The Role of Stem Cell Markers in the Diagnosis of Periodontitis

Ainar A Bannani1*, AbdulAzizSGul2**, Mohammad N Redwan1**, Wed A. Baghdadi1*, Amal M. Eldeeb3*

1Post Graduate Student, 2Demonstrator, 3Professor,
Oral Pathology Department, Oral Surgery Department,
Faculty of Dentistry, Umm Al-Qura University, Saudi Arabia.

Corresponding Author: Ainar A Bannani

ABSTRACT

Aim: This study was conducted to compare the levels of salivary CD44 in periodontally diseased smokers and non-smokers, and compare them with healthy subjects.

Materials and methods: A total of 40 patients, were divided according the presence or absence of the disease into two groups: Group I (Control group): 10 patients. Group II (Periodontally diseased): 30 patients, divided into two subgroups: Group II A (Smokers): 15 and Group II B (Non-Smokers): 15.

Patients who presented with chronic periodontitis identified via the plaque index, gingival index (GI), probing depth and clinical attachment level using Williams periodontal probe, along with the intraoral periapical radiographs.

The saliva samples were collected from each patient using Navazesh's Unstimulated method and testing the CD44 levels using ELISA. SPSS software was used for all statistical analyses.

Results: The higher mean of CD44 13.5 was recorded in (Group I) followed by (Group II B) 10.3, and the lower mean was recorded in (Group II A) 6.0. The difference in mean CD44 between the groups was found to be statistically non-significant (P > 0.01).

Conclusion: Based on the finding of the present study it could be concluded that the mean level of CD44 was higher in healthy group more than that in non-smokers chronic periodontitis group, and the lower mean was recorded in smokers-chronic periodontitis group. It may be suggested that smoking increase periodontal destruction and decrease the regeneration capacity of the tissue and this may lead to decrease the level of salivary CD44.

Key Words: Stem cell marker, Periodontitis.

INTRODUCTION

Recent interesting new findings helped the dentists to focus on the patients’ benefits from their own stem cells derived from deciduous and permanent dentition.

There are two major types of stem cells, embryonic and adult stem cells. A single embryonic stem cell can be differentiated into 220 types of specialized cells that make up the human body. Adult stem cells are responsible for the regeneration and replacement of tissue damaged by disease or injury. (1)

Sources of Dental Stem Cells.

The periodontal ligament serves as a reservoir for many cell types, including fibroblasts, osteoblasts/ clasts, cementoblasts/ clasts, and odontoclasts. The remarkable characteristic of the periodontal ligament is its ability to regenerate and repair virtually every other tissue type that comprises the periodontium. Undifferentiated mesenchymal cells of the periodontal ligament can differentiate into osteoblasts, chondrocytes and adipocytes. (1)
The periosteum is a very dense, tough layer of fibrous tissue intended to act as a covering for bone and provide progenitor cells for bone growth and repair of buccal mucosa and gingiva, muscle and Alveolar bone (1)

**Stem Cell Markers:**

The stem cells markers used to identify and characterize the number of adult stem cells and they have been given short-hand names according to the molecules that attach to the stem cell receptors. (1)

**CD44 Marker:**

Human CD44 is a widely expressed family of glycoproteins, can predict the risk of certain diseases (e.g., diabetes mellitus, cardiovascular, oncology, endocrinology, and psychiatric diseases), also can be found in saliva and used for periodontal disease prognosis. (2)

**REVIEW OF THE LITERATURE**

Periodontal disease has been diagnosed to be a breakdown of the supporting structures of the teeth and involve numerous and complex causes and symptoms. And that comes from the interactions of numerous factors, such as exposure to bacteria and viruses, inflammation and genetic factors. (3) In turn, periodontal diseases have powerful, multiple influences on the occurrence and severity of systemic disorders, such as cardiovascular and respiratory conditions and diabetes mellitus (DM) (4)

The inflammatory periodontal diseases are mainly based on clinical assessment in their diagnosis like pocket depth measurement, attachment level, bleeding index, plaque index or radiographic assessment and there is some other qualitative and quantitative assessment of serum or saliva or sub gingival micro flora or gingival crevicular fluid (GCF) may give information about the periodontal diseases like type or location or severity of pocket depth (PD) and may help in the choice of treatment and follow up of the diseases. (5)

**Effect of smoking on periodontal health:**

Smoking is the major risk factor in the development of sever periodontal diseases, (6-9) in comparison between smokers and nonsmokers cross sectional studies have shown that the smokers are up to seven times more susceptible to periodontitis. (6,10,11)

Clinical studies have demonstrated that smokers have more severe periodontal disease, with increased bone loss, (12,13) greater periodontal attachment loss, more gingival recession and periodontal pocket formation. (14) Patients history of smoking has been associated with early loss of periodontal attachment in adults. (15)

The influence of smoking on experimental gingivitis was evaluated in a group of dental students (16) This study revealed that the number of gingival bleeding sites, the amount of gingival exudate and the number of gingival sites with distinct redness were significantly lower in smokers than in nonsmokers with comparable levels of plaque indexes. The preponderance of evidence suggests that smoking may decrease gingival bleeding and that this may occur owing to changes in the proportion of blood vessels in the periodontal tissues. (17)

Although a number of studies have considered smoking as a true risk factor for periodontitis, the mechanisms involved are still not clear. (18)

**Biological markers and oral diseases:**

A biologic marker is an objectively measured substance which is an indicator of normal biologic processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention. Biomarkers of disease in succession play an important role in life sciences and have begun to assume a greater role in diagnosis, monitoring of therapy outcomes, and drug discovery. The challenge for biomarkers is to allow earlier detection of disease evolution and therapy efficiency measurements. (19)

Saliva and gingival crevicular fluid (GCF) are containing locally and systemically derived markers of periodontal
disease. Oral fluid biomarkers that have been studied for the diagnosis of periodontal diseases include proteins of host origin, enzymes and immunoglobulins, phenotypic markers, host cells, hormones, bacteria and bacterial products, ions, and volatile compounds. Salivary immunocomponents have also been studied at length in oral health, including immunoglobulin subclass immunoglobulin isotypes, and antibody levels. \(^{(19)}\)

Saliva contain markers for periodontal diseases like Immunoglobulins (Igs) one of the major defensive factor of saliva is Secretory IgA (sIgA). Enzymes in the saliva are produced from oral microorganism and leukocyte and salivary glands and from epithelial cell of the (GCF). The ions found in salivalike calcium (Ca) ion is consider as a marker for periodontal diseases. Sewon et al \(^{(19)}\) concluded that patients with periodontitis have high concentration of calcium in their stimulated saliva.

Some studies tried to find the relation between nonspecific markers in saliva (non enzymatic, non immunoglobulin) and its relation to periodontal diseases, this like the number of leukocytes in saliva, specific species of bacteria. \(^{(21)}\) Growth factors as epidermal growth factor (EGF). \(^{(22)}\) Epithelial keratins; Epithelial cells of the lining of the oral cavity are found in saliva. \(^{(21)}\)

Understanding the biomarkers in the saliva give a valuable laboratory information about many of the body and mouth diseases like periodontal diseases. Oxidative stress markers, protein markers and enzymes are saliva biomarkers which help to detect periodontal diseases and its progress, also many other diseases like cardiovascular and diabetes mellitus and some benign and malignant tumor has biomarkers in saliva. \(^{(19,23)}\)

**Stem Cells:**

Stem cells are the master cells of the body they are undifferentiated cells with a remarkable capability to self-renew, able to differentiate into one or more specialized cell types that play a role in homeostasis and tissue repair.

Subsequent to injury, the stem cell self-renews – undergoes cell division and produce one daughter stem cell and one progenitor cell. A progenitor cell is an intermediate cell type formed before it achieves a fully differentiated state.

Stem cells are classified according to their origin either as embryonic stem cells (ESCs) or as postnatal stem cells/somatic stem cells/adult stem cells (ASCs). Totipotency which generate all types of cells including embryonic cells (ESCs), The Pluripotency generate all types of cells except cells of the embryonic membrane, Multipotency differentiate into more than one mature cell. \(^{(23)}\)

**Stem cell types:**

1- **Stem cells of dental origin**

a- **Periodontal ligament (PDL) -derived mature cell (MSCs)**

The PDL is a fibrous connective tissue that surrounds and supports the tooth; it is located between the cementum of the root and the inner wall of the alveolar bone socket. The PDL consist of small subpopulation of stem cells that maintain and regenerate periodontal tissue structure and function. These cells are multipotential, which differentiate into osteoblasts, fibroblasts and tooth cementoblasts and form cementum- and PDL-like tissues.

Byoung-Moo Seo, et al, 2004 \(^{(5)}\) studied the use of PDL’s stem cells in the regeneration of periodontal tissues. Their results showed that PDL carry stem cells that have the ability to produce cementum/PDL-like tissue in vivo. Transplantation of these cells, may facilitate the regeneration of the tissues destroyed by periodontal diseases. \(^{(5)}\)

Wei Zheng, et al 2015, \(^{(25)}\) studied the effect of periodontitis on the number, proliferation and differentiation potential of human periodontal ligament stem cells (PDLSCs). Their finding showed that periodontitis affects the proliferation and differentiation potential of human PDLSCs in vitro and in vivo. \(^{(25)}\)
2- Oro-maxillofacial region: Stem cells from oral and maxillofacial region can derived from

a. The periodontal ligaments:
The periodontal ligament can differentiate into osteoblasts, chondrocytes and adipocytes.

b. Periosteum:
The periosteum is a thick, tough layer of fibrous tissue cover the bone and facilitates bone growth and repair. Periosteum contains fibrous layer that differentiate into fibroblasts and the cambium layer that contain progenitor cells which develop into osteoblasts.

c. Buccal mucosa and Gingiva:
Gingival keratinocyte stem cells, may help in the studies on stem cell differentiation, developing gene therapy procedures, and testing oral hygiene products.

d. Muscle:
There is different types of stem cells beside the satellite cells that have been identified in skeletal muscles.

e. Alveolar bone:
The bone graft that contains stem cells is useful for bone reconstruction and regeneration. (1)

CD44:
The CD44 antigen is a glycoprotein that is found on the surface of many cell types, including the basal layers of oral mucosa, resting T cells, and is involved in cell-cell interactions and cell adhesion. CD44 acts as a receptor for hyaluronic acid and plays an important role in cell migration, including the recruitment of effector T cells and other leukocytes to infection sites. CD44 also participates in lymphocyte activation, recirculation and homing, as well as hematopoiesis and tumor metastasis. The function of CD44 is controlled by posttranslational modifications including proteolytic cleavage, N- and O-glycosylation and phosphorylation. (26)

Human CD44 is an adhesion molecules contain a chain of glycoproteins, encoded by a single gene containing 20 exons. All CD44 isoforms came from alternate splicing of pre-mRNA and share the same N- and C-terminal sequences. The hematopoietic, or standard form, of CD44 (CD44H; CD44s) is a 248 amino acid protein that contains no variant exon-encoded peptide, CD44 is a tumor initiation marker that is appear in the earliest stages of carcinogenesis. (27) Soluble CD44 (solCD44), released by proteinases, the level of (solCD44) can be raised in the subject with inflammatory condition and is detectable in body fluids. (2) CD44 is involved in cell proliferation, cell differentiation, cell migration, and angiogenesis, it also helps in presentation of cytokines, chemokines, and growth factors to corresponding receptors, as well as in signaling for cell survival. (28)

Some researches which studied the use of CD44 isoforms in disease diagnosis didn’t consider the smoking situation of the subjects. However, other studies concluded that salivary CD44 shown to be higher in smokers than non-smokers, stopping smoking for long time lower the level of CD44 to levels near of non smoker level. Soluble CD44 was found in serum 20 years ago, but it’s physiological importance still not fully clear. (21)

Consideration of the available data shows that there is a need to further develop our understanding of the influence of tobacco smoking on the CD44 gene, to evaluate any diagnostic and prognostic significance to CD44 molecules in periodontal diseases.

AIM OF THE WORK
The aim of this study was to compare the levels of salivary CD44 in periodontally diseased smokers and non-smokers, and compare them with healthy subjects (control group).

MATERIALS AND METHODS
Selection of the patients: The study was conducted in Umm-Al Qura University. Patients were recruited from the regular pool of patients visiting Umm-Al Qura
University Dental Hospital. **Inclusion criteria:** The selected patient were systemically free of any disease, have no allergic reaction, and between 18-60 years of age. **Exclusion criteria:** Pregnant females, patients taking antibiotics or corticosteroids at the time of treatment, immune-compromised patients, patients with complicating systemic disease, or under 18 and above 60 years of age were excluded. **Sample Size:** A total of 40 patients were collected and divided according the presence or absence of the disease into two groups; **Group I (Control group):** 10 patients **Group II (patients with chronic periodontitis):** 30 patients; divided into two subgroups; **Group II A (Smokers):** 15Group II B (Non-smokers): 15. **Procedures:** The relevant features and conditions of each case were collected from patients’ electronic files in to data collection forms not showing any nominative information. Patient identified by serial study code and initials. That linked to patient’s name in a separate identification log sheet, which kept in a safe locked place. Two persons performed data entry. After verification, data transferred to statistical database directly. Patients who presented with chronic periodontitis identified via the plaque index (PI), gingival index (GI), probing depth and clinical attachment level (CAL) using Williams periodontal probe, along with the intraoral periapical radiographs. The saliva sample was collected by Navazesh's Unstimulated method. Briefly the saliva was allowed to accumulate in the floor of the mouth and expectorated in a test tube to collect 5 ml. After collection the samples were stored at −80°C until testing the CD44 levels using ELISA.SPPS software used for all statistical analyses. Comparison between groups was made by Bonferroni, Kruskal–Wallis test, Mann–Whitney test and the Correlation test to evaluate the results. Chi squared test for categorical values and for comparison of survival a log rank test was used. **RESULTS** Three patients were excluded from the analysis of the results, as they did not give the right amount and type of saliva. Of the remaining thirty seven patients, ten patients were in control group (Group I) and fourteen patients were in smokers-chronic periodontitis group (Group II A) the last thirteen patients were in nonsmokers-chronic periodontitis group (Group II B). The overall mean [PI, Table 2] at baseline in (Group I) was 0.80 in (Group II A) was 1.50 in (Group II B) was 1.45. In (Group II A) it was highest with a mean of 1.50, the difference between them was significant. Higher mean [GI, Table 3] was recorded in (Group II B) followed by (Group II A) and the control group, the difference between them was significant. Highest mean probing depth [PD, Table 4] was recorded in (Group II B) (3.78) followed by (Group II A) (3.45) and healthy group (1.99), the difference between them was significant. Higher mean clinical attachment loss [CAL, Table 5] was recorded in (Group II A), (3.90) followed by (Group II B) (3.10), but the difference between them was not statistically significant (P > 0.05).
Table 2: Comparison of mean Gingival Index between the different groups

<table>
<thead>
<tr>
<th>#</th>
<th>Healthy Non-Smokers (G I)</th>
<th>Periodontitis Smokers (G II A)</th>
<th>Periodontitis Non-Smokers (G II B)</th>
<th>One way ANOVA F (p value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean ± Standard Deviation</td>
<td>0.47± 0.24</td>
<td>1.50 ± 0.29</td>
<td>1.65 ± 0.29</td>
<td>45.615 &lt;0.001*</td>
</tr>
<tr>
<td>Minimum</td>
<td>0.22</td>
<td>1.19</td>
<td>1.28</td>
<td></td>
</tr>
<tr>
<td>Maximum</td>
<td>1.16</td>
<td>2.07</td>
<td>2.02</td>
<td></td>
</tr>
</tbody>
</table>

* Significant at p value less than 0.05

Table 3: the mean probing pocket depth between the three groups.

<table>
<thead>
<tr>
<th>#</th>
<th>Healthy Non-Smokers (G I)</th>
<th>Periodontitis Smokers (G II A)</th>
<th>Periodontitis Non-Smokers (G II B)</th>
<th>One way ANOVA F (p value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean ± Standard Deviation</td>
<td>1.99 ± 0.41</td>
<td>3.75 ± 0.80</td>
<td>3.78 ± 0.69</td>
<td>23.764 &lt;0.001*</td>
</tr>
<tr>
<td>Minimum</td>
<td>1.09</td>
<td>3.05</td>
<td>2.10</td>
<td></td>
</tr>
<tr>
<td>Maximum</td>
<td>2.60</td>
<td>5.60</td>
<td>5.04</td>
<td></td>
</tr>
</tbody>
</table>

* Significant at p value less than 0.05

Table 4: Comparison of probing Clinical Attachment Lose between the groups.

<table>
<thead>
<tr>
<th>#</th>
<th>Healthy Non-Smokers (G I)</th>
<th>Periodontitis Smokers (G II A)</th>
<th>Periodontitis Non-Smokers (G II B)</th>
<th>One way ANOVA F (p value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean ± Standard Deviation</td>
<td>2.80 ± 0.46</td>
<td>3.90 ± 0.69</td>
<td>3.10 ± 0.30</td>
<td>0.477 &lt;0.788</td>
</tr>
<tr>
<td>Minimum</td>
<td>1.35</td>
<td>2.35</td>
<td>1.60</td>
<td></td>
</tr>
<tr>
<td>Maximum</td>
<td>7.50</td>
<td>9.31</td>
<td>4.20</td>
<td></td>
</tr>
</tbody>
</table>

non significant p value more than 0.05

Table 5: CD44 Value of The Patients.

<table>
<thead>
<tr>
<th>CD44 Marker</th>
<th>#</th>
<th>Healthy Non-Smokers (G I)</th>
<th>Periodontitis Smokers (G II A)</th>
<th>Periodontitis Non-Smokers (G II B)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>17.1</td>
<td>12.0</td>
<td>16.3</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>17.3</td>
<td>17.4</td>
<td>7.0</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>9.0</td>
<td>0.4</td>
<td>2.3</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>14.9</td>
<td>0.4</td>
<td>16.5</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>11.2</td>
<td>17.4</td>
<td>5.0</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>16.8</td>
<td>0.8</td>
<td>17.3</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>1.9</td>
<td>14.5</td>
<td>17.4</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>17.3</td>
<td>1.5</td>
<td>0.2</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>16.9</td>
<td>2.1</td>
<td>17.0</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>12.1</td>
<td>2.6</td>
<td>17.3</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>1.3</td>
<td>0.5</td>
<td>0.5</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>2.0</td>
<td>12.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>13</td>
<td></td>
<td></td>
<td>0.5</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td></td>
<td></td>
<td>14.1</td>
<td></td>
</tr>
<tr>
<td>Mean ± Standard Deviation</td>
<td>13.5 ± 5.0</td>
<td>6.0 ± 7.4</td>
<td>10.3 ± 7.1</td>
<td></td>
</tr>
<tr>
<td>Minimum</td>
<td>1.9</td>
<td>0.4</td>
<td>0.2</td>
<td></td>
</tr>
<tr>
<td>Maximum</td>
<td>17.3</td>
<td>17.4</td>
<td>17.4</td>
<td></td>
</tr>
<tr>
<td>One way ANOVA (F)</td>
<td>2.335</td>
<td>0.4²</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

O non significant difference

Table 6: Correlations Between the Healthy, Periodontitis Non-Smokers and Periodontitis Smokers groups

<table>
<thead>
<tr>
<th>Comparing Groups</th>
<th>Correlation</th>
<th>Sig</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy &amp; Periodontitis Non-Smokers</td>
<td>-0.056</td>
<td>.878²</td>
</tr>
<tr>
<td>Healthy &amp; Periodontitis Smokers</td>
<td>-0.252</td>
<td>.482²</td>
</tr>
<tr>
<td>Periodontitis Non-Smokers &amp; Periodontitis Smokers</td>
<td>0.05</td>
<td>.987²</td>
</tr>
</tbody>
</table>

O non significant difference
DISCUSSION

The result of the present study demonstrated that there were significant differences between the three groups in PI, GI and CAL with the highest mean in the Periodontitis Smokers group which is matching with the result of Kaur Sumeet et al (3).

The objective of this study was to compare the levels of salivary CD44 in periodontally diseased smokers and non-smokers and compare them with healthy subjects (control group).

The result of the present study demonstrated that the highest levels of salivary CD44 (ng/ml) in healthy group ranging from 1.9 (ng/ml) to 17.3 (ng/ml) with a mean value of 13.5 in comparison to smokers-chronic periodontitis group; 10.3 ng/ml. This result was in opposite to the previous study done by Ghallab and Shaker, (30) in which they were recorded the higher levels of CD44 in chronic periodontitis cases. They also explained that CD44 is implicated in cellular matrix changes by the cell to cell and cell matrix interaction. It induces adhesive interaction between lymphocytes and gingival fibroblasts that is mediated by the CD44 – hyaluronic acid (HA) complex, which regulates cell - cell adhesion and cell migration and proliferation, required for tissue morphogenesis and repair. Thus, salivary CD44 – HA complex plays an important role in chronic inflammation. Increased salivary CD44 can be attributed to increased inflammatory response and the chronicity of the disease. (2,5)

In order to further throw light on the role of smoking on levels of salivary CD44, the comparison between smokers (6.3ng/ml) and nonsmokers (9.7ng/ml) was done, but the finding in our study did not match with the previous reports (2,4,30) where in this study CD44 levels was higher in nonsmokers as compared to smokers. Moreover, in this study self-reported smoking habits were taken into consideration which may be unreliable and analysis of dose-dependent relationships may be further complicated because of variable factors in the smoking habits of the individual, such as frequency and depth of inhalation. This is supported by the finding in a study done by Scott et al (4) where they found that both the subjects with highest and the lowest level of cotinine smoked equal number of cigarettes daily. Cotinine, a major catabolite of nicotine is shown to be a specific and accurate biomarker of current smoking status.

Elevated concentrations of soluble adhesion molecules reflect ongoing inflammatory processes; however, increasing evidence suggests that specific soluble adhesion molecules are also immunomodulatory. (2)

The result of the present study demonstrated that the CD44 levels in smoker chronic periodontitis group were lower than Group II (nonsmoker chronic periodontitis), and also lower than the healthy group (Group I). This can be explained on the basis of genetic
susceptibility and hence altered immunologic responses to specific bacterial pathogens. Thus, emphasizing that these individuals may have pathogenic mechanisms other than pathways mediated by salivary CD44.

Kaur Sumeet 2014 (3) concluded that longitudinal as well as interventional studies with adequate sample size and age and gender distribution are necessary to validate substantial changes in salivary CD44 levels. They added that these studies will help in assessing periodontal disease severity and monitor its progression and also determine the therapeutic significance of CD44 levels as a diagnostic marker for periodontitis. (3)

Other studies (31) were concluded that differences observed between smokers and nonsmokers with regard to periodontal condition are attributable to differences in oral hygiene.

Fakulte Medicinski et al 2002, (31) examined the oral hygiene and periodontal status in smokers and compared them with non smokers, they found that periodontal destruction, alveolar bone loss and gingival recession were significantly increased in smokers compared to nonsmokers (p < 0.001). Lucarini Guendalina 2009 (32) investigated the relationship between expression of angiogenic and regeneration markers (VEGF, CD44 and CD133) and periodontal disease in subjects with/without diabetes mellitus .They suggested that the high expression of VEGF, CD44 and CD133 in periodontal disease may predict a greater regeneration capacity of gingival tissue. (32) This could explain the lower level of CD44 in smoker chronic periodontitis group in this study when compared with other groups. It may be suggested that smoking increase periodontal destruction and decrease the regeneration capacity of the tissue and this may lead to decrease the level of CD44.

CONCLUSION

Based on the finding of the present study it could be concluded that the mean level of CD44 was more than that in nonsmokers-chronic periodontitis group, and the lower mean was recorded in smokers-chronic periodontitis group. It may be suggested that smoking increase periodontal destruction and decrease the regeneration capacity of the tissue and this may lead to decrease the level of CD44.

RECOMMENDATION

1. Further study should be conducted with larger sample size with fixed variable factors to have accurate analysis.
2. Furthermore, smoking status of an individual should have been assessed by cotinine levels, which would be more reflective and precise.
3. This study has not considered the effect of periodontal therapy on salivary CD44 levels.

ACKNOWLEDGEMENT

We would like to express our acknowledgements to Dr. Amal El-deeb, Professor, Oral Pathology Faculty of dentistry Umm Al-Qura university for her kind supervision, advice and support.

Many thanks to Dr. Hind Abdellatif, Assistant Professor, Removable Prosthodontics Faculty of Dentistry Umm Al-Qura university for her guidance and patience.

Finally, We would like to thanks Dr. Abdulrahman Yousef, Assistant Professor, Microbiology Faculty of Dentistry Umm Al-Qura university for his great help with the experiment in the research lab and Dr. Rabab Salama Assistant Professor, Public Health faculty of dentistry Umm Al-Qura university for her help with the data analysis.

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