

Comparative Evaluation of Serum Tumor Necrosis Factor α in Health and Chronic Periodontitis: A Case–Control Study

Abstract

Background: Tumor necrosis factor-alpha (TNF- α), 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- α in serum and its relationship to periodontal disease. **Aim:** Our study aimed to compare the serum TNF- α among the two study groups, namely, healthy controls and chronic periodontitis patients and establish a correlation between serum TNF- α and various clinical parameters. Hence, an attempt is made to estimate the level of TNF- α in serum, its relationship to periodontal disease and to explore the possibility of using the level of TNF- α in serum as a biochemical “marker” of periodontal disease. **Materials and Methods:** Forty individuals participated in the study and were grouped into two subgroups. Group A – 20 systemically and periodontally healthy controls. Group B – twenty patients with generalized chronic periodontitis. The serum samples were assayed for TNF- α levels by enzyme-linked immunosorbent assay method. **Results:** The mean serum TNF- α cytokines for Group B Generalized chronic periodontitis (GCP) was 2.977 ± 1.011 , and Group A (healthy) was 0.867 ± 0.865 . The range of serum TNF- α was from (0.867 to 2.977). Serum TNF- α cytokines had highly significant correlation with all clinical parameters (plaque index, probing pocket depth, clinical attachment loss, and gingival index) among all study participants ($P = 0.001$). **Conclusion:** These observations suggest a positive association between periodontal disease and increased levels of TNF- α in serum. It can be concluded that there is a prospect of using the estimation of TNF- α in serum as a “marker” of periodontal disease in future. However, it remains a possibility that the absence or low levels of TNF- α 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- α in serum as an “Indicator” of periodontal disease.

Keywords: Generalized chronic periodontitis, Inflammation, Tumor necrosis factor alpha

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 complex pathogenesis with both bacterial and host factors contributing to the destruction of periodontium. The role of host immune response is most important factor in periodontitis as it determines both disease progression and severity.^[2]

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

traditional diagnostic aids such as probing depth and attachment loss has proved them to be inadequate in the modern era of periodontal therapeutics.^[3-5] 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- α) 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- α have been observed in rheumatoid arthritis (RA)

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How to cite this article: Jain P, Ved A, Dubey R, Singh N, Parihar AS, Maytreeye R. Comparative evaluation of serum tumor necrosis factor α in health and chronic periodontitis: A case–control study. *Contemp Clin Dent* 2020;11:342-9.

Prince Jain,
Aditi Ved¹,
Rajat Dubey²,
Neha Singh³,
Anuj Singh Parihar⁴,
Rochira Maytreeye⁵

Consultant Periodontist
and Implantologist, Vidisha,

¹Dental Surgeon, Dental Planet
Clinic, Vidisha, ²Department
of Dentistry, Government
Hospital, Ratlam, ³Consultant
Periodontist, Bhopal, ⁴People’s
Dental Academy, Bhopal
Madhya Pradesh, ⁵Dental
Surgeon, Apollo Hospital,
New Delhi, India

Submitted : 16-Feb-2018

Accepted : 08-Apr-2020

Published : 20-Dec-2020

Address for correspondence:

Dr. Anuj Singh Parihar,
People’s Dental Academy,
Bhopal – 462037, Madhya
Pradesh, India.
E-mail: dr.anujparihar@gmail.
com

Access this article online

Website:

www.contempclindent.org

DOI: 10.4103/ccd.ccd_97_18

Quick Response Code:



and chronic obstructive pulmonary disease. In addition, upregulation of its expression in keratinocytes in chronic inflammatory skin diseases such as psoriasis has also been observed.^[10] This clearly indicates the role of TNF- α in chronic inflammation. In addition, 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 can stimulate the production of a number of pro-inflammatory cytokines, including interleukin (IL)-1b and IL-6 and TNF- α , which coordinate a local inflammatory response.^[12,13]

The role of TNF- α 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]

In addition, TNF- α 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.^[15-20]

Hence, the present study was carried out to compare and to correlate TNF- α levels between chronic periodontitis patients and healthy individuals.

Materials and Methods

A total of forty patients were selected from the outpatient department of Periodontology, People's College of Dental Sciences and Research Centre, Bhanpur, Bhopal. They were divided into four groups of patients aged between 24 and 60 years.

The total study population was divided into two groups:

- Group A – 20 systemically and periodontally healthy controls
- Group B – twenty patients with generalized chronic periodontitis.

Inclusion criteria

Patients should have at least 20 permanent teeth.

For chronic periodontitis – periodontal pockets ≥ 4 mm as well as clinical attachment loss (CAL) and bleeding on probing at more than 30% of sites (Armitage 1999)

For healthy periodontium – periodontal probing depth as well as clinical attachment level ≤ 3 mm.

Exclusion criteria

- Patients suffering from chronic systemic diseases
- Pregnant and lactating females
- Patients taking any medication 6 months before 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 informed consent was obtained from every patient. A detailed case history was recorded in a prepared pro forma which included information regarding the patient's age, gender, medical history and dental history including various clinical parameters.

Collection of blood

From the selected patients, 5 ml of blood was withdrawn from the antecubital vein to evaluate the levels of TNF- α in serum through human serum enzyme-linked immunosorbent assay (ELISA) detection.

Biochemical analysis

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

Enzyme-linked immunosorbent assay KITS

- TNF- α is a potent lymphoid factor which exerts cytotoxic effects on a wide range of tumor cells and certain other target cells. Human TNF- α 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- α , 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 were subdivided and distributed meaningfully and presented as individual tables along with graph.

Statistical procedures were carried out in two steps:

1. Data compilation and presentation
2. Statistical analysis.

Statistical analysis was done using Statistical Package of Social Science (SPSS Version 20; Chicago Inc., IL, 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.

The significance level was fixed at $P < 0.05$.

Interpretation of the results

Periodontitis is a chronic bacterial infection characterized by persistent inflammation, connective tissue breakdown, and alveolar bone destruction mediated by pro-inflammatory mediators. TNF- α is a pro-inflammatory cytokine released by macrophages which is known for its substantial role in periodontitis mediated bone loss.^[6] This

Table 1: Comparison of mean serum tumor necrosis factor-alpha cytokines among healthy and chronic generalized periodontitis patients

Groups	n	Serum TNF-α cytokines		
		Mean±SD	Median	Range
Healthy	20	0.867±0.865	0.47	0.116-2.87
CGP	20	2.977±1.011	3.26	1.11-4.50
Kruskal-Wallis test: χ^2		49.979		
Significance P		0.001 (HS)		

TNF-α: Tumor necrosis factor-alpha; CGP: Chronic generalized periodontitis; SD: Standard deviation; HS: Highly significant

Table 2: Comparison of mean serum TNF-α cytokines among smokers & non-smokers healthy subjects

Groups	n	Serum TNF-α cytokines		
		Mean±SD	Median	Range
Healthy	15	0.867±0.865	0.47	0.116-2.87
CGP	15	2.977±1.011	3.26	1.11-4.50
Mann-Whitney U-test value		28.000		
Significance P		0.001 (HS)		

TNF-α: Tumor necrosis factor-alpha; CGP: Chronic generalized periodontitis; SD: Standard deviation; HS: Highly significant

Table 3: Spearman’s correlation of serum tumor necrosis factor-alpha cytokines with all clinical parameter among healthy study participants

Clinical parameter	Serum TNF-α cytokines		
	Correlation coefficient (ρ)	P	Inference
GI	-0.245	0.297	Weak correlation
PI	0.059	0.804	No linear relationship
PPD	0.672**	0.001	Strong correlation
CAL	0.672**	0.001	Strong correlation

TNF-α: Tumor necrosis factor-alpha; GI: Gingival index; PI: Plaque index; PPD: Probing pocket depth; CAL: Clinical attachment loss; Significance P 0.001 (HS)

Table 4: Spearman’s correlation of serum tumor necrosis factor-alpha cytokines with all clinical parameter among chronic generalized periodontitis patients

Clinical parameter	Serum TNF-α cytokines		
	Correlation coefficient (ρ)	P	Inference
GI	-0.095	0.691	No linear relationship
PI	0.264	0.260	Weak correlation
PPD	-0.068	0.777	No linear relationship
CAL	-0.196	0.408	Weak correlation

TNF-α: Tumor necrosis factor-alpha; GI: Gingival index; PI: Plaque index; PPD: Probing pocket depth; CAL: Clinical attachment loss

can be detected in saliva, GCF, and serum in both health and periodontitis.^[7] Increased concentration observed in periodontitis correlate closely with the tissue destruction and immune response.^[8]

Table 5: Spearman’s correlation of serum tumor necrosis factor-alpha cytokines with all clinical parameter among all study participants

Clinical parameter	Serum TNF-α cytokines		
	Correlation coefficient (ρ)	P	Inference
GI	0.441*	0.001 (HS)	Moderate correlation
PI	0.597**	0.001 (HS)	Strong correlation
PPD	0.711**	0.001 (HS)	Strong correlation
CAL	0.702**	0.001 (HS)	Strong correlation

TNF-α: Tumor necrosis factor-alpha; GI: Gingival index; PI: Plaque index; PPD: Probing pocket depth; CAL: Clinical attachment loss; HS: Highly significant; Significance P 0.001 (HS)

Table 6: Comparison of mean gingival index among healthy and chronic generalized periodontitis patients

Groups	n	GI		
		Mean±SD	Median	Range
Healthy	20	0.032±0.011	0.030	0.02-0.06
CGP	20	1.32±0.37	1.42	0.67-1.88
Kruskal-Wallis test: χ^2		49.116		
Significance P		0.001 (HS)		

GI: Gingival index; CGP: Chronic generalized periodontitis; SD: Standard deviation; HS: Highly significant

Table 7: Comparison of mean plaque index among healthy and chronic generalized periodontitis patients

Groups	n	PI		
		Mean±SD	Median	Range
Healthy	20	0.029±0.013	0.030	0.01-0.06
CGP	20	2.76±0.39	2.72	2.05-2.85
Kruskal-Wallis test: χ^2		53.103		
Significance P		0.001 (HS)		

PI: Plaque index; CGP: Chronic generalized periodontitis; SD: Standard deviation; HS: Highly significant

Table 8: Comparison of mean probing pocket depth among healthy and chronic generalized periodontitis patients

Groups	n	PPD		
		Mean±SD	Median	Range
Healthy	20	1.49±0.18	1.46	1.15-1.87
CGP	20	5.33±0.51	5.09	4.73-6.51
Kruskal-Wallis test: χ^2		61.909		
Significance P		0.001 (HS)		

PPD: Probing pocket depth; CGP: Chronic generalized periodontitis; SD: Standard deviation; HS: Highly significant

When the mean of serum TNF-α values was compared between healthy and generalized chronic periodontitis patients, it was found that the values were significantly higher for patients with GCP than for healthy controls as shown in Table 1 and Graph 1. Increased level of TNF-α 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- α on stimulation by the bacterial products^[67]

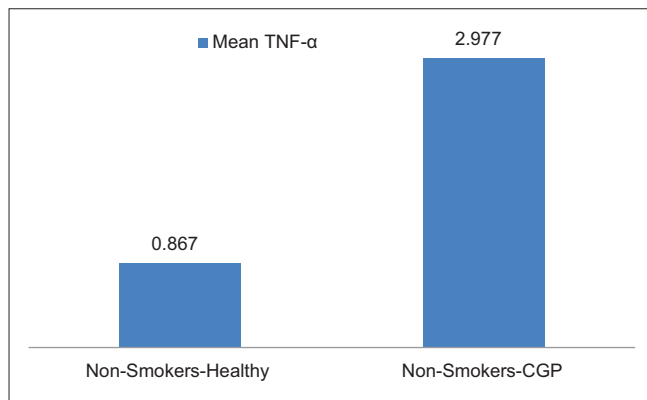
Stashenko *et al.*^[70] have reported that there were increased levels of TNF- α in gingival tissues of periodontitis patients. They suggested that TNF- α is related with the inflammatory condition of the periodontium. TNF- α may be synthesized and secreted by the local periodontal connective tissue cells, such as fibroblasts and endothelial cells or by infiltrating leukocytes, i.e., mononuclear cells, macrophages, and neutrophils.

The results of our study was in accordance with a study conducted by Varghese *et al.* (2015), to estimate the

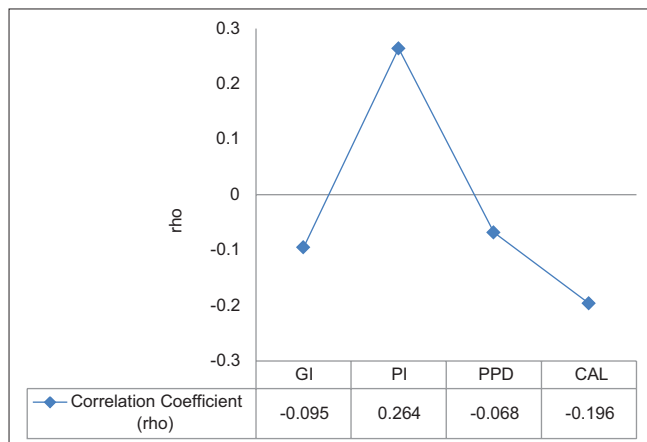
Table 9: Comparison of mean clinical attachment loss among healthy chronic generalized periodontitis patients

Groups	n	CAL		
		Mean \pm SD	Median	Range
Healthy	20	1.49 \pm 0.18	1.46	1.15-1.87
CGP	20	4.97 \pm 0.53	4.94	4.01-6.0
Kruskal-Wallis test: χ^2		61.766		
Significance P		0.001 (HS)		

CAL: Clinical attachment loss; CGP: Chronic generalized periodontitis; SD: Standard deviation; HS: Highly significant



Graph 1: Comparison of mean serum tumor necrosis factor-alpha cytokines among healthy and chronic generalized periodontitis patients



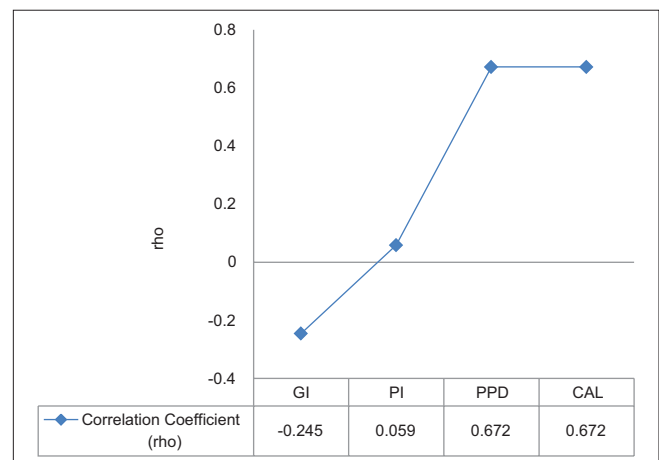
Graph 3: Spearman's correlation of serum tumor necrosis factor-alpha cytokines with all clinical parameter among chronic generalized periodontitis patients

salivary TNF- α in chronic and aggressive periodontitis and control participants. They concluded that salivary TNF- α levels are significantly higher in chronic periodontitis than in healthy controls; however, there was no significant correlation with the clinical parameters.

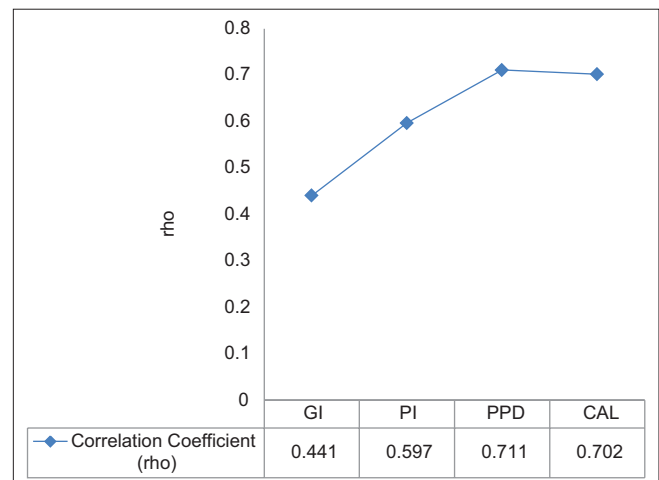
Correlation of tumor necrosis factor alpha with various clinical parameters

In our study, the results show that serum TNF- α cytokines had a strong positive highly significant correlation with plaque index (PI), probing pocket depth (PPD), and CAL and there was a moderate positive significant correlation between TNF- α and gingival index (GI). Serum TNF- α cytokines had a highly significant correlation with all clinical parameters among all study participants ($P = 0.001$) as shown in Graph 2-4 and Tables 1-5.

In a previous study by Engebretson *et al.*,^[47] TNF- α showed a significant positive correlation with attachment loss, but not probing depth and PI. A dose-response relationship was observed between periodontitis severity and TNF- α .



Graph 2: Spearman's correlation of serum tumor necrosis factor-alpha cytokines with all clinical parameter among healthy study participants



Graph 4: Spearman's Correlation of serum tumor necrosis factor-alpha cytokines with all clinical parameter among all study participants

Ikezawa *et al.* in their study reported a significant positive correlation between GCF TNF- α levels with pocket depth in chronic periodontitis patients.^[71] Kurtiş *et al.* also reported a positive correlation between salivary TNF- α levels and clinical parameters such as probing depth, CAL, PI, and GI in GCF samples of patients with chronic and aggressive periodontitis.^[72]

Mean gingival index score

Mean GI for chronic generalized periodontitis (CGP) patients was (1.32 ± 0.37) and among healthy controls were (0.032 ± 0.011) There was statistically highly significant difference in mean gingival index among both the groups ($P = 0.001$) as shown in Table 6.

Mean plaque index score

Mean PI were highest among CGP patients. It was (2.76 ± 0.39) among CGP, and (0.029 ± 0.013) among healthy controls. There was statistically highly significant difference in mean PI among both the groups ($P = 0.001$) as shown in Table 7.

Mean probing pocket depth

The mean PPD were (5.33 ± 0.51) among CGP, and (1.49 ± 0.18) among healthy controls as shown in Table 8. There was statistically highly significant difference in mean PPD among both the groups ($P = 0.001$).

Mean clinical attachment loss

The mean CAL was (4.97 ± 0.53) among CGP, and (1.49 ± 0.18) among healthy controls as shown in Table 9. There was statistically highly significant difference in mean CAL among both the study groups ($P = 0.001$).

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.^[21]

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 the destruction of bone and the attachment apparatus of the teeth.^[22-34] It has also been considered a risk for a variety of systemic conditions, including cardiovascular disease, diabetes mellitus, RA, and respiratory disorders.^[35-42] When the relationship between periodontitis and RA was examined, the findings suggested that circulating TNF- α is related to periodontal inflammation with regard to tissue destruction and vascular reaction in patients with RA.^[41]

Role of tumor necrosis factor-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 IL-1, IL-6, IL-8, IL-10, IL-12, and TNF- α , 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^[43-52] (Genco 1992).

TNF- α is also a monocyte-derived protein that has a wide range of pro-inflammatory and immunomodulatory effects on a number of different cell populations. TNF- α can stimulate fibroblasts including gingival fibroblasts, to produce collagenase (Meikle *et al.* 1989), an enzyme implicated in the tissue destruction of periodontal disease, and to stimulate bone resorption (Bertolini *et al.* 1986). TNF- α activates monocytes and stimulates the production of IL-1b, platelet activating factor, and prostaglandins (Decker 2000). Monocyte stimulation by lipopolysaccharide enhances the production of TNF- α , which has also been shown to induce collagenase release and bone resorption *in vivo* (Erdemir EO).^[52-62]

Pro-inflammatory cytokines (TNF- α , IL-1 α , and IL-1 β) are necessary for initiating an effective inflammatory process against infection. TNF- α also activates osteoclasts and thus induces bone resorption and has synergistic effects with the bone-resorptive actions of IL-1 β .^[41]

Studies have shown a positive correlation between IL-1 β and TNF- α in chronic periodontitis patients (Vahabi *et al.*)^[63]

Reddy *et al.* have shown a positive correlation of TNF- α concentration with the extent of periodontal destruction.^[64] Level of circulating TNF- α in serum has been seen to have decreased following periodontal therapy.^[65] The concentration of IL-1 β was reported to be higher in GCF in cases of chronic periodontitis patients than in gingivitis and control group.

Tumor necrosis factor 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 β and TNF- α were higher in serum of diabetic patients with periodontal disease (Dag A).^[66] IL-6 and TNF- α concentrations were little higher in the serum of patients with type-2 diabetes mellitus than that of the control group (Monea A).^[67]

The level of TNF- α level was significantly higher in patients with osteoporosis. It was concluded that osteoporosis patients are prone to overproduce TNF- α , which also activates the B-cells and promotes the B-cell activity in the periodontal inflammatory sites, aggravating the periodontal disease^[68]

TNF- α level in serum act as diagnostic marker of periodontal disease in patients with Alzheimer disease (Kanakdande V).^[69]

Limitations

Although bacteria are the primary etiologic factors in periodontal disease, the patient’s host response is a determinant of disease susceptibility. The presence of excessive amount of subgingival and supragingival plaque makes the evaluation of the effect of smoking on periodontal health extremely difficult.^[43]

Difficulty in determining active disease and ongoing destruction in periodontal tissue by traditional diagnostic aids such as 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- α in serum. It can be concluded that there is a prospect of using the estimation of TNF- α in serum as a “marker” of periodontal disease in the future.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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