Estimation and Comparison of Salivary Immunoglobulin A levels in Tobacco Chewers, Tobacco Smokers and Normal Subjects

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Abstract

Aims: To estimate the salivary immunoglobulin A (IgA) levels in tobacco chewers, tobacco smokers and normal subjects and to compare the salivary IgA levels among tobacco chewers and tobacco smokers.

Methods: The study group consisted of 80 subjects (tobacco users), 40 tobacco chewers and 40 tobacco smokers. Unstimulated whole saliva was collected from all tobacco users and 40 healthy age- and gender-matched non-tobacco users as control group. The study and control groups were divided into four subgroups based on age range. Salivary IgA levels were estimated by single radial immunodiffusion assay (SRID). All data were analysed using statistical software and to compare the results in three groups, single-factor analysis of variance was applied.

Results: The mean salivary IgA level in control group was 16.76 ± 1.37 mg/dl (SD); in tobacco chewers it was 7.89 ± 0.61 mg/dl (SD) and in tobacco smokers it was 6.55 ± 0.99 mg/dl (SD). The salivary IgA levels were decreased in tobacco chewers and tobacco smokers compared with the controls. Among the tobacco users, tobacco smokers had much reduced salivary IgA levels compared to tobacco chewers. All of these results were highly significant (P<0.001).

Conclusions: The present study showed that tobacco chewers and tobacco smokers had decreased salivary IgA levels and among tobacco users, tobacco smokers had much reduced salivary IgA levels compared to tobacco chewers in unstimulated whole saliva.

Key Words: Salivary IgA, Tobacco Chewers, Tobacco Smokers, Controls, Tobacco Users

Introduction

The effects of tobacco on the oral tissues have been an area of interest to researchers for a long time [1]. Tobacco is addictive, and its use is harmful to health in many ways [2]. Lack of awareness of the effects of tobacco use and the difficulty to discontinue the habit (psychology and nicotine dependence of an individual) has led to the increased incidence of tobacco use [3]. Tobacco habit encountered around the world is mainly in the form of tobacco smoking, tobacco chewing and tobacco snuff use but in India, tobacco is used in the form of bidis (34%), cigarettes (30%), chewing tobacco (19%), hookah (9%), cigars and cheroots (5%), and snuff (2%) [4]. Tobacco was responsible for an estimated three million annual deaths in the world during early 1990s and with the current consumption trends it is expected to rise to ten million annual deaths during the 2020s. About 70% of these deaths are expected to occur in developing countries [5,6]. A potentially dangerous association exists between tobacco smoking and health. Smoking is a complex external and internal stimulus consisting of visual, tactile, mechanical (mouth movement), olfactory, gustatory, and irritational factors [7]. Tobacco smoke contains a major class of organic chemical compounds that includes chemical asphyxiants, irritants, ciliastatic compounds, carcinogens and co-carcinogens [5]. Its use is known to be associated with cancer of the lungs, larynx, oesophagus and lips, chronic bronchitis, emphysema, coronary artery disease (arteriosclero-

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sis), peripheral vascular disease, and peptic ulceration [8].

As a substitute for a smoking habit, because of its effect on the soft tissues of the oral cavity and respiratory tract, the use of chewing tobacco (smokeless tobacco) has become increasingly popular [9]. The prevalence of tobacco chewing is higher in India compared to other parts of the world. Both tobacco smoking and tobacco chewing have noticeable effects on the ecology of mouth [8,10]. Smoking produces bad breath, gum disease, hoarse voice, cough and stained teeth. In addition to these problems, smoking has been associated with acute necrotising ulcerative gingivitis (ANUG) and an increased risk of neoplasms [8]. Smokeless tobacco can stain the teeth, cause bad breath, tooth erosion, dental caries, tooth loss, a decreased sense of taste, alveolar bone destruction and gingival recession [11].

Antibodies found in the oral cavity comprise three major classes i.e., immunoglobulin (Ig) A, IgG and IgM. In normal circumstances IgA and IgG are found in saliva in concentrations of 19.4 5.37 mg/dl and 1.44 0.9 mg/dl, respectively [12]. Salivary IgA, the predominant immunoglobulin is the characteristic humoral factor of the local immune system of the oral cavity [13]. It is produced by plasma cells locally in salivary glands and can transverse mucous membrane [12,14,15].

It has been demonstrated that mucosal immunity is depressed among tobacco users (tobacco chewers and tobacco smokers). The local influence of tobacco smoking and chewing can alter the levels of IgA in saliva. Various studies have been carried out to estimate salivary IgA levels in smokers [15-21], those with periodontal diseases [22,23], those with recurrent aphthous ulcers [15,16,24], those who have stopped smoking [14,25], and in dental caries [13,26,27]. On the other hand, very few studies have been carried out to estimate salivary IgA levels in smokeless tobacco users [28]. Furthermore, to our knowledge, there are no studies to compare salivary IgA levels among tobacco chewers and tobacco smokers. This study was first of its kind to be carried out among tobacco chewers and tobacco smokers in Indian subjects.

Aims

To estimate the salivary IgA levels in tobacco chewers, tobacco smokers and normal subjects and to compare the salivary IgA levels among tobacco chewers and tobacco smokers.

Methods

A comparative descriptive cross-sectional study was conducted in the Department of Oral Medicine and Radiology, PMNM Dental College, Hospital and Research Centre Bagalkot, North Karnataka, India. Ethical clearance to undertake the study was obtained from the Institutional Ethical Committee. After a complete and detailed explanation about the nature of study and its objective, written consent was obtained from all subjects recruited for the study. The study population was selected from consecutive patients between the period as and when they presented and as long as they satisfied the inclusion criteria.

A power analysis was performed which gave a total of 90 cases (30 per group). In order to increase the power, 120 cases were selected for the study, with 90% power at a two-tailed alpha of 0.05 to detect on an average of 01 mg/dl of salivary IgA between the groups assuming a common within-group standard deviation of 0.6 to 1.4.

The study group consisted of 80 subjects (tobacco users), 40 tobacco chewers (not having a tobacco smoking habit) and 40 tobacco smokers (not having a tobacco chewing habit). In the study, tobacco chewers were defined as users of tobacco chewing in their various forms and tobacco smoking was defined as use of cigarettes or bidis (a small quantity of shredded, sun-cured tobacco which is hand rolled into a piece of dried tendu or temburni leaf) or a combination of both. The demographic data were entered into a pro forma after seating the subject on a well-illuminated dental chair. Duration, frequency and type of tobacco use habit were recorded. Only male subjects above 20 years with the habit of either tobacco chewing or tobacco smoking for a minimum of five years were included in the study. Subjects who had the habit of tobacco chewing and tobacco smoking together, overt salivary gland dysfunction and salivary flow rate of less than 2 ml/10 minutes were excluded from the study.

The control group consisted of 40 age-matched healthy male subjects above 20 years, who had either a tobacco-chewing or a tobacco-smoking habit. The study was carried out over six-month period. The data and saliva sample collection were carried out by the first author (BRD) with the assistance of the second author (SP).

Saliva collection procedure

Unstimulated whole saliva samples were collected

as previously described [24,29,30]. All subjects were advised to gargle their mouth with water before collection of saliva and had not been eating, drinking or smoking for at least one hour [20,26,29,31]. This was to avoid the contamination of saliva sample with local factors such as food debris, tobacco and other particles. The saliva sample was collected at one occasion under resting conditions in the outpatient department during the morning hours, between 9 am and 12 noon, to minimise the effects of circadian rhythms [32,33]. Subjects were asked to collect saliva in their mouths for ten minutes and then spit into a sterile wide mouthed calibrated cup as previously described [22,24,29,32]. The concentration of salivary IgA is influenced by stimulation and minor salivary glands are also a major source of salivary IgA [24,34-36], so to eliminate this variability we preferred to work on unstimulated whole saliva. The samples were transferred to wide mouth sterile containers, which were capped and stored at -20°C until used for the assay.

Estimation of salivary IgA

Prior to assay, saliva samples were thawed, then centrifuged at 3500 rpm for ten minutes and supernatant fluid was used for estimation of salivary IgA levels by a single radial immunodiffusion (SRID) technique [37]. In this assay the saliva samples were applied directly to the immunodiffusion agarose gels containing antibodies against human IgA raised in rabbits. A Tripartigen ruler was used to measure the diameter of the precipitation rings. Quantitation of salivary IgA was performed by comparing the square of the diameter of the test rings with that of World Health Organization standards, which are internationally accepted. Antiserum standard IgA used in this study was obtained commercially (Lupin Laboratories Ltd, Mumbai, India) and a protein reference set was obtained from Orion Diagnostica, Espoo, Finland.

Statistical analysis

All the collected data were tabulated and subjected to statistical analysis. The data were analysed using statistical software (Statistical Package for the Social Sciences Version 13.0; SPSS Inc, Chicago, IL, USA). Descriptive statistics, including mean, standard deviation and minimum and maximum value, were calculated for the control and each of the study groups. To compare the results in the three groups, single-factor analysis of variance (ANOVA) was applied. An unpaired Student's *t*-test was used to determine whether significant difference was present in the results between the two groups. The significance for all statistical tests was predetermined at $P<0.05$ using Fisher's exact probability test.

Results

Age range of tobacco chewers, tobacco smokers and controls

The age of 40 tobacco chewers who participated in the study ranged from 25 to 70 years. Their age range was divided into four groups. There were six subjects in the 25-34 year group, 20 subjects in the 35-44 year group, nine subjects in the 45-54 year group, and five subjects in the 55-70 year group, with a mean age of 41.4 ± 9.6 years (SD). The age of 40 tobacco smokers who participated in the study ranged from 35 to 70 years. There were zero subjects in the 25-34 year group, 14 subjects in the 35-44 year group, 15 subjects in the 45-54 year group and 11 subjects in the 55-70 year group, with a mean age of 48.2 ± 8.7 years (SD). The age of 40 controls who participated in the study ranged from 25 to 70 years. There were eight subjects in the 25-34 year group, 11 subjects in the 35-44 year group, ten subjects in the 45-54 year group, and 11 subjects in the 55-70 year group, with a mean age of 46.2 ± 11.6 years (SD) (*Table 1*).

Table 1. *Age range of tobacco chewers, tobacco smokers and controls*

Age (years)	Tobacco smokers (n=40)	Tobacco chewers (n=40)	Control group (n=40)
25-34	0	6	8
35-44	14	20	11
45-54	15	9	10
55-70	11	5	11
Mean ± SD	48.2 ± 8.7	41.4 ± 9.6	46.2 ± 1.6
Age range	35-70	25-70	25-70

Distribution of salivary IgA levels in tobacco chewers, tobacco smokers and controls

In tobacco chewers, salivary IgA levels ranged between 6.41-9.70 mg/dl with a mean of 7.89 ± 0.61 mg/dl (SD). In tobacco smokers, salivary IgA levels ranged between 4.00-8.14 mg/dl with a mean of 6.55 ± 0.99 mg/dl (SD). In the controls, salivary

IgA levels ranged between 14.10-19.01 mg/dl with a mean of 16.76 ± 1.37 mg/dl (SD) (*Table 2*).

Salivary IgA levels between tobacco users and controls

The mean salivary IgA level among tobacco smokers was less, 6.6 mg/dl ± 1.0 (SD), when compared to tobacco chewers, 7.9 mg/dl ± 0.6 (SD) and controls, 16.8 mg/dl ± 1.4 (SD) (*Figure 1*).

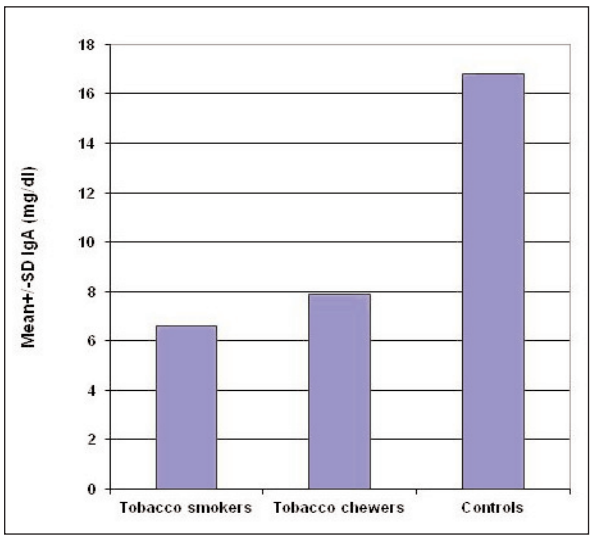

Figure 1. *Salivary IgA levels between tobacco users and controls.*

Comparison of S-IgA levels between tobacco chewers, tobacco smokers and controls using Student's *t*-test and single-factor ANOVA

Table 3 shows the comparison of salivary IgA levels between (a) tobacco chewers and controls, (b) tobacco smokers and controls, and (c) tobacco chewers and tobacco smokers. The mean difference of salivary IgA between tobacco chewers and controls was 8.87 mg/dl, between tobacco smokers and controls it was 10.21 mg/dl, and between tobacco chewers and tobacco smokers it was 1.34 mg/dl, with a *t*-value of 37.4, 38.1, and 7.2, respectively, which is highly significant at *P*<0.001.

Discussion

This study revealed that the salivary IgA levels of tobacco chewers and tobacco smokers were significantly different from those of controls (*P*<0.001).

In the present study the concentration of salivary IgA in unstimulated whole saliva revealed a decrease of 53% in tobacco chewers when compared to controls, which was statistically a highly significant difference noted between them (tobacco chewers and controls). This is consistent with a previously reported study [38]. In disagreement with results of our study, a previous study, which was performed over 20 years ago and which investigated the effect of smokeless tobacco use in humans on mucosal immune factors, reported a significantly higher salivary IgA concentration of total whole saliva in smokeless tobacco users, suggesting that smokeless tobacco has a greater effect on the secretory epithelial cells in minor, submandibular and sublingual glands responsible for producing and packaging secretory component on to IgA J-chain complexes synthesised in plasma cells [28]. However, the decrease in salivary IgA levels in this study can be explained on the basis of differences in sample size, age range and geographical variation. The salivary IgA levels of control subjects were within normal range in the present study, which is similar to those from a previous study [12].

Table 2. *Descriptive statistics of salivary IgA levels in tobacco chewers, tobacco smokers and controls*

Study population	Salivary IgA levels (mg/dl)		
	Minimum-Maximum	Mean	SD
Tobacco chewers (n=40)	6.41-9.70	7.89	0.61
Tobacco smokers (n=40)	4.00-8.14	6.55	0.99
Control group (n=40)	14.10-19.01	16.76	1.37

Table 3. *Comparison of S-IgA levels between tobacco chewers, tobacco smokers and controls using Student's t-test and one factor ANOVA*

Study group	S-IgA level (mg/dl)	Groups compared	Significance		
			Mean difference	*t*-value*	*P*-value
1: Tobacco chewers	7.89 ± 0.61	1-3	8.87	37.4	<0.001
2. Tobacco smokers	6.55 ± 0.99	2-3	10.21	38.1	<0.001
3. Controls	16.76 ± 1.37	1-2	1.34	7.2	<0.001

Single-factor ANOVA (F=359.2, *P*<0.001), *Student's test (*P*<0.001, highly significant)

In the current study, the difference in salivary IgA levels of tobacco smokers and controls was highly significant. The salivary IgA in unstimulated whole saliva of tobacco smokers was decreased by 61% when compared with the controls, a finding that agrees with many studies previously reported [16,18,19,21,25]. One of the earlier studies noted a decrease in salivary IgA concentration in chronic tobacco smokers (smoking more than 20 cigarettes a day for at least 40 years). Its authors suggested that this could be due to an immunosuppressive effect of the combustion products of tobacco and the possibility of the incidence of intra-oral neoplastic disease being increased in tobacco smokers by this effect [16]. Another study observed a striking and reproducible influence of cigarette smoking on salivary immunoglobulin in healthy smokers, found reduced concentration of IgA in pure parotid saliva, compared with non smokers, and showed that smokers had a dose dependent and probably reversible humoral mucosal immunodeficiency, as reflected either directly or otherwise by salivary IgA concentrations [21]. A few investigators have studied the effect of smoking on serum and salivary immunoglobulin of healthy individuals and found decreased salivary and serum IgA levels [18] and some have studied the effect of cigarette smoking on the immune system and found an increase in immunoglobulin levels (IgA and IgG) in saliva after cessation of smoking [19]. A few researchers have observed a significant decline in sIgA relative to pre-smoking cessation levels following one day of smoking cessation, and opined that this could be due to transient tobacco withdrawal effect, but levels returned to pre-abstinence values after one week. They stated that for a pre-smoking cessation measure, a longer time since the last cigarette was significantly related to lower salivary IgA levels; therefore to overcome this, the analysis was confined to those who had smoked within 0.5 to 1.5 hours of the pre-cessation measure [25].

Variation in the level of immunoglobulin may be due to smoking, having several toxic effects. So the dramatic reduction in salivary IgA levels in the present study may be due to chronic upper respiratory tract symptoms or the immunosuppressive effect of the combustion products of tobacco or it may be due to impairment of T-cell immuno-regulation of B-cell differentiation and maturation.

However, results of the present study are contradictory with those from some previous studies. Some investigators studied the effect of tobacco smoking on salivary immunoglobulin levels in immunodeficiency and observed that the increased concentration of salivary IgA may be due to protection of oral mucosa secondary to an immune response to tobacco antigens in the smoking population [20]. A previous study reported high mixed salivary IgA levels in a group of smokers, drinkers, and head and neck cancer patients [21]. But studies have reported that many smokers are heavy alcohol drinkers [17,39] and alcohol consumption increases the salivary IgA concentration [17,40], and is an independent risk factor for squamous cell cancer of the head and neck [17,41]. However, the present study did not assess alcohol consumption.

Some studies have found no statistical significant difference in salivary IgA levels between smokers and non-smokers [3,22,23]. Differences in the age, sample size and smoking history of the participants may account for these discrepancies.

In the present study, the concentration of salivary IgA in unstimulated whole saliva revealed a decrease of 20% in tobacco smokers when compared to tobacco chewers, which is highly significant statistically.

This difference in the results can be explained in the following ways. First, this decrease in salivary IgA in tobacco smokers can be explained on the basis of immunosuppressive effects of combustion products of tobacco [16]. Secondly, smoking markedly increases the flow rate of saliva leading to increased calcium levels in the oral cavity during smoking [10]. However, an earlier study reported a level of salivary IgA reduced by 56% after physiological stimulation and its authors opined that these findings indicated that a considerable portion of salivary IgA is produced locally, depending on selective transport and the release from the local storage sites [35]. Another study found that there was a decrease in the concentration of salivary IgA in parotid and whole saliva after stimulation [24]. On the other hand, another previous study reported a significantly lower salivary flow rate in smokeless tobacco users and opined that it could likely be due to a negative effect of nicotine on exocrine glands, which consists of an initial stimulation followed by an inhibition of saliva flow. They concluded that a lower salivary flow rate was compensated for by a significantly higher concentration of total whole salivary IgA that could be ascribed to the IgA_2 subclass [28]. Considering these studies, it could be hypothesised that the decrease in salivary IgA in smokers is not solely due to the tobacco

itself but may be partially contributed by its stimulant action i.e., increased flow rate of saliva.

There are no studies available in the literature about the comparison of salivary IgA levels between tobacco smokers and tobacco chewers. No research is ever quite complete and innovative studies can provide new insights, which encourage new lines of investigation. Thus, the field remains open for future investigation with a larger sample size among tobacco chewers and tobacco smokers.

Conclusions

In the group studied, salivary IgA levels were lower in tobacco users than in non-tobacco users. Of the tobacco users, tobacco smokers had more reduced salivary IgA levels than tobacco chewers. This finding suggests that smoking has a greater effect on the mucosal immune system than smokeless tobacco.

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Contributions of each author

- BD designed the study and carried out data collection.
- SP assisted in data collection and coordinated all aspects of this study.
- BVP compiled the clinical data and carried out the laboratory analysis.
- HK carried out editing of the manuscript.
- KG coordinated all aspects of this study, carried out the laboratory analysis and assisted in proof-reading.

Statement of conflict of interest

As far as the authors are aware there was no conflict of interests.

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