|| Print ISSN: 2589-7837 || Online ISSN: 2581-3935 ||
International Journal of Medical Science and Diagnosis Research (IJMSDR)
Available Online at www.ijmsdr.com
PubMed (National Library of Medicine ID: 101738825)
Volume 3, Issue 10; October: 2019; Page No. 47-54
Original Research Article
crossref DOI: https://dx.doi.org/10.32553/IJMSDR/v3i10.8

COMPARATIVE ESTIMATION OF LEVEL OF TNF- α IN GINGIVAL CREVICULAR FLUID AND BLOOD SERUM IN PATIENT WITH CHRONIC PERIODONTITIS BEFORE AND AFTER SCALING AND ROOT PLANING: A BIOCHEMICAL STUDY

Dr. Nitesh Anand[1], Dr. Pradeep Shukla[2], Dr. Prerna Kataria[3], Dr. Gaurav Malhotra[3], Dr.Varun Dahiya[3]

[1]Post Graduate Student, Department of Periodontology & Implantology, Divyajyoti College of Dental Sciences & Research

[2]Professor & HOD, Department of Periodontology & Implantology, Divyajyoti College of Dental Sciences & Research

[3]Professor, Department of Periodontology & Implantology, Divyajyoti College of Dental Sciences & Research

Conflicts of Interest: Nil

Corresponding author: Dr. Nitesh Anand

Abstract:

TNF-α is produced by various cells and is involved in normal inflammatory and immune responses. TNF-α is secreted by macrophages, monocytes, neutrophils and T-cells.TNF is also a potent trigger for cellular apoptosis, or programmed cell death. Thus, TNF is critical to wound debridement either under septic or aseptic circumstances. TNF- α induces bone resorption but it is much less potent.

Aim-The Aim of the present study is to Estimate the level of TNF -α in Gingival Crevicular Fluid and Blood Serum in patient with Chronic Periodontitis before and after Scaling and Root Planing (SRP).

Materials and Methods-25 subjects within age range of 25-65 years both male and female were selected on basis of inclusion criteria and were categorized into two treatment groups.

GROUP I (n=25): Chronic Generalized Periodontitis patients before scaling and root planing

GROUP II (n=25): Chronic Generalized Periodontitis (group1) patients after one month of scaling and root planing. Biochemical analysis of GCF and serum samples were done to estimate the level of TNF- α at baseline and after 1 month of SRP using ELISA kit (BOSTER IMMUNOLEADER HUMAN TNFα ELISA KIT).

Results-Showed elevated levels of TNF-a in group I subjects as compared to Group II subjectsin both GCF and serum and showed an association between periodontal disease and levels of TNF- α.

Conclusion-Increase in level of cytokines, includingTNF-α and IL-6 has been seen in close association with periodontal disease.Level of cytokines before and after periodontal therapy reflects the response to periodontal treatment.Clinical parameters and level of TNF-α decreased after scaling and root planing. These observations indicates positive association between periodontal disease and increased level of TNF-α in GCF and Serum.

Introduction

Periodontitis is defined as an inflammatory disease of the supporting tissues of the teeth caused by specific microorganisms or groups of specific microorganisms, resulting in progressive destruction of periodontal ligament and alveolar bone with pocket formation, recession or both.The primary etiologic factor in periodontal disease is the accumulation of bacteria in the gingival sulcus.

The clinical signs of periodontitis are changes in the morphology of gingival tissues, bleeding on probing, as well as periodontal pocket formation. This pocket provides an ideal environment for the growth and proliferation of anaerobic pathogenic bacteria. The microflora found in periodontitis is complex and composed mainly of gram negative anaerobic bacteria. Periodontal pathogens has been seen to produce a large number of biological molecules that may act directly on host tissues and destroy its integrity. Bacteria and their products accumulate in the gingival sulcus and mediate connective tissue destruction through the ability of the antigens from their cell walls to stimulate pro-inflammatory cytokines such as Interleukin-1 (IL-1) and Tumour necrosis factor–alpha(TNF-α) production by circulating mononuclear cells. Production of numerous pro-inflammatory cytokines is amplified by several bacteria-derived virulence factors, that leads to the destruction of soft tissues and bone.

In periodontitis, numerous substances associated with the host immunological response are found in

Gingival Crevicular Fluid (GCF), including various inflammatory mediators like prostaglandins (PGE2) and pro-inflammatory cytokines such as Interleukin-1α (IL-1α)and TNF-α which play a major role in the progression of periodontal tissue destruction.

TNF alpha was co-discovered by '**Beutler and Cerami' .**TNF-α is produced by various cells and is involved in normal inflammatory and immune responses. TNF-α is secreted by macrophages, monocytes, neutrophils and t-cells. It is in two forms:-TNF-α and TNF-β.

There are 2 types of receptors for TNFα, i.e. TNFα R1 and TNFα R 2. It has been reported that TNFα R1 activation is responsible for mediatingLipoplysacchride(LPS) toxicity and cell toxicity, and an activation of TNFα R2 is responsible for cellular proliferation. TNF-α is the extremely pleiotropic nature of its action, which could be ascribed to the presence of TNF receptors virtually on all cells leading to an activation of multiple signal transduction pathways, kinases and transcription factors.

'KULL and JACOBS'have reported that there are about 1000 to 3000 receptors virtually on all cells. TNF-α once produced and secreted, will bind to TNF receptor present in all plasma membrane of most of the cells through out the body.

TNF is also a potent trigger for cellular apoptosis, or programmed cell death. Thus, TNF is critical to wound debridement either under septic or aseptic circumstances. TNF- α is a induces of bone resorption but it is much less potent.

Chronic low levels of TNF within the bloodstream have been attributed to cause the formation ofatheromatous plaques in the large vessels and to serve as an inducer of a chronic hypercoagulative state, ultimately leading to thromboembolic phenomena such as stroke and myocardial infarction.

When infectious challenge leads to systemic elevations of TNF this can result in a life threatening condition. High levels of TNF are extremely toxic to the host and it has been termed the "suicide hormone".

The suitability of using cytokine TNF-α in GCF as a possible indicator of periodontal disease was first assessed by '**ROSSOMANDO'.**

TNF- α is a proinflammatory cytokine and has been implicated in the destruction of periodontal tissues in periodontitis, it induces the secretion of collagenase by fibroblasts, resorption of cartilage and bone [11] .TNF- α also activates osteoclasts and thus induces the bone resorption and also induces the synthesis of IL–1 and PGE 2. TNF- α has synergistic effects with the bone resorptive actions of IL– 1b.

Hybridization and immunohistochemistry were used to show that TNF- α mRNA was abundant in macrophages and T-cells of the gingival tissues of patients with moderate to severe periodontitis. These findings support the hypothesis that TNF α could have some possible role in the inflammatory process and subsequent tissue destruction in periodontal disease.

Evaluation of the contents of GCF is a promising, non–invasive method to determine the tissue changes in periodontium.ThusAn aim of this study is to estimate the level of TNF-α in GCF and serum, and to explore its relationship to periodontal disease.

MATERIALS AND METHODS

Patients with in age range of 25-65 years both male and female were selected from the Out Patient Department of Periodontology, after the approval of the ethical committee of the D.J. College of Dental Sciences and Research, Modinagar. Systemically healthy patients with chronic periodontitis having a pocket depth of 4-8 mm .No history of antibiotic or periodontal therapy in the preceding 6 months.Age group of 25 – 65 years.

Patients with aggressive periodontitis, smokers, alcoholics, diabetes, hypertension, immunocompromised patients, and pregnant or lactating mothers.

Patients with dental infections like chronic peri-apical lesions, apthous stomatitis, and oral lichen planus.Patients on systemic statin therapy or antibiotic therapy were excluded from study.

Twenty five subjects were selected on basis of inclusion criteria were categorized into two treatment groups.

GROUP I (n=25): Chronic Generalized Periodontitis patients before scaling and root planning.GROUP II (n=25): Chronic Generalized Periodontitis (group1) patients after one month of scaling and root planing. At each patient's initial appointment, baseline data were obtained on modified Sulcus Bleeding Index (mSBI) by the method of **Mombelli** A on four posterior teeth in each quadrant (1^{st} premolar, 2^{nd} premolar, 1^{st} molar and 2^{nd} molar). Probing depth

(PD), Relative attachment level (custom made occlusal stent) was measured with a UNC-15 periodontal probe for same teeth. GCF samples were taken at 16 sites for each patient. These sites were the mesio-buccal surfaces of the above stated 4 posterior teeth in each upper quadrant and at the mesio-lingual surfaces of same in each lower quadrant**.** SRP performed until the root surface is considered smooth and clean by the operator. No Antibiotics or Anti-plaque and Anti-inflammatory agents were prescribed after treatment. Serum and GCF sampling were repeated after one month were as these measurements (mSBI, PD, RAL) were repeated after one month and three months.

Each GCF sample was collected for 15 seconds by calibrated volumetric microcapillary pipettes which was inserted in the Gingival Sulcus immediately after the area has been isolated with cotton rolls, dried, and supragingival plaque has been removed with a sterile Gracey curette. The calibrated volumetric microcapillary pipettes was placed in a polypropylene tube and immediately transferred to plastic vial then stored at -70°C till the time of ASSAY. Microcapillary Pipettes contaminated with blood and saliva were excluded from the sampled group. GCF sample were collected again after one month.

Collection of blood serum:2 ml of blood was collected from the Antecubital fossa by venipuncture using 20 gauge needle and 2ml syringes and immediately transferred to the laboratory. Samples were allowed to clot for 1 hour at room temperature centrifuged for 10 minutes (4°C) and Serum was extracted. Collected serum samples were stored at –70°C before used for ASSAY procedure. . Serum sample were collected again after one month..

GCF and Serum Analysis:

Biochemical analysis of GCF and serum samples were done to estimate the level of TNF- α using ELISA kit (BOSTER IMMUNOLEADER HUMAN TNFα ELISA KIT).

RESULTS

The Aim of this study was to Estimate the level of TNF -α in Gingival Crevicular Fluid and Blood Serum in patient with Chronic Periodontitis before and after Scaling and Root Planing (SRP).

Control of plaque and gingivitis is important in clinical studies process because both vary in their association with periodontitis and both affect the response to therapy. Since probing depth and loss of relative attachment are pathognomic for periodontitis, so pocket probing is a crucial and mandatory procedure in diagnosing periodontitis and evaluating the success of periodontal therapy.

The patients selected were subjected to assessment of modified sulcus bleeding index, probing depth and relative attachment level. UNC-15 probe and occlusal stent were used as a reference point.

TNF-α Level was measured by collecting GCF and blood serum samples of individuals in study group .The clinical parameters were assessed at baseline, 1month and 3 months postoperatively whereas TNF-α was assessed at baseline and 1 month postoperatively.

In this present study, GCF and serum Tumour Necrosis Factor- Alpha level (TNF-α) estimation has been performed since it is a potential prognostic biomarker of periodontal disease activity.

Comparison of Group 1(before Scaling and root planning) and Group 2 (after Scaling and root planing) between the different intervals shows that there is significant reduction in mean scores of mSBI, probing depth and gaining RAL at baseline, 1 month and 3 months. Thus, it shows that Scaling and Root-Planing is efficient in reducing gingival bleeding, probing depth and gaining RAL.

Study shows significant percentage change in the score of GCF TNF-α in Group 2(after SRP) as compared to Group 1(before SRP) was measured.

Study shows significant percentage change in the score of Serum TNF-α in Group 2(after SRP) as compared to Group 1(before SRP) was measured

Gender Distribution and mean age study subjects

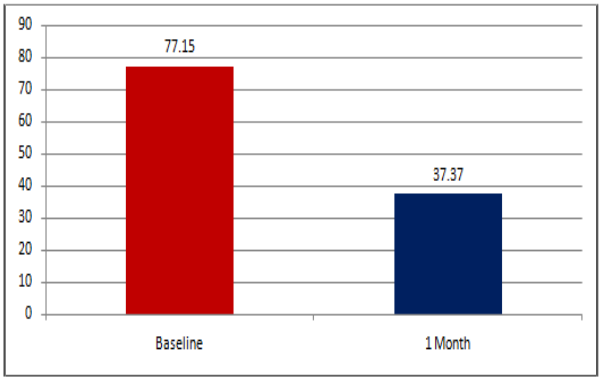
Table 1: Shows the descriptive for subjects

	Frequency	Percent
Female	10	40.0
Male	15	60.0
Mean Age of Study Subjects	41.51±9.52	

Comparison of GCF TNF – α Between Baseline and 1 Months

Table 2: Shows the descriptive analysis for level of GCF TNF – α at baseline and 1 month

	Mean	SD	P value	Significance
Baseline	77.15	5.77	0.001	Significant
1 Month	37.37	7.50		



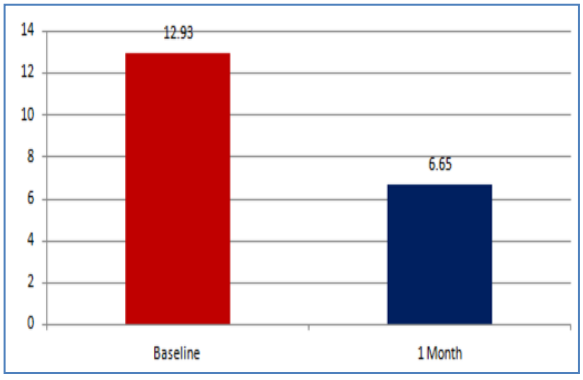
Graph 1: Shows the comparison of GCF TNF – α for group I (before scaling and root-planning) and group II (after scaling and root planning)

Comparison of serum TNF – α between baseline and 1 months

Table 3: Shows descriptive analysis of Serum TNF – α level at baseline and 1 month

	Mean	SD	P value	Significance
Baseline	12.93	2.07	0.001	Significant
1 Month	6.65	1.19		

- Baseline-12.93±2.07
- 1 Month-6.65±1.19

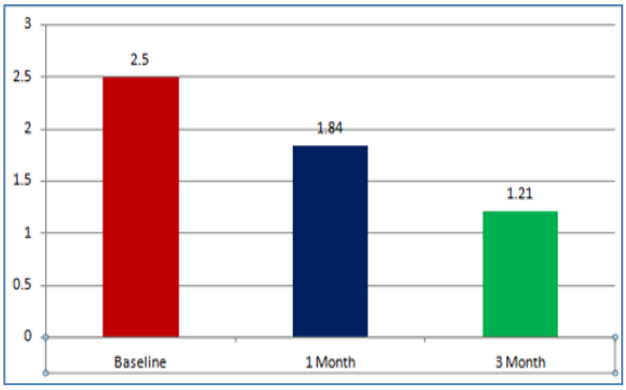


Graph 2: Shows the comparison of serum TNF – α for group I (before scaling and root planning) and group II (after scaling and root planning)

Comparison of sulcular bleeding index between baseline and different time intervals

Table 4: Shows comparison of sulcular bleeding index between baseline and different time intervals

Between Baseline and 1 Month				
	Mean	SD	P value	Significance
Baseline	2.50	0.550	0.001	Significant
1 Month	1.84	0.365		
Baseline and 3 Month				
Baseline	2.50	0.550	0.001	Significant
3 Month	1.21	0.352		
Between 1 Month and 3 Months				
1 Month	1.84	0.365	0.001	Significant
3 Month	1.21	0.352		

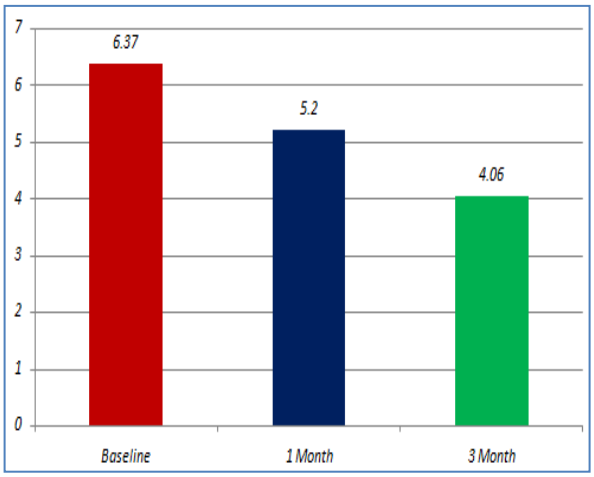


Graph 3: Shows comparison of sulcular bleeding index between baseline and different intervals

Comparison of probing depth between baseline and different time intervals

Table 5: Shows Comparison of probing depth between baseline and different time intervals

Between Baseline and 1 Month				
	Mean	SD	P value	Significance
Baseline	6.37	0.501	0.001	Significant
1 Month	5.20	0.302		
Baseline and 3 Month				
Baseline	6.37	0.501	0.001	Significant
3 Month	4.06	0.513		
Between 1 Month and 3 Months				
1 Month	5.20	0.302	0.001	Significant
3 Month	4.06	0.513		

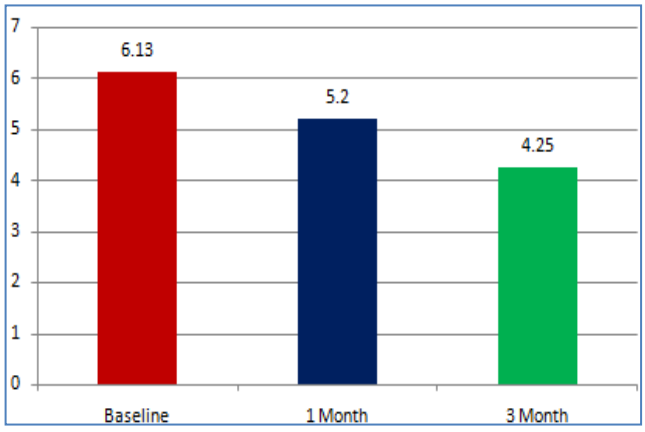


Graph 4: Shows Comparison of probing depth between baseline and different time intervals

Comparison of RAL between baseline and different time intervals

Table 6: Shows Comparison of RAL between baseline and different time intervals

Between Baseline and 1 Month				
	Mean	SD	P value	Significance
Baseline	6.13	0.482	0.001	Significant
1 Month	5.20	0.523		
Baseline and 3 Month				
Baseline	6.13	0.482	0.001	Significant
3 Month	4.25	0.575		
Between 1 Month and 3 Months				
1 Month	5.20	0.523	0.001	Significant
3 Month	4.25	0.575		



Graph 5: Shows Comparison of RAL between baseline and different time intervals

DISCUSSION

Periodontal diseases are inflammation, destruction and bacterial infections of the attachment apparatus, often leading to tooth loss.

It is a multifactorial disease with the presence of pathogenic bacteria which is necessaryfor the initiation of inflammation, but the progression of periodontal disease also depends on the host response to various pathogenic bacterial products and components.The bacterial products initiate a local host response in gingiva that involves recruitment of inflammatory cells, generation of prostanoides and cytokines, elaboration of lytic enzymes and activation of osteoclasts.[38]

Cytokines are main regulators of the host response to infection, tissue degradation and repair. cytokines play an important role in the maintenance of tissue homeostasis. Excessive and Continuous production of cytokines at the site of inflamed periodontal tissues are responsible for the progression of periodontal distruction.Among the cytokines at the site of inflamed periodontal tissues,TNF-α act to be important candidate for periodontal tissue distruction[41].Many cytokines has its own bioactivities that plays a consistent and contributory role in the destruction of bone and connective tissue in Periodontitis.These include IL-1, IL-l-α, and TNF-α[41].The tumor necrosis factor family has two members TNF alpha(cachectin) and TNF beta(lymphotoxin).TNF alpha is mainly produced by monocytes and macrophages and TNF beta is produced by lymphoid cells.TNF alpha has been grouped among major inflammatory cytokines because it is found at the site of inflammation and produced by mononuclear cells[7].

TNF can stimulate fibroblast **(Dayer, Beutler and Cerami,1985),** including gingival fibroblast to produce collagenase **(Rossomando et al,1987)**,an enzyme responsible for tissue distruction in periodontal disease and stimulates bone resorption **(Bertolini et al,1986).**

Increase in the levels of tumor necrosis factor-α also increases the production of prostaglandin E_2 and matrix-metalloproteinases. Matrix-metalloproteinases stimulate osteoclastic activity and results in bone destruction, which is the prominent pathogenesis of periodontal diseases[18].

PatrizziaM et al[38]conducted study to evaluate level of cytokines in periodontitis patients,levels of TNF alpha decreased after non surgical periodontal therapy.

The Aim of this study was to Estimate the level of TNF -α in Gingival Crevicular Fluid and Blood Serum in patient with Chronic Periodontitis before and after Scaling and Root Planing (SRP).

TNF-α Level was measured by collecting GCF and blood serum samples of individuals in study group at baseline and 1 month .The clinical parameters were assessed at baseline, 1month and 3 months postoperatively.

In this present study, GCF, serum Tumour Necrosis Factor- Alpha level (TNF-α) estimation has been performed since it is a potential prognostic biomarker of periodontal disease activity.

Heralgi R et al[38]**,** in a clinical study concluded that TNF-α plays a key role in the progression of periodontal disease and also provides site specific information on changes in TNF-α levels serving as a strong clinical marker of disease activity.

A similar study by **Gokul K**[33]**,** suggested a positive association between periodontal disease and increased levels of TNF-α in GCF and serum and a possibility of using the estimation of TNF-α in GCF as a "marker" of periodontal disease.

Study shows significant percentage change in the score of GCF TNF-α in Group 2(after SRP) as compared to Group 1(before SRP) was measured.

Study shows significant percentage change in the score of Serum TNF-α in Group 2(after SRP) as compared to Group 1(before SRP) was measured.

Hence the study shows that there was higher level of TNF-α in GCF and Serum found in periodontitis patients(Group I)before scaling and root planing. which significantly reduced after scaling and root planing in(GroupII) Patients.

This is similar to the results presented by **Meyle J**[13]**,** who reported elevated levels of TNF-α in serum of periodontitis patients as compared to healthy controls.

The results obtained in our study are closes to the results obtained by **Gorska et al** [22], who estimated low TNF-α values from healthy sites and high TNF- α values from periodontitis sites from gingival tissue biopsies suggesting that high concentration of TNF-α in periodontitis group strongly correlated with severity of periodontitis.

Results are inconsistent with the data reported by **Shapira, Van Dyke**[39], who showed no increase in serum levels of TNF-α in LJP patients.

This study states that TNF alpha plays a significant role in progression of periodontal disease and also provide site specific information on change in TNF alpha level as a strong clinical marker of disease progression.

Based on these facts, it seems reasonable to state that TNF alpha may be relevant in initiation and progression of periodotitis.

Summary and conclusion

Increased in level of cytokines, including TNF alpha and IL-6 has been seen in close association with periodontal disease. Chronic periodontal disease can be treated effectively by non-surgical or surgical therapy to reduce diseased condition. Level of cytokines before and after periodontal therapy reflects the response to periodontal treatment.

Comparison of Group 1(before Scaling and root planning) and Group 2 (after Scaling and root planing) between the different intervals shows that there is significant reduction in mean scores of mSBI, probing depth and gaining RAL at baseline, 1 month and 3 months. Thus, it shows that Scaling and Root-Planing is efficient in reducing gingival bleeding, probing depth and gaining RAL.

Study shows significant percentage change in the score of GCF TNF-α in Group 2(after SRP) as compared to Group 1(before SRP) was measured.

Study shows significant percentage change in the score of Serum TNF-α in Group 2(after SRP) as compared to Group 1(before SRP) was measured.

With the results of the present study, following conclusions were drawn: Clinical parameters and level of TNF alpha decreased after scaling and root planing. These observations indicates positive association between periodontal disease and increased level of TNF alpha in GCF and Serum.

The limitation to this study could be small sample size and observation period. Hence, more rigorous work needs to be done to confirm the effect of scaling and root planing in reducing the level of TNF alpha in GCF and Blood serum of periodontitis patients.

Reference

1. Carranza FA, editor: Clinical Periodontology, ed -10 ,Philadelpiia 1996, Saunders.
2. Shapira L, Stabholz A, Rieckmann P, Kruse N: Genetic polymorphism of the tumor necrosis factor (TNF)-a promoter region in families with localized early-onset periodontitis. J Periodont Res 2001; 36: 183±186.
3. Sugumari E., Thanga Kumaran S.:Statins: A new era in local drug deliveryJournal of Pharmacy and Bioallied Sciences.2012;4:2 - Part 2
4. Mc farlaneCG,reynoldsJJ,MeikleMC:,The release of interleukin 1-beta,tumor necrosis factor alpha and interferon-gamma by culured peripheral blood mononuclear cells from patients with periodontitis. J periodont Res 1990;25:207-214.
5. Morimoto Y, Kawahara K-I, Tancharoen S, Kikuchi K, Matsuyama T, Hashiguchi T, Izumi Y, Maruyama I:Tumor necrosis factor-a stimulates gingival epithelial cells to release high mobility-group box 1. J Periodont Res 2008; 43: 76–83. 2007.
6. Akihiko S,Mitsuhiro O, Naoyuki , Kichibee O, and Koichi: Profiling the Cytokines in Gingival Crevicular Fluid Using a Cytokine Antibody ArrayJ Periodontol 2006;77:856-864.
7. Jan Vilaek and Tae H. Lee:Tumor Necrosis Factor NEW INSIGHTS INTO THE MOLECULAR MECHANISMS OF ITS MULTIPLE ACTIONS THE JOURNAL OF BIOLOGICAL CHEMISTRY,Vol. 266, No. 12, April 25, 1991, 7313-7316,
8. Bruce B and Anthony C: THE BIOLOGY OF CACHECTIN/ TNFA PRIMARY MEDIATOR OF THE HOST RESPONSE. Ann. Rev. Immunol. 1989. 7:625-55
9. P Stashenko, F E Dewhirst, W J Peros:Synergistic interactions between interleukin 1, tumor necrosis factor, and lymphotoxin in bone resorption March 1, 1987, 138 (5) 1464-1468;
10. Steven Offenbacher: Periodontal Diseases: Pathogenesis.Ann Periodontol 1996;1:821-878.
11. Thomas E. Van D, Mary Ann L, and Lior S:The Role of the Host Response in Periodontal Disease Progression: Implications for Future Treatment Strategies,J Periodontol 1993; 64:792-806.
12. Donald R,Glenn E:,Stimulation of bone resorption and inhibition of bone formation in vitro by human tumor necrosis factors. vol. 319, 6 feb 1986. Nature publishing group
13. Meyle J:neutrophil chemotaxis and serum concentration of tumor necrosis-facor-alpha.J.periodontal Res 1993;28:491-493.
14. Ira B. Hamster: The Host Response in Gingival Crevicular Fluid: Potential Applications in Periodontitis Clinical Trials,J Periodontol 1992; 63:1117-1123.
15. E. F. Rossomando, J.E. Kennedy, J Hadjimichael: Tumor necrosis factor alpha in gingival crevicular fluid as a possible indicator of periodontal disease in humans..4r&v oral Biol. Vol. 35, No. 6, pp. 431434, 1990
16. Anirud, Srinath Thakur, et al: Tumor necrosis factor-α, interleukin-4 and -6 in the serum of health, chronic periodontitis, and type 2 diabetes mellitus.v.20(5); Sep-Oct 2016
17. N. Ravindra Reddy, D. S. Madhu Babu1,et al: Estimation of tumor necrosis factorα in chronic periodontitis and its corelation with preterm gestation: A Clinico biochemical study.Journal of Orofacial Sciences 108 Vol. 4 • Issue 2 • December 2012.
18. Shirin: The effect of chronic periodontitis on serum levels of tumor necrosis factor-alpha in Alzheimer diseas.Dent Res J (Isfahan). 2014 Sep-Oct; 11(5): 549–552.
19. Bruce and anthonyCerami: Cachectin and tumor necrosis factor as two sides of the same biological coin. nature vol.320.17 april 1986.
20. Ali cekici, Alpdogan K, Hatice H et al: Inflammatory and immune pathways in the pathogenesis of periodontal disease. Periodontology 2000, Vol. 64, 2014, 57–80.
21. Charles A. Dinarello:impact of basic research on tomorrow's medicine.(CHEST 2000; 118:503–508).
22. Go´rska R, Gregorek H, Kowalski J:Relationship between clinical parameters and cytokine profiles in inflamed gingival tissue and serum samples from patients with chronic periodontitis.J Clin Periodontol 2003; 30: 1046–1052.
23. D.T. Graves and D. Cochran: The contribution of Interleukin-1 and Tumor necrosis factor to periodontal tissue destruction ;J Periodontal 2003;74:391-401.
24. Dana Graves: Cytokines That Promote Periodontal Tissue Destruction. J Periodontol 2008;79:1585-1591.
25. Jonathan Lam et al: TNF-α induces osteoclastogenesis by direct stimulation of macrophages exposed to permissive levels of RANK ligand.journal of clnical investigation 10.1172/JCI11176,December 15, 2000.
26. Kinney JS, Morelli T, Oh M, Braun TM et al: Crevicular fluid biomarkers and periodontal disease progression. J Clin Periodontol 2014; 41: 113–120
27. Negar Falahi, et al: The Effect of Scaling and Root Planning on Salivary TNF-α and IL-1α Concentrations in Patients with Chronic Periodontitis Open Dent J. 2017; 11: 573–580.
28. A Dag, ET Fırat, S¸ Arıkan,etal:The effect of periodontal therapy on serum TNF-a and HbA1c levels in type 2 diabetic patients,Australian Dental Journal 2009; 54: 17–22
29. Çiğdem C Türer,DuyguDurmuş et al :Effect of Non-surgical Periodontal Treatment on Gingival Crevicular Fluid and Serum Endocan, Vascular Endothelial Growth Factor-A and Tumor Necrosis Factor Alpha Levels, Journal of Periodontology:2016

30. V Dodwad,ShubhraV,Aakriti M et al: Local drug delivery in periodontics:A Strategic Intervention,Int J Pharm Pharm Sci, Vol 4, Issue 4, 30-34,2012

31. Heasman PA,G Collins et al:Changes in crevicular fluid levels of interleukin-1β, leukotriene B_4, prostaglandin E_2, thromboxane B_2 and tumour necrosis factor α in experimental gingivitis in humans,Volume 28, Issue 4, Pages: 233-311,July 1993.

32. Hojatollah, RobatiMaryam,et al: Evaluation of salivary tumor necrosis factor-alpha in patients with the chronic periodontitis: A case-control study,J Indian 2013 Nov-Dec; 17(6): 737–740.

33. KalpanaGokul:Estimation of the level of tumor necrosis factor-a in gingival crevicular fluid and serum in periodontal health and disease:A biochemical study,Indian Journal of Dental Research, 23(3), 2012

34. Pierre Vassalli:The Pathophysiology of tumor necrosis factor,Annu. Rev. Immunol. 1992. JOAII-52.

35. Page RC:The role of inflammatory mediators in the pathogenesis of periodontal disease. J Periodont Res 1991;26:23042.