ISSN: 2581-5989
PubMed - National Library of Medicine - ID: 101738774

International Journal of Dental Science and Innovative Research (IJDSIR)

IJDSIR : Dental Publication Service

Available Online at: www.ijdsir.com

Volume – 4, Issue – 1, February - 2021, Page No. : 628 - 642

Assessment of levels of tumour necrosis factor alpha in saliva and gingival crevicular fluid in chronic periodontitis

[1]Dr. Harikrishnan Balachandran Pillai, Senior Lecturer, Department of Periodontology, Azeezia College of Dental sciences and research, Kollam, Kerala

[2]Prof. Dr. K. Nandakumar, Dean, Azeezia College of Dental sciences and research, Kollam, Kerala

[3]Prof. Dr. Padmakumar T P, Head of the Department, Department of Periodontology, Azeezia College of Dental sciences and research, Kollam, Kerala

[4]Dr. Devisree Naveen, Reader, Department of Periodontology, Azeezia College of Dental sciences and research, Kollam, Kerala

[5]Dr. Mithulaj Azeez, Reader, Department of Periodontology, Azeezia College of Dental sciences and research, Kollam, Kerala

Corresponding Author: Dr. Harikrishnan Balachandran Pillai, Senior Lecturer, Department of Periodontology, Azeezia College of Dental sciences and research, Kollam, Kerala

Citation of this Article: Dr. Harikrishnan Balachandran Pillai, Prof. Dr. K. Nandakumar, Prof. Dr. Padmakumar T P, Dr. Devisree Naveen, Dr. Mithulaj Azeez,"Assessment of levels of tumour necrosis factor alpha in saliva and gingival crevicular fluid in chronic periodontitis", IJDSIR- February - 2021, Vol. – 4, Issue - 1, P. No. 628 – 642.

Type of Publication: Original Research Article

Conflicts of Interest: Nil

Introduction

Background: Periodontitis is a chronic inflammatory condition of the periodontium involving interactions between bacterial products, numerous cell populations and, inflammatory mediators. It is generally accepted that periodontitis is initiated by dental plaque. Substances released from this biofilm such as lipopolysaccharides, antigens and, other virulence factors, gain access to the gingival tissue and initiate an inflammatory and immune response, leading to the activation of host defense cells. As a result of cellular activation, inflammatory mediators, including cytokines, chemokines, arachadonic acid metabolites and proteolytic enzymes which collectively contribute to tissue destruction and bone resorption are produced. Tumor necrosis factor-alpha and Interlukene-1β are mainly produced by tissue monocytes / macrophages. All these cytokines play a crucial role in immune and inflammatory responses tissue destruction.

Aims and objectives: To determine and compare the level of pro-inflammatory cytokine tumor necrosis factor-alpha (TNF-α) in saliva and gingival crevicular fluid (GCF) in patients with chronic periodontitis.

Methodology: 60 samples which were divided into two groups of 30 sample each. Of these 30 samples, 15 samples were gingival crevicular fluid and 15 samples were saliva. About 1.5 ml of saliva was collected and the samples were stored at -40ºC. 1- 5µl of gingival crevicular fluid was collected using calibrated, volumetric micro capillary pipette and was mixed with 100 µl of elution buffer and stored at -80ºC. The stored samples were centrifuged at 4000 r.p.m for 10 minutes at 4ºC. Tumour necrosis factor- α was estimated by solid phase sandwich Enzyme-Linked Immuno Sorbent Assay.

Results: The levels of tumour necrosis factor- α in gingival crevicular fluid were significantly higher in chronic periodontitis. The study also showed that the levels of tumour necrosis factor- α in saliva even though elevated in chronic periodontitis, was not significant.

Interpretation and conclusion: Tumour necrosis factor- α were elevated in many inflammatory conditions and periodontitis being an inflammatory condition, tumour necrosis factor- α was elevated. Thus the result of the present study can be used to interpret the potential use of tumour necrosis factor- α as a biomarker in chronic periodontitis.

Keywords: Tumour necrosis factor alpha, gingival crevicular fluid, Saliva, Chronic periodontitis.

Introduction

Chronic Periodontitis is defined as an infectious disease resulting in inflammation within the supporting tissues of the teeth, progressive attachment loss, and bone loss. It is characterized by pocket formation and/or gingival recession[1]. The tissue destruction in periodontal disease appears as a result of the interplay between the pathogenic bacteria and the host's immune and inflammatory responses that result in the loss of the collagenous structures supporting the teeth [2]. Periodontal disease involves a number of inflammatory mediators, such as interleukin (IL)-1, IL-6, IL-8, tumor necrosis factor-α (TNF-α), prostaglandins and matrix metalloproteinases (MMPs) [3]. These mediators may affect the activities of leukocytes, osteoblasts, and osteoclasts and promote the tissue remodeling process systemically and locally[2]. The collagenolytic enzymes, including MMPs, are mediated by a variety of inflammatory cytokines, such as IL-1, IL-6, IL-8 and TNF-α [4, 5].

Due to their role in the immunopathology of periodontal infections, different cytokines have been measured in gingival crevicular fluid (GCF) and their levels related to the disease status of sites and subjects. For the most part, these studies have found that with worsening in clinical parameters of periodontal disease an increase in levels of several GCF cytokines was noted [6]. In addition, treatment and improvement of the periodontal condition have been associated with decreases in the GCF levels of certain cytokines [6- 8]. These findings suggest that cytokines could be putative biomarkers of periodontal disease initiation and progression.

TNF-α is a proinflammatory cytokine that is often overexpressed in periodontitis and is responsible for alveolar bone resorption during periodontitis [9, 10].

TNF-α, secreted predominantly by monocytes and macrophages, is a potent inflammatory cytokine that upregulates the production of collagenases, prostaglandin (PG) E2, chemokines and cytokines, cell adhesion molecules and bone resorption- related factors. [2]

The aim of the current study is

1. To determine the level of pro-inflammatory cytokine Tumour necrosis factor-alpha (TNF-α) in Saliva and Gingival crevicular fluid (GCF) in patients with

chronic periodontitis.

2. To compare the level of a pro-inflammatory cytokine (TNF-α) in saliva and GCF in patients with chronic periodontitis.

Materials And Methods

This descriptive cross-sectional study was carried out during the period of July 2014 to June 2015 in the patients availing treatment in the Out Patient Department of Periodontology, Azeezia College of Dental Sciences And Research, Meeyannoor, Kollam. The study was commenced upon obtaining clearance from the institutional ethical committee. Prior to initiating the study, written informed consent in accordance with ethical codes adopted by the National Committee For Medical Research Ethics was completed by all participants. Subjects within the age group 20 to 65 years and both sexes were included in the study. After obtaining informed consent, subjects were thoroughly examined and detailed case history was taken from each subject as per the attached Proforma. The samples were selected by simple random sampling according to inclusion and exclusion criteria.

Inclusion criteria

- Subjects who are willing to take part in the study.
- Patients with chronic periodontitis based on criteria established in 1999 at the International Workshop for Classification of Periodontal Diseases and Conditions.

Exclusion criteria

- Patients with a history of any systemic diseases.
- Pregnant women, lactating mothers.
- Patients with a history of antibiotic therapy in the past 6 months.
- Smokers.
- Previous history of any periodontal therapy within last 5 years.

Study Design

A total of 60 samples were collected, divided into two groups;

Group I): Chronic Periodontitis group, based on criteria established in 1999 at the International Workshop for Classification of Periodontal Diseases and Conditions.

15 gingival crevicular fluid (GCF) samples and 15 saliva samples were collected.

Group II): Healthy subjects group

15 CF samples and 15 saliva samples were collected

16 **Armamentarium**

For clinical examination

Kidney trays, Sterile mouth mirrors, Williams probe, Straight probes, sterile gloves, and Mask.

For gingival crevicular fluid and saliva collection and analysis

Sterile saliva collecting vials, Hirschmann® µL- Sigma-Aldrich Germany, collection vials- 1.5ml Eppendorf tubes, Centrifuge-ANM Personal microfuge – Amkette India, Cooling Centrifuge- Remi R24, Micropippete-Thermo scientific F1, Multichannel pipette-Thermoscientific, Micropipette tips –Axygen, USA, Test tubes (15 ml) — Borosil, Orbital Shaker ,Multiplate reader (Erba, Germany), TNF-α (human) EIA Kit Item No. 589201 Cayman chemical CO, USA.

Saliva sample collection

Whole unstimulated (resting) saliva was collected by passive drool (Navazesh et al).[11]Saliva samples were collected before breakfast between to 8-10 am. The patients were seated in an upright relaxed position with the head bent forward. The subjects were asked to swallow the saliva first. Then the subject lets the saliva passively drip into saliva collecting vial over a period of 5-10 minutes till approximately 1.5 ml of saliva was collected. Saliva samples were stored at -40°C until analysis.[12]

Gingival crevicular fluid collection

Multiple test sites were dried and isolated with cotton rolls to prevent any contamination from saliva and blood. Prior to GCF sampling, supragingival calculus was removed using sterile Gracey curette. A standard volume of 5µl was collected extracrevicularly using a calibrated, volumetric, Hirschmann® microcapillary pipette, measuring 1 to 5 µl for 5 to 30 minutes.[13] The GCF obtained was mixed with 100 µl elution buffer containing 50 mM $CaCl_2$ and 0.01% Triton X-100 and stored at -80°C until the assay was performed.[14]

Estimation of TNF –α

The stored samples were centrifuged at 4000 r.p.m for 10 minutes at 4ºC. TNF-α was estimated using Cayman TNF-alpha (human) EIA kit (Item no. 589201) by solid phase sandwich Enzyme Linked-Immuno-Sorbent Assay.

Results

In this study, 15 patients with chronic periodontitis and 15 healthy controls were considered for comparisons with respect to TNF-α in gingival crevicular fluid (GCF) and TNF-α in saliva.

In the descriptive part, percentage, mean value and standard deviation (SD) depending on whether the variable is qualitative or quantitative (continuous) was used. In the inferential part, for the comparison of TNF-α values of the patients with healthy controls, students 't' test was used. The correlation between TNF-α from GCF and saliva was assessed by using Pearson's correlation coefficient, r, and the prediction, if possible was assessed by regression analysis after adjusting the difference between patients and controls in the baseline characteristics especially age, because the patients were significantly older than controls. The effect of this age difference was removed by a multivariable regression analysis by including the variable "age" also in the regression equation. The comparability of patients with controls was assessed by using the chi-square test (χ^2) and modified 't' test because the variances of the group were unequal.

Data were entered in the computer using the software Microsoft Excel and analyzed using SPSS / Epi. Info. (Statistical Programme for Social Sciences).

Discussion

Periodontal diseases are caused by gram-negative bacterial infections, which synthesize lipopolysaccharides (LPS). TNF-α is considered to be a major cytokine involved in the pathogenesis of periodontal disease and results in tissue destruction and the erosive reaction in periodontitis [15]. TNF-α is produced mainly by monocytes and macrophages in response to LPS. Increased levels of TNF-α also may promote the release of collagenase from human gingival fibroblasts, leading to cartilaginous collagen destruction and bone resorption [15]. There is also substantial evidence to prove that the elevated levels of TNF-α in chronic periodontitis serves as a link in the development of cardiovascular diseases. [16]

In this study, 15 patients with chronic periodontitis and 15 healthy controls were considered for comparisons with respect to TNF-α in gingival crevicular fluid (GCF) and TNF-α in saliva. Both the groups were compared in their baseline characteristics. In this study among the 15 chronic periodontitis and 15 control groups, 8 were males and 8 were females and both the groups were comparable with respect to gender as shown in table 5.

The mean age of chronic periodontitis group was 40.13 (± 7.46) and healthy controls were 30.27 (± 1.94). There was a significant difference in mean ages between the groups as shown in table 6. To adjust the difference between groups with respect to age, a multivariable regression modeling was used by putting the TNF- α values as the dependent variable and age and group as independent variables. As shown in table 7, age was not significant

indicating that age is not a predictor of GCF TNF- α values but the groups, which was significant. But for saliva TNF- α values, both variables age and group were not significant.

The mean value of TNF- α in saliva for chronic periodontitis group was 81.40 picogram /ml (± 3.73) and that of healthy control was 79.82 picogram/ml (± 4.15) which was not statistically significant as shown in table 4. This is in accordance with the study by R.P. Teles et al (2009) .[6]The rationale for assessing cytokines present in saliva is based on the concept that these mediators come into the whole saliva through GCF. The GCF levels of cytokines are elevated in chronic periodontitis sites compared to healthy sites it was hypothesized that they would also be elevated in the whole saliva of periodontitis individuals.[17] Additionally, there is increased secretion of an inflammatory cytokine from buccal and gingival epithelial cells which would be another source of cytokines.[18, 19] In contrast to this there was no significant difference in the TNF- α levels. This may be due to extensive dilution of GCF in saliva and also due to the presence of putative inhibitors in the whole saliva mainly large salivary proteins such as mucins which cleaves these cytokines.[20] The results of this study are however contradictory to what was obtained by Frodge et al in 2008, who showed a statistically significant difference in TNF- α value in saliva in chronic periodontitis patients compared to healthy controls.[21] Gumus et al. in their study in 2014 found similar results as in this study. There was an increase in salivary TNF-α in chronic periodontitis when compared to healthy controls but was not statistically significant. Similar results were obtained in a study done by Pritma Singh et al. in 2013.[22] Varghese et al. in their study found out contradictory results in 2015 in which significantly higher levels of salivary TNF-α value in chronic periodontitis compared to healthy controls.[23]

The mean value of TNF- α in GCF for chronic periodontitis group was 86.25 picogram / ml (± 2.71) and that of healthy control was 80.74 picogram / ml (± 3.33) which was highly statistically significant (p = 0.0001) as in table 3. The study has similar results to the study done by Kurtis et al in 2005 who showed a statistically significant difference of TNF- α in GCF in chronic periodontitis patients compared to healthy controls.[24] In a study by Fentoglu et al. in 2011, the level of TNF- α in GCF was not statistically significant in chronic periodontitis group in comparison to healthy controls.[3] Alves et al. in 2013 found a result similar to this study that is a statistically significant difference in GCF TNF-α in chronic periodontitis patients compared to healthy controls.[25] The relation between increased TNF-α and inflammation was early established. IL-1 and TNF are produced by many cell types and have been shown to contribute to several events that are essential for the initiation of an inflammatory response, sustaining of inflammation and, ultimately, tissue destruction. They induce upregulation of adhesion molecules on leukocytes and cells of endothelium, also stimulate the production of chemokines, which are needed for recruiting leukocytes. IL-1 and TNF induce expression of mediators that amplify or sustain the inflammatory response like prostaglandins, and the synthesis of lytic enzymes such as matrix metalloproteinases; they also can enhance bacterial killing and phagocytosis. Moreover, IL-1 and TNF are synergistic in their capacity to enhance bone resorption. Although the periodontium has a high capacity for repair, cytokines limit repair by inducing apoptosis of matrix-producing cells.[26]

There was no significant correlation detected between TNF- α saliva values and TNF- α GCF values in chronic periodontitis patients as shown in table 8. This may be due to the increased amount of TNF-α released during

inflammation which may be elevated in both saliva and GCF.[17-19] Thus there was no significant prediction of one from the other as shown in graph 3.

Conclusion

The following conclusions were drawn from the study:

1. The levels of Tumour necrosis factor- α is significantly elevated in Gingival crevicular fluid of Chronic Periodontitis patients when compared to healthy controls.
2. The levels of Tumour necrosis factor- α is elevated but not significantly in Saliva of Chronic Periodontitis patients when compared to healthy controls.

References

1. Consensus Report: Chronic Periodontitis. Ann Periodontol December 1999; Volume 4, Number 1.
2. Min Ki Noh, Min Jung, Soo Hwan Kim, Seo Ra Lee, Ki Ho Park, Dong Hwan Kim, Hanna Hyun Kim, and Young Guk Park. Assessment of IL-6, IL-8 and TNF-α levels in the gingival tissue of patients with periodontitis. Experimental and Therapeutic Medicine 2013; 6: 847-851.
3. Fentoglu O, Koroglu BK, Hic, Yilmaz H, Sert T, Ozdem M, Sutc uR, Tamer MN, Orhan H, Ay ZY, Ozturk Tonguc¸ M, Krzoglu FY. Pro-inflammatory cytokine levels in association between periodontal disease and hyperlipidaemia. J Clin Periodontol 2011; 38: 8–16.
4. Veli-juka, Christopher M Overall, Christopher McCulloch. Proteolytic host cell enzymes in gingival crevice fluid. Periodontology 2000, 2003; 31, 77–104.
5. H. Okada, S. Murakami. Cytokine expression in Periodontal health and disease. Crit Rev Oral Biol Med 1998; 9(3), 248-266.
6. R.P. Teles, V. Likhari, S.S. Socransky, and A.D. Haffajee. Salivary Cytokine Levels in Chronic Periodontitis and Periodontally Healthy Subjects. A Cross-sectional Study. J Periodontal Res. 2009; 44(3): 411–417.
7. J. Gamonal, A. Acevedo, A. Bascones, O. Jorge, and A. Silva. Levels of Interleukin-1, -8, and -10 and RANTES in Gingival Crevicular Fluid and Cell Populations in Adult Periodontitis Patients and the Effect of Periodontal Treatment. J Periodontol 2000; 71, 1535-1545.
8. Holmlund A, Hanstrom L, Lerner UH. Bone resorbing activity and cytokine levels in the gingival crevicular fluid before and after treatment of periodontal disease. J Clin Periodontol 2004; 31: 475–482.
9. Philip Stashenko, Thomas Van Dyke, Patrice Tully, Ralph Kent Jr., Stephen Sonis, and Anne C.R. Tanner. Inflammation and Genetic Risk Indicators for Early Periodontitis in Adults. J Periodontol. 2011; 82(4): 588–596.
10. Raed AlRowis, Hani S AlMoharib, Abdulrahman AlMubarak, Jagankumar Bhaskardoss, R S Preethanath, Sukumaran Anil. Oral Fluid-Based Biomarkers in Periodontal Disease – Part 2. Gingival Crevicular Fluid; Journal of International Oral Health 2014; 6(5):126-135.
11. M. Navazesh and C.M. Christensen. A Comparison of the Whole Mouth Resting and Stimulated Salivary Measurement Procedures. J DENT RES 1982; 61: 1158.
12. Pınar Gu¨mu¨sx, Nejat Nizam, David F. Lappin, and Nurcan Buduneli. Saliva and Serum Levels of B-Cell Activating Factors and Tumor Necrosis Factor-α in Patients With Periodontitis. J Periodontol 2014; 85: 270-280.
13. Rashmi Srinath, Anirudh B. Acharya, and Srinath L. Thakur. Salivary and Gingival Crevicular Fluid Melatonin in Periodontal Health and Disease, J Periodontol 2010; 81: 277-283.

14. Nicolas Dutzan, Rolando Vernal, Marcela Hernandez, Andrea Dezerega, Oriana Rivera, Nora Silva et al, Levels of Interferon-Gamma and Transcription Factor T-Bet in Progressive Periodontal Lesions in Patients With Chronic Periodontitis, J Periodontol 2009;80:290-296.
15. Bostrom L. Linder LE. Bergstrom J. Clinical expression of TNF-α in smoking Associated periodontal disease. J clin Periodontol 1998; 25, 767-773.
16. James Beck, Raul Garcia, Gerardo Heiss, Pantel S. Vokonas and Steven Offenbacher. Periodontal Disease and Cardiovascular Disease. J Periodontol 1996; 67:1123-1137.
17. S. Offenbacher, S.P. Barros, R.E. Singer, K. Moss, R.C. Williams, and J.D. Beck. Periodontal Disease at the Biofilm–Gingival Interface, J Periodontol 2007; 78:1911-1925.
18. Panagiota G. Stathopoulou, Manjunatha R. Benakanakere, Johnah C. Galicia, and Denis F. Kinane. Epithelial cell pro-inflammatory cytokine response differs across dental plaque bacterial species. J Clin Periodontol. 2010; 37(1): 24–29.
19. M. R. Milward, I. L. C. Chapple, H. J. Wright, J. L. Millard, J. B. Matthews, and P. R. Cooper. Differential activation of NF-kB and gene expression in oral epithelial cells by periodontal pathogens. Clinical and Experimental Immunology, 148: 307–324.
20. Patricia Yen Bee Ng, Maureen Donley, Ernest Hausmann, Alan D. Hutson, Edward F. Rossomando, and Frank A. Scannapieco. Candidate salivary biomarkers associated with alveolar bone loss: cross-sectional and *in vitro* studies, FEMS Immunol Med Microbiol. 2007; 49(2): 252–260.
21. William V. Giannobile, Thomas Beikler, Janet s. Kinney, Christoph A. Ramseier, Thiago Morelli & David T. Wong. Saliva as a diagnostic tool for periodontal disease: current state and future directions. Periodontology 2000; Vol. 50, 2009, 52–64.
22. Pritma Singh, Narender Dev Gupta, Afshan Bey, Saif Khan. Salivary TNF-alpha: A potential marker of periodontal destruction. Journal of Indian Society of Periodontology 2014; 18(3), 306-310.
23. Varghese SS, Thomas H, Jayakumar ND, Sankari M, Lakshmanan R. Estimation of salivary tumor necrosis factor alpha in chronic and aggressive periodontitis patients. Contemp Clin Dent 2015; 6: S152-6.
24. Gareth S, Griffith S. Formation, collection and significance of gingival crevice fluid. Periodontology 2000 2003; Vol. 31, 32–42.
25. Vanessa Tubero Euzebio Alves, Henrique Aparecido Bueno da Silva, Bruno Nunes de França, Rosangela Santos Eichler, Luciana Saraiva, Maria Helena Catelli de Carvalho, Marinella Holzhausena. Periodontal Treatment Downregulates Protease-Activated Receptor 2 in Human Gingival Crevicular Fluid Cells. Infection and Immunity 2013; 81(12), 4399–4407.
26. D.T. Graves and D. Cochran. The Contribution of Interleukin-1 and Tumor Necrosis Factor to Periodontal Tissue Destruction. J Periodontol 2003; 74(3), 391- 401.

Legend Table and Figure

Table 1:Tumour necrosis factor- α level in Gingival crevicular fluid and Saliva in picogram/milliliter

No	GCF TNF-α in pg/ ml	Saliva TNF-α in pg/ ml
1	82.345	80.92
2	86.255	76.08
3	80.44	76.245
4	86.68	82.885
5	88.85	76.075
6	87.37	83.235
7	81.58	79.745
8	88.6	86.225
9	88.43	84.435
10	87.305	86.075
11	86.36	81.575
12	88.6	86.36
13	87.54	77.92
14	88.2	79.15
15	85.192	84.01

Table1 includes Group 1 patients with chronic periodontitis from whom levels of TNF-α is assessed in Gingival crevicular fluid and Saliva using ELISA (Enzyme-Linked Immunosorbent Assay).

Table 2: Tumour necrosis factor- α level in Gingival crevicular fluid and Saliva in picogram/milliliter

No	GCF TNF-α in pg/ ml	Saliva TNF-α in pg/ ml
1	81.925	83.8
2	78.93	74.8
3	79.255	75.65
4	75.25	81.45
5	78.75	73.65
6	80.625	79.3
7	80.305	80.15
8	86.1	85.85
9	84.66	83.8
10	86.1	85.65
11	79.85	79.5
12	81.575	81.5
13	74.96	73.15
14	79.71	77.6
15	83.125	81.45

Table 2 includes Group 2 healthy subjects from whom levels of TNF-α is assessed in Gingival crevicular fluid and Saliva using ELISA (Enzyme-Linked Immunosorbent Assay).

Table 3: Comparison of TNF-α value in GCF between the study groups

TNF- α in GCF	Chronic Periodontitis	Healthy Controls	Test of significance and P-Value
Mean	**86.25**	**80.74**	**t = 4.97**
Standard deviation (SD)	**2.71**	**3.33**	**P value= 0.0001***

*highly statistically significant

Mean values of TNF- α in GCF in chronic periodontitis group was 86.25 (± 2.71) pg/ ml whereas for healthy controls it was 80.74 (± 3.33) pg/ ml which was highly statistically significant (p = 0.0001).

Table 4: Comparison of TNF-α value in Saliva between the study groups

TNF- α in Saliva	Chronic Periodontitis	Healthy Controls	Test of significance and P-Value
Mean	81.40	79.82	t = 1.094
Standard deviation (SD)	3.73	4.15	P value= 0.283

Mean values of TNF- α in saliva in chronic periodontitis group was 81.40 (± 3.73) pg/ ml whereas for healthy controls it was 79.82 (± 4.15) pg/ ml which was not statistically significant.

Table 5: Distribution of the groups with respect to gender

Gender	Chronic Periodontitis group		Healthy controls		Test of significance and p value
	Number	%	Number	%	
Male	8	53.3	8	53.3	χ^2 = 0.00
Female	7	46.7	7	46.7	p = 1.00
Total	15	100.0	15	100.0	

Comparability of the study groups (test and control) showing no statistically significant difference between the groups with respect to gender.

Table 6: Distribution of the groups with respect to age

Age	Chronic Periodontitis	Healthy Controls	Test of significance and P- Value
Mean	40.13	30.27	Modified t = 4.96
Standard deviation (SD)	7.46	1.94	p value= 0.0001*

*statistically significant

Comparability of the study groups (test and control) showing a statistically significant difference between the groups with respect to age.

Table 7: Multivariable regression modelling

	AGE		GROUP	
	p		p	
TNF- α in GCF	0.0826	Not significant	0.002	significant
TNF- α in Saliva	0.405	Not significant	0.821	Not significant

Multivariable regression modelling using TNF- α value as dependent variable and Age and Group as independent variable.

Table 8: Correlation between TNF- α values in GCF and Saliva in Chronic Periodontitis

Correlations			
		TNF- α GCF	TNF- α SALIVA
TNF- α GCF	Pearson Correlation	1.000	.363
	Significance	.	.183
	N, Sample size	15	15
TNF-α SALIVA	Pearson Correlation	.363	1.000
	Significance	.183	.
	N, Sample size	15	15

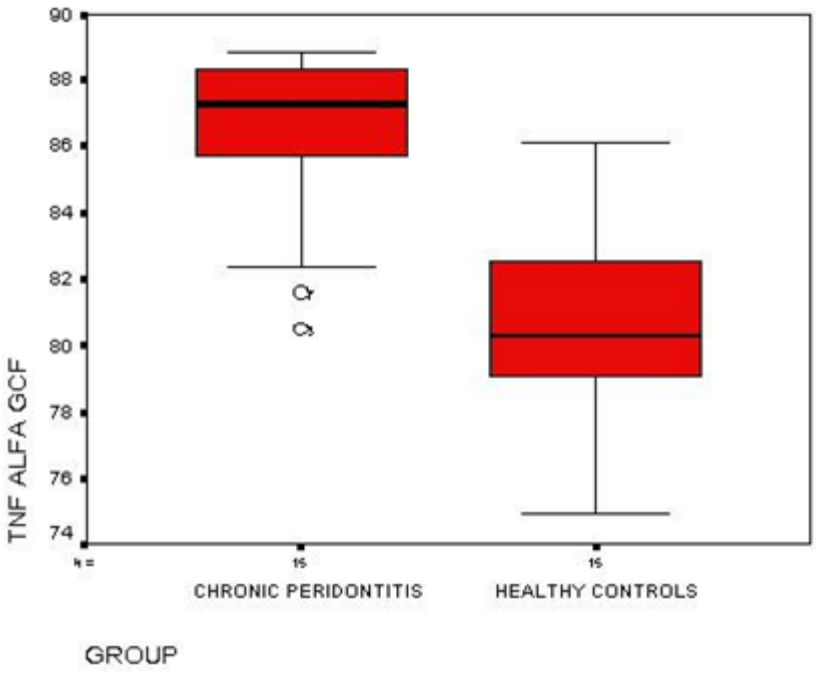
There was no significant correlation between TNF- α GCF and TNF- α saliva values.

Table 9: Correlation between TNF- α values in GCF and Saliva in Healthy controls

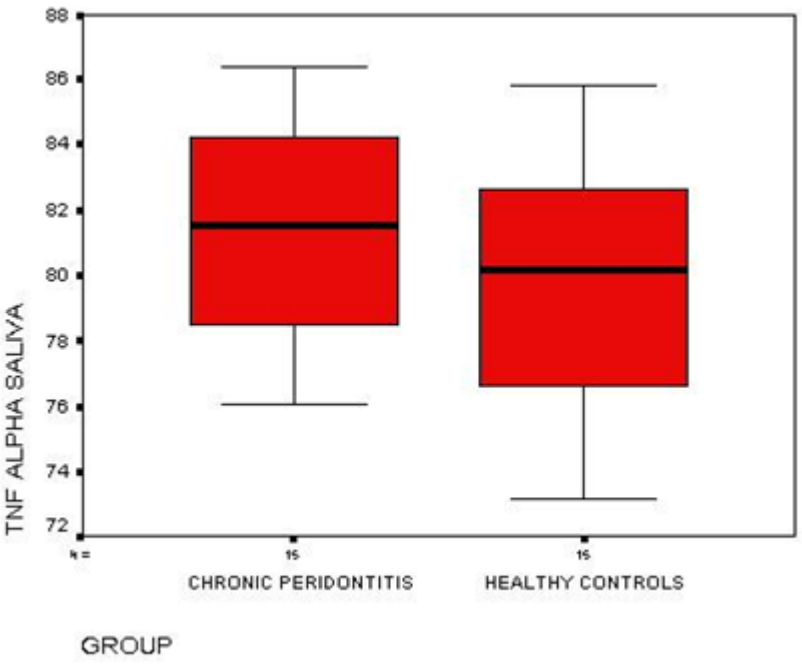
Correlations			
		TNF- α GCF	TNF- α SALIVA
TNF- α GCF	Pearson Correlation	1.000	.769
	Significance	.	.001
	N, Sample size	15	15
TNF- α SALIVA	Pearson Correlation	.769	1.000
	Significance	.001	.
	N, Sample size	15	15

There was a highly significant correlation between TNF- α GCF and TNF- α saliva values

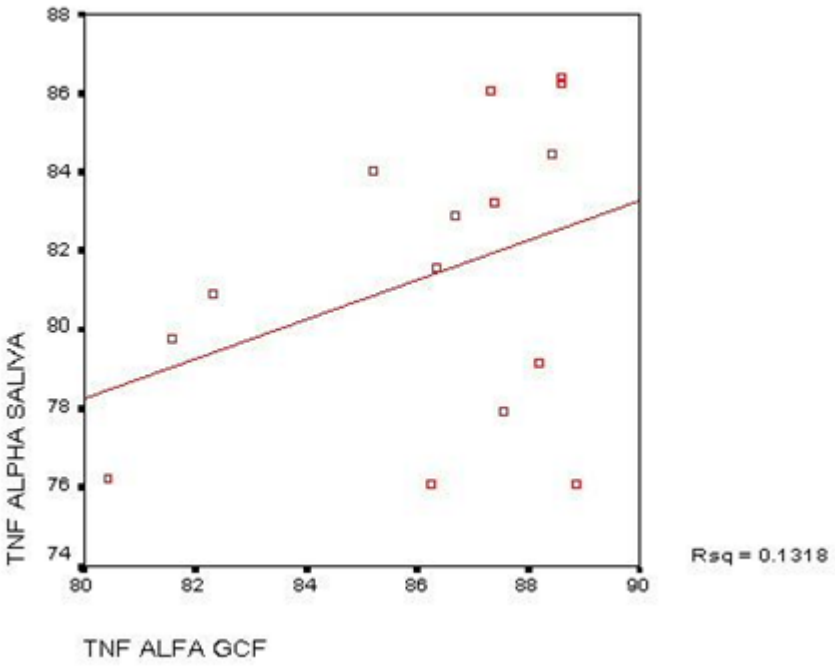
Graph 1: Comparison of TNF-α value in GCF between the study groups



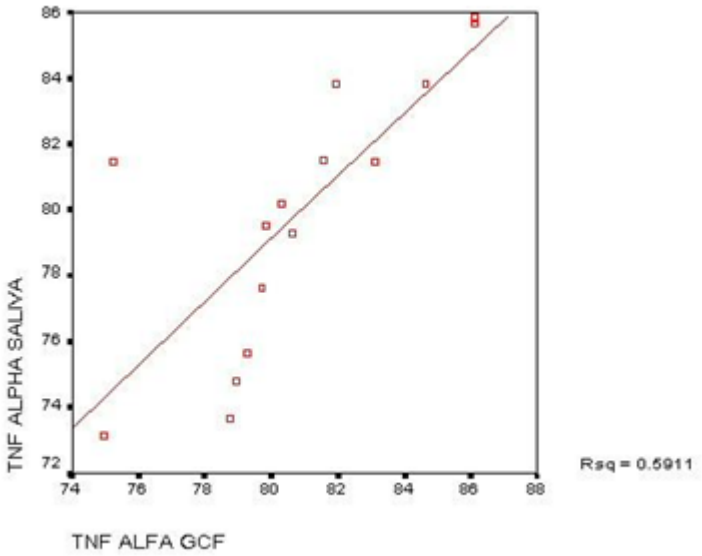
Graph 2: Comparison of TNF-α value in Saliva between the study groups



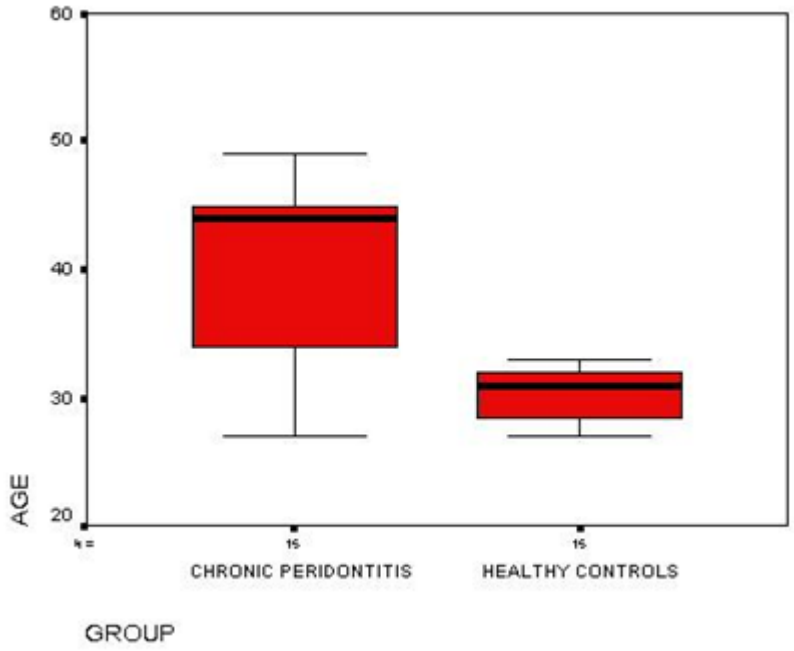
Graph 3: Correlation between TNF- α values in GCF and Saliva in Chronic Periodontitis



Graph 4: Correlation between TNF- α values in GCF and Saliva in Healthy controls



Graph 5: Distribution of the groups with respect to age



Graph 6: Distribution of the groups with respect to gender