ORIGINAL ARTICLE

Association between salivary sialic acid and periodontal health status among smokers

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Abstract *Background:* Smoking is an environmental risk factor causing poor dental health. Sialic acid is a salivary marker of oxidative stress for research of periodontal diseases.

Aims: To identify diagnostic sialic acid fraction and its scavenger effect for periodontal diseases among smokers and periodental health status.

Subject and method: This study carried out in the Khanzad specialized dental center – Erbil city. The study population is composed of 62 convenient samples. A structured interview questionnaire form was used to collect data about socio-demographic properties and smoking history. Clinical measurements were carried out to measure periodontal health status. Un-stimulated whole saliva samples were collected for measuring sialic acid fractions. Statistical package for social science (SPSS, version 18), was used for analysis and odds ratio.

Results: Risk of smoking increased significantly in young to mid ages, which included most of the current smokers, with periodontal diseases, and high total free sialic acid. Risk of periodontitis and teeth missing increased significantly by long duration of smoking, bad tooth brushing, and poor eating habits. Risk of teeth mobility and loss decreased significantly by early smoking cessation and low income. High levels of free sialic acid correlated significantly in current smokers with medium and deep pocket depth.

Conclusion: Salivary free sialic acid may be used as a diagnostic oxidative stress biomarker for periodontal diseases among young current smokers. Cumulative destructive effect of long duration of smoking on the periodontum can be controlled by smoking cessation, good oral hygiene and diet habit in early old ages.

1. Introduction

Periodontal diseases (gingivitis and periodontitis) are the most prevalent chronic diseases affecting population worldwide. Gingivitis is inflammation of the gum due to the accumulation of plaque, and affects 50% of the adult population (Sculley and Langley-Evans, 2003). Periodontitis affects the supporting structures of the teeth and if not promptly recognized and

correctly managed can ultimately lead to gum recession, loss of gingival tissue, underlying alveolar bone and tooth, resulting in reduced masticatory function and subsequent alterations in dietary intake and nutritional status (Milward and Chapple, 2013).

Periodontal disease is initiated by the colonization of the gum by specific bacteria and their products which causes abnormal host response, involving the release of excess proteolytic enzymes and reactive oxygen species (ROS), that cause increased levels of biomarkers for host tissue damage (Chapple et al., 2007). Tissue injury from free radical production in periodontitis is related to low antioxidant (AO) capacity and may be caused by a number of factors including smoking and poor nutritional status (Sculley and Langley-Evans, 2003).

Smoking is a single, modifiable environmental risk factor responsible for excess prevalence of periodontal disease in the population and has a direct influence on periodontal variables (Sceedev et al., 2012). Smoking effects include chronic reduction of blood flow, altered neutrophil function, cytokine and growth factor production, inhibition of fibroblast growth and attachment, and decreased collagen production and vascularity (Naresh Kumar, 2012). It was demonstrated that smoking increases the levels of free radicals and lipid peroxidation in periodontal tissues. In addition to decreased antioxidant levels in blood, gingival tissue, saliva, and gingival crevicular fluid (GCF) of periodontitis and gingivitis smokers (Kurtul and Gökpinar, 2012). Smejkalova et al. (2012) reported that socioeconomic disadvantages, poor oral hygiene habit, and bad eating behavior associated with smoking and smoking related diseases.

Laboratory tests of samples from plaque, saliva or gingival crevicular fluid are more accurate than clinical measurements and are developed to measure biomarkers (derived from bacterial structure or the host inflammatory system) of periodontal diseases to detect of 'high-risk' individuals and an increased probability of disease (Beltrán-Aguilar et al., 2012).

Saliva is the first defense fluid and an important salivary biomarker is sialic acids, they are family of nine carbon acidic monosaccharide, systemic inflammatory marker, and component of salivary glycolipids, glycoproteins including IgA and other immunological and acute phase proteins (Sculley and Langley-Evans, 2003). Sialic acid levels increased in periodontitis, because it is protective constituent of human salivary mucin, and lipid bound sialic acid fraction can be used as diagnostic parameter for periodontitis (Jawzaly, 2010). Ogasawara et al. (2007) concluded that sialic acids of mucin acts as scavengers for hydroxyl (OH) free radical and react directly with it. Therefore this study was conducted to identify sialic acid fractions levels among smokers as biomarkers for periodontal diseases and its prognoses.

2. Material and methods

2.1. The study population

Sixty-two (62) convenient samples attending Khanzad specialized dental center/ Erbil city were recruited to this descriptive study after their consent had been taken.

Inclusion criteria: Patients should have been smoking at least one year and more, should not have any systemic condition and should not have be subjected to periodontal therapy or any antibiotic medication during the last 3 months.

Exclusion criteria: Patients just received dental treatment were included to avoid contamination.

The studied population ages were between 21 and 75 years with a mean of 48.3 ± 13.5 years, composed from 52 males and 10 females and were divided to two groups after clinical measurements of dental condition;

Group I: 41 smoker patients with periodontal diseases, consist of 35 periodontitis patients and 6 gingivitis patients.

Group II: 21 non periodontal smokers consist of 6 patients with simple caries, 7 with partial edentulous patients (loss of teeth as a result of previous caries and periodontities) and 8 individuals with healthy dental condition, regarded as the control group.

2.2. Data collection and measures

A structured interview questionnaire form was used to collect data, which are composed of three parts:

2.2.1. First part

Clinical measurements were carried out by the trained dentist. Periodontal examinations were repeated in 10% of the sample for calibration by the heads of the periodontal department in the center to measure the number of teeth remaining, and the indices of; gingivitis, periodontitis and caries.

Oral examination:

Bleeding on probing for gingivitis (Saxer and Muhlemann, 2004), Probing Pocket depth (PPD): Ramjford teeth were selected for pocket depth measurement as there was high agreement between these index teeth and full mouth situation concluded by Mumghamba et al. (2004). These teeth are (16, 21, 24, 36, 41, and 44). If one of these teeth is missing its distal neighbor (17, 11, 25, 37, 42, or 45, respectively) may be substituted. Probing pocket depth was done by measuring the distance with Williams periodontal probe from the gingival margin to the bottom of the pocket for each tooth and at the six sites (mesial, middle, and distal area of the facial and lingual surfaces). The greatest single measurement determines the pocket score for the tooth. Navy Periodontal Diseases Index (NPDI) second component (pocket scores) criteria were used for periodontal destruction diagnosis (Grossman, 1974) and classification of (Silvestre et al., 2009) was used for identifying severity of disease:

0 probing depth not over 3 mm, (1–3 mm mild pockets).
5 probing pocket depth greater than 3 mm but not over 5 mm, (4–5 mm medium pockets).
8 probing pocket depth greater than 5 mm ($\geqslant 6$ mm deep pockets).

Mobility degree of teeth (Fermin and Henry, 2005), missing of teeth and caries by World Health Organization diagnostic criteria was used for determining the decayed, missing, filled teeth (DMFT) index.

2.2.2. Second part

Socio-demographic data and smoking history, collected by asking the studied population about social behavior factors, included; ages, sex, familial history of oral diseases, eating

habit of sweet foods, and oral hygiene habit (frequency of tooth brushing and use of dental floss). Smoking history included: Smoking status, former (had used cigarettes in the past) and current smokers (who were continuous in cigarettes smoking). Dose of cigarette was determined on the bases of sticks of cigarette per day and they were classified into two categories; (1–10 cigarettes/day) smokers, and (>10 cigarette/day) high smokers. Duration of smoking (the number of years of smoking) was computed by subtracting the participant's age at smoking initiation from their age at smoking cessation for former smokers, or subtracting the participant's age at smoking initiation from their current age for current smokers and categorized to; 1–5, 6–10, 11–15, and >15 years. Duration of smoking cessation for former smokers is computed by subtracting participant's age at smoking cessation from their current ages and categorized as; <5 years, 5–10 years, 11–15 years and >15 years of cessation.

2.2.3. Third part

Biochemical measurements were done for saliva for all subjects in the laboratory of basic science – College of Nursing/Hawler Medical University. Un-stimulated whole saliva samples (prior to the clinical measurements) were collected from all subjects between 9 and 11 h AM., by the spitting method. The samples were stored at −20 °C for one hour, then centrifuged immediately at (10,000)g and at 4 °C for 20 min to obtain a clear supernatant. The remaining sediment (precipitate) was washed and then re-suspended to the original volume with saline solution (7% pH 7.0) and stored at −20 °C for analysis of total sialic acid (TSA) and free sialic acid (FSA) and measured with the modified thio-barbituric acid method of Skoza and Mohos, 1976). Extraction and determination of lipid -bound sialic acid (LSA) (Masami, 1989). Determination of sialic acid bound to proteins (PSA) (Shetty and Pattabiraman, 2004).

2.3. Statistical analysis

The collected data were analyzed statistically by using statistical package for social science (SPSS, version 18) that included descriptive tests for frequency, and means with standard deviation. Independent *T*-Test was used to compare between two means and a one way ANOVA, to compare three or more means. Chi – square, Eta-test and Pearson's R to test relations; between nominal with nominal, nominal with interval and interval with interval (data scale) respectively. A *P*-value less than 0.05 was considered as statistically significant, and less than 0.01 highly significant. Logistic regression was used for variables to calculate odds ratio and adjust of cofounders effects.

3. Results

3.1. Dental health status of the studied smokers

Fig. 1 represents distribution of the studied smokers in different status of; dental health (cases) and smoking. They are composed of 35 (56.5%), 33 male and 2 female periodontitis patients, 6 (9.7%) gingivitis (5 male and 1 female), 6 (9.7%) caries (4 male and 2 female), 7 (11.3%) of partial edentulous (4 male and 3 female), and 8 (12.3%) of the control group (6 male and 2 female) that were free from oral diseases. Majority of periodontitis 62.86%, and 83.3% of gingivitis were current smokers, while 50%, 58.1%, 62.5.5% of caries, partial edentulous, and controls respectively were former smokers.

3.2. Characteristic of periodontal health status among smokers

Figs. 2 and 3, show significant differences of dental parameters between cases. Periodontitis smokers had significantly highest score 5.43 ± 1.6 mm of probe pocket depth, it is in the third criteria of (NPDI) and regarded approximately as deep pocket according to (Silvestre et al., 2009) classification, while smokers in other dental health status had medium means of pocket depth (3.44) mm. There was also significant difference in the degree of teeth mobility between periodontitis (1.3) score and cases except partial edentulous smokers and did not differ in teeth mobility (0.86) score with periodontitis.

3.3. Relation between dental oral health and smoking parameters

Duration of smoking and its cessation with cases (dental oral health): Table 1 shows high relation between durations of smoking and cases, because majority of studied smokers (36) 58.1% had longest duration of smoking and 21, 58.3% of these smokers had periodontitis diseases. Table 1 also shows low relation between cases and duration of smoking cessation. The highest percentage (42.3%) of smokers were in ⩾10 years of smoking cessation and 9 (81.8%) of these smokers had periodontitis and were partially edentulous, while 62.5% of caries and control groups were in short duration of smoking cessation <5 years.

3.3.1. Smoking duration and cessation with teeth missing

Table 2 shows significant relations between duration of smoking and duration of smoking cessation with teeth missing. There was also significant difference in the means of teeth missing among smokers with different smoking duration and duration of smoking cessation. The highest number (36) 58.1% of smokers had teeth missing in longest (>15) year duration of smoking and (15) smokers with high number (>6) of teeth missing occupies 41.7%. Also high number (11), 42.3% of smokers had teeth missing in >10 years of duration of smoking cessation and smokers with high number (>6) of teeth missing occupy 72.7%. Multinomial regression analysis showed increase duration of smoking caused significant increased risk of teeth missing (3- to >6) teeth. Smoking cessation showed a significantly increased risk of low number of teeth missing (3–6) teeth in groups <5 and 5–10 years of smoking cessation comparing to the high number of teeth missing (>6 teeth) as reference in >10 years duration of cessation.

3.3.2. Smoking duration and teeth mobility

Table 3 shows significant correlations between duration of smoking and degree of teeth mobility. There was a significant difference between duration of smoking with different scores of teeth mobility. The highest number (36) 58.1% of smokers with teeth mobility were in the group >15 years of smoking. Logistic regression analysis showed that low duration of smoking (1–5) years caused a significant decrease in teeth mobility among the studied smokers.

3.4. Relation between socio-demographic properties with smoking parameters and dental health status

3.4.1. Smoking status, cases and ages

The studied population ages ranged between 21 and 75 years with mean 48.3 ± 13.5 years and were divided into five age groups. Table 4 shows a high and significant relation of age groups with smoking status and cases respectively. There was a high significant difference in ages of smokers among cases. Also between means of former smoker's ages (53.9 ± 12.4) years and current smokers (44.3 ± 12.9) years. The highest number (18) and percentage 69.2% of former smokers were in old age groups (51 to ⩾61) years, and included all partial edentulous (7 smokers). While (17), 47% of current smokers were in young age (21–40) years. Logistic regression analysis showed significant increase
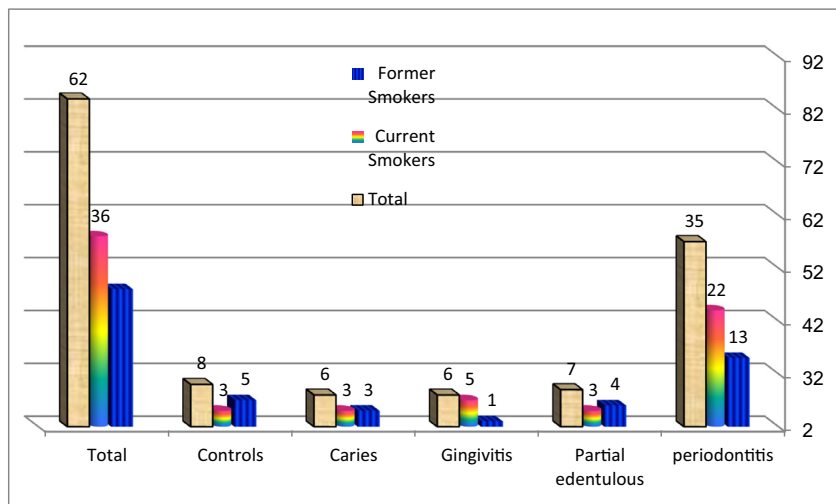
Figure 1 Distribution study sample in different cases with different smoking status.
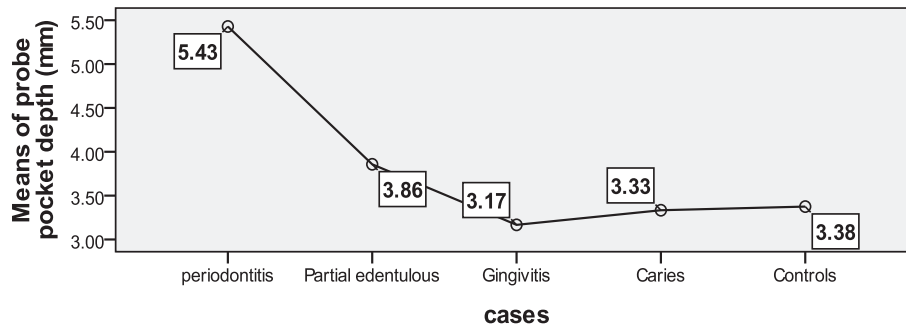

Figure 2 Significant difference between smokers in the means of teeth probe pocket depth.
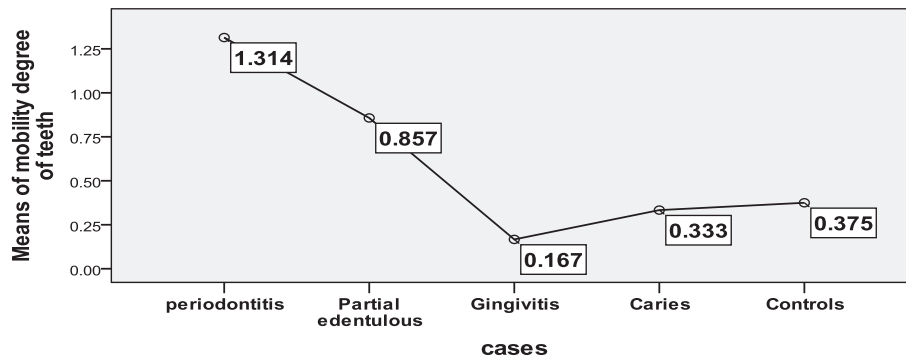
Figure 3 Significant difference between smokers in the means of teeth mobility degree.

in the risk of smoking by (10.7) times at age group (31–40) years, 80% (12) smokers of it were current smokers, and 66.7% of this age had periodontitis.

3.4.2. Smoking duration, smoking cessation and ages

Table 5 shows significant relation between age groups with duration of smoking, and duration of smoking cessation. The highest percentage 32.2.1% of smokers were in age group (51–60) years and longest duration of smoking (> 15) years occupy 85% of it. Also the highest percentage 38.5% of smokers who ceased smoking was in the age group 51–60 years. While the age group ⩾61 years included large number

(6) smokers with longest duration of smoking cessation (> 10) years. There was also a significant difference between groups of ages in the duration of smoking and duration of smoking cessation.

3.4.3. Education and dose of smoking:

Table 6 shows a significant relation between dose of cigarette intake and education levels. There was a significant difference in dose of cigarette intake among smokers with different education levels. Majority 38.7% were illiterate and 22 smokers, 91.7% of illiterate had a high dose of cigarette intake. Lowest intake 1.6% was among smokers with institute education levels.

Table 1 Relations between smoking parameters and cases.

Cases	Duration of smoking (years)						Eta-value	Degree
	1–5	6–10	11–15	>15	T.	%		
Periodontitis	2	10	2	21	35	56.5	0.53	High
Gingivitis	0	1	2	3	6	9.7		
Caries	0	0	3	3	6	9.7		
Partial edentulous	0	2	1	4	7	11.3		
Controls	0	2	1	5	8	12.9		
Total	2	15	9	36	62	100		
%	3.2	24.2	14.5	58.1	100			

Cases	Duration of cessation (years) in former smokers					Eta-value	Degree
	< 5	5–10	⩾10	T.	%		
Periodontitis	3	5	5	13	50	0.37	Low
Partial edentulous	0	0	4	4	15.4		
Gingivitis	0	0	1	1	3.8		
Caries	2	1	0	3	11.5		
Controls	3	1	1	5	19.3		
Total	8	7	11	26	100		
%	30.8	26.9	42.3				

T. = Total.

Table 2 Relation of teeth missing with smoking duration and cessation.

Teeth missing Groups	Duration of smoking (years)						P-Value of χ^2	Mean ± SD teeth missing	F-Value	P-Value	OR	95% CI
	1–5	6–10	11–15	>15	T.	%						
0–2	1	8	3	4	16	25.8	<0.05[*]	3.5 ± 3.5	3.7	<0.05[*]	First missing reference	
3–6	1	4	5	17	27	43.5		3.3 ± 3.9			1.14[*]	1.0–1.3
>6	0	3	1	15	19	30.7		3.9 ± 3.5			1.2[*]	1.1–1.4
Total	2	15	9	36	62			6.3 ± 3.4				
%	3.2	24.2	14.5	58.1	100			5.1 ± 3.7				

Teeth missing Groups	Duration of cessation (years) in former smokers					P-Value	Mean ± SD of teeth missing	F-Value	P value	OR	95% CI
	< 5	5–10	> 10[a]	Total	%						
0–2	1	2	1	4	15.3	0.028[*]	2.1 ± 0.64	4.9	<0.05[*]	4.0 / 16 / >6 teeth reference	0.2–95.7 / 0.67–383
3–6	5	5	2	12	46.2		1.7 ± 0.48			10[*]	1.1–95.5
>6	2	0	8	10	38.5		2.6 ± 0.67			20[*]	1.4–283
Total	8	7	11	26	100		2.2 ± 0.7 777			>6 missing reference	
%	30.8	26.9	42.3	100							

χ^2 = Chi square test. % = percentage, > 10[a] smoking cessation = Multinomial result of this group was redundant. OR = odds ratio.
[*] = significant result for P value.

Table 3 Relation between teeth mobility and duration of smoking.

Duration of smoking G. (year)	Mobility degree of teeth				T.	%	R.	P-Value	Mean ± SD of mobility	F. value	P value	Odds ratios	95% 95% CI
	0	1	2	3									
1–5	1	1	0	0	2	3.2	0.30	<0.05[*]	0.5 ± 0.7	2.9	<0.05[*]	0.84[*]	0.72–0.98
6–10	7	5	3	0	15	24.2			0.73 ± 0.8			0.94	0.81–1.08
11–15	5	4	0	0	9	14.5			0.33 ± 0.5			0.93	0.80–1.1
>15	7	18	8	3	36	58.1			1.19 ± 0.85			Third mobility reference	
Total	20	28 28	13	3	62	100			0.94 ± 0.84				

G = Groups R. = persons correlation, T, Total.

Table 4 Relation of ages with smoking status and cases.

Age groups (years)	Smoking status		Relation					Differences			95% CI	
	Former smokers	Current smokers	T.	Eta-test	Value			T-Value	P-Value	Odds ratio	Low	Up
21–30	2	5	7	0.8	High	Smoking status	Means ± SD of ages	8.7	<0.01**	6.7	0.8–54.9	
31–40*	3	12	15			Former smokers	53.9 ± 12.4			10.7*	1.7–66.7	
41–50	3	5	8			Current smoker	44.3 ± 12.9			4.4	0.6–31.3	
51–60	10	11	21							2.9	0.6–14.2	
≥61	8	3	11							0.4		
Total	26	36	62									

Age G (years)	Dental health status (cases)					T.	Relation		Differences		
	Periodontitis 1	P .ed. 2	Gingivitis 3	Caries 4	Control 5		P-χ^2	Cases	Means ± SD of ages (y)	F. value	P value
21–30	5	0	1	0	1	7	0.05*	1	45.7 ± 13.5	3.9	<0.01*
31–40*	10	0	2	1	2	15		2	63.4 ± 9.9		
41–50	5	0	2	1	0	8		3	40.5 ± 11.5		
51–60	11	2	1	2	5	21		4	53.8 ± 9.2		
≥61	4	5	0	2	0	11		5	48.6 ± 10.9		
Total	35	7	5	6	8	62		Total	48.3 ± 13.5		

χ^2 = Chi square value, P .ed. = Partial edentulous, T. = Total.

Table 5 Relations between Smoking duration and cessation with age groups.

Age G. (years)	Duration of smoking categories (years)						χ^2	P-value	Mean ± SD of duration	F-Value	Sig.
	1–5	6–10	11–15	>15	T.	(%)					
21–30	1	6	0	0	7	11.3	32.6	<.01**	7.6 ± 2.6	7.9	<.01**
31–40	0	4	4	7	15	24.2			14.9 ± 4.1		
41–50	0	3	1	5	9	14.5			17.4 ± 7.5		
51–60	0	0	3	17	20	32.2			22.5 ± 6.9		
≥61	1	2	1	7	11	17.8			17.8 ± 8.6		
Total	2	15	9	36	62	100			17.5 ± 7		
%	6.5	24.2	14.5	58.1							

Smoking cessation (years)	Age groups (years)							Eta test	D.	Mean ± SD of ages	F value	Sig
	21–30	31–40	41–50	51–60	>61	T.	%					
<5	1	1	0	4	2	8	30.8	0.9	High	51.9 ± 12.5	3.2	≥.05*
5–10	1	2	1	3	0	7	26.9			46.6 ± 10.8		
>10	0	0	2	3	6	11	42.3			60.1 ± 10.9		
Total	2	3	3	10	8	26	100			53.9 ± 12.4		
%	7.7	11.5	11.5	38.5	30.8	100						

% = Percentage, T. = Total, χ^2 = Chi square test, D. = Degree. * = significan.

3.4.4. Occupation and smoking status

Table 7 shows a significant relation between occupation and smoking status. There was a significant difference in smoking status among smokers with various occupations status. Most 33.9% were government employed and 14, (66.7%) of them were current smokers, while the highest 85.7% percentage of retired smokers were former smokers. Logistic regression showed that risk of current smoking decreased by income, significantly among retired smokers.

3.4.5. Oral hygiene habit with smoking duration and cases

Table 8 shows high relation of oral hygiene habit (tooth brushing frequency) and duration of smoking as data scale variables. The highest percentage 44.4%, 16 smokers among the longest duration >15 years of smoking had no brushing habit of teeth. Logistic regression showed increased frequency of tooth brushing significantly among smoking duration 11–15 years, 55% of these smokers had once/day tooth brushing compared to smokers which had >15 years duration of smoking.

Relation of oral hygiene with cases: Table 9 shows low relation of oral hygiene habit (tooth brushing frequency) with cases. High number of smokers 15, 62.5% with no brushing habit of teeth had periodontitis disease. Logistic regression showed that tooth brushing frequency decreased in all cases except caries smokers and increased significantly compared to control, 50% of controls had once/day teeth brushing.

Table 6 Relation between education levels and dose of cigarette/day.

Education status	(1–10) cigarette/d	> 10 cigarette/d	Total	%	Chi squire value	P-Value	F-Value	P-Value
Illiterate	2	22	24	38.7	11.33	< .05[*]	2.45	< .05[*]
Primary	1	13	14	22.6				
Intermediate	2	10	12	19.4				
Secondary	0	8	8	12.9				
Institute	1	0	1	1.6				
University	0	3	3	4.8				
Total	6	56	62	100				

Table 7 Relation between occupations status and smoking status.

Occupation	Former smoker	Current smoker	Total	%	χ^2	P-Value	F-Value	P-Value	Odds ratio	95% CI
Self employed	10	10	20	3.2	10.2	< 0.05[*]	2.8	< 0.05[*]		
Government employed	7	14	21	33.9					0.13	0.013–1.2
Students	2	3	5	8.1					0.25	0.03–2.4
Retired	6	1	7	11.3					0.19	0.012–2.9
House wife	1	8	9	14.5					0.02[*]	0.001–0.4
Total	26	36	62	100					Last as reference	

Table 8 Relations between duration of smoking and tooth brushing.

Duration of smoking (years)	Tooth brushing frequency/week					Eta-test value	Degree of relation	Odds ratio	95% CI
	Not	1–4	> 7	Total	%				
1–5	0	1	1	2	3.2	0.6	High	1.2	0.9–1.7
6–10	6	1	8	15	24.2			1.1	0.92–1.3
11–15	2	2	5	9	14.5			1.8[*]	1.0–1.4
> 15	16	10	10	36	58.1			> 15 is reference	
Total	24	14	24	62	100				
%	38.7	22.6	38.7	100					

Table 9 Relations between dental health status (cases) and tooth brushing.

Cases	Tooth brushing frequency/week					Eta-test value	Degree of relation	Odds ratio	95% CI
	Not	1-4	> 7	Total	%				
Periodontitis	15	8	12	35	56.5	0.31	Low	.964	0.8–1.16
Gingivitis	3	1	3	7	11.3			.976	0.76–1.3
Caries	1	3	2	6	9.7			1.104[*]	0.87–1.4
Partial edentulous	2	1	3	6	9.6			.952	0.74–1.2
Controls	3	1	4	8	12.9			Control is reference	
Total	24	14	24	62					

3.4.6. Diet habit and dental oral health

Table 10 shows relation of sweet diet intake and dental health status (cases). There was low relation and significant difference in sweet diet intake among cases. Caries smokers had significantly low to medium intake of sweet diet compare to periodontitis, while controls (healthy dental smokers) ingested significantly larger amount of medium content of sweet diet compared to periodontitis.

3.5. Sialic acid, smoking and dental health

3.5.1. Levels of total sialic acid and their fractions among smokers

Figs. 4–6 show difference in the mean levels of total sialic acid (TSA), total free sialic acid (TFSA), and total protein bound sialic acid (TPSA) respectively in different dental health status. Statistical analysis showed high significant difference between periodontal groups (periodontitia and gingivitis) and groups; partial edentulous, caries and controls in the level of total free sialic acid, while TSA and TPSA, were significantly different between periodontitis and cases.

3.5.2. Relation between salivary sialic acid and smoking status

Table 11 shows significant correlation between TFSA and smoking status, and distribution of current smokers among cases. There was a significant difference of means of total free sialic acid (TFSA) between current smokers and former smokers. Logistic regression showed increase in risk of high levels of total free sialic acid significantly in current smoking. Total protein bound sialic acid (TPSA) showed only a

Table 10 Relation between oral health status and sweet diet intake.

Parameters	Degree of sweet diets						F	P value	Intake	OR	95% CI
Cases	No	Low	Med	High	T.	Eta value					
Periodontitis	9	11	4	11	35	0.4	5.0	< 0.01**			
Partial edentulous	2	2	1	2	7						
Gingivitis	1	1	2	2	6						
Caries	1	2	3	0	6				Not	5.7	
									Little	9.2*	1.1–7.4
									Med	3.8	
									High		
Controls	0	2	5	1	8				Not		
									Little		
									Med	13.8*	1.2–156.7
									High		
Total	13	18	15	16	62						

Med = Medium.
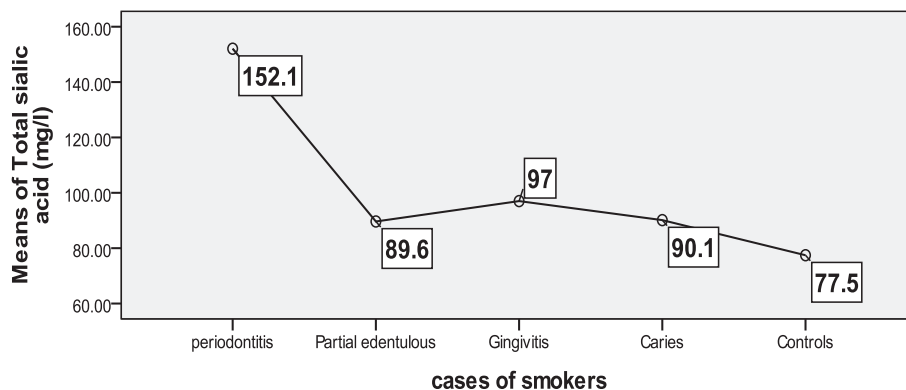
Figure 4 Significant difference among smokers in the mean of total sialic acid.
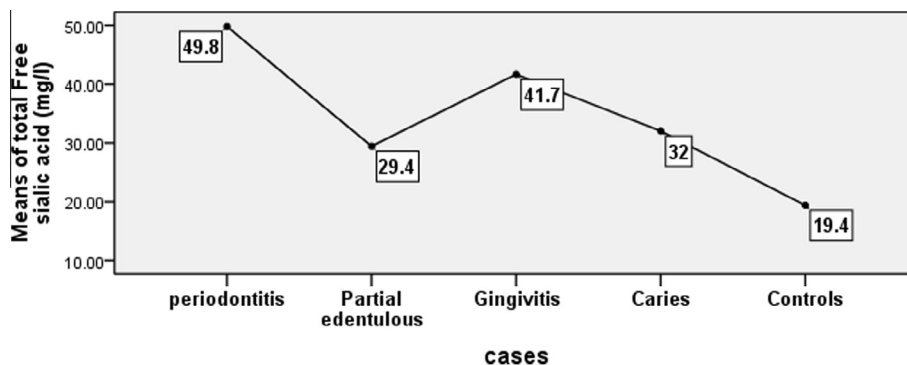
Figure 5 Significant difference among smokers in the mean of total free sialic acid.

significant positive relation with duration of smoking, while total sialic acid and other fractions of sialic acid showed no significant relations.

3.5.3. Relation between salivary free sialic acid and periodontal pocket depth

Total free sialic acid (TFSA) fraction and current smokers were used for identifying relation between salivary sialic acid and dental parameters as a result of significant increase (TFSA) level in current smoking (Table 11). Levels of free sialic acid were divided to two groups ($\leqslant 27$

to > 27) mg/l depending on the normal range of free sialic acid in healthy dental status of non smokers, concluded by (Jawzaly, 2010). Also probe pocket depths were divided to two groups ($\leqslant 3$ mm) and (4 to > 6 mm) depending on NPDI. 75% of current smokers had periodontal diseases composed of 61.1% periodontitis and 13.9% gingivitis (Table 11). Also 75% of current smokers had high levels (> 27 mg/l) of (TFSA) and 63.9% of them had ($\geqslant 4$ mm) probe pocket depth (Table 12). Statistical analysis showed significant relation between TFSA and probe pocket depth, and significant difference (P
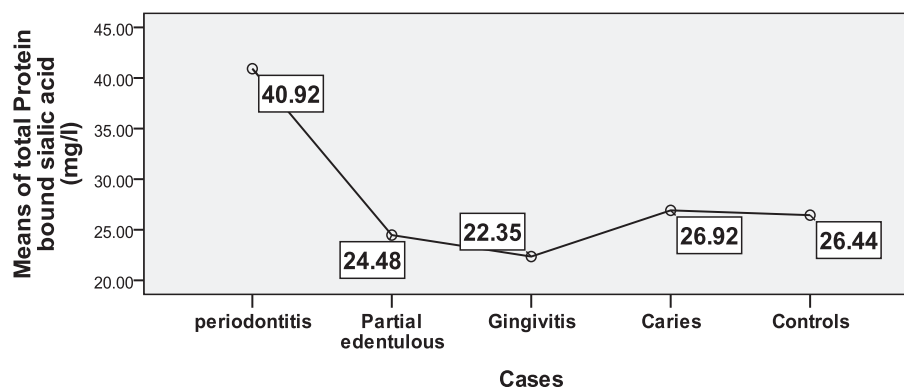
Figure 6   Significant difference among smokers in the means of total protein sialic acid.

Table 11   Relation between salivary sialic acid fraction and smoking parameters.

Smoking parameter Status	Cases	N.	%	R value	P-Value	Means ± SD of TFSA (mg/l)	T-test value	P-Value	OR	95% CI
Current	Periodontitis	22	61.1	0.28	⩽0.05*	45.3 ± 19.4	2.3	⩽0.05*	1.04*	1.0–1.1
	P. edentulous	3	8.3							
	Gingivitis	5	13.9							
	Caries	3	8.3							
	Controls	3	8.4							
	Total	36	100							
Former		26				35.2 ± 14.9				

Duration of smoking (y)	R value	P-Value	Means ± SD of TPSA	F-	P-Value
1–5	0.28	⩽0.05*	34.4 ± 25.7	0.57	Ns.
5–10			31.0 ± 12.6		
11–15			29.7 ± 21.8		
>15			36.4 ± 17.3		

Table 12   Relation between salivary free sialic acid (mg/l) and pocket depth.

Dental parameter	Total free sialic acid mg/l				χ^2	P value	Means ± SD of TFSA	Odds ratio	95% CI
Pocket depth (mm)	⩽27	>27	T.	%					
⩽3	7	6	13	36.1	9.1	<0.01**	31.05 ± 15.7	13.6*	1.4–129.7
⩾4	2	21	23	63.9			53.4 ± 16.5		
Total	9	27	36	100			45.3 ± 19.4		
%	25	75							

** = high significant of P value.

value = 0.003) in means of TFSA among smokers with various pocket depth. Odds ratio showed significant increases in the risk of medium and deep periodontal pocket depth among smokers in high levels of TFSA.

4. Discussion

4.1. Dental health status of the studied smokers

Periodontal diseases account for more than half (66.1%) of the study population (Fig. 1) and prevalence of current smokers among periodontal patients indicated that tobacco is a risk factor for poor dental health compared to former smokers

who had lower rates of periodontal diseases. This agrees with (Kaye et al., 2006) who suggested that smoking is a significant risk factor of periodontal diseases and quitting smoking results in reduced risk as stated by (Smejkalova et al., 2012). Also with (Bloom et al., 2012) who concluded that current smokers had a poorer oral health status and more oral health problems (periodontal, dental caries, oral cancer, and other diseases) than either former smokers or never smokers.

4.2. Characteristic of periodontal health status among smokers

Highest score of probe pocket depths (deep pockets) and teeth mobility scores were among periodontitis smokers (Figs. 2 and

3), this indicates ligament and alveolar bone destruction with pocket formation and it is in agreement with (Sceedev et al., 2012) who reported that smokers had significantly more sites with probing depths 4–7 mm, and (Smejkalova et al., 2012) who found shallow or deep gingival pocket in all sextants of smokers. Bergstrom et al. (2000) found that smokers not only have significantly increased probing depths and alveolar bone loss, but also increased tooth mobility. High teeth mobility score in partial edentulous smokers who lost teeth as a result of peridontitis or caries may due to their old ages ($\geqslant 50$ years) and extended tobacco use which cause permanent loss of the alveolar bone. This in line with (Jette et al., 1993) who confirmed that exposure to tobacco is a risk factor for tooth loss in older adults.

4.3. Dental health status and smoking parameters (Table 1–3)

Most studied smokers (current and former smokers) had longest duration of smoking (>15) years especially periodontitis smokers, who had highest teeth missing and teeth mobility scores. This was apparent by a significant association of teeth missing and teeth mobility with duration of smoking and included a large number smokers with highest teeth missing, (Tables 2 and 3). Therefore long duration of smoking may be a risk factor for poor oral health; periodontitis and teeth loses. This result is consistent with (Jette et al., 1993) who found that duration of tobacco use among current and past tobacco users is a significant risk factor for tooth loss.

Majority (81.1) of former smokers with the longest duration of smoking cessation >10 years were periodontitis and partially edentulous (Table 1) and included approximately 3/4 of smokers with high teeth missing (>6) and increased risk of teeth missing in contrast to short duration of smoking cessation and decreased risk of low number of teeth missing (Table 2). These results indicate lifelong permanent cumulative effect of smoking on periodontum destruction, which increases by duration of smoking, and requires more years to reduce tooth loss. This result is in consistence with (Kaye et al., 2006) who concluded that smoking cessation is beneficial for tooth retention, but long term abstinence is required to reduce the risk to the level of people who have never smoked, because of irreversible alveolar bone loss.

4.4. Smoking parameter, dental health status and socio-demographic properties

4.4.1. Relation smoking status and dental health status with ages (Table 4)

Age group 31–40 years, increased the risk of smoking because 80% of this age group were current smokers, and more than half had periodontitis diseases. While 3/4 of old ages groups 51 to $\geqslant 61$ years were former smokers, included all partially edentulous, simple caries diseases, and healthy dental group (control). These results indicate that current smoking in young ages have a higher than average risk of periodontal disease and declined with age as concluded by (Wayne, 2007).

4.4.2. Smoking duration and cessation with ages (Table 5)

Longest duration of smoking which increased teeth missing and mobility was in age group 51–60 years. So at this age group; year's exposure to tobacco accumulated and increase risk of missing and mobility as in periodontitis which occupies 11 smokers 53.4% of this age (Table 4). Short cessation of smoking which decreased the risk of low number of teeth missing was also in this age group and included control and caries groups (Table 1) especially 5 smokers represent 62.5%, of the control at age group 51–60 years (Table 4). This may be due to a protective effect of smoking cessation in early old ages not >60 years because older included more than half of smokers that had the longest duration of smoking cessation with the highest teeth missing, which were former periodontitis and partial edentulous smokers. These results are in agreement with (Mai et al., 2013) who concluded that smoking may be a major factor in tooth loss especially in the older population due to persistent periodontitis, and may decrease in young adults and it is less important factor in tooth loss due to caries which occur in all age groups.

4.4.3. Smoking with education and occupation (Tables 6 and 7)

The result indicates that low levels of education, cause less awareness to high dose of cigarette intake in contrast to high levels of education. Smoking risk is associated positively with occupation status and income, which increased in government employers, and decreased in retired former smokers as confirmed by logistic analysis and showed a significantly decreased risk of current smoking in retired old age smokers compared to house wives, majority were current smokers. This result is in consistence with (Smejkalova et al., 2012) that found a prevalence of current smokers in the group of respondents with the highest income and is in contrast with (Wayne, 2007) in income but agrees with him in the levels of education and age who stated that the prevalence of current smoking declined with advancing age and was inversely associated with household income and level of education.

4.4.4. Duration of smoking and dental health status with oral hygiene habit and diet habit (Tables 8–10)

The result indicates protective effect of tooth brushing habit (once/day), short duration (<15 years) of smoking and low to medium content of sweet diets, especially among patients who quit smoking which includes simple caries and control because; most of them were former smokers (Fig. 1) and 50% of caries and 32.7% of controls quit smoking after a short duration of smoking 11–15 years (Table 1) and most of them were at age groups <61 years (Table 4). These results indicated that, smoking cessation before age of 61 years, oral hygiene and medium content of sweet diet may decrease oxidative stress and conserves oral health. These results confirm the results of Arowojolu et al. (2013) who concluded that smokers should be instructed to detailed oral hygiene and encouraged to quit smoking. Öztürk et al. (2008) who reported that good oral hygiene may contribute to saliva composition as an oxidative stress-decreasing factor in dental diseases. Smejkalova et al. (2012) who found that current smokers consumed sweetened diet more frequently than non smokers and former smokers and reported that daily smoking was associated with increased use of sugar in tea and coffee, and with (Milward and Chapple, 2013) who suggested that oxidative stress that was generated from frequency intake of simple sugar, carbohydrate and fat is the mechanism by which smoking increases the risk of periodontitis.

4.4.5. Levels of sialic acid and its fraction among the studied sample (Figs. 4–6)

High significant levels of total sialic acid (TSA), protein bound sialic acid (PSA), among periodontitis and free sialic acid (FSA) among periodontitis and gingivitis smokers (most were current smokers) compared to partial edentulous, caries and controls,(majority were former smokers Fig. 1), may indicate difference in inflammation, and oxidative stress status, free radical production and destructive process .These results may be due to differences; in ages, smoking habit, adequate antioxidant defense and social behaviors and are in consistence with (Dhotre et al., 2012) who revealed that smoking increases the level of free radicals in periodontal tissues, which may be responsible for the destruction seen in periodontal diseases and with (Kurtul and Gökpinar, 2012) who concluded increased salivary total sialic acid (TSA) and malonyl aldehyde (MDA) levels in smokers and contribute it to lipid peroxidation.

No significant difference between caries and control group in; TSA, and its fraction (FSA, TPA) was confirmed by no significant difference of periodontal indexes (probe depth and mobility) and indicated to no periodontal damage and oxidative stress in these groups. This result may be due to smoking cessation and similarity of control with caries in ingestion of sweet diet and tooth brushing. It is online with (Öztürk et al., 2008) who found similar LPO levels for both the caries and caries-free groups and was attributed to tooth brushing. Mai et al. (2013) concluded that smoking appears to be a less important factor in tooth loss due to caries. Smejkalova et al. (2012) reported that association between caries and smoking due to other contributing factors; ages, oral hygiene and eating habit and caries is well documented in old age groups.

4.4.6. Relation between salivary free sialic acid with smoking status and periodontal pocket depth: (Tables 11 and 12)

Significant relation of high levels of free sialic acid (>27) with current smoking (47% were in young ages Table 4) and probe pocket depth $\geqslant 4$ mm, indicate to risk of smoking and sever periodontitis disease. Free sialic acid may be released from hydrolysis of glycosidic linkage of terminal sialic acid of mucin by reactive oxygen species (ROS) resulted from current smoking, as indicated by (Eguchi et al., 2005), and cause injury, lipid peroxidation, and destruction of periodontal tissues. ROS may be produced from defects of the inflammatory response, lack of adequate antioxidant (AO) defence in young ages, smoking (which cause change in neutrophil function and cytokine) and poor dietary habit as reported by (Sculley and Langley-Evans, 2003). These results confirm that sialic acid besides being an inflammation marker may be an alternative oxidative stress marker in tobacco exposure, and agree with (Bloomer, 2007) who indicated that young, novice cigarette smokers have lower blood antioxidant capacity and higher lipid peroxidation levels compared to nonsmokers, and revealed that smoking increases the level of free radicals and periodontal diseases. Also with (Cavas et al., 2005) who concluded that over-excreted sialic acids to saliva might have an important role in the removal of hydrogen peroxide and increase in FSA levels in saliva, and has been found to be in good accordance with antioxidant enzymes and concluded that FSA may be an alternative oxidative stress marker.

5. Conclusion

Salivary free sialic acid can be used as diagnostic oxidative stress bio-market for periodontal disease among young to mid ages of current smokers. Cumulative destruction effect of long duration of smoking on periodontal tissue can be decreased by early smoking cessation, accompanied with good oral hygiene, eating habits and low income, especially in ages not more than 60 years.

Conflicts of interest

There was no conflict of interest to declare.

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