

## Effect of non surgical periodontal treatment on TNF- $\alpha$ levels in serum of patients with chronic periodontitis

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### Abstract

**Background and objective:** Chronic periodontitis is a multifactorial inflammatory disease characterized by destruction of tooth supporting tissues. Tumor necrosis factor- $\alpha$ , a key inflammatory cytokine, plays an important role in periodontal destruction. This study aimed to analyze the effect of non-surgical periodontal treatment on tumor necrosis factor- $\alpha$  levels in serum of patient with chronic periodontitis (with and without using chlorhexidine 0.2% mouthwash).

**Methods:** A total of 44 patients with moderate to severe localized chronic periodontitis were divided into: Group I (n=22) without using of chlorhexidine mouthwash and Group II (n=22) with 0.2% chlorhexidine mouthwash. Serum samples collected from each subjects at baseline and four weeks after scaling and root planning treatment were quantified for tumor necrosis factor- $\alpha$  levels using ELISA technique.

**Results:** The mean concentration of TNF- $\alpha$  were significantly reduced one month after treatment in sera of patients with chronic periodontitis for group I when compared to its mean concentration before treatment ( $P < 0.05$ ), while in group II a highly significant reduction after one month treatment ( $P < 0.001$ ) was observed.

**Conclusion:** The effect of non-surgical periodontal therapy with chlorhexidine mouth wash showed more reduction in the levels of serum tumor necrosis factor- $\alpha$ .

**Keywords:** Chronic periodontitis, Tumor necrosis factor- $\alpha$ , Chlorhexidine mouth wash, Non-surgical periodontal treatment

### Introduction

Chronic periodontitis is a chronic infectious disease characterized by degradation and destruction of periodontal supporting tissue of the teeth.<sup>1</sup> The occurrence and progression rate of periodontal disease depend on complex interaction between periodontopathic bacteria include oral microorganisms such as *Porphyromonas gingivalis* (*P. gingivalis*) and cells of host immune system. These interactions are mediated by cytokines and chemokines, which are produced by both resident and emigrant cells at the site of inflammation.<sup>2</sup> There is good effect of non-surgical treatment like scaling on chronic periodontitis which is removal of the biofilm and calculus from both supragingival and subgingival tooth surfaces. No deliberate

attempt is made to remove tooth substance along with the calculus. Root planning is the process by which residual embedded calculus and portions of cementum are removed from the roots to produce a smooth, hard, and clean surface. The main objective of scaling and root planning is to restore gingival health by completely removing elements that provoke gingival inflammation from the tooth surface.<sup>3</sup> After scaling and root planning, the periodontal tissues require approximately four weeks healing sufficiently to be probed accurately. Patients also need the opportunity to improve their plaque control skills to both reduce inflammation and adopt new habits.<sup>3</sup> Chlorohexidinedigluconate (CHX) is a potent antibacterial substance that binds strongly to bacterial cell membrane.

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In periodontal treatment, the use of CHX in periodontal pockets as an adjunct non-surgical periodontal treatment produces important improvement in clinical parameters compared with conventional treatment.<sup>4</sup> Chlorohexidine mouthwash can be used as an adjunct to oral prophylaxis in reducing pro-inflammatory cytokines such as interleukine-2 (IL-2) and interferon gamma (IFN- $\gamma$ ) in patients with chronic periodontitis.<sup>5</sup> Cytokines change in periodontitis which is soluble proteins that serve as mediators of cell function and are produced by various cell types. Several lines of evidence have discovered that cytokines play significant roles not only in tissue homeostasis but also in the pathogenesis of many infectious diseases. Cytokines play crucial roles in the maintenance of tissue homeostasis, a process which requires a delicate balance between anabolic and catabolic activities. In particular tumor necrosis factor alpha (TNF- $\alpha$ ) which is a pro-inflammatory cytokine, that has been detected in gingival crevicular fluid and gingival tissues from individuals with periodontitis.<sup>6,7</sup> This cytokine is a potent immunologic mediator, in addition to its inflammatory effects, increases bone resorption and regulates fibroblast proliferation.<sup>8,9</sup> TNF- $\alpha$  activity is regulated by interleukin-10 and other anti-inflammatory molecules. The balance between these and other cytokines regulate the homeostasis of the immune system. Thus, in any inflammatory disease, the predominance of pro-inflammatory cytokines is expected, leading to an unbalanced response and further tissue destruction.<sup>10</sup> Thus, in view of the aforementioned findings, this study was undertaken to assess whether non-surgical periodontal treatment: scaling and root planning with and without using 0.2% CHX mouthwash is associated with changes in immunoserological marker of systemic inflammation like (TNF- $\alpha$ ).

## Methods

### Study subjects

Participants were selected from subjects who attended the Department of Periodontology, College of Dentistry, Hawler Medical University in Erbil city for the period from March to June 2013.

### Study design

Forty four patients (28 males and 16 females) were selected to participate in this comparative study by using convenience sampling method. Patients aged from 30 to 50 years, diagnosed as localized chronic periodontitis with moderate clinical attachment loss (3-4mm) or severe ( $\geq 5$ mm) with the presence of probing pocket depth (PPD)  $\geq 4$ mm were enrolled in this study. Patients were divided into two groups; Group I: Twenty two patients were treated by scaling and root planning without using of CHX and followed up after one month. Group II: Twenty two patients were treated by scaling and root planning with 0.2% CHX mouth wash and followed up after one month. The patients were asked to do mouth rinsing twice daily every 12 hours with 0.2% CHX for one month. All patients were systemically healthy and had not received periodontal treatment for at least 6 months prior to the clinical examination and sampling. Exclusion criteria were subjects with pregnancy/lactating females, deleterious habits like smoking/alcohol consumption, subjects with antibiotics, anti-inflammatory agents, immunosuppressant, or systemic contraceptive for the past six months. Subjects who satisfied the inclusion criteria of the study were selected and ethical approval was obtained from the Ethics Committee and the Scientific Committee of the College of Dentistry, Hawler Medical University. Each patient received a detailed explanation regarding the study procedure, and written informed consent was obtained from those who agreed to participate voluntarily in the study.

### Stent preparation

The purpose of using the occlusal stent in

this clinical trial study was to permit and guide the entry of periodontal probe into probing locations at measured sites (buccal, lingual, mesial, distal). First, for each patient an upper and lower alginate impression was taken to make stone – casts. Then, an individually customized transparent hard acrylic occlusal stent was constructed for each single arch of the patients. Stents were trimmed to the height of contour of the buccal and lingual surfaces with a periphery away from the cement-enamel junction and the gingival margins shown in Figure 1. In addition, fixed guiding steering grooves (buccal and lingual/palatal) and interproximal notches were made on the stent for each tooth included adjacent to the measured sites. The stents were used at baseline and after one month of treatment for follow up. Scaling and root planning were performed under local anesthesia (in some exceptional cases when we had some tooth sensitivity). After samples were collected basically performed in the same visit following the baseline data collection, a full mouth scaling and root planning was carried out as follows:

Gingival infiltration by local anesthesia (Ultracain D-S forte Karpul 40mg/ml; 1,7ml streil-apirojen). Full mouth scaling and root planning using electrical scaler in one visit. It was carried out until the root surface felt smooth with the tip of a periodontal probe. After scaling and root planning was completed the teeth were polished with prophylaxis paste containing pumice.

For Group II 0.2% CHX mouthwash (chorsodyle) twice daily for one month in addition to 0.2% CHX solution as a coolant with electric scaler, while Group I electric scaler used without adding CHX. A second visit was scheduled for all patients one week after the first visit to emphasize the need to follow the oral hygiene instructions. Chronic periodontitis patients were diagnosed based on the criteria of American Academy of Periodontology classification of periodontal diseases (2000). Periodontal evaluation included gingival index, bleeding on probing (BOP), probing pocket depth (PPD), and clinical attachment level (CAL).<sup>3,11,12</sup>

#### **Blood collection and processing**

Three ml of blood sample were drawn from the ante-cubital vein from each patient twice times before and after periodontal treatment. The blood was allowed to clot at room temperature which helps the plasma separate from cells and after two hours serum was separated from blood by centrifuging at 3000 rpm for 10 minutes. The extracted serum was immediately transferred to a plastic vial and stored at - 20° C in deep freezer,<sup>13</sup> at Blood Bank Unite of Nanakaly Hospital for Blood Diseases. Sera were testes for TNF- $\alpha$  by enzyme-linked immunosorbent assay (ELISA) according to the manufacturer instruction kit (Koma Biotech INC Human TNF- $\alpha$  /ELISA Kit: Cat# K0331131 U.S.A.) at Virology Unite of the Nanakaly Hospital for Blood Diseases.



**Figure 1:** Method of periodontal examination.

**Statistical analysis**

The data was analyzed using the statistical package for the social sciences (version 20). Microsoft Word and Excel have been used to generate figures, tables etc. Mean and standard deviation (SD) were calculated for all the parameters (PPD, CAL, BOP and TNF- $\alpha$ ,) of both groups at baseline and after four weeks. Continuous data were expressed as mean $\pm$ SD. Mean values of each parameter are compared between the groups using paired sample t test. A *P* value of  $\leq 0.05$  was considered statistically significant.

**Results**

Forty four patients with chronic periodontitis (28 males and 16 females aged from 30 to 50 years with the mean age 36.75 $\pm$ 6.12 years) were deprived of the exclusion criteria and completed the one month clinical trial.

**Clinical effects of periodontal treatment**

The mean value of periodontal parameters (BOP, PPD and CAL) for group I patients before treatment (46.700  $\pm$  24.023, 4.349  $\pm$  0.633 and 3.595  $\pm$  0.719, respectively) showed highly significant (*P* <0.001) decrease after one month treatment (11.825  $\pm$  10.190, 2.886  $\pm$  0.951 and 2.255  $\pm$  0.642, respectively) as shown in Table 1.

**Table 1:** Descriptive statistics for mean  $\pm$  SD and paired t-test for mean values of (BOP, PPD, CAL, and TNF- $\alpha$ ) in group I and II before and one month after treatment in patients with chronic periodontitis.

| Variables             |        | Group I: N=22<br>(without chlorhexidine) |                | Group II: N=22<br>(with chlorhexidine) |                |
|-----------------------|--------|--|----------------|--|----------------|
|                       |        | Mean $\pm$ SD                            | <i>P</i> value | Mean $\pm$ SD                          | <i>P</i> value |
| BOP %                 | Before | 46.700 $\pm$ 24.023                      | < 0.001        | 56.740 $\pm$ 24.660                    | < 0.001        |
|                       | After  | 11.825 $\pm$ 10.190                      |                | 9.079 $\pm$ 9.841                      |                |
| PPD (mm)              | Before | 4.349 $\pm$ 0.633                        | < 0.001        | 4.369 $\pm$ 0.469                      | < 0.001        |
|                       | After  | 2.886 $\pm$ 0.951                        |                | 2.833 $\pm$ 0.667                      |                |
| CAL (mm)              | Before | 3.595 $\pm$ 0.719                        | < 0.001        | 3.874 $\pm$ 0.655                      | < 0.001        |
|                       | After  | 2.255 $\pm$ 0.642                        |                | 2.486 $\pm$ 0.663                      |                |
| TNF- $\alpha$ (pg/ml) | Before | 31.079 $\pm$ 17.690                      | 0 <05*         | 40.583 $\pm$ 21.523                    | 0 <001**       |
|                       | After  | 27.298 $\pm$ 20.254                      |                | 30.198 $\pm$ 12.576                    |                |

SD- Standard deviation

In group II patients with the use of 0.2% CHX mouthwash, the mean value of periodontal parameters (BOP, PPD and CAL) before treatment ( $56.740 \pm 24.660$ ,  $4.369 \pm 0.469$  and  $3.874 \pm 0.655$ , respectively) showed highly significant ( $P < 0.001$ ) decrease after one month treatment ( $9.079 \pm 9.841$ ,  $2.833 \pm 0.667$  and  $2.486 \pm 0.663$ , respectively) as demonstrated in Table 1.

**Changes in circulating level of TNF-  $\alpha$**

As revealed in Table 1, the mean concentration of TNF- $\alpha$  in sera of patients with chronic periodontitis for the Group I showed significant decrease in mean concentration ( $27.298 \pm 20.254$ pg/ml) after one month treatment, as compared to its concentration ( $31.079$  pg/ml) in serum of the same patients before treatment ( $P < 0.05$ ). However, our results showed a highly significant decrease in mean concentration of TNF- $\alpha$  ( $30.198 \pm 12.576$  pg/ml) in sera of Group II patients with chronic periodontitis after one month treatment, as compared to its concentration ( $40.583 \pm 21.523$ pg/ml) before treatment ( $P < 0.001$ ). On clarifying the role of using 0.2% CHX mouthwash, a comparison in the mean value of periodontal parameters (BOP, PPD and CAL) and pro inflammatory cytokine (TNF- $\alpha$ ) between group I and II patients one month after treatment was done using paired t-test. CHX mouthwash caused more obvious reduction in all studied parameters although all the changes were statistically non-significant ( $P > 0.05$ ) as shown in Table 2.

**Discussion**

In this study, the periodontal parameters; BOP, PPD and clinical attachment loss (CAL) showed high significant reduction in their mean scores one month after periodontal treatment in Group I and II, in comparison to their mean scores before treatment. The results are in agreement with those obtained by George and Janam,<sup>14</sup> who showed a highly significant reduction in the mean value of PPD and CAL two months after treatment. The present study also supported by Radafshar et al,<sup>15</sup> who suggested that non-surgical periodontal treatment was significant in improvement of the PPD, CAL and bleeding scores. Furthermore, statistically significant reduction in clinical index scores (CAL and PPD) were noted in chronic periodontitis patients after non-surgical periodontal treatment.<sup>16</sup> These results were also in agreement with the findings of Radafshar et al,<sup>17</sup> who stated that patients with chronic periodontitis treated by full mouth scaling and root planning within 24 hours plus a local application of antiseptic and prescription of 0.2 % chlorhexidine mouth wash once daily for 14 days without any intra pocket disinfecting irrigation conducted highly significant reduction of clinical parameters (BOP, PPD and CAL) after one month of the treatment ( $P < 0.001$ ). Our results were in the same direction with Chauhan et al,<sup>18</sup> who observed that a significant reduction in (PPD) and gain in (CAL) between base line and three months follow up ( $P < 0.05$ ). In a randomized, control clinical trial,

**Table 2:** Paired t-test for mean values of (BOP, PPD, CAL, and TNF- $\alpha$ ) between group I and II one month after treatment in patients with chronic periodontitis.

| Parameters            | Group I: N=22<br>(Without chlorhexidine)   | Group II: N=22<br>(With chlorhexidine)     | P value |
|-----------------------|--|--|---------|
|                       | After one month treatment<br>Mean $\pm$ SD | After one month treatment<br>Mean $\pm$ SD |         |
| BOP (%)               | 11.825 $\pm$ 10.190                        | 9.079 $\pm$ 9.841                          | 0.361   |
| PPD (MM)              | 2.886 $\pm$ 0.951                          | 2.833 $\pm$ 0.667                          | 0.827   |
| CAL (mm)              | 2.255 $\pm$ 0.642                          | 2.486 $\pm$ 0.663                          | 0.225   |
| TNF- $\alpha$ (pg/ml) | 27.298 $\pm$ 20.254                        | 30.198 $\pm$ 12.576                        | 0.519   |

45 patients were assigned to four weeks rinsing with a 0.05 CHX /herbal extract combination or a 0.1 % CHX solution. The results were significant decrease in PPD ( $P < 0.001$ ).<sup>19</sup> In contrast to our findings a systematic review on the effects of local antimicrobials as adjuncts to subgingival debridement, compared with subgingival debridement alone, in the treatment of chronic periodontitis demonstrated that the application of CHX and metronidazole showed a minimal effect when compared with placebo on BOP, CAL and PPD.<sup>20</sup> A systematic review of the effect of full mouth debridement with and without antiseptics in patients with chronic periodontitis by Lang et al,<sup>21</sup> reported that despite the significant differences of modest magnitude, full mouth debridement with antiseptic or full mouth scaling and root planning without antiseptic don't provide clinically relevant advantages over conventional staged debridement in BOP, CAL and PPD. The explanation for this variation may be due to the effectiveness of scaling and root planning because restoration and epithelialization of the sulcus generally require two to seven days and restoration of the junctional epithelium occurs as early as five days after treatment. Immature collagen fibers appear within 21 days. Healthy gingival fibers inadvertently severed from the tooth and tears in the epithelium are repaired in the healing process.<sup>22</sup> Several investigators have reported that in monkeys and humans treated by scaling and root planning, healing results in the formation of a long, thin junctional epithelium with no new connective tissue attachment. In some cases, this long epithelium is interrupted by "windows" of connective tissue attachment.<sup>3</sup> This study found that the mean concentration of TNF- $\alpha$  was highly significantly decreased in sera of patients with chronic periodontitis one month after treatment, when compared to its concentration in sera of the same patients before treatment for both groups, particularly for group II. A study done by

Dag et al.,<sup>23</sup> showed that significant decreases determined in the TNF- $\alpha$  level between the base line and three month measurements. Periodontal therapy was shown to reduce the serum levels of IL-6, TNF- $\alpha$  and CRP.<sup>24,25</sup> Periodontitis may conceivably contribute to circulating TNF- $\alpha$  in at least three ways. First, enhanced the production of TNF- $\alpha$  by monocytes, with periodontitis may indicate a hyper responsiveness of monocytes to bacterial challenge.<sup>26</sup> Second, the translocation of Gram -negative species from the periodontal biofilm into the circulation may cause TNF- $\alpha$  level to become elevated. Geerts et al,<sup>27</sup> demonstrated that plasma endotoxin levels increased following mastication in subject with periodontitis. As systemically administered endotoxin is known to cause TNF- $\alpha$  level to become elevated,<sup>28</sup> it is plausible that endotoxin entering the circulation from the periodontal biofilm may account for elevated TNF- $\alpha$ . Both attachment loss and plasma endotoxin are significant independent predictors of elevated plasma TNF- $\alpha$ .<sup>27</sup> A third possible mechanism may result from a direct cytokinaemia from the gingival crevicular fluid (GCF) i.e., a translocation of cytokines from the periodontal ligament into the circulation. Scaling and root planning result in a short-term increase in circulating TNF- $\alpha$ ,<sup>29</sup> that may be due to septicemia. Subjects with mild periodontitis had significantly lower TNF- $\alpha$  level than those with moderate or severe periodontitis. Our results suggest that periodontitis may influence serum TNF- $\alpha$  level by any of these three mechanisms. Interestingly, TNF- $\alpha$  induces the secretion of collagenase by fibroblast, stimulates the resorption of bone and has been implicated in the destruction of periodontal tissue in periodontitis.<sup>30</sup> In contrast to our results, Yamazaki et al,<sup>31</sup> explored that TNF- $\alpha$  and IL-6 level didn't change following periodontal treatment and there was no difference in the serum levels of these inflammatory cytokines between patients either at base line or at reassessment and control subjects.

**Conclusion**

Non-surgical periodontal treatment (scaling and root planning) with using of 0.2% chlorohexidine mouth wash leads to the recovery of inflammation by reducing the serum level of TNF- $\alpha$  with highly significance difference in patients with chronic periodontitis after one month treatment. Therefore, it is suggested to design further longitudinal studies with larger sample sizes on other different groups of periodontal diseases to evaluate this biomarker and also measure this biomarker in gingival crevicular fluid, saliva and compare with serum in different groups.

**Conflicts of interest**

The authors report no conflicts of interest.

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