Periodontics

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

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Abstract

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

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

Results: Salivary flow rate was not altered regarding to periodontal health status. Salivary pH was lower in smokers than in non-smokers, while it was not affected by periodontal health status. TP, Alb and Alb/Glo ratio were higher in CP patients. Saliva Glo and TF levels increased in both CP and smokers with CP, while salivary PBF level decreased in both CP and smokers with CP comparing to healthy control. The concentrations of these parameters did not affect by smoking status except for TP. Regarding CRP, in general, its level was higher in smokers than in non- smokers, while it was not affected by periodontal health status.

Conclusion: 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 smokers 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.

Introduction

Chronic periodontitis (CP) can be defined as an infectious disease that, results in inflammation within supporting structure of the tooth, progressive attachment loss, and bone loss[1]. 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 bacteria 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 aggressive[6].

Smoking is very strong behavioral risk factor for CP. Cigarette smokers are 2.5 - 6 times more likely to develop CP than non-smokers[7]. Chronic periodontitis is more prevalent and more severe in smokers, characterized by deeper periodontal pockets, greater attachment loss and more fraction defects. Smoking is considered as an independent risk factor for periodontitis[8].

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 plague index, calculus index, periodontal pocket depth, bleeding index and clinical attachment loss (CAL) [11].

Saliva plays an important role in the protection of periodontium. It also affected by smoking[12, 13]. Analysis of saliva can be contributed in the periodontal disease diagnosis[14].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) 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[18] .

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[6, 19].

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 [20].

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) [11].

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 [21].Spitting method was used for collecting unstimulated whole saliva (UWS) [22]. 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 later, 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 (Flink,2005). Any blood contaminated saliva was discarded. The samples were centrifuged for ten minutes at 3000 r.p.m.[23].

Laboratory methods

Unstimulated salivary flow rate was defined as the total volume of saliva produced per unit time (ml/mint) [24]. The pH values of the saliva were immediately measured by using pH meter. Afterward, saliva samples were stored at (-20ºC) until analysis[23].

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[25], 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 cited in Al-Sarrag[26]. The estimation of CRP was performed by Latex slide agglutination method (Qualitative Measurement) recorded as a negative or positive results[25].

Statistical analysis

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

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 sta-

tistically significant difference (p>.001) in smoking exposure measured in PK in GII compared to GIV. GII had lower smoking exposure in their life time than GIV.

Regarding flow rate (FR), non significant changes were observed among the groups.

There was a statistically significant decrease in the salivary pH in both GII and GIV when compared to GI. In general smokers had lower salivary pH than non-smokers. No change in pH value was found in GIII.

Regarding salivary TP, increase in its mean value was seen in GII, GIII, and GIV when compared with GI. The results showed that there was a high significant increase in the salivary albumin in GIII when compared to GI, GII and GIV (p> 0.001). Non-significant differences between GI and GII and between GI and GIV were observed.

There was a statistically significant increase in the salivary globulin in both GIII and GIV when compared to GI (p> 0.05). Non-significant differences between GII and GI and between GIII and GIV were seen.

The result indicated a statistically significant increase in the ratio of salivary albumin to globulin in GIII when compared to GI, GII and GIV (p> 0.05). Non-significant differences among GII, GIV and GI were seen. GII had the lowest mean value.

There was a high significant increase in the salivary TF in both GIII and GIV when compared to GI (p> 0.001). Patient with CP had higher salivary TF concentration than subjects with clinically healthy periodontium.

The result showed a high significant decrease in the salivary PBF in both GIII and GIV when compared to GI. There was also a highly significant decrease in the salivary PBF in both GIII and GIV comparing to GII (p> 0.001), while a non-significant difference between GI and GII and between GIII and GIV was found. Patient with CP had lower PBF concentration than subjects with clinically healthy periodontium.

There was a statistically significant increase in salivary CRP in GII comparing to GI and GIII, while a significant increase was found in GIV comparing to GI and GIII (p> 0.05). Non significant differences

between GII and GIV and between GIII and GI were observed. In general smoker groups had significantly higher salivary CRP than non- smoker groups (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 the groups. This result was in agreement with other studies[27-31], who found that UWS flow rate was not affected by periodontal health status, while the result showed a disagreement with Sculley and Langley-Evans, who found that UWS flow rate significantly increased in severe CP[32]. The result also was in disagreement with Aziz and Askari, who observed that UWS flow rate was significantly lower in smokers compared with non-smoker[33].

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 authers[27, 28], while it was in disagreement with Gonzaalez et al[34]. This disagreement might be resulted from using low sample numbers in their studies. Low salivary pH value in smokers comparing to non smokers might be due to the acidity effect of cigarette smoke components that may be dissolved in saliva. There was a non significant difference in pH values between patient with CP and subjects with clinically healthy periodontium. This result was in line with some other studies[33, 35], while the result was in disagreement with Bezerra-Junior et al, who found that salivary pH value was higher in CP patient comparing to control[29].

According to the results of this work, patients with CP had higher salivary total protein concentration than clinically healthy subjects. This result might be due to periodontal tissue destruction, thus releasing of periodontal proteins into oral cavity. 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. The high albumin

level in CP patients may be due to periodontal tissue destruction, bleeding status, bacteria growth and/or ulceration in sulcular epithila [36]. 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[37].

In the present study, there was a statistically significant increase in salivary globulin concentration in GIII and GIV comparing to GI, while a statistically no significant difference was found among the other groups. This result might be due to the increase in inflammatory proteins infiltrated through sulculer epithelia into gingival sulcus, then into saliva in CP patients6, whereas the inflammatory proteins will decrease in saliva of smokers[38, 39].

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.

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 periodontal tissue destruction in CP and increase in glycosidase activity, which is responsible for glycoprotein degradation[37]. The results showed that cigarette smoking has no significant influence on salivary TF and PBF levels.

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 CP.

Conclusions

Smoking, CP, and both smoking and Cp in combination can affect the chemical components of saliva; mostly proteins, glycoprotiens and their related parameters. Some of these salivary components may be used as indicators in the diagnosis and prognosis of CP and smokers with CP. It is necessary that, both periodontal health and smoking status be considered during study of salivary composition.

Table (1): The mean ± SD values of all the parameters in saliva of the groups

Parameters	GI	GII	GIII	GIV
SH (PK)	_	254 ± 202.7	_	642.5 ± 411.4
BOP	0.087 ± 0.0575	0.061 ±0.0655	0.9945 ± 0.708	0.5795 ± 0.931
CI	0.3525 ± 0.4078	0.584 ± 0.735	1.4675 ± 0.911	1.7135.± 1.023
PI	1.289 ± 0.845	1.6275 ± 0.871	2.0735 ± 0.663	2.2745 ± 0.931
CAL (severity)	_	_	5.0535 ± 0.584	5.126 ± 0.8749
CAL(extension)	_	_	0.306 ± 0.214	0.560 ± 0.412
PD (severity)	_	_	4.735 ± 0.151	4.9 ± 0.32
PD (extension)	_	_	0.135 ± 0.036	0.311 ± 0.078
FR (ml/min)	0.61 ± 0.452	0.83 ± 0.5	0.54 ± 0.376	0.61 ± 0.37
pH	7.498 ± 0.51	7.17 ± 0.4	7.492 ± 0.25	7.07 ± 0.63
TP (mg/dl)	178.1 ± 13.97	224.65 ± 20.62	270.2 ± 93.7	248.1 ± 76.9
Alb (mg/dl)	21.56 ± 8.61	21.55 ± 8.69	48.2 ±13.27	27.67± 4.87
Glo (mg/dl)	116.4 ± 61.97	202.4 ± 86.7	221 ± 90.3	220.4 ±76.9
Alb/Glo	0.1563 ± 0.0936	0.1622 ± 0.2402	0.2785 ± 0.1936	0.1489 ± 0.1635
TF (mg/dl)	11.67 ± 4.164	14.37 ± 3.51	18.73 ± 4.24	20.95 ± 5.17
FBP (mg/dl)	3.793 ± 0.193	3.813 ± 0.193	2.368 ± 0.43	2.342 ± 0.55

Table (2):-Statistically significances for the salivary parameters among the groups.

Parameters	GI-GII	GI-GIII	GI-GIV	GII-GIII	GII-GIV	GIII-GIV
SH (PK)	_	_	_	.0001**	_	_
TBF	.150	022*	.045*	.791_	.919	.755
BOP	.884	_	_	_	_	.022*
CI	.365	.0001**	.0001**	.001**	.0001**	.336
PI	.203	.004*	.0001**	.095	.016*	.448
CAL (severity)	_	_	_	_	.664	_
CAL(extension)	_	_	_	_	.017*	_
PD (severity)	_	_	_	_	.647	_
PD (extension)	_	_	_	_	.220	_
FR	.872	.198	.291	.248	.370	.794
pH	.030*	.968	.005*	.033*	.506	.006*
Alb	. 819	.0001**	.212	.0001**	.307	.0001**
TP	. 069	.001**	.005*	.162	.483	.414
Glo	. 076	.021*	.012*	. 57	. 563	.941
Alb/Glo	. 917	.036*	.897	.045*	.816	.026*
TF	.052	.0001**	.0001**	.002*	.0001**	.108
PBF	. 648	.0001**	.0001**	.0001**	.0001**	.948
CRP	.028*	.846	.006*	.028*	.526	.006*

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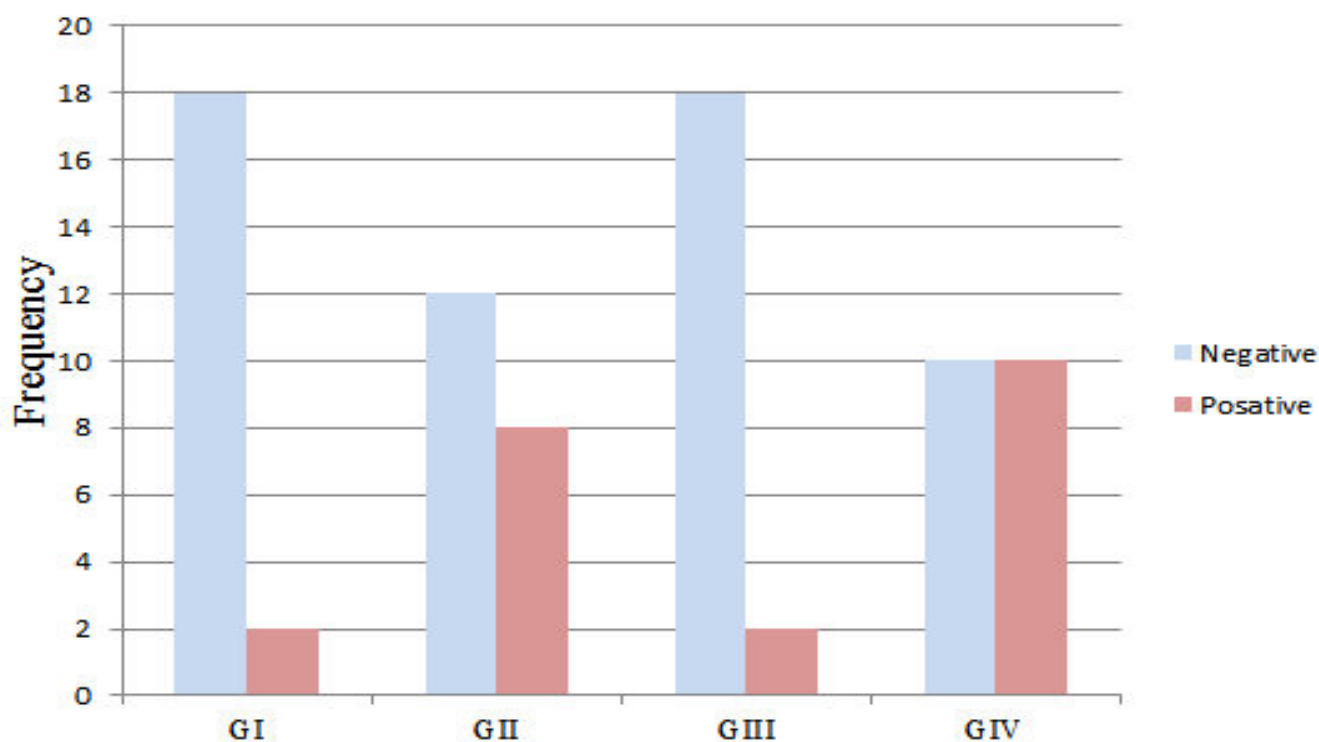(**) mean that there were highly significant differences between groups .

Figure (1): Salivary CRP values in all groups; GI, GII, GIII, GIV

Periodontics

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Periodontics