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 PLANNING: A BIOCHEMICAL STUDY

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Conflicts of Interest: Nil

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

Conclusion-Increase in level of cytokines, including TNF-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 mediating Lipopolysaccharide (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 inducer of bone resorption but it is much less potent.

Chronic low levels of TNF within the bloodstream have been attributed to cause the formation of atheromatous 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. TNF-α also activates osteoclasts and thus induces the bone resorption and also induces the synthesis of PGE1 and PGE2. TNF-α has synergistic effects with the bone resorptive actions of IL-1.

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. Thus, 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, aphthous 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 (group 1) 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 (1st premolar, 2nd premolar, 1st molar and 2nd molar). Probing depth.
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>
<thead>
<tr>
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<th>Frequency</th>
<th>Percent</th>
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<tbody>
<tr>
<td>Female</td>
<td>10</td>
<td>40.0</td>
</tr>
<tr>
<td>Male</td>
<td>15</td>
<td>60.0</td>
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Mean Age of Study Subjects 41.31±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

<table>
<thead>
<tr>
<th></th>
<th>Mean</th>
<th>SD</th>
<th>P value</th>
<th>Significance</th>
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</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>77.15</td>
<td>5.77</td>
<td>0.001</td>
<td>Significant</td>
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<tr>
<td>1 Month</td>
<td>37.37</td>
<td>7.50</td>
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**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

<table>
<thead>
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<th>Mean</th>
<th>SD</th>
<th>P value</th>
<th>Significance</th>
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<tbody>
<tr>
<td>Baseline</td>
<td>12.93</td>
<td>2.07</td>
<td>0.001</td>
<td>Significant</td>
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<tr>
<td>1 Month</td>
<td>6.65</td>
<td>1.19</td>
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- 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

<table>
<thead>
<tr>
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<th>Mean</th>
<th>SD</th>
<th>P value</th>
<th>Significance</th>
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<tbody>
<tr>
<td>Baseline</td>
<td>2.50</td>
<td>0.550</td>
<td>0.001</td>
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<td>1 Month</td>
<td>1.84</td>
<td>0.365</td>
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<td>3 Month</td>
<td>1.21</td>
<td>0.352</td>
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**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

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<thead>
<tr>
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<th>Mean</th>
<th>SD</th>
<th>P value</th>
<th>Significance</th>
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<tbody>
<tr>
<td>Baseline</td>
<td>6.37</td>
<td>0.301</td>
<td>0.001</td>
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<td>1 Month</td>
<td>5.20</td>
<td>0.302</td>
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<tr>
<td>3 Month</td>
<td>4.06</td>
<td>0.513</td>
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**Graph 4:** Shows comparison of sulcular bleeding index between baseline and different 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 necessary for 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 prostanoids and cytokines, elaboration of lytic enzymes and activation of osteoclasts.\(^\text{38}\)

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 destruction. Among the cytokines at the site of inflamed periodontal tissues, TNF-\(\alpha\) act to be important candidate for periodontal tissue destruction\(^{41}\). 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-\(\beta\)-\(\alpha\), and TNF-\(\alpha\)\(^{41}\). 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 distraction in periodontal disease and stimulates bone resorption (Bertolini et al, 1986).

Increase in the levels of tumor necrosis factor-\(\alpha\) 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\(^{18}\).
PatriziaM et al 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, 1 month 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, 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, 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 (Group II) Patients.

This is similar to the results presented by Meyle J, 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, 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, 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 perioditis.

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 disease condition. Level of cytokines before and after periodontal therapy reflects the response to periodontal treatment.

Comparison of Group 1(before Scaling and root planing) 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