0.265
G-I PPD 3M - G-II PPD 3M	.403	.262	.133	.940	1.538	28	0.067
G-I CAL 3M - G-II CAL 3M	.419	.288	3.601	4.782	14.549	28	.000
G-I PI 3M - G-II PI 3M	.020	.077	.137	.178	0.267	28	0.395

Table 3: CORRELATION BETWEEN CLINICAL PARAMETERS AND TNF-α LEVEL AT BASELINE AND 3MONTHS AFTER PHASE-I THERAPY

GROUP-I

CLINICAL PARAMETERS		TNF-α
BASELINE GBI	Pearson Correlation	-.483
	Sig. (2-tailed)	.068

BASELINE PPD	**Pearson Correlation**	**.990**
	Sig. (2-tailed)	**.000**
BASELINE CAL	**Pearson Correlation**	**.990**
	Sig. (2-tailed)	**.000**
BASELINE PI	**Pearson Correlation**	**.416**
	Sig. (2-tailed)	**.123**

CLINICAL PARAMETERS		**TNF-α**
3 MONTHS GBI	**Pearson Correlation**	**-.280**
	Sig. (2-tailed)	**.313**
3 MONTHS PPD	**Pearson Correlation**	**.677**
	Sig. (2-tailed)	**.006**
3 MONTHS CAL	**Pearson Correlation**	**.682**
	Sig. (2-tailed)	**.005**
3 MONTHS PI	**Pearson Correlation**	**.073**
	Sig. (2-tailed)	**.795**

Table 4: CORRELATION BETWEEN CLINICAL PARAMETERS AND TNF-α LEVEL AT BASELINE AND 3MONTHS AFTER PHASE-I THERAPY

GROUP-II

CLINICAL PARAMETERS		**TNF-α**
BASELINE GBI	**Pearson Correlation**	**-.027**

	Sig. (2-tailed)	**.923**
BASELINE PPD	**Pearson Correlation**	**.970**
	Sig. (2-tailed)	**.000**
BASELINE CAL	**Pearson Correlation**	**.970**
	Sig. (2-tailed)	**.000**
BASELINE PI	**Pearson Correlation**	**-.357**
	Sig. (2-tailed)	**.191**

CLINICAL PARAMETERS		**TNF-α**
3 MONTHS GBI	**Pearson Correlation**	**-.075**
	Sig. (2-tailed)	**.790**
3 MONTHS PPD	**Pearson Correlation**	**.916**
	Sig. (2-tailed)	**.000**
3 MONTHS CAL	**Pearson Correlation**	**.920**
	Sig. (2-tailed)	**.000**
3 MONTHS PI	**Pearson Correlation**	**-.179**
	Sig. (2-tailed)	**.523**

Table 5: COMPARISON OF SALIVARY TNF-α LEVEL BETWEEN GROUP-I AND GROUP-II

AT BASELINE

Variables	Differences				t	df	Sig. (2tailed)
	Mean	Std. Error Mean	95% Confidence Interval				
			Lower	Upper			
G-I TNF-α BL vs. G-II TNF-α BL	9.730	1.330	7.005	12.456	7.313	28	.000

AT 3MONTHS

Variables	Differences				t	df	Sig. (2tailed)
	Mean	Std. Error Mean	95% Confidence Interval				
			Lower	Upper			
G-I TNF-α 3M vs. G-II TNF-α 3M	10.114	.925	8.218	12.009	10.931	28	.000

Discussion: According to Marugame et al. (2003)[12], periodontitis is an inflammatory condition of the tissues supporting the teeth brought on by various bacteria. If left untreated, it progresses to the deterioration of the alveolar bone and finally results in tooth loss. One of the pathophysiological routes leading to the degeneration of periodontal tissues is hypothesized to include an "inappropriate" cytokine milieu in that tissues[13]. This is another crucial physiologic mechanism through which periodontal disease exerts its systemic consequences. It is generally accepted that an imbalance between harmful proinflammatory cytokines and beneficial antiinflammatory cytokines causes inappropriate cytokine release**.** It has been discovered that smoking is linked to elevated TNF- levels, which alter the host response and the severity and scope of periodontal disease. The preservation of the teeth is the primary goal of periodontal therapy. Periodontal treatment should ideally reduce inflammation, halt the spread of the disease, restore lost periodontium, and foster an environment that discourages recurring illness. Both surgical and non-surgical methods are used to achieve these objectives. Scaling and root planing have been shown to halt the progression of periodontitis in several clinical trials that evaluated the effectiveness of non-surgical treatment [14-17]. In individuals with chronic periodontitis, scaling and root planing lowered gingival inflammation and reduced bleeding during probing. Since saliva is a non-invasive medium that contains a variety of protein indicators, its application as a

diagnostic tool has received a lot of interest over the years. However, it was believed that its drawback as a diagnostic tool was a lack of site-specificity and increased intra-individual variability. Because these illnesses might change the amount of TNF-, which may produce a confounding impact on the study, participants with any acute or chronic inflammatory disorders were excluded from the current investigation. Patients who had received periodontal therapy within the previous six months and those who had taken anti-inflammatory drugs, corticosteroids, or antibiotics within the previous three months were also excluded from the study because these treatments can suppress the inflammatory process and have confounding effects. The current investigation assessed 15 individuals from two groups with chronic periodontitis. Patients in group I was nonsmokers, whereas those in group II were smokers. Patients in both groups received phase-I periodontal treatment. At baseline and three months after treatment, clinical measures were assessed for each group, including gingival bleeding index, plaque index, pocket probing depth, and clinical attachment level. Additionally, the salivary TNF levels before and after treatment were compared between the two groups. Periodontal disorders are frequently diagnosed using the criteria of bleeding during probing[21]. This study's mean gingival bleeding index score (%) was 86.671 1.997 at baseline and decreased to 19.503 1.652 after phase-I therapy in group-I and to 83.521 1.239 in group II after phase-I therapy. Overall, the findings of this study show that salivary indicators should be read cautiously since they cannot precisely represent serum levels. However, since a statistically significant difference in TNF alpha levels was found, salivary cytokines may be reasonably accurate in detecting periodontal disease. Periodontal illnesses must be recognized to entail interactions between several cytokines participating in the inflammatory process. Environmental variables like smoking can change the amount of these cytokines and accelerate the course of periodontal disease. The study of a few cytokines cannot thus show the development of the inflammatory process in the periodontal tissues. To map the etiology of periodontal disorders, various other cytokines produced in the periodontal environment should also be examined. Therefore, a panel of salivary cytokines may be employed for diagnostic and prognostic reasons and may offer an accurate prediction of periodontal disease activity. The cross-sectional research design may reduce the quality of the evidence since the small sample size may not accurately reflect the community from which it is collected, it is susceptible to Neyman bias, and it may provide different results if a different period is used. Therefore, more research is necessary to evaluate the different cytokine profiles in periodontal disorders to forecast the course of the disease and the therapeutic success of the periodontal intervention.

Conclusion: Smokers with chronic periodontitis have higher baseline levels of salivary TNF- than nonsmokers with the same condition. Following phase-I periodontal treatment, there was a significant decline in clinical parameter values in both groups I and II. Both groups I and II saw a significant decrease in salivary TNF- levels following phase-I periodontal treatment. Compared to smokers, nonsmokers much more dramatically reduced their clinical parameters and salivary TNF levels after receiving phase-I treatment. Within the confines of this investigation, the amount of TNF- in saliva can be used as a biomarker for generalized chronic periodontitis diagnosis, assessment of therapy response, and prognosis. Salivary TNF- has been highlighted as a possible diagnostic tool; however, longer-term research with larger sample sizes are needed to validate these findings and explore if salivary TNF- estimate may be utilized as a chair-side diagnostic tool for periodontitis diagnosis in a clinical environment.

References

1. Page RC, Schroeder HE; Pathogenesis of inflammatory periodontal disease – A summary of current work Laboratory Investigation; Journal of Technical Methods and Pathology 1976; 34(3):235-249.
2. William V, Giannobile, Thomas Beikler, Janet S. Kinney, Christophar Amseier, Thiago morelli & David T. Wong; Periodontology 2000, 2009; 50(1):52-64.
3. Okada N, Kobayashi M, Mugikura K, Okamatsu Y, Hanazawa S, Kitano S, Interleukin-6 production in human fibroblasts derived from periodontal tissues is differentially regulated by cytokines and a glucocorticoid. J Periodontal Res 1997; 32, 559-569.
4. Bergstrom. J & Preber. H (1994) Tobacco use as a risk factor. Journal of Periodontology 65, 545–550.
5. Kinane D. F. & Chestnutt I. G. (2000) Smoking and periodontal disease. Critical Reviews in Oral Biology and Medicine 11, 356–365.
6. Soames JV, Entwisle DN, Davies RM; The progression of gingivitis to periodontitis in the beagle dog: a histological and morphometric investigation. Journal of Periodontology 1976; 47(8):435-439.
7. S. Chiappin, G. Antonelli, R. Gatti, and E. F. De Palo, “Saliva specimen: a new laboratory tool for diagnostic and basic investigation,” Clinica Chimica Acta, 2007;383(1-2):30–40.

8. Frodge, B. D., Ebersole, J. L., Kryscio, R. J., Thomas, M. V. & Miller, C. S. Bone remodeling biomarkers of periodontal disease in saliva. Journal of Periodontology 2008;79:1913–1919.
9. Silness J, Loe H. Periodontal disease in pregnancy. II. Correlation between oral hygiene and periodontal conditions. Acta Odontol Scand. 1964; 22: 121-35.
10. Ainamo J, Bay.I. Problems and proposals for recording gingivitis and plaque. Int. Dent. J 1975; 25: 229-235.
11. Carranza's Clinical Periodontology. Eleventh edition 2012.
12. Marugame T, Hayasaki H, Lee K, Eguchi H, Matsumoto S. Alveolar bone loss associated with glucose tolerance. Japanese men. Diabet Med 2003; 20:746- 751.
13. Kelso A. Cytokines in infectious disease. Aust Microbiol 1990;11:372-376 .
14. Badersten A, Nilveus R, Egelberg J. 4 year observations of basic periodontal therapy. J Clin Periodontol 1987; 14:438-444.
15. Philstrom B, Ortiz-Campos C, McHugh R. A randomized four-year study of periodontal therapy. J Periodontal 1981;52:227-242.
16. Philstrom B, McHugh R, Oliphant T, Ortiz-Campos C. Comparison of surgical and nonsurgical treatment of periodontal disease. A review of current studies and additional results after 6 1/2 years. J Clin Periodontol 1983;10:524-541.
17. Philstrom B, Oliphant T, McHugh R. Molar and nonmolar teeth compared over 6-1/2 years following two methods of periodontal therapy. J' Periodontol 1984;55:499-504.
18. Singletary MM, Crawford JJ, Simpson DM. Dark-field microscopic monitoring of subgingival bacteria during periodontal therapy. J Periodontal 1982; 53:671- 681.
19. Greenwell H, Bissada NF. Variations in subgingival microflora from healthy and intervention sites using probing depth and bactériologie identification criteria. J Periodontal 1984; 55:391-397.
20. Lavanchy D, Bickel M, Bachni P. The effect of plaque control after scaling and root planing on the subgingival microflora in human periodontitis. J Clin Periodontal 1987; 14:295-299.
21. Bergstrom, J., S. Eliasson, et al. (2000). "Exposure to tobacco smoking and periodontal health." J Clin Periodontol 27(1): 61-8.