Assessment of some salivary biochemical parameters in cigarette smokers with chronic periodontitis

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ABSTRACT

Background: Cigarette smoking is an important risk factor that has a direct association with the prevalence and severity of chronic periodontitis (CP). Salivary biochemical parameters may be affected by both smoking and CP together.

Materials and methods: Eighty systematically healthy male patients were included in this study. They were grouped based on their periodontal and smoking status. Unstimulated whole saliva (UWS) was collected from all subjects. Salivary flow rate (FR) was measured during sample collection. Parameters such as salivary pH, total protein (TP), albumin (Alb), total fucose (TF), protein bound fucose (PBF) and C-reactive protein (CRP) were estimated.

Results: Salivary flow rate was not altered regarding to smoking status or periodontal health status. Salivary pH was lower in smokers comparing to non-smokers, while salivary pH was not affected by periodontal health status. TF, TP and Alb were higher in CP and PBF was lower in CP comparing to healthy control, while these parameters concentrations did not affect by smoking status except for Alb (smokers with CP had lower Alb concentration comparing to non-smokers with CP). CRP was higher in smokers compared to non-smokers, while its value was not affected by periodontal health status. Both smoking and chronic periodontitis together affect some salivary biochemical parameters, thus the concentrations of these parameters could be used as indicators for periodontal disease progression and severity in smoker with CP. Both smoking and periodontal health status together should be taken in consideration when salivary composition is studied.

Key words: Salivary biochemical compositions, Saliva, Smokers, Chronic Periodontitis, salivary flow rate, salivary glycoproteins, salivary fucose. (J Bagh Coll Dentistry 2014; 26(1):144-149).

INTRODUCTION

Chronic periodontitis (CP) is an infectious disease that results in inflammation within supporting structure of the tooth, progressive attachment loss, and bone loss1. Advanced form of the disease affects about 10% - 15% of adult population worldwide 2. Although, its occurrence normally involved adult individual, chronic periodontitis can appear at any age 3.

Periodontitis are considered as an outcome of an imbalance in the host parasite interaction. Although the microbial etiology of periodontitis is well established, the extent and severity of the disease depend upon the interaction between pathogenic bacterial challenge and host response 4,5. In the presence of systemic or environmental factors, which may modify the host response to plaque accumulation, such as, diabetes, smoking or stress, the disease progression may become more aggressive6.

Smoking is very strong behavioral risk factor for CP. Cigarette smokers are 2.5 - 6 times more likely to develop CP than non-smokers7.

Chronic periodontitis is more prevalent and more severe in smokers, characterized by deeper periodontal pockets, greater attachment loss and more furcation defects. Smoking is considered as an independent risk factor for periodontitis8.

The precise mechanisms whereby cigarette smoking can exert an effect on periodontal tissues are not completely understood, it is clear that it is still the most significant preventable risk factor for CP. Its effects are related to the duration and number of cigarettes consumed 9,10.

The diagnosis of periodontal disease usually accomplished through clinical periodontal parameters including plaque index, calculus index, periodontal pocket depth, bleeding index and clinical attachment loss (CAL) 11.

Saliva, which plays an important role in the protection of periodontium, also affected by smoking 11,12. Analysis of saliva can be contributed in the periodontal disease diagnosis14. Saliva can be easily collected, it contained locally derived and systemically derived markers of periodontal diseases 15. However, their exact value or the optimal markers combination has not been defined 16,17. Furthermore, the analysis of saliva may be offer a cost-effective approach to assess periodontal disease incidence in large population 14.

The purpose of this study was to analysis some salivary parameters in smokers with CP. Most studies, done on salivary compositions in chronic
periodontitis patient, excluded smoker as it might affect the salivary compositions. Little information is available on salivary compositions in smokers with chronic periodontitis patients, while no study was found included Kurdistan population.

**SUBJECTS AND METHODS**

**Subjects**

Eighty systematically healthy male, their age ranged between (30-60) years old, were enrolled in the study. They were subdivided into four equal groups: Non-smokers with clinically healthy periodontium (GI), Smokers with clinically healthy periodontum (GII), Non-smoker with CP (GIII) and Smoker with CP (GIV). Chronic periodontitis was defined as a patient who had two or more interproximal sites with CAL of 4mm or more (not in the same tooth), while clinically healthy periodontium was defined as subjects with mean bleeding on probing index (BOP) ≤ than 0.11 and they had no CAL.

**Exclusion criteria:** cardiovascular disease, diabetes mellitus, hypertension, liver disease, endocrine disorders, immunodeficiency diseases, subjects had less than 20 teeth retained in their mouth, former smokers, alcohol drinkers, patients on medical treatment or had history of pervious periodontal therapy, were excluded.

The clinical periodontal examinations used in this study were periodontal Pocket depth (PD), CAL, BOP, plaque index (PI), Calculus index (CI), in four surfaces of all tooth. Periodontal tissue destruction was determined by CAL which was measured from cementoenamel junction to the base of the periodontal pocket (Varma and Nyake, 2009). Periodontal pocket depth was measured from gingival margin to the base of the periodontal pocket.

Severity of PD and CAL was estimated (total PD /CAL divided by affected surfaces) and extension of PD and CAL was calculated (number of affected tooth surfaces divided by total tooth surfaces).

Personal information was collected by including social and behavioral factors such as age, address, smoking status (measured by Pack year (PY); number of cigarette smoked in a day multiplied by number of years of smoking) and tooth brushing frequency (TBF).

**Saliva collection**

Unstimulated saliva samples were collected from all subjects in the morning (9-11 a.m.), in order to minimize the effect of diurnal variation on flow and composition. Spitting method was used for collecting unstimulated whole saliva (UWS). All subjects instructed to brush their teeth and refrained from drinking, eating or smoking two hour before saliva collection. Subjects was asked to rinse the mouth with distilled water for three minute to remove any food debris, then 10 minutes latter, all subjects was directed to accumulate saliva in their mouth until the desire to swallow occurred, then they spitted saliva into a sterilized graduated plastic test tube until four to five milliliter of saliva was collected. Any blood contaminated saliva was discarded. The samples were centrifuged for ten minutes at 3000 r.p.m.

**Laboratory methods**

Unstimulated salivary flow rate was defined as the total volume of saliva produced per unit time (ml/mint). The pH values of the saliva were immediately measured by using pH meter. Afterward, saliva samples were stored at (-20°C) until analysis.

Salivary total protein concentration was estimated using biuret reaction; salivary albumin concentration was estimated using Bromocresol green method. Salivary globulin concentration (Glo) was estimated by subtracting salivary albumin concentration from salivary total protein, then albumin/globulin ratio (Alb/Glo) was calculated. Salivary total fucose (TF) and salivary protein bound fucose (PBF) were determined by using Dische and Sheetels method. The estimation of CRP was performed by Latex slide agglutination method (Qualitative Measurement) recorded as a negative or positive results.

**Statistical analysis**

The study variables were statistically analyzed using Post Hoc test, t-test and Pearson Chi Square.

**RESULTS**

Table (1) shows the mean ± SD (stander deviation) for all the parameters which have been measured in this study, while table (2) shows statistically significance differences among the groups. There was a statistically significant difference (p<0.001) in smoking exposure measured in PY in GII compared to GIV. GII had lower smoking exposure in their life time than GIV.

There was a statistically significant increase in the salivary pH in GI when compared to both GII and GIV. There was also a significant increase in the salivary pH in GIII when comparing to both GII and GIV (p> 0.05), while there was a non-significant difference in the salivary pH among
the other groups. In general smokers had lower salivary pH than non-smokers, thus GIV had the lowest pH, followed by GII.

There was a highly significant decrease in the salivary TF in GI when compared to both GIII, and GIV (p> 0.001). There was also significant decrease in the salivary TP in GII when compared to GIII (p> 0.05), while there was a non-significant difference between GI and GII, neither between GIII and GIV. Patient with CP had higher salivary TF concentration than subjects with clinically healthy periodontium.

There was a high significant increase in the salivary PBF in GI when compared to both GIII and GIV. There was also highly significant increase in the salivary PBF in GII comparing to GI and GIII (p> 0.001), while a non-significant difference between GI and GII, neither between GIII and GIV was found. Patient with CP had lower protein bound fucose concentration than subjects with clinically healthy periodontium.

There was a high significant decrease in the salivary TP in GI when compared to both GIII and GIV (p>0.05), while a non-significant difference among the other groups was found.

The results showed that there was a statistically high significant decrease in the salivary albumin in GI when compared to GIII, and in GII when compared to GIV, and in GIII when compared to GIV (p> 0.001), while a non-significant difference between GI and GII, GI and GIV was observed. GI had the lowest salivary albumin concentration while GIII had the highest salivary albumin concentration.

There was a statistically significant decrease in the salivary globulin in GI when compared to GIII and GIV (p> 0.05), while non-significant differences among the other groups were seen. GI had the lowest salivary globulin concentration.

There was a statistically significant difference in the ratio of salivary albumin to globulin in GIII when compared to GI, GII and GIV (p> 0.05), while non-significant difference among the other groups was seen. GIII had the highest ratio of salivary albumin to globulin, while GII had the lowest value.

There was a statistically significant increase in salivary CRP in GII comparing to GI and GIII, and a significant increase in GIV comparing to GII, GIII (p> 0.05), while statistically non-significant differences between GII and GIV, GI and GIII was observed. In general smoker groups had significantly higher salivary CRP than non-smoker groups, as shown in figure (1).

DISCUSSION

In this study, the results showed that there was a high significant difference in smoking exposure in term of PY between GII and GIV. This result is indicated that there is a dose response relationship between smoking and periodontal health status.

In the present study, there were statistically non significant differences in UWS flow rate among either groups. This result was in agreement with other studies who found that UWS flow rate was not affected by periodontal health status, while this result showed a disagreement with Aziz and Askari who observed that UWS flow rate was significantly lower in smokers compared with non-smoker. The result also was in disagreement with Scolley and Langley-Evans, who found that UWS flow rate significantly increased in severe CP.

In this work, there was a statistically significance decrease in salivary pH in smokers when compared with non smokers. This result was in agreement with some authors, while it was in disagreement with Gonzalez et al. This disagreement might be resulted from using low sample numbers in their studies.

There were statistically non significant differences between subjects with clinically healthy periodontium comparing to patients with CP, this result was in line with some studies, while the result was in disagreement with Bezerra-Junior et al, who found that salivary pH value was higher in CP when compared to control. Their result might be due to the collection of saliva on fasting state in morning.

Low salivary pH value in smokers comparing to non smokers might be due to the higher percentage of periodontal pathogene in smokers since pH level negatively correlated with the proportion of periodontal pathogenes.

According to this study, salivary TF was increased, while salivary PBF decreased in patients with CP compared with clinically healthy groups. This result might be due to increase in glycosidase activity and periodontal tissue destruction in CP. Salivary TF and PBF were not affected by smoking.

According to the results of this work, clinically healthy subjects had lower salivary total protein concentration than patients with CP. This result might be due to that these studies used saliva taking from both gender, and there were differences in age range between study groups and control in their work. Smoking had statistically non significant effect on salivary TP.

The result showed that there was a high significant increase in salivary albumin concentration in GIII, comparing to the other groups. This indicates that CP patients had higher salivary albumin concentration than clinically healthy groups. The high albumin level in CP
patients may be due to ulceration in sulcular epithila 45

In this study, it was also found that, smokers with CP had lower salivary albumin concentration compared with non smokers with CP. This result might be due to the thickening of the basement membrane in blood vessels, so reducing gingival blood flow in smokers compared with non smokers 46.

In the present study, there was a statistically significant decrease in salivary globulin concentration in GI comparing to GIII and GIV, while a statistically non significant difference was found among the other groups. GI had the lowest salivary globulin concentration. This result might be due to the increase in inflammatory proteins infiltrated through sulcular epithilia into gingival sulcus, then into saliva in CP patients, while inflammatory proteins in saliva may decrease in saliva of smokers 46,47.

The result showed that, salivary albumin/globulin ratio was statistically higher in GIII when compared with the other groups. This result might be due to higher salivary albumin levels in non smokers with CP compared with the other groups.

In the present study, smokers had higher salivary CRP value than non smokers, while salivary CRP value was not altered in periodontal health status. This result indicated that smoking has more effect on salivary CRP than CR.

Both smoking and chronic periodontitis (in combination) can affect the physical properties and chemical composition of saliva. During study of salivary composition proteins, glycoproteins and their related parameters, these parameters may be used as indicators in the diagnosis and progosis of CP and smokers with CP. It is necessary that, both periodontal health and smoking status should be considered during study of salivary compositions.

REFERENCES
27- Rosa M, Lucas Q, Lucas N. Cigarette smoker and alveolar bone and alveolar bone in young adults: A
34- Aziz A, Askari S. Comparison of unstimulated saliva secretion between heavy smokers and non-smokers subject. J Guilan University of Medical Sciences 2008; 69:11-16.

Figure1: Salivary CRP values in all group; GI, GII, GIII, GIV
Table 1: The mean ± SD of all the parameters in saliva of the groups.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>GI</th>
<th>GII</th>
<th>GIII</th>
<th>GIV</th>
</tr>
</thead>
<tbody>
<tr>
<td>GI (PY)</td>
<td>254 ± 202.69</td>
<td></td>
<td></td>
<td>642.5 ± 411.44</td>
</tr>
<tr>
<td>BOP</td>
<td>0.074 ± 0.0575</td>
<td>0.061 ± 0.0655</td>
<td>0.9945 ± 0.7080</td>
<td>0.5795 ± 0.93099</td>
</tr>
<tr>
<td>CI</td>
<td>0.3525 ± 0.40779</td>
<td>0.5841 ± 0.7356</td>
<td>1.4675 ± 0.911</td>
<td>1.7135 ± 1.0228</td>
</tr>
<tr>
<td>PI</td>
<td>1.289 ± 0.845</td>
<td>1.6275 ± 0.8708</td>
<td>2.0735 ± 0.6628</td>
<td>2.2745 ± 0.93099</td>
</tr>
<tr>
<td>CAL (severity)</td>
<td>_</td>
<td>_</td>
<td>5.053 ± 5.838</td>
<td>5.126 ± 8.7489</td>
</tr>
<tr>
<td>CAL (extension)</td>
<td>_</td>
<td>_</td>
<td>0.306 ± 0.2443</td>
<td>0.560 ± 0.41149</td>
</tr>
<tr>
<td>PD (severity)</td>
<td>_</td>
<td>_</td>
<td>4.7350 ± 5.09099</td>
<td>4.8993 ± 3.2121</td>
</tr>
<tr>
<td>PD (extension)</td>
<td>_</td>
<td>_</td>
<td>0.13459 ± 0.03597</td>
<td>0.31074 ± 0.0768</td>
</tr>
<tr>
<td>FR (ml/min)</td>
<td>0.6095 ± 0.45187</td>
<td>0.8295 ± 0.4946</td>
<td>0.5410 ± 0.37597</td>
<td>0.6060 ± 0.37021</td>
</tr>
<tr>
<td>pH</td>
<td>7.4982 ± 5.0847</td>
<td>7.17 ± 3.966</td>
<td>7.4925 ± 2.547</td>
<td>7.071 ± 6.2944</td>
</tr>
<tr>
<td>TP (mg/dl)</td>
<td>178.09 ± 13.969</td>
<td>224.65 ± 20.6175</td>
<td>270.20 ± 93.7433</td>
<td>248.09 ± 76.9053</td>
</tr>
<tr>
<td>Glo (mg/dl)</td>
<td>116.43 ± 61.962</td>
<td>202.44 ± 86.068</td>
<td>220.96 ± 90.289</td>
<td>220.42 ± 76.0829</td>
</tr>
<tr>
<td>Glo/Alb</td>
<td>0.1563 ± 0.09357</td>
<td>0.1622 ± 0.2403</td>
<td>0.2785 ± 0.19362</td>
<td>0.1489 ± 0.16348</td>
</tr>
<tr>
<td>FBF (mg/dl)</td>
<td>3.7930 ± 1.9257</td>
<td>3.7930 ± 1.9257</td>
<td>2.3680 ± 0.5305</td>
<td>2.342 ± 0.5543</td>
</tr>
</tbody>
</table>

Table 2: Statistically significancies for the salivary parameters among the groups.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>GI-GII</th>
<th>GI-GIII</th>
<th>GI-GIV</th>
<th>GI-GII</th>
<th>GI-GIII</th>
<th>GI-GIV</th>
</tr>
</thead>
<tbody>
<tr>
<td>SH (PY)</td>
<td>_</td>
<td>_</td>
<td>_</td>
<td>0.001**</td>
<td>_</td>
<td>_</td>
</tr>
<tr>
<td>TBF</td>
<td>0.150</td>
<td>0.022*</td>
<td>0.045*</td>
<td>0.791</td>
<td>0.919</td>
<td>0.755</td>
</tr>
<tr>
<td>BOP</td>
<td>0.884</td>
<td>_</td>
<td>_</td>
<td>_</td>
<td>_</td>
<td>0.022*</td>
</tr>
<tr>
<td>CI</td>
<td>0.365</td>
<td>0.001**</td>
<td>0.001**</td>
<td>0.011**</td>
<td>0.001*</td>
<td>0.336</td>
</tr>
<tr>
<td>PI</td>
<td>0.203</td>
<td>0.004*</td>
<td>0.001**</td>
<td>0.095</td>
<td>0.016*</td>
<td>0.448</td>
</tr>
<tr>
<td>CAL (severity)</td>
<td>_</td>
<td>_</td>
<td>_</td>
<td>_</td>
<td>_</td>
<td>0.664</td>
</tr>
<tr>
<td>CAL (extension)</td>
<td>_</td>
<td>_</td>
<td>_</td>
<td>_</td>
<td>_</td>
<td>0.017*</td>
</tr>
<tr>
<td>PD (severity)</td>
<td>_</td>
<td>_</td>
<td>_</td>
<td>_</td>
<td>_</td>
<td>0.647</td>
</tr>
<tr>
<td>PD (extension)</td>
<td>_</td>
<td>_</td>
<td>_</td>
<td>_</td>
<td>_</td>
<td>0.220</td>
</tr>
<tr>
<td>FR</td>
<td>0.872</td>
<td>0.198</td>
<td>0.291</td>
<td>0.248</td>
<td>0.370</td>
<td>0.794</td>
</tr>
<tr>
<td>pH</td>
<td>0.030*</td>
<td>0.068</td>
<td>0.005*</td>
<td>0.033*</td>
<td>0.506</td>
<td>0.006*</td>
</tr>
<tr>
<td>Alb</td>
<td>0.819</td>
<td>0.001**</td>
<td>0.212</td>
<td>0.001**</td>
<td>0.307</td>
<td>0.001**</td>
</tr>
<tr>
<td>TP</td>
<td>0.069</td>
<td>0.001**</td>
<td>0.005*</td>
<td>0.162</td>
<td>0.483</td>
<td>0.414</td>
</tr>
<tr>
<td>Glo</td>
<td>0.076</td>
<td>0.021*</td>
<td>0.012*</td>
<td>0.57</td>
<td>0.563</td>
<td>0.941</td>
</tr>
<tr>
<td>Alb/Glo</td>
<td>0.917</td>
<td>0.036*</td>
<td>0.897</td>
<td>0.045*</td>
<td>0.816</td>
<td>0.026*</td>
</tr>
<tr>
<td>TF</td>
<td>0.052</td>
<td>0.001**</td>
<td>0.001**</td>
<td>0.002*</td>
<td>0.001**</td>
<td>0.108</td>
</tr>
<tr>
<td>PBF</td>
<td>0.648</td>
<td>0.001**</td>
<td>0.001**</td>
<td>0.001**</td>
<td>0.001**</td>
<td>0.948</td>
</tr>
<tr>
<td>CRP</td>
<td>0.028*</td>
<td>0.846</td>
<td>0.006*</td>
<td>0.028*</td>
<td>0.526</td>
<td>0.006*</td>
</tr>
</tbody>
</table>

(*) mean that there were significant differences between groups at p>0.05.
(**) mean that there were highly significant differences between groups.