EFFECT OF NON SURGICAL PERIODONTAL THERAPY ON LEVELS OF INFLAMMATORY BIOMARKERS IN STAGE III/IV GENERALIZED PERIODONTITIS PATIENTS VERSUS PERIODONTALLY HEALTHY INDIVIDUALS

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ABSTRACT

Aim of the present study: To assess the levels of Interleukin-17 and Interleukin-18 in the gingival crevicular fluid (GCF) of stage III/IV generalized periodontitis patients compared to their levels in periodontally healthy individuals.

Subjects and methods: Fifty subjects were included; twenty five of them are diagnosed with stage III/IV generalized periodontitis and the other twenty five are periodontally healthy subjects. Probing depth (PD), clinical attachment level (CAL), plaque index (PI) and gingival index (GI) were recorded for all the enrolled subjects. GCF levels of Il-17 and IL-18 were analyzed by enzyme-linked immunosorbent assay (ELISA).

Results: Clinical parameters and GCF levels of Interleukin -17 and Interleukin -18 were higher in stage III/IV generalized periodontitis than periodontally healthy subjects and both Il-17 and Il-18 are positively correlated.

Conclusion: Higher levels of Interleukin -17 and Interleukin -18 in stage III/IV generalized periodontitis and their decrease after non surgical treatment show that they may have a role in pathogenesis of aggressive periodontitis.

KEYWORDS: Stage III/IV generalized periodontitis, interleukin-17, interleukin-18, GCF
INTRODUCTION

Periodontitis is one of the inflammatory diseases related to many factors as bacterial agents present in the dental plaque biofilm, host susceptibility, and various environmental factors. The clinical characteristics of periodontitis involve gingival inflammation, clinical attachment loss (CAL), alveolar bone resorption, periodontal pocket formation (PD), and gingival recession. (Flemmig, 1999, Kinane, 1999, Tonetti and Mombelli, 1999).

In 2009, the incidence and intensity of periodontal tissue destruction were described to be around 47% among the United States mature population as documented by Eke’s research at 2012. The result of periodontal diseases is periodontal inflammation (Seymour et al., 2007) which finally leads to bone resorption, mobile teeth, and quick teeth loss (Poorsattar et al., 2014).

Therefore, phase 1 periodontal therapy comprising scaling and root planing with manual or powered instruments is considered as the main and typical treatment resulting in removal of calculus and disruption of any remaining biofilms (Axelsson and Lindhe 1981). Due to the increased incidence of periodontal disease, it is needed to identify new approaches for diagnosis of such diseases. Many biologic markers in body fluids such as gingival crevicular fluid (GCF) can be used as markers for observing first alterations in periodontal tissues and can also govern the usefulness of the periodontal therapy (Luke et al., 2015).

IL-17 can be synthesized by many cells as macrophages, natural killer cells and mast cells (Yao et al., 1995). Powerful intracellular biologic effects can occur when IL-17 combine with other cytokines (Korn et al., 2009).

The RANKL gene appearance was found to be increased by IL-17 which also decreases the osteoprotegerin gene expression in osteoblasts, together with increasing the bone resorption (Lubberts et al., 2003).

Similar to other inflammatory cytokines, IL-17 helps in bone remodeling and shows an intrabony defensive role against periodontal disease organisms such as porphyromonas gingivalis (PG) (Yu et al., 2007). Accordingly, the role of IL-17 remains uncertain (Takahashi et al., 2005, Kramer and Gaffen 2007, Kadkhodazadeh et al., 2013).

IL-1 cytokine family include many cytokines as Interleukin 18 (IL-18) that can be synthesized by keratinocytes, macrophages, epithelium cells of intestine, bone forming cells, and adrenal gland cortex cells (Dinarello 1999).

Together with IL-1b, IL-18 has a proinflammatory role and increases immune responses by stimulating production of additional cytokines as, IL-8, TNF-a, and IL-1b and also increases the reaction of T helper 1 (Th1) and T helper 2 (Th2) cells (Dinarello 2000, Gracie et al., 2003). It is also able to increase neutrophil chemotaxis and bone resorption; so it can be considered as a significant factor for cleaning of viruses and intracellular pathogens (Netea et al., 2002, Arend et al., 2008). The increased levels of IL-18 in host body fluid samples from diseased patients confirmed its role in pathogenesis of periodontal disease (Orozco et al., 2006 and 2007, Figueredo et al., 2008).

Due to the importance of early diagnosis of periodontal disease and the deficiency of data regarding the relationship between IL-17 and IL-18 and these diseases, the present study was performed.

SUBJECTS AND METHODS

Ethics approval

Research ethical committee in Faculty of Oral and Dental medicine, Future University in Egypt has accepted this clinical study with number (FUE. REC(2)/2-2023) and was also listed at https://clinicaltrials.gov/ under the number (NCT05295576).

Oral consent was taken from patients as many of them can’t read or write.
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1-Populations:
A total of 50 individuals were enrolled and categorized as;

Group A that included 25 stage III/IV generalized periodontitis patients and

Group B which included 25 periodontally healthy controls.

2-Inclusion criteria: Medically free subjects from both genders with age range between 30 to 60 years old were included (Kerr and Millard 1965).

3-Exclusion criteria: Patients with periodontal surgeries in the last 6 months, Subjects with prior usage of antibiotics in the last 6 months, Smokers, Pregnant and lactating females were excluded

4-Clinical parameters:

- **Plaque Index (PI):** as discussed by Silness and Loe, 1964.

- **Gingival Index (GI):**

  The degree of gingival inflammation was recorded by means of gingival index of (Löe and Silness, 1963), which divided tissues surrounding each tooth into four gingival scoring units: Disto-facial papilla, facial margin, mesio-facial papilla, and the entire lingual gingival margin.

- **Probing depth (PD) and Clinical Attachment level (CAL):**

  Both were recorded in 6 points (3 buccal and 3 lingual/palatal) using William’s graduated periodontal probe. Pocket depth was measured from the gingival margin to the base of the pocket while clinical attachment level was measured from the cemento-enamel junction (CEJ) to the base of the periodontal pocket (Glavind and Löe 1967).

5. GCF sampling:
In group A, the site with deepest probing depth was selected for GCF sampling while in group B, any site was sampled using PerioPaper strips according to guidelines provided by Bıyıkoğlu et al., 2013 and Kinney et al., 2014.

6. Phase I periodontal therapy
After baseline GCF sample collection and clinical measurements, group A patients received phase I periodontal therapy.

7. Analysis of IL-17 and IL-18 levels in GCF:
GCF samples were examined by enzyme-linked immunosorbent assay (ELISA). Procedures were achieved according to the manufacture directions in the kits.

**Statistical Analysis**
Numerical data were explored for normality by checking the distribution of data and using tests of normality (Kolmogorov-Smirnov and Shapiro-Wilk tests). IL-17 and IL-18 levels data showed normal (parametric) distribution while PI, GI, PD, CAL and amounts of change in all parameters showed non-normal (non-parametric) distribution. Numerical data were presented as mean and standard deviation (SD) values. For parametric data; Student’s t-test was used to compare between mean age values in the two groups. Repeated measures ANOVA test was used to compare between the groups and study the changes within group regarding IL-17 and IL-18 levels. Qualitative data were presented as frequencies and percentages. Chi-square test was used to compare between gender data in the two groups. The significance level was set at P ≤ 0.05. Statistical analysis was performed with IBM SPSS Statistics for Windows, Version 23.0. Armonk, NY: IBM Corp.

* PerioPaper, ProFlow, Amityville, NY, USA
RESULTS

Base line demographics

There was no significant statistical difference between mean age values in the two groups. There was also no statistically significant difference between gender distributions in the two groups.

Plaque Index (PI)

At base line; stage III/IV generalized periodontitis group showed higher statistical significant mean PI than control group ($P$-value <0.001, Effect size = 1.598). After three months; there was no statistical significant difference between the two groups ($P$-value = 0.209, Effect size = 0.003). There was a statistical significant decrease in mean PI in stage III/IV generalized periodontitis group after three months ($P$-value <0.001, Effect size = 0.834).

Gingival Index (GI)

At base line; stage III/IV generalized periodontitis group showed higher statistical significant mean GI than control group ($P$-value <0.001, Effect size = 2.243). After three months; there was no statistical significant difference between the two groups ($P$-value = 0.386, Effect size = 0.215). There was a statistical significant decrease in mean GI in stage III/IV generalized periodontitis group after three months ($P$-value <0.001, Effect size = 0.822).

Probing Pocket Depth (PD)

At base line as well as after three months; stage III/IV generalized periodontitis group showed statistically significantly higher mean PD than control group ($P$-value <0.001, Effect size = 3.313) and ($P$-value <0.001, Effect size = 2.3), respectively. There was a statistically significant decrease in mean PD in stage III/IV generalized periodontitis group after three months ($P$-value <0.001, Effect size = 0.891).

Clinical Attachment Level (CAL)

There was a statistical significant decrease in mean CAL in stage III/IV generalized periodontitis group after three months ($P$-value <0.001, Effect size = 0.854).

Interleukin-17 (IL-17) level

At base line as well as after three months; stage III/IV generalized periodontitis group showed higher statistical significant mean IL-17 level than control group ($P$-value <0.001, Effect size = 0.843) and ($P$-value = 0.001, Effect size = 0.21), respectively. There was a statistical significant decrease in mean IL-17 after three months in stage III/IV generalized periodontitis group ($P$-value <0.001, Effect size = 0.689).

Interleukin-18 (IL-18) level

At base line as well as after three months; stage III/IV generalized periodontitis group showed statistically significantly higher mean IL-18 level than control group ($P$-value <0.001, Effect size = 0.685) and ($P$-value <0.001, Effect size = 0.428), respectively. There was a statistically significant decrease in mean IL-18 after three months in stage III/IV generalized periodontitis group ($P$-value <0.001, Effect size = 0.657).

Correlation between IL-17 and IL-18 levels in stage III/IV generalized periodontitis group

There was a statistically significant direct correlation between IL-17 and IL-18 levels at base line as well as after three months ($r = 0.295$, $P$-value = 0.038) and ($r = 0.417$, $P$-value = 0.003), respectively.

**TABLE (1) Descriptive statistics and results of Student’s t-test and Chi-square test for comparison between base line characteristics in the two groups**

<table>
<thead>
<tr>
<th></th>
<th>Stage III/IV periodontitis (n = 25)</th>
<th>Control (n = 25)</th>
<th>$P$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td></td>
<td></td>
<td>0.492</td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>35.2 (6.2)</td>
<td>36.2 (3.7)</td>
<td></td>
</tr>
<tr>
<td>Gender [n (%)]</td>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Male</td>
<td>8 (32%)</td>
<td>8 (32%)</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>17 (68%)</td>
<td>17 (68%)</td>
<td></td>
</tr>
</tbody>
</table>

*: Significant at $P \leq 0.05$
**TABLE (2)** The mean, standard deviation (SD) values, results of Mann-Whitney U test and Wilcoxon signed-rank test for comparison between clinical parameters in the two groups and the changes within each group.

<table>
<thead>
<tr>
<th>Clinical parameter</th>
<th>Time</th>
<th>Stage III/IV periodontitis (n = 25)</th>
<th>Control (n = 25)</th>
<th>P-value</th>
<th>Effect size (d)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PI</td>
<td>Base line</td>
<td>2.28</td>
<td>0.74</td>
<td>1.059</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td></td>
<td>Change</td>
<td>0.80</td>
<td>0.28</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td></td>
<td>P-value (Effect size)</td>
<td>&lt;0.001*</td>
<td>(d = 0.834)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GI</td>
<td>Base line</td>
<td>0.72</td>
<td>0.61</td>
<td>0.560</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td></td>
<td>Change</td>
<td>1.24</td>
<td>0.78</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td></td>
<td>P-value (Effect size)</td>
<td>&lt;0.001*</td>
<td>(d = 0.822)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PD (mm)</td>
<td>Base line</td>
<td>2.96</td>
<td>0.73</td>
<td>1.360</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td></td>
<td>Change</td>
<td>2.44</td>
<td>1.04</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td></td>
<td>P-value (Effect size)</td>
<td>&lt;0.001*</td>
<td>(d = 0.891)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CAL (mm)</td>
<td>Base line</td>
<td>6.28</td>
<td>1.28</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Change</td>
<td>1.84</td>
<td>0.107</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td></td>
<td>P-value (Effect size)</td>
<td>&lt;0.001*</td>
<td>(d = 0.854)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*: Significant at P ≤ 0.05

**TABLE (3)** The mean, standard deviation (SD) values, results of repeated measures ANOVA for comparison between Interleukin 17 and 18 levels in the two groups and the changes within each group.

<table>
<thead>
<tr>
<th>Interleukins</th>
<th>Time</th>
<th>Stage III/IV periodontitis (n = 25)</th>
<th>Control (n = 25)</th>
<th>P-value</th>
<th>Effect size (Partial η^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-17</td>
<td>Base line</td>
<td>42.40</td>
<td>4.28</td>
<td>21.520</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td></td>
<td>Change</td>
<td>26.44</td>
<td>4.82</td>
<td>21.520</td>
<td>0.001*</td>
</tr>
<tr>
<td></td>
<td>P-value (Effect size)</td>
<td>&lt;0.001*</td>
<td>(Partial η^2 = 0.689)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-18</td>
<td>Base line</td>
<td>280.4</td>
<td>95.6</td>
<td>13.430</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td></td>
<td>Change</td>
<td>117.22</td>
<td>68.48</td>
<td>13.430</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td></td>
<td>P-value (Effect size)</td>
<td>&lt;0.001*</td>
<td>(Partial η^2 = 0.657)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*: Significant at P ≤ 0.05
TABLE (4) Results of Pearson’s correlation coefficient for the correlation between IL-17 and IL-18 levels

<table>
<thead>
<tr>
<th>Time</th>
<th>Correlation coefficient (r)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Base line</td>
<td>0.427</td>
<td>0.033*</td>
</tr>
<tr>
<td>3 months</td>
<td>0.470</td>
<td>0.018*</td>
</tr>
</tbody>
</table>

*: Significant at $P \leq 0.05$

DISCUSSION

The current study showed that all periodontal clinical parameters (e.g., PI, GI, PPD, CAL) were higher in the periodontitis group more than the healthy group. Also, IL-18 and IL-17 GCF concentrations were reported to be higher in patients with periodontitis more than the healthy controls. This was in agreement with studies of Ozçaka et al., 2011 and Banu et al., 2015.

The current study was performed on stage III/IV generalized periodontitis patients (previously known as chronic periodontitis (Armitage 1999)). Staging in the new periodontal disease classification refers to degree of periodontal disease severity or amount of periodontal tissue destruction. Stage III/IV periodontitis patients suffer from interdental clinical attachment loss $\geq 5$mm with teeth loss ranging from 4 teeth (Stage III) to 5 teeth or more (Stage IV). Extent in the new classification refers to distribution of the periodontal disease. Generalized pattern means that more than 30% of the existing dentition is affected (Caton et al., 2018).

Current study outcomes revealed greater levels of IL-17 in GCF of stage III/IV generalized periodontitis patients more than the periodontally healthy subjects. This was in accordance with Awang et al., 2014 who reported increased salivary and gingival serum concentrations of IL-17 in the group of chronic generalized periodontitis patients more than the healthy controls and they also confirmed that IL-17 concentration is directly correlated to clinical criteria as CAL and PPD. Also, Chitrapritya et al., 2015; Yang et al., 2016 documented lower levels of IL-17 in the healthy group compared to chronic generalized periodontitis group. However, some studies reported decreased levels of IL-17 in chronic generalized periodontitis versus periodontally healthy subjects which may be due to inconsistent case selection (Yetkin et al., 2009; Ozçaka et al., 2011).
Due to IL-17 inflammatory and bone resorption effects in many inflammatory bone diseases, it is proposed that this biomarker has a major role in periodontal disease pathogenesis (Kramer and Gaffen, 2007).

Moreover, The results of the present study showed higher levels of IL-18 in GCF of periodontitis patients, which were in accordance with the studies of Orozco et al., 2006; Figueredo et al., 2008; Pradeep et al., 2009 and Nair et al., 2016; so IL-18 is recognized as an important biomarker of periodontal tissues destruction. In contrast to the current study results about IL-18, Chitrapiyriya et al., 2015 reported variable concentrations of IL-18 and they attributed this to sampling regions with less inflammatory changes in the periodontitis group.

CONCLUSION

The current study confirms positive role of IL-17 and IL-18 in pathogenesis of periodontal disease due to the reported higher concentrations of both markers in stage III/IV generalized periodontitis patients compared to the periodontally healthy subjects and the decrease after non surgical periodontal treatment for the periodontitis group. The current study also confirms that IL-17 and IL-18 are directly correlated; when one of them increases, the other marker increases.

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