

Self-perceived oral health and whole salivary immunoglobulin G levels in habitual *gutka*-chewers and nonchewers

FA Alshehri, AA Al-Kheraif¹, KM Aldosary², F Vohra^{3,4}, H Malmstrom⁵, GE Romanos⁶, F Javed⁵

Department of Periodontics and Community Dentistry, College of Dentistry, ¹Department of Dental Health, Dental Biomaterials Research Chair, College of Applied Medical Sciences, ²Department of Dental, University Hospitals, King Saud University, ⁴Department of Prosthetic Dental Sciences, College of Dentistry, King Saud University, Riyadh, Saudi Arabia, ³Department of Dentistry, Jinah Hospital, Karachi, Pakistan, ⁵Division of General Dentistry, Eastman Institute for Oral Health, University of Rochester, Rochester, ⁶Department of Periodontology, School of Dental Medicine, Stony Brook University, NY, USA

Abstract

Objective: To assess self-perceived oral health and whole salivary immunoglobulin G (IgG) levels among habitual *gutka*-chewers and nonchewers (controls).

Materials and Methods: Fifty *gutka*-chewers and fifty controls were included. Demographic data and self-perceived oral health status (pain in teeth, pain on chewing, bleeding gums (BG), bad breath, loose teeth and daily oral hygiene protocols) were collected using a questionnaire. Unstimulated whole saliva (UWS) was collected and unstimulated whole salivary flow rate (UWSFR) was determined. Whole salivary IgG levels were determined using standard techniques. Odds ratios were calculated for oral symptoms and group differences in protein levels were compared using one-way analysis of variance ($\alpha < 5\%$).

Results: BG was more often reported by *gutka*-chewers than controls ($P < 0.05$). There was no significant difference in UWSFR and self-perceived pain in teeth, pain on chewing, bad breath and loose teeth among *gutka*-chewers and controls. IgG levels were significantly higher among *gutka*-chewers than controls ($P < 0.01$). Among *gutka*-chewers, whole salivary IgG levels were comparable individuals with and without self-perceived oral symptoms. Among controls, IgG levels in UWS were significantly higher among individuals who had BG than those who did not ($P < 0.05$).

Conclusions: Self-perceived oral health is worse and whole salivary IgG levels are higher in *gutka*-chewers compared to controls.

Key words: Alveolar bone loss, areca, inflammation, smokeless, tobacco

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Introduction

Gutka is a form of smokeless tobacco (ST) and is chiefly an amalgam of powdered tobacco, areca nut (fruit of *Areca catechu* tree), slaked lime (aqueous calcium hydroxide paste) and artificial fragrances (such as menthol and sandalwood).^[1,2] Several studies have associated habitual *gutka* usage with the development of oral premalignant and malignant lesions such as oral submucous fibrosis and oral squamous cell carcinoma, respectively.^[3-6] In addition,

periodontal inflammatory conditions (such as plaque index, bleeding on probing, probing depth, clinical attachment loss and marginal bone loss as measured on radiographs) have also been reported to be worse in habitual *gutka*-chewers as compared to individuals not using tobacco in any form (controls).^[1,7,8] However, to our knowledge from indexed literature, there is a dearth of studies that have

Address for correspondence:

Dr. F Javed,
Division of General Dentistry, Eastman Institute for Oral Health,
University of Rochester, Rochester, NY 14642, USA.
E-mail: Fawad_Javed@urmc.rochester.edu

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compared self-perceived oral health among *gutka*-chewers and controls.

Questionnaires are an efficient approach to evaluate self-perceived oral health;^[9-11] however, they are less reliable about specific periodontal variables.^[12] Moreover, it has also been reported that self-reported periodontal health is unreliable particularly under situations when respondents with periodontal disease (PD) are unaware of their oral condition.^[13] Furthermore, the manner in which questions are administered to participants and education of respondents may also influence the outcome scores derived.^[14] Although clinical assessment is usually performed to verify data obtained from questionnaires,^[15] laboratory based investigations may also be used to verify self-reported oral health.^[11]

Unstimulated whole saliva (UWS) is a complex oral fluid that can be easily collected for investigative purposes using noninvasive techniques. Studies^[16-18] have shown that various types of inflammatory biomarkers associated with oral and systemic disorders exist in UWS, which make it a potential analytic fluid for monitoring oral inflammatory conditions, including PD. Several studies have been performed to determine the immunoglobulin (Ig) levels in UWS.^[9,19-21] It has been proposed that whole salivary IgG is an important biomarker of periodontal inflammation.^[9,22]

Since clinical periodontal status is worse in *gutka*-chewers as compared to controls,^[7,8] it is hypothesized that (a) self-perceived oral health is poorer among *gutka*-chewers as compared to controls; and (b) whole salivary IgG levels are significantly higher among *gutka*-chewers than controls. The aim of this study was to assess self-perceived oral health and whole salivary Ig levels among habitual *gutka*-chewers and controls.

Materials and Methods

Ethical approval

The study was approved by the research ethics review committee at the Dental Department of Jinah Hospital, Karachi, Pakistan. Participation was voluntary, and written informed consent was obtained from all individuals prior to their inclusion in this study.

Inclusion and exclusion criteria

Gutka-chewers were defined as individuals who had been chewing at least one *gutka* sachet for at least 1-year. Nonchewers (controls) were defined as individuals who reported to have never used any form of tobacco products. Exclusion criteria comprised the following: Tobacco smoking, alcohol consumption, systemic diseases such as diabetes, renal disorders, cardiovascular disorders and acquired immune deficiency syndrome, edentulism,

overlapping teeth and use of antibiotics/steroids within the past 6 months.

Study participants

Participants were residents of a local suburban area of Karachi, Pakistan. Individuals who fulfilled our eligibility criteria were invited to the Dental Department, Jinah Hospital, Karachi, Pakistan for questionnaire administration and collection of UWS samples.

Interview questionnaire

Information regarding age, gender, education status, socioeconomic status (SES), *gutka* usage (yes/no), duration of *gutka* chewing habit (in years), daily frequency of *gutka* intake, duration of *gutka* placement in the buccal vestibule and self-perceived oral symptoms (pain in teeth, pain on chewing, bleeding gums [BG], bad breath and loose teeth) was recorded using a questionnaire. Data regarding daily tooth brushing (once, twice, three times or more) was also recorded. The questionnaire was administered to all participants by a trained and calibrated interviewer (FV) ($\kappa = 0.80$).

Collection of unstimulated whole saliva samples and measurement of unstimulated whole salivary flow rate

Unstimulated whole saliva samples were collected by one investigator (FV) by a method as described elsewhere.^[23] In summary, for UWS collection, all participants ($n = 100$) were seated on a chair and requested to spit (without swallowing) for 5 continuous minutes into a gauged measuring cylinder. Unstimulated whole salivary flow rate was measured and recorded in milliliters per minute (mL/min). Immediately after collection, UWS samples were placed on ice and aliquoted before being frozen at -70°C . UWS samples were analyzed within 6 months of collection.

Assessment of whole salivary immunoglobulin G levels

Levels of IgG in UWS were assessed by enzyme-linked immunosorbent assay using a fivefold dilution pattern in duplicate wells. In summary, 96-well microtiter plates (Corning Inc., NY, USA) were coated with 100 μL /well of anti-human IgG (DAKO A/S, Denmark) in coating buffer (0.05 M carbonate-bicarbonate buffer, pH 9.6) and incubated at room temperature for 24 h. After washing, 100 μL /well of appropriately diluted IgG standard, positive control (saliva from an individual from the control group), negative control (distilled water) and saliva samples were added to the respective microplate wells. After incubation at room temperature, the microplates (Corning Inc., NY, USA) were washed to remove unbound proteins. Purified alkaline phosphatase conjugated anti-human IgG (DAKO A/S, Denmark) were added (100 μL /well), and the microplates were incubated for 3 h at room temperature. After washing, 100 μL /well of substrate (*p*-nitrophenyl phosphate) in 1.0 M diethanolamine, 0.5 mM MgCl_2 , pH 9.8, was added. The absorbance was read at 405 nm in a microtiter plate photometer (Molecular devices, Vmax, Sunnyvale, CA, USA).

Statistical analysis

Statistical analysis was performed using a statistical software (SPSS, Version 18, IL., USA). For self-perceived oral symptoms among *gutka*-chewers and controls, odds ratios (OR) with 95% confidence intervals were calculated. Whole salivary IgG levels were statistically assessed among *gutka*-chewers and controls using one-way analysis of variance. For multiple comparisons, Bonferroni *post-hoc* test was used. $P < 0.05$ were considered statistically significant.

Results

Characteristics of the study population

One hundred individuals (50 *gutka*-chewers and 50 controls) volunteered to participate in the present study. Among *gutka*-chewers and controls, numbers of male participants were 44 and 40, respectively. Mean ages of *gutka*-chewers and controls were comparable. Thirty-two percent *gutka*-chewers and 72% controls reported to have attained graduate level education. SES was significantly higher among controls as compared to *gutka*-chewers ($P < 0.01$). *Gutka*-chewers reported to have been consuming 6.3 ± 3.1 *gutka* sachets daily since 9.1 ± 4.5 years. *Gutka*-chewers reported to have been placing *gutka* in the buccal vestibule for 20.5 ± 10.2 min. Sixty-six percent *gutka*-chewers and 70% controls reported to brush their teeth once daily [Table 1].

Self-perceived oral health among *gutka*-chewers and controls

Self-perceived BG was more often reported by *gutka*-chewers than controls (OR 3.27 [range 2.3–3.7]) ($P < 0.05$). There was no statistically significant difference in self-perceived pain in teeth, pain on chewing, bad breath and loose teeth among *gutka*-chewers and controls [Table 2].

Unstimulated whole salivary flow rate

Unstimulated whole salivary flow rate was comparable among *gutka*-chewers (0.55 ± 0.2 mL/min) and controls (0.54 ± 0.1 mL/min).

Whole salivary immunoglobulin G levels among *gutka*-chewers and controls

In general, whole salivary IgG levels were significantly higher among *gutka*-chewers (62.3 ± 5.5 mg/L) as compared to controls (16.3 ± 2.1 mg/L) ($P < 0.01$) [Figure 1].

Whole salivary IgG levels were significantly among *gutka*-chewers with self-perceived oral symptoms (pain in teeth, pain on chewing, BG, bad breath and loose teeth) as compared to controls with and without self-perceived oral symptoms ($P < 0.05$). Whole salivary IgG levels were significantly among *gutka*-chewers without self-perceived oral

symptoms (as stated above) as compared to controls with and without self-perceived oral symptoms ($P < 0.05$) [Table 3]. Whole salivary IgG levels were comparable among *gutka*-chewers with and without self-perceived oral symptoms.

Among controls, whole salivary IgG levels were significantly higher among individuals who perceived to exhibit BG as compared to those who did not ($P < 0.05$). There was no significant difference in whole salivary IgG levels among controls with and without self-perceived pain in teeth, pain on chewing, bad breath and loose teeth [Table 3].

Table 1: Characteristics of the study population

	<i>Gutka</i> chewers	Non- chewers
Number of individuals (n)	50	50
Gender (male : female)	44:6	40:10
Age in years (mean \pm SD)	46.5 \pm 4.4	43.4 \pm 2.6
Graduate Education status (%)	32%	72%
Socioeconomic status (monthly income in USD, mean \pm SD)	190.5 \pm 34.2*	475.7 \pm 55.7
Daily frequency of <i>gutka</i> consumption (mean \pm SD)	6.3 \pm 3.1	-
Duration of chewing habit in years (mean \pm SD)	9.1 \pm 4.5	-
Duration of placing <i>gutka</i> in the buccal vestibule in minutes (mean \pm SD)	20.5 \pm 10.2	-
Daily tooth brushing protocol (%)		
Once daily	66	70
Twice daily	34	30
Three times or more daily	-	-

SD=Standard deviation; USD=United States Dollars. *Compared to non-chewers ($P < 0.05$). Data regarding monthly income was collected in Pakistani rupees and converted to United States dollars for international readers. One United States Dollar was equivalent to 102.37 Pakistani rupees)

Table 2: Group comparisons for self-perceived oral symptoms among *gutka*-chewers and non-chewers

Self-perceived oral symptoms (n=50)	Yes	No	Odds ratio (95% confidence intervals)	P value
Bleeding gums				
Gutka-chewers	28	22	3.27 (2.3-3.7)	<0.05
Non-chewers	14	36		
Pain in teeth			0.57 (0.2-0.7)	>0.05
Gutka-chewers	3	47		
Non-chewers	5	45		
Pain on chewing			0.78 (0.3-0.8)	>0.05
Gutka-chewers	4	46		
Non-chewers	5	45		
Bad breath			0.3 (0.1-0.7)	>0.05
Gutka-chewers	4	46		
Non-chewers	11	39		
Loose teeth			0.73 (0.4-0.8)	>0.05
Gutka-chewers	3	47		
Non-chewers	4	46		

Whole salivary immunoglobulin G levels among gutka-chewers and controls after stratification for brushing habits

Whole salivary IgG levels related to self-perceived oral symptoms were significantly higher among gutka-chewers

Table 3: Whole salivary IgG levels (in mg/L) among gutka-chewers and controls with and without self-perceived oral symptoms

Self-perceived oral symptoms	Whole salivary IgG levels (in mg/dl)			
	Gutka-chewers		Gutka-chewers	
	Yes	No	Yes	No
Pain in teeth	68.6±6.5**†	50.1±12.1§§	10.4±1.5	9.1±2.3
Pain on chewing	64.5±5.5**†	48.2±9.5§§	12.5±2.4	10.9±2.1
Bleeding gums	65.1±13.3**†	49.8±11.5§§	23.6±6.1 [†]	10.6±1.4
Bad breath	65.5±3.2**†	50.8±8.1§§	12.8±0.4	10.7±1.4
Loose teeth	64.5±7.1**†	51.2±11.4§§	11.1±2.6	12.4±1.1

*Compared to controls with self-perceived oral symptoms (P<0.05), †Compared to controls without self-perceived oral symptoms (P<0.05), ‡Compared to controls with self-perceived oral symptoms (P<0.01), §Compared to controls without self-perceived oral symptoms (P<0.05), †Compared to controls without self-perceived oral symptoms (P<0.001)

Table 4: Whole salivary IgG levels (in mg/L) among gutka-chewers and controls after stratification for brushing habits

Self-perceived oral symptoms	Whole salivary IgG levels (in mg/dl)			
	Gutka-chewers		Controls	
	Once daily	Twice daily	Once daily	Twice daily
Pain in teeth	51.5±6.5**†	47.2±7.1§§	13.5±5.1	10.5±2.6
Pain on chewing	54.2±3.5**†	43.1±5.1§§	12.4±1.7	11.6±3.6
Bleeding gums	58.5±8.1**†	50.2±4.8§§	17.8±2.5 [†]	8.4±1.5
Bad breath	50.6±10.2**†	47.1±9.9§§	13.3±2.1	9.6±3.3
Loose teeth	53.4±6.4**†	50.2±8.2§§	14.2±3.1	10.7±1.8

*Compared to controls brushing their teeth once daily (P<0.01), †Compared to controls brushing their teeth twice daily (P<0.01), ‡Compared to controls brushing their teeth once daily (P<0.01), §Compared to controls brushing their teeth twice daily (P<0.01), †Compared to controls brushing their teeth twice daily (P<0.05)

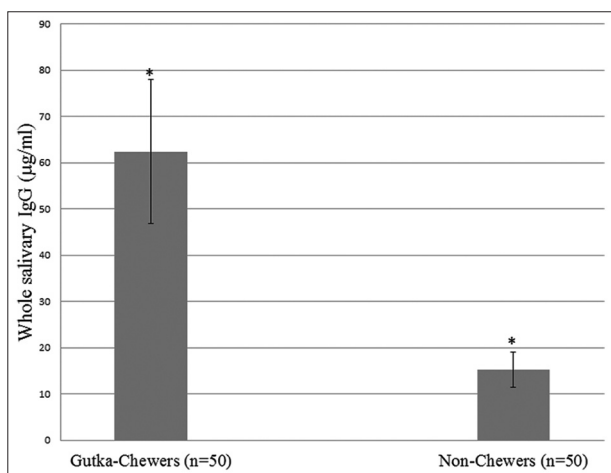


Figure 1: Whole salivary immunoglobulin G levels among gutka-chewers and controls *P < 0.01

who brushed once daily as compared to controls who brushed their teeth once a day (P < 0.01). Whole salivary IgG levels were significantly higher among gutka-chewers who reported to brush twice daily as compared to controls, who brushed their teeth once a day (P < 0.01). Whole salivary IgG levels were comparable among gutka-chewers who brushed their teeth once and twice daily [Table 4].

Among controls, whole salivary IgG levels related to self-perceived BG were significantly higher among controls who brushed their teeth once daily compared to controls who brushed twice a day (P < 0.05). There was no significant difference in whole salivary IgG levels among controls with and without self-perceived pain in teeth, pain on chewing, bad breath and loose teeth [Table 4].

Discussion

This study was based on the hypotheses that self-perceived oral health is worse and whole salivary IgG levels are higher among habitual gutka-chewers as compared to controls. The results showed that self-perceived BG were more often reported by gutka-chewers as compared to controls. These results are in accordance with previous clinical studies^[1,7] in which, scores of bleeding on probing were significantly higher among gutka-chewers than controls. An explanation for this may be derived from the study by Mavropoulos et al.^[24] in which, the effect of ST on gingival blood flow (GBF) was assessed using laser-Doppler flowmetry. The results showed that application of ST increases GBF in and around exposed areas.^[24] However the present results showed that other oral symptoms (pain in teeth, pain on chewing, bad breath and loose teeth) were comparable among gutka-chewers and controls. This could be associated with age of participants, duration of gutka chewing habit and daily gutka consumption. In this study, gutka-chewers and controls were ~ 45 years old and were daily consuming ~ 6 gutka sachets since nearly a decade. It may be argued that the periodontal damage induced by habitual gutka-consumption was not intense enough to elicit symptoms such as pain in teeth, pain on chewing and loose teeth. It is also pertinent to mention that gutka contains artificial fragrances (such as menthol) as integral components.^[7] This could have biased the results among gutka-chewers and controls in terms of perception of bad breath. It is speculated that excessive daily gutka consumption and its placement in the buccal vestibule over durations longer than those reported in this study may elicit other oral symptoms such as pain in teeth, pain on chewing and loose teeth.

Most IgG in UWS is derived from blood circulation and reaches the oral cavity by passively leaking mainly via gingival crevicular epithelium;^[25] however, some IgG production may also occur locally in the gingiva or salivary glands.^[25] The normal concentration of IgG in UWS is low (~20 mg/L).^[21,25] Raised levels of IgG in UWS have been reported in patients with oral inflammatory (predominantly

PD).^[11,19] It has been reported that significantly higher volume of gingival crevicular fluid (GCF) is produced in patients PD than those without periodontal inflammation.^[26] Therefore, it is likely that among controls who perceived to have BG had significantly higher GCF leakage into the oral cavity, which in turn could have led to the expression of raised IgG levels in UWS as compared to those who did not have self-reported BG.

An interesting finding in the present study was that there was no significant difference in whole salivary IgG levels among *gutka*-chewers with and without self-perceived oral symptoms. This could possibly be associated with the socioeconomic and education status of these individuals. It has been reported that self-rated oral health status is dependent on various factors including life style and education status.^[27,28] In the present study, SES and education status were significantly higher among controls as compared to *gutka*-chewers [Table 1]. It is therefore speculated that because of a poorer education status, *gutka*-chewers misrecognized their oral symptoms and hence misevaluated their self-perceived oral health status. The fact that whole salivary IgG levels were comparable among *gutka*-chewers with and without self-perceived oral symptoms and were significantly higher than controls ($P < 0.05$) reflects that *gutka*-chewers were more susceptible to PDs than controls (regardless of their perception of oral health). It is therefore emphasized that laboratory-based investigations can be used to verify self-perceived oral health particularly in populations with underprivileged living condition and poor education status. The authors applaud the study by Sandholm *et al.*^[22] in which, whole salivary IgG was considered as a parameter of PD activity.

Studies have reported that oral hygiene maintenance protocols (such as regular tooth brushing and nonsurgical periodontal therapy) reduce periodontal inflammation and GCF volume.^[26,29,30] The authors support these results since whole salivary IgG levels were significantly higher among controls who brushed teeth once daily when compared to those who brushed twice a day. Interestingly, there was no significant difference in whole salivary IgG levels among *gutka*-chewers who either brushed once or twice daily. It is likely that the underprivileged SES and poor education had compelled *gutka*-chewers to neglect oral hygiene maintenance and primarily focus on the attainment of basic standards of living. Moreover, education status was also compromised among *gutka*-chewers than controls [Table 1]; it is therefore likely that information regarding self-reported tooth brushing reported by these individuals was blemished. It is recommended that educational programs about health issues should routinely be conducted particularly in vulnerable populations (such as among patients with deprived living conditions and poor education) in order to educate targeted populations about the importance of

oral hygiene maintenance and deleterious effects of ST products on health.

A limitation of the present study is that all individuals perceived to be systemically healthy. It has been reported that areca-nut (an integral component of *gutka*) is a significant risk factor for systemic conditions such as metabolic syndrome, renal disorders and cardiovascular diseases.^[31-33] Such systemic conditions are also associated with the etiology of PD. It is possible that there might have been a contribution of latent systemic disorders in aggravating PD activity and increased IgG expression in UWS in some *gutka*-chewers who might have been unaware of their poor systemic health (most likely due to their poor socioeconomic and education status). Another limitation of the present study is that mean ages of *gutka*-chewers and controls were comparable. In the present study, data was not adjusted with reference to age as there was no significant difference in mean ages among *gutka*-chewers and nonchewers. It is known that advancing age is a major risk factor of PD. It may therefore be hypothesized that self-perceived oral symptoms are worse and whole salivary IgG levels are higher in older *gutka*-chewers (for e.g., those >60 years) as compared to relatively young *gutka*-chewers (for e.g. <30 years old). Further studies are needed to test this hypothesis.

Conclusion

Self-perceived gingival bleeding scores and whole salivary IgG levels are higher among *gutka*-chewers than controls.

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