Study the effects of smoking on selected oral biomarkers levels in chronic periodontitis cases in Al-Hilla city

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Abstract
Alkaline phosphatase (ALP) is a hydrolase enzyme, a membrane-based glycoprotein produced by many cells within the area of the periodontium and gingival crevice. Granulocyte-macrophage colony-stimulating factor (GM-CSF) is a cytokine that functions as a white blood cell growth factor. GM-CSF stimulates stem cells to produce granulocytes (neutrophils, eosinophils, and basophils) and monocytes.

Subjects and method: the study comprised 40 participate, 25 smoker chronic periodontitis, 15 normal subject as control, age ranged between (20-50) years old, this study carried out in periodontal department clinic/ college of dentistry / university of Babylon, Hilla city, Iraq, 5 ml of unstimulated whole saliva was collected from all subjects to measure Alkaline phosphatase (ALP) and Granulocyte-macrophage colony-stimulating factor (GM-CSF) by Enzyme-linked immunosorbent assay technique (ELISA).

Results: the result show there are highly significant differences in the levels of ALP and GM-CSF between smoker and control

Conclusion: Smoking in chronic periodontitis patients has positive effects on levels of ALP and GM-CSF in saliva.

Keyword: Alkaline phosphatase, GM-CSF, saliva, chronic periodontitis

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INTRODUCTION
The Chronic Periodontitis (CP) has been defined as “an infectious disease resulting in inflammation within the supporting tissues of the teeth, progressive attachment loss, and bone loss(1) Cigarette smoking has a significant impact on the risk for developing periodontal disease and considered to be one of the most important environmental risk factors (2) Cigarette smoking is considered to be one of the most important environmental risk factors, which is closely related not only with the risk but also the prognosis of periodontitis (3) Biochemical mediators in oral fluids like saliva and gingival crevicular fluid (GCF) are highly beneficial in the determination of current periodontal status. These substances are known as biomarkers (4) These substances are known as biomarkers. They help in determination of inflammatory mediator levels, as they are good indicators of inflammatory activity. (5) Biomarkers, whether produced by normal healthy individuals or by individuals affected by specific systemic diseases are telltale molecules that could be used to monitor health status, disease onset, treatment response and outcome (6). Oral fluid biomarkers that have been studied for periodontal diagnosis include proteins of host origin (e.g., enzymes and immunoglobulins), phenotypic markers, host cells (7)(8). Among these enzymes of host origin are further divided into proteolytic and hydrolytic enzymes. Intracellular enzymes are increasingly released from the damaged cells of periodontal tissues into the GCF and saliva. Several enzymes that are evaluated for the early diagnosis of periodontal disease are aspartate and alanine, aminotransferase (AST, ALT), alkaline and acid phosphatase (ALP, ACP)(9) Advantages of saliva as diagnostic fluid abundant, noninvasive diagnosis and
monitoring of disease, simple in collection with a modest trained assistant and applicable in remote areas, painless, cost effective applicability for screening large population, patient suffers no discomfort, cheap technology as compared to other tests, little anxiety in the collection process (Malamud, 2006). (10).

Alkaline phosphatase (ALP) is a hydrolase enzyme, a membrane-based glycoprotein produced by many cells within the area of the periodontium and gingival crevice. It is released from polymorphonuclear neutrophils during inflammation, osteoblasts during bone formation, and periodontal ligament fibroblasts during periodontal regeneration. (11,12) ALP is one of the potentially powerful markers of periodontal disease activity. This was first recognized by Ishikawa and Cimasoni, (13) who demonstrated levels of enzyme in GCF three times those of serum and showed a significant correlation between ALP concentration in GCF and pocket depth. Binder et. al. (14) Granulocyte-macrophage colony-stimulating factor (GM-CSF) is a cytokine that functions as a white blood cell growth factor. GM-CSF stimulates stem cells to produce granulocytes (neutrophils, eosinophils, and basophils) and monocytes (15). (CSF)-1 is a growth factor that stimulates the survival, proliferation and differentiation of mononuclear phagocytes, which has been implicated in several inflammatory diseases. (16)

**MATERIAL AND METHOD**

This study was conducted in the Department of periodontology at the college of Dentistry, Babylon University, Hilla City Iraq, chronic periodontal disease diagnosis by periodontologist. This research was approved by Committee of Ethics at Research of the University of Babylon, signed forms was obtained from all participants before conducting the study. All subjects in this study were males, the age of subjects between (20-50) years, only those patients who were diagnosed sever chronic periodontitis (30% of teeth with clinical attachment level ≥5mm) [17].

The determinations of disease severity were based on criteria established in 1999 at the International Workshop for classification of periodontal disease and conditions [18]. All smoker subjects regularly smoked at least 10 cigarettes per day for at least 5 years. While we excluded the followings: -Female gender. All individuals with medical disorders such as diabetes mellitus, herps disease, thyroid disorder, etc. Former smoker, Receive antibiotic or anti-inflammatory drug and history of using mouth wash and use dental treatment at last three month, saliva sample mixed with blood or bloody sample was exclude.

**Collection of Saliva**

Unstimulating whole saliva collection according to modification of the method described by [19]. The assay of ALP and GM-CSF was performed by quantitative sandwich immunoassay technique (ELISA) using human (ALP and GM-CSF) kit and according to instruction of manufactured companies (Elabsience).

Analysis was done using statistical software, Statistical Package for Social Science (SPSS) version 17 for windows. an independent T test was used for analysis of Immunological parameters were used to compared between patients and control groups.

**RESULTS**

40 subjects were included in this study divided into two groups, The results of Alkaline phosphatase (ALP) shoe that show that the mean ± S. D level in smoker CP patients is \(27.136 ± 11.134\) where is Mean ± S. D of controls group \(19.676 ± 6.691\), there is highly significant differences among the groups \(P≤0.05\), as shown in Table (1), Figure (1)

<p>| Table 1. Mean and Standard deviation of alkaline phosphatase in smoker CP, patients’ group and control group |
|---|---|---|---|---|</p>
<table>
<thead>
<tr>
<th>character</th>
<th>Patients group No.</th>
<th>Control No. (15)</th>
<th>t - test</th>
<th>P</th>
<th>Significant*</th>
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<td>Alkaline phosphatase</td>
<td>Mean±S.D</td>
<td>Mean±S.D</td>
<td>27.136 ± 11.134</td>
<td>19.676 ± 6.691</td>
<td>2.7843</td>
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* Significant less than \(≤0.05\)
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Figure 2. Level of ALP in smoker CP. patients’ group and control group

- Evaluation of Granulocyte-macrophage colony-stimulating factor (GM-CSF)

The result of the study shows that the mean ± SD level in smoker CP. patients is \(22.81 \pm 4.94\) where is Mean ± S.

D of controls group \(15.44 \pm 6.44\), there is high significant differences among the groups \((P \leq 0.05)\), as shown in Table 2, Figure (2)

Table 2. Mean and Standard deviation of GM-CSF in smoker CP. patients’ group and control group

<table>
<thead>
<tr>
<th>character</th>
<th>Patients group No. (25)</th>
<th>Control No. (15)</th>
<th>t - test</th>
<th>P</th>
<th>Significant*</th>
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</thead>
<tbody>
<tr>
<td>GM-CSF</td>
<td>Mean±SD</td>
<td>Mean±S.D</td>
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<tr>
<td></td>
<td>22.81 ± 4.945</td>
<td>15.44 ± 6.44</td>
<td>4.0711</td>
<td>0.0002</td>
<td>HS</td>
</tr>
</tbody>
</table>

* Significant less than \(\leq 0.05\)
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Figure 3. Level of GM-CSF in smoker CP, patients’ group and control group

DISCUSSION
Chronic periodontal disease stimulates the secretion of several proinflammatory proteins that promote bone resorption, resulting in loss of tooth matrix and support (21). Salivary cytokines are produced during periodontal inflammation and tissue destruction (22), many markers have been used for evaluation of the diseased periodontal tissue. In our study the result of (ALP) showed that there was high-significant difference occurred between disease group and control group, this result was agree with( Fathima & Harish (23), who showed that ALP level were significantly higher in patient group more than in control group, their study in India and included 80 subjects were selected for the study divided to four group ( group1: healthy periodontium, group 2- gingivitis, group3 – periodontitis, group 4- periodontitis with smoker ), also this results of our study agreed with Ban & Leku’a (24) who showed that ALP level were significantly higher in smoker than non-smoker, this study was carried out in Baghdad and included 50 subjects(smoker group 25 ) and (nonsmoker group 25 ), our study disagreement with Ameer& Ali (25) , who showed non-significant difference between smoker and nonsmoker , this study carried out in Baghdad , (55 subjects patient groups) and (21 subjects study groups).While the results of GM-CSF in our study showed that there was high significant difference occurred between disease group and control group, this result was agree with( Ronaldo Lira-Junior, et al.(26), who showed that level GM-CSF were significantly higher in patient group more that control group, their study included (400 patient group , and 41 control group ) our study disagreement with (Teles et al. (27) who showed nonsignificant difference between smoker and control, this study carried out in Boston 74 subjects with chronic periodontitis and 44 periodontally healthy individuals.

CONCLUSION
1. Smoking it a risk factor associated with periodontitis
2. the influence of smoking cigarettes on level of oral biomarkers has positive effects on levels of (ALP and GM-CSF in saliva

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REFERENCES
2. Barbour, S. E. et al. (1997) 'Tobacco and smoking: environmental factors that modify the host response (immune system) and have an impact on periodontal health', Critical Reviews in Oral Biology & Medicine. International and American Associations for Dental Research, 8(4), pp. 437–460.
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