Salivary IL-6 and TNF-α in patients with periodontitis

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Abstract:
Background: Periodontitis is a (chronic) bacterial infection represented by connective tissue breakdown persistently, inflammation and destruction of alveolar bone interfered by pro-inflammatory mediators. As a diagnostic non-invasive fluid; saliva could be used in diagnosis of systemic and oral diseases. The salivary biomarkers levels like cytokines could certainly be applied as an alternate to separate periodontal healthy from periodontitis subjects. Objective: Aim of the study is to evaluate the salivary (TNF-α and IL-6) in control participants and chronic periodontitis then further identify with clinical parameter levels such as the plaque index (PI), the gingival index (GI), the clinical attachment loss (CAL) and the probing pocket depth (PPD). Subjects and methods: In present study (75) patients with the age ranging from (36-65) years were registered. The sample was categorized into two main categories (25) of them were healthy (control) and other (50) have chronic periodontitis (CP). All of the attendants from the department of Periodontics of al-Shaheed Nasser al-Mosawi Specialist center in Al-Najaf city. All individuals that participate in this study were healthy and not receiving any periodontal treatment or antibiotic or anti-inflammatory remedies in the past three months prior to research. Clinical Parameters of Periodontal include Gingival Index (GI), Plaque index (PLI), the clinical attachment level (CAL) and the probing Pocket depth (PPD). Samples of the participants’ saliva were used to assess levels of the IL-6 and TNF-α by using the enzyme-linked immunosorbent assay (ELISA). Results: There were highly significant differences among subjects suffering from periodontitis when compared to the control measured with periodontal criteria and parameters, like: (indices of plaque, Gingiva, in addition to measurement for the depth of pocket (PPD) with attachment loss (L.A) (p-value ≤0.001). The concentration of salivary interleukin-6 and tumor necrosis factor-α were highly significant for chronic periodontitis group (10.15± 3.03)(13.04±18.04), than control group (3.25±1.92) (2.32±3.72) respectively. In addition, the mean of periodontal pocket depth (3.84 ±0.19) and clinical attachment loss (2.65±0.21), were highly significant for group of chronic periodontitis when compared to the control. Conclusion: Salivary interleukin-6, with TNF-α were highly in patients with periodontitis in comparison to the control, which could be explained, as indicator might clarified the idea about the advance periodontitis.

Keywords: TNF-α, Interleukin-6, cytokine, Chronic Periodontitis

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Introduction:
Periodontal disease is the inflammatory disease that affect any segment of the periodontium, which includes a periodontal ligament, gingiva and an alveolar bone (1). It was divided in to major types: gingivitis and periodontitis (2,3). The gingivitis is defenders a process of the gingival tissues marginally inflammation without apparent loses of attachment to connective tissue or bone, laid by irritation of substance locally that came from accumulation of plaque (microbial) on the tooth surface (4). Untreated gingivitis may cause apical extension of the inflammation and result in periodontitis which is more destructive form of disease (5). Periodontitis is a bacterial chronic infection of gingiva defined by losing attachment with the jaw bone and the tooth (6). This disease also specified by breakdown of connective tissue, persistent inflammation and destruction of alveolar bone. The products of tissue breakdown and inflammatory mediators have been more often recognized in crevicular fluid of gingiva, tissues, saliva and serum of gingiva(7). Periodontitis causes increase in locally pro-inflammatory mediator of cytokines which may play a critical role in chronic inflammatory process in
periodontitis. Cytokines are small and loose polypeptides of immunomodulatory, metabolic, and inflammatory properties. They're produced by lymphocytes, neutrophils, macrophage, dendritic cells, fibroblasts, monocyte, and endothelial cells. Cytokines are considered an interaction between non-immune and immune cells. The inflammatory mediators may be used as diagnostic markers as they are important to the pathogenesis of diseases.

Interleukin-6 (IL-6) is a basic cytokine utilized in host response regulation to the tissue damage and infection. It is sensitized by different cells, fibroblast, monocyte osteoblast and endothelial cells of vessels in regard to inflammation. It takes a crucial part in differentiation of B-cell and maturation of T-cell. Coordination among interleukin -6 (IL-6) and IL-1β leads to resorption of bone. The Interleukin 6(IL-6) is a particular and specific parameter in researches related to periodontics due to its' acting in inflammatory process and resorption of the bone with the action of osteoclasts activity. Remarkable level of the Interleukin-6(IL-6) has linked to the periodontal disease severity and the age.

One of the pro-inflammatory cytokine that causes periodontal tissue destruction is Tumor necrosis factor TNF-α and TNF-β. TNF-α is synthesized by keratinocyte, activated macrophage, neutrophil, mast cell, monocyte and in regarding to lipopolysaccharides (LPSs). TNF-β is generated by TH1 subsets of CD4+ T cells that activated by antigens or mitogens. TNF-α has a varied action, broadly pro-inflammatory. Vascular permeability and Leukocyte recruitment are expedited by the stimulated expression of selectins and adhesions by TNF-α. Macrophage prompted angiogenesis is also induced by TNF-α and has a critical role in the periodontal granulation tissue formation in vascular proliferation. In response to bacterial LPS, TNF-α activates osteoclast proliferation, activation and differentiation leads to bone resorption.

Saliva has been presumed to be a beneficial in the diagnosis of systemic and oral disorders non-invasively. Salivary biomarkers levels like cytokines should certainly be used up an alternate that differentiated periodontal healthy subjects from those have periodontitis. Saliva has long been considered to assess periodontitis and its role as a diagnostic mean for subject of reliable research activity. Proposed mediators for the disease include proteins of host origin (immunoglobulin, enzymes and cytokines), phenotypic markers (epithelial keratins), hormones (cortisol), host cells, bacteria and its products. The biomarkers help in recognize susceptible patients and serve as surrogate endpoints for monitoring the end of therapy. It therefore, meets the demands of being an inexpensive and noninvasive collection technique.

Current study was conducted to determine the levels of Interleukin-6 (IL6) and tumor necrosis factors (TNF-α) in saliva and correlate their levels with clinical parameters in patients with chronic periodontitis (CP) and compared to subjects with healthy periodontium.

Aims of the study
To detect the levels of tumor necrosis factor (TNF-α) and Interleukin-6 (IL6) in subject with normal periodontium when compared to patients with chronic periodontitis.

To correspond the levels of tumor necrosis factor (TNF-α) and Interleukin-6 (IL6) with the clinical parameter (gingival index (GI), plaque index (PI), probing pocket depth (PD), and clinical attachment loss (CAL)).

Materials and Methods
Subjects
Seventy-five (75) patients, (25) healthy subjects (control group) and (50) of patients having chronic periodontitis(CP) with ages between (36-65) years that were grouped at department of the periodontics of al-Shaheed Nasser al-Mosawi Specialist center in Al-Najaf city. All subjects were in healthy condition systematically and without receiving any past periodontal management or taken anti-inflammatory, immunomodulatory, antibiotics treatment about three (or less) months earlier to the research. Review committee of official ethics consent for the study was obtained. Every patient was given a written explanation for the means of this research and asked for signing the consent.

2 Exclusion Criteria
Any subject receiving systemic treatment like antibiotic or anti-inflammatory within the past three months, patients that have any medical condition including bacterial, fungal and viral infections, diabetes, Smokers, alcoholics, and persons with less than 20 teeth precluded from the research.

3 Design of the Study

The patients were told about the purpose of the research and consent to be a part in this study was signed. All the patients were questioned about their name, age, dental, history of medical and drug conditions and smokers or alcoholics. After taking examinations, collecting saliva with registering the parameters of clinical periodontics (PPD, CAL, GI and PLI) accomplished.

4. Plaque index (PI)

Oral hygiene was first examined by application of plaque index (33). Six indices were assessed for specifying all teeth, by using sharp straight explorer. The study involved only fully erupted teeth and it is not replaced by adjacent tooth if missed or partially erupting index tooth was found out.

Gingival Index (GI):

The condition of gingiva was evaluated by applying gingival index (34). Examination sequels were similar to that of dental plaque; the same Ramfjord index teeth were examined.

5. Probing Pocket Depth (PPD)

William’s periodontal probe was used, length was registered between the border of gingiva and the periodontal pocket base or sulcus of gingiva in millimeters. The probe was inserted into pocket of gingiva and sulcus near to the tooth's long axis at each tooth’s surfaces (labial/ buccal, mesial, lingual /palatal and distal) surfaces, during inserting the William’s probe, no pressure was applied (35).

6. Clinical attachment level (CAL)

Again William's periodontal graduated probe was inserted to each tooth's the lingual (palatal), buccal (labial), distal and mesial surfaces; the length along the cemento-enamel junction [CEJ] in an upward to apex and basal of the gingival sulcus or pocket to calculate the proximate millimeter (36).

7. Saliva samples

Whole unstimulated saliva (Five milliliters) was collected from the individuals. The material was gathered at the morning (between 08.00 and 12.00), at least two hours postprandial, responding to the procedure explained by Navazesh (37). The samples that were collected were frozen at –80°C, and the analysis was applied for a time not expanded more than six months of freezing time.

8. Cytokine test:

The level of cytokine IL-6 and TNF-α in saliva where measured by utilizing enzyme linked immune sorbent assay [ELISA] process related to the Bio-Source Europe S.A. kits.

Calculation of results

The results of total patients and control groups where measured by interposing from standard curve applying the curve equation fit for IL-6 and TNF-α.

Statistical analysis: Values of all parameters were performed as mean , Standard Deviation (SD) and the remarkable differences between the means were evaluated by (ANOVA test) , Duncan's test or the least significant differences (LSD ) by employing the computer programmed social package for statistical analysis (SPSS) , version 7.5 where the probability [P] less than or equals to ( 0.05) was considered significant .

Result :

Table (1) illustrate the distribution of the examined groups divided according to the age groups in years. These samples were subdivided into the main two groups (25) healthy subject, and (50) patients diagnosed as periodontitis.

In table (2) The results of these statistical findings of these periodontal parameters, were shown the mean of the study groups with the standard deviation for indices like, plaque and Gingival, pocket
depth (PPD) with loss of attachment (L.A), are well defined in table (2), these findings manifested that higher values for these all samples shown between study groups in comparison to findings of control groups, with significance of highly difference (P ≤ 0.01), PI in chronic periodontitis (0.98±0.34) than control group (0.25±0.08), GI in CP (1.13±0.39) when compared to the control group (0.26±0.13), further more mean for probing pocket depth (3.84 ±0.19 ) in CP group while in the control group (2.42±0.21), the mean of clinical attachment loss (2.65±0.21), and in the control group (0.93±1.09).

In table (3), the findings manifested high significance differences in concentration for the salivary (IL-6) and TNF-α between CP(10.15± 3.03)(13.04±18.04) and control group(3.25±1.92) (2.32±3.72) respectively.

Table (4), demonstrate the correlation coefficient among salivary IL-6 and periodontal parameters in control group. The findings of our study illustrate, weak positive not correlational significant among the index of plaque with level of salivary Interleukin 6 r (0.021), p-value was (0.891), While there were, negative not significantly correlation among GI with level of salivary IL6 r (-0.160) p-value (0.490), all others correlation shown weak positive with no significance (P≥0.05).

Table (5), illustrate correlation coefficient, among salivary IL-6 with mentioned periodontal parameters, in group of periodontitis, there were clearly weak, positive correlation with non-significantly among PLI, GI with level of salivary Interleukin 6 as shown r (0.276)and the value of p (0.224), r was (0.394) while p-value was (0.087), in addition the correlation were moderate, positive, highly significant among pocket depth, attachment loss with level of salivary Interleukin 6 as demonstrated r (0.599), p-value was (0.006), r (0.681), p-value was (0.003) respectively.

Table (6), shows correlation between salivary TNF-α and clinical periodontal parameters among control group. The findings demonstrate a significant negative correlation among TNF-α with PI (r = −0.35, while the value of was P = 0.03), while not significant correlation were found for the other parameters.

Table (7), demonstrate the correlation among salivary TNF-α with clinical parameters among study group. The direction of correlations, varied between negative and positive not significant correlation, recorded for the periodontal parameters (P≥0.05).

**Table (1): Age distribution of the examined groups**

<table>
<thead>
<tr>
<th>Age groups</th>
<th>Control</th>
<th>Patient</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>%</td>
<td>No.</td>
</tr>
<tr>
<td>36-45</td>
<td>32%</td>
<td>15</td>
</tr>
<tr>
<td>46-55</td>
<td>52%</td>
<td>20</td>
</tr>
<tr>
<td>56-65</td>
<td>20%</td>
<td>9</td>
</tr>
<tr>
<td>Total</td>
<td>100%</td>
<td>50</td>
</tr>
</tbody>
</table>
Table (2) Mean and standard deviation of a study and control group (plaque, gingival index (PI) (GI) and (PPD) Periodontal pocket depth with loss of attachment (L.A), IL-6 and TNF-α

<table>
<thead>
<tr>
<th>Variable</th>
<th>Groups</th>
<th>Descriptive Statics</th>
<th>Groups Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mean</td>
<td>SD±</td>
</tr>
<tr>
<td>GI</td>
<td>Control</td>
<td>0.26</td>
<td>0.13</td>
</tr>
<tr>
<td></td>
<td>Study</td>
<td>0.13</td>
<td>0.39</td>
</tr>
<tr>
<td>PPD</td>
<td>Control</td>
<td>2.42</td>
<td>0.21</td>
</tr>
<tr>
<td></td>
<td>Study</td>
<td>3.84</td>
<td>0.19</td>
</tr>
<tr>
<td>PI</td>
<td>Control</td>
<td>0.25</td>
<td>0.08</td>
</tr>
<tr>
<td></td>
<td>Study</td>
<td>0.98</td>
<td>0.34</td>
</tr>
<tr>
<td>L.A</td>
<td>Control</td>
<td>0.93</td>
<td>1.09</td>
</tr>
<tr>
<td></td>
<td>Study</td>
<td>2.65</td>
<td>0.21</td>
</tr>
<tr>
<td>IL-6</td>
<td>Control</td>
<td>3.25</td>
<td>1.92</td>
</tr>
<tr>
<td></td>
<td>Study</td>
<td>10.15</td>
<td>3.03</td>
</tr>
<tr>
<td>TNF-α</td>
<td>Control</td>
<td>2.32</td>
<td>3.72</td>
</tr>
<tr>
<td></td>
<td>Study</td>
<td>13.04</td>
<td>18.04</td>
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</table>

Table (3) correlation between variables for CP with IL-6

<table>
<thead>
<tr>
<th>Variables</th>
<th>IL-6</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(r)</td>
</tr>
<tr>
<td>GI</td>
<td>0.394</td>
</tr>
<tr>
<td>PPD</td>
<td>0.599</td>
</tr>
<tr>
<td>PI</td>
<td>0.276</td>
</tr>
<tr>
<td>L.A</td>
<td>0.681</td>
</tr>
</tbody>
</table>

Table (4) correlation between variables for the group of control with IL-6

<table>
<thead>
<tr>
<th>Variables</th>
<th>IL-6</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(r)</td>
</tr>
<tr>
<td>GI</td>
<td>0.160</td>
</tr>
<tr>
<td>PPD</td>
<td>0.070</td>
</tr>
<tr>
<td>PI</td>
<td>0.021</td>
</tr>
<tr>
<td>L.A</td>
<td>0.292</td>
</tr>
</tbody>
</table>
Table (5) correlation among variables in CP and TNF-α

<table>
<thead>
<tr>
<th>Variables</th>
<th>TNF-α</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(r)</td>
<td>(P)</td>
<td></td>
</tr>
<tr>
<td>GI</td>
<td>0.03</td>
<td>(N.S) 0.72</td>
<td></td>
</tr>
<tr>
<td>PPD</td>
<td>-0.21</td>
<td>(N.S) 0.49</td>
<td></td>
</tr>
<tr>
<td>PI</td>
<td>-0.23</td>
<td>(N.S) 0.401</td>
<td></td>
</tr>
<tr>
<td>L.A</td>
<td>-0.17</td>
<td>(N.S) 0.49</td>
<td></td>
</tr>
</tbody>
</table>

Table (6) correlation among variables in control groups and TNF-α

<table>
<thead>
<tr>
<th>Variables</th>
<th>TNF-α</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(r)</td>
<td>(P)</td>
<td></td>
</tr>
<tr>
<td>GI</td>
<td>0.03</td>
<td>(N.S) 0.88</td>
<td></td>
</tr>
<tr>
<td>PPD</td>
<td>/</td>
<td>/</td>
<td></td>
</tr>
<tr>
<td>PI</td>
<td>-0.35</td>
<td>0.03*</td>
<td></td>
</tr>
<tr>
<td>L.A</td>
<td>/</td>
<td>/</td>
<td></td>
</tr>
</tbody>
</table>

Discussion:

Periodontitis; is inflammatory process causing local arise in actual level for some important cytokines that play a crucial factor in destruction of periodontium these results are supported by Teny (2006) and Raher et al. (2007) (38;39) who reported that the immune response are different in these patients.

Saliva assume as non-invasive diagnosis fluid which can use for diagnosis of some cytokines and oral diseases. The salivary biomarker level like a cytokines could employ to distinguish between health periodontium and periodontitis (40).

salivary interleukin 6, produce by a number of cell type, manifest in a variety of situations as inflammatory reactions and host immune responses, our results clarify a highly significant difference in chronic periodontitis in comparison with control groups. The findings agree with with many studies reported that were increasing levels of interleukin 6 in periodontitis patients. Our results disagree with the results of (41;42;43;44;45;46;47) On the contrary Goutoudi (2012) and Frodge et al., (48;49) showed weakly correlated among quantities of (IL-6) in saliva.

The inflamed Periodontal tissue, destruction, together with IL-6 concentration in healthy sites was significantly higher when compared with diseased places after therapy, these results could be due to decrease in the volume of saliva after therapy, it propose that the cytokines quantity may be a good representative for assessment the situation of disease, as compare their concentration IL-6 has an effective and stimulatory role on desorption of bone, new researches with multiple large samples are necessary for registering more perfect in cytokine expression could be responsible for future studying of the variance.

TNF-α has high, significantly differences between the chronic compared to control subjects. TNF-α regarded as the most important cytokine in the periodontitis pathogenicity could be explained by their role in the destruction and erosive reaction of periodontal tissue, TNF-α was secreted by both monocytes and macrophages as a result to bacterial components, like lipopolysaccharide found in the
mouth, high concentration of the TNF-α could promote release of collagenase from fibroblasts of infected human gingiva, resulting in destruction of collagen of the cartilage with resorption of the bone (52;53). It was manifested, there was none correlation among concentration of salivary TNF-α and explanation for the chronic, degenerative changes, like indices of plaque and gingival in addition to the probing pocket depth, (54) therefore, TNF-α considered as a good indicator inflammatory process.

Conclusion:-

The values of plaque, gingival, pocket depth, attachment loss, concentration of; (IL-6) in addition to (TNF-α), which were, recorded high in the group of periodontal disease in comparison to control, with significant differences.

The productiveness of both salivary (TNF-α) and (IL-6), may labeled like indicator signifies important obstructor around periodontal health situation, furthermore high levels for both salivary (IL-6) plus (TNF-α) might give important implement for the early diagnosis, with more clearly screening of disease.

References:

48. Goutoudi, Paschalina; Diza, Evdoxia; Arvanitidou, Malamatenia Effect of Periodontal Therapy on Crevicular Fluid Interleukin-6 and Interleukin-8 Levels in Chronic Periodontitis International Journal of Dentistry; 2012.