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Research Article

Evaluation of Salivary Immunoglobulin A Levels in Tobacco Smokers, Tobacco Chewers and Healthy Individuals – A Comparative Study

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ABSTRACT

Aim: To evaluate the salivary immunoglobulin A (IgA) levels in tobacco smokers, tobacco chewers and normal subjects and to compare the salivary IgA levels among all of them.

Methods: The study cluster consisted of 90 subjects who were divided into 3 groups as 30 tobacco chewers, 30 tobacco smoker and 30 normal individuals. Saliva was collected by unstimulated spitting method. Based on age range study and control groups were divided into four subgroups. Salivary IgA levels were estimated by Sandwich ELISA technique. All data were analysed using statistical software SPSS version-17 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 104.33 ± 12.16 µg/ml (SD), in tobacco chewers it was 77.59 ± 9.39 µg/ml (SD) and in tobacco smokers it was 58.86 ± 9.56 µg/ml (SD). Among tobacco chewers and tobacco smokers Salivary IgA levels were decreased compared with the controls. Among the tobacco users, tobacco smokers had a lot of reduced secretion immune serum globulin levels compared to tobacco chewers. Results of study was highly significant at $P<0.001$.

Conclusions: salivary IgA levels among tobacco chewers and tobacco smokers had decreased in the present study. This makes subjects more susceptible to oral diseases. Thus, tobacco chewing and tobacco smoking is injurious to health.

Keywords: Salivary Iga, Tobacco Chewers, Tobacco Smokers, Controls, Tobacco Users.

INTRODUCTION

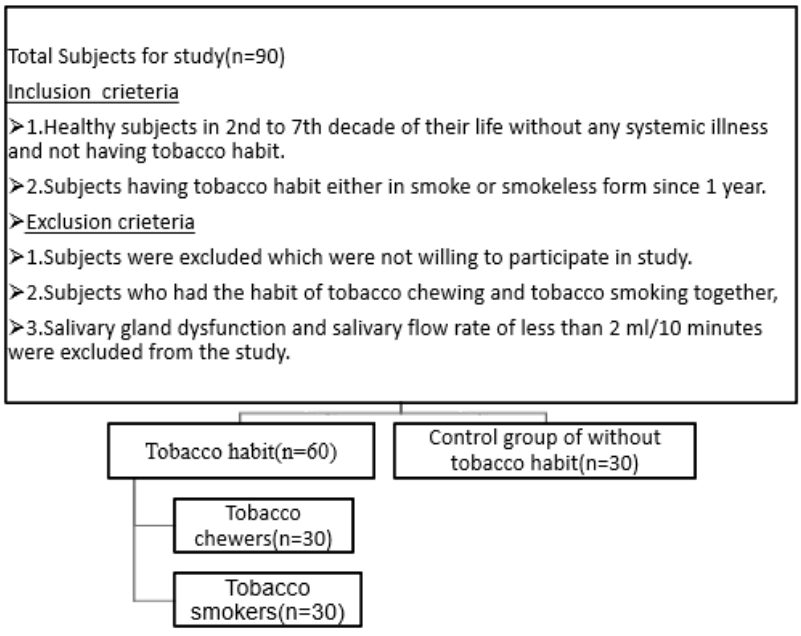
It has been demonstrated that mucosal immunity is depressed among tobacco smokers and chewers as tobacco impairs immune function which is carried out by immunoglobulin (1). Cotinine is a sensitive and specific quantitative indicator of the uptake of nicotine over the past few days(2).Tobacco smoking affects a wide range of immunological functions in human and experimental animals including both humoral and cell mediated immune responses.(3) Five classes of immunoglobulins synthesized by plasma cells and lymphocytes to some extent are have been recognized as IgG, IgA, IgM, IgD, and IgE (WHO 1964) out of them IgG, IgA and IgM are major and IgD and IgE are minor immunoglobulins(4).Salivary IgA the predominant immunoglobulin is the characteristic humoral factor of the local immune system of the oral cavity(5).The local influence of tobacco smoking and chewing can alter the levels of IgA in saliva(6).Secretory IgA (SIgA) constitutes the predominant immunoglobulin isotype in secretions, including saliva. It is considered to be the first line of defence of the host against pathogens, which colonize or invade surfaces bathed by external secretions (7). The single biggest contributor and most important preventable cause of death is Tobacco use. In addition, the risk of oral cancer and potentially malignant lesions is higher among smokers compared with those who have ever smoked and had profound effect on oral tissue (8). Cigarette smoke is a mixture of thousands of chemical agents, including nicotine, acrolein, and phenyl acetylene. It also contains free radicals which can cause cellular damage and alter the antioxidative potential of the saliva (9).

Understanding which tobacco products affect secretory immune system is important since it is a key defence line against mucous pathogens. IgA agglutinates and inhibits bacterial adhesion to mucous surfaces by bonding. Periodontal tissues destruction inhibited by the activity of some hydrolases produced by bacteria. There are contradictory results about the effect of tobacco use on salivary antioxidant system (10). The Aim of the study is to evaluate and compare the Salivary Immunoglobulin A (IgA) levels in tobacco chewers, tobacco smokers and normal subjects.

MATERIALS AND METHODS

The study was included 90 subjects from the routine OPD of Department of Oral Medicine and Radiology of our institute. The protocol with informed consent of study participants of the present cross-sectional case– control study was approved by the Institutional Ethics Committee. Out of 90 participants, 30 healthy individuals were assigned to group A. Group A cluster had no periodontal disease and never smoked. Thirty participants were allocated to group B. They had no participants periodontal disease, never used smokeless tobacco, but had 3 years history of smoking 10 cigarettes daily. The 30 participants in group C had no participants periodontal disease and had 1 year history of chewing about 10-g tobacco packet daily. Exclusion criteria for group participants having any systemic conditions or having a history of aphthous stomatitis or any other oral disease, being pregnant or breastfeeding, taking vitamin supplements during the previous 3 months, participants taking any medications for longer than 3 months and subjects who were not willing to participate in the study.

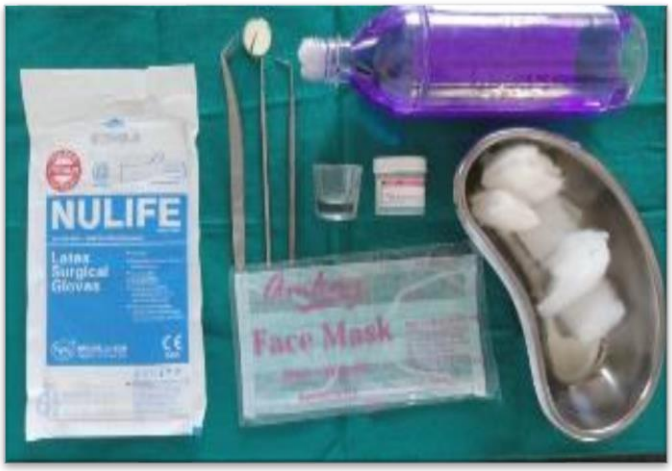
All three clusters were homogenous in terms of age and gender. Salivary samples were collected from participants in the morning. Before the sampling procedure participants rinsed their oral cavities with physiologic serum. Then, each subject's non-stimulatory saliva was collected in special containers and immediately centrifuged and transferred into freezer at -20°C.Estimation of salivary IgA Prior to assay, saliva samples were thawed, then centrifuged at 3500 rpm for fifteen minutes and supernatant fluid was used for estimation of salivary IgA levels by Sandwich ELISA by Photo analytic analyser by using salivary ELIZA Kits manufactured by DRG international Inc. USA. (11)



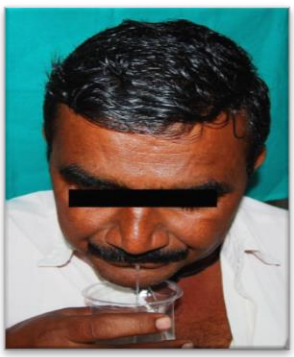
Statistical Analysis

Data was entered into Microsoft excel sheet and analysed using SPSS Inc. 21.0 software. Frequency, mean, standard deviation and percentages (descriptive statistics) were calculated. Single-factor analysis of variance (ANOVA) was applied to compare three groups and to determine whether significant difference was present in the results between the two groups, an unpaired Student's t-test was used. For all statistical tests P-value < 0.05 was considered statistically significant.

Armamentarium



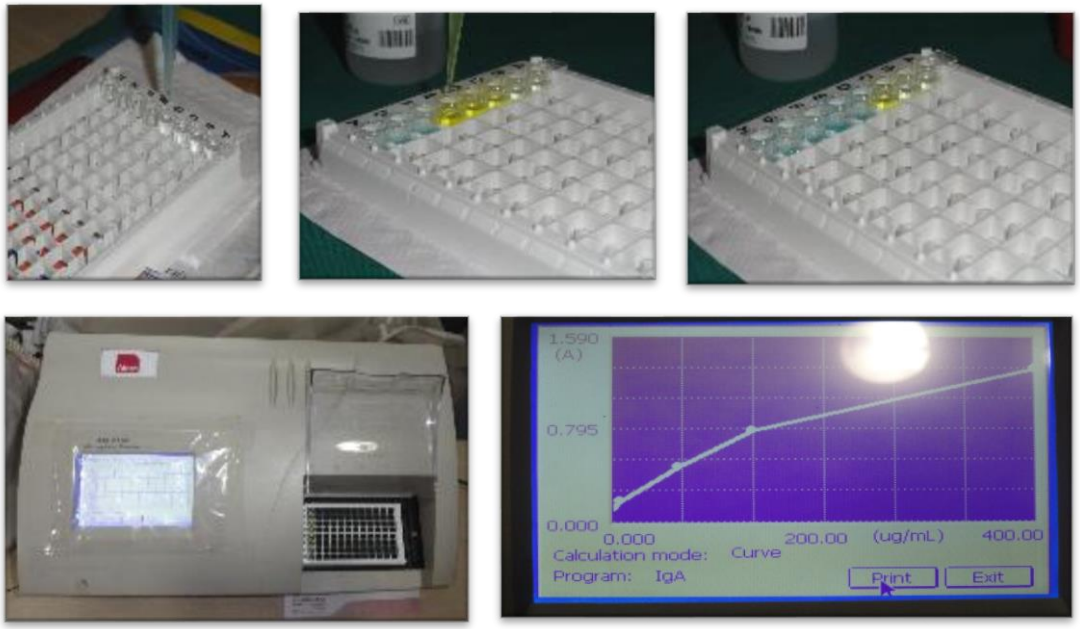
1. Armamentarium for Chair Side Investigation



A. Saliva Collection by Spitting Method **B.** Saliva Samples **C.** Centrifuge Machine

2. Sandwich ELISA Test for Salivary IgA

3. Sandwich ELISA By Photo Analytic Analyser



RESULTS

Table 1. Mean and S.D. Of Salivary Iga Levels in Age Range of Tobacco Chewers, Tobacco Smokers and Controls.

Age in years	Tobacco Chewers Salivary IgA levels mg/dl (Mean± S.D.)	Tobacco smokers Salivary IgA levels mg/dl (Mean± S.D.)	Control group Salivary IgA levels mg/dl (Mean± S.D.)
25-34	3 (70.08 ± 4.67)	4(55.51 ± 9.94)	6(91.95 ± 14.95)
35-44	11(81.67 ± 8.01)	14(60.71 ± 9.63)	8(104.72 ± 9.01)
45-54	11(76.53 ± 3.07)	8(59.13 ± 10.28)	8(109.35 ± 8.30)
55-70	5(75.44 ± 10.52)	4(55.17 ± 9.34)	8(108.22 ± 11.18)
Total	30 (77.59 ± 9.39)	30(58.86 ± 9.56)	30(104.33 ± 12.16)

Total 90 study participants who have been divided into three groups of tobacco chewers(n=30), tobacco smokers(n=30) and control group(n=30) and their age range was divided into four groups as depicted in the above table. Among tobacco chewers group; three subjects in the 25–34-year group showing lowest level of mean salivary IgA levels which is 70.08 ± 4.67. In the tobacco smokers' group, there were four subjects in the 25–34-year group, 14 subjects in the 35–44-year group, 8 subjects in the 45–54-year group and 4 subjects in the 55–70-year group, with highest mean salivary IgA levels 60.71± 9.63 among 35-44 years group. Mean Salivary IgA levels among control group is more compared to tobacco chewers and Tobacco smoker's group in all age groups.

Table 2. Descriptive Statistics of Salivary Iga Levels in Tobacco Chewers, Tobacco Smokers and Controls

	Salivary IgA levels (mg/dl)		
Study population	Minimum	Maximum	Mean ± SD
Tobacco chewers (n=30)	63.78	95.14	77.59 ± 9.39
Tobacco smokers (n=30)	41.13	82.27	58.86 ± 9.56
Control group (n=30)	67.82	127.32	104.33 ± 12.16

In tobacco chewers, salivary IgA levels ranged between 63.78 – 95.14 mg/dl with a mean of 77.59 ± 9.39 mg/dl (SD). In tobacco smokers, mean salivary IgA levels ranged between 41.13-82.27 mg/dl with a mean of 58.86 ± 9.56 mg/dl (SD). Among the control group Mean Salivary IgA levels are high compared to other two groups which is 104.33 ± 12.16.

Table 3. Comparison of S-Iga Levels between Tobacco Chewers, Tobacco Smokers and Controls Using

Study group	**S-IgA level (mg/dl)**	**Groups compared**	**Significance**		
			Mean difference	t-value*	P-value
1: Tobacco chewer	77.59 ± 9.39	1-2	1.71	7.65	<0.0001
2: Tobacco Smoker	58.86 ± 9.56	2-3	1.74	16	<0.0001
3: Control	104.33 ± 12.16	1-3	2.22	9.53	<0.0001

Student's T-Test and One Factor ANOVA

One way ANOVA (F=143.5, P<0.001), *Student's test (P<0.001, highly significant)

As depicted in the table 3 salivary IgA levels between (a) tobacco chewers and controls, (b) tobacco smokers and controls, and (c) tobacco chewers and tobacco smokers are compared. The mean difference of salivary IgA between tobacco chewers and tobacco smokers was 1.71 mg/dl, between tobacco smokers and controls was 1.74 mg/dl, and between tobacco chewers and control was 2.22 mg/dl, with a t-value of 7.65,16 and 9.53 respectively, which is statistically significant P<0.001.

DISCUSSION

IgA is the principal immunoglobulin present in secretions such as milk, saliva, tears, sweat, nasal fluids, colostrum. It provides protection against microorganisms to mucous membranes including oral cavity through saliva (12). IgA antibodies can neutralize viruses, bind toxins, agglutinate bacteria, prevent bacteria from binding to cells (13). Decrease in salivary IgA in tobacco smokers can be explained on the basis of immunosuppressive effects of combustion products of tobacco(14).Smoking markedly increases the flow rate of saliva leading to increased calcium levels in the oral cavity during smoking which ultimately result in reduced salivary IgA levels(15)(16).Smokeless tobacco contains nicotine and aromatic benzene containing products which directly affects plasma cells of salivary gland and oral mucosa, which shows changes in salivary IgA levels(17).Smoking, which has several toxic effects, so it is possible that the immune system is also effected in some way, leading to decreased antibody production(18)

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In the present study we have found the mean salivary IgA level in control group was 104.33 ± 12.16 µg/dl (SD), in tobacco chewers it was 77.59 ± 9.39 µg/dl (SD) and in tobacco smokers it was 58.86 ± 9.56 µg/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 ($P<0.0001$). Our findings were consistent with Bennet KR et al 1982(19), Ussher M et al 2004(20), Gupta et al in 2011(21) which indicates salivary IgA levels reduced in tobacco smokers than tobacco chewers. Other studies conducted by Olson B Let al1985 (22), Zuabi O et al 1999(23), Lie MA et al 2002(24) which shows salivary IgA levels in tobacco smokers and tobacco chewers without any remarkable difference. The field remains open for future investigation with a larger sample size among tobacco chewers and tobacco smokers.

CONCLUSION

Present study showed that chewing as well as smoking tobacco habit remarkably decreases salivary IgA levels. Thus, making individuals more prone to Oro-mucosal diseases.

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