Estimation of Salivary Immunoglobulin A and Serum Immunoglobulin A in Smokers and Nonsmokers: A Comparative Study

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Abstract:
Background: Cigarette smoke has been shown to cause immune modulatory effects making the individual susceptible to various diseases. Immunoglobulin A (IgA) forms the first line of defense against pathogens at the mucosal surfaces and smoking might interfere with its production and function. By understanding how cigarette smoking causes reduced serum and salivary IgA levels, increasing their levels may be therapeutic in the prevention of oral diseases.

Materials and Methods: The study group comprised 60 smokers and the control of 60 nonsmokers of age group 18-45 years. Case histories were elicited, informed consent was taken, and serum and saliva samples were collected. Salivary and serum IgA were detected using the turbidimetric IgA immunoassay. The presence of IgA in the test specimen resulted in the formation of an insoluble complex producing a turbidity, which was measured at a wavelength of 340 nm. IgA concentration for all serum and saliva samples was calculated as per the manufacturer’s guidelines. Data obtained was statistically analyzed.

Results: Serum and salivary IgA levels were significantly higher in nonsmokers as compared to smokers. Statistical analysis showed a significant difference between the serum and salivary IgA levels in smokers and nonsmokers.

Conclusion: There is strong evidence that smoking precipitates many orogastrointestinal diseases due to its profound effects on the immune system. Estimation of the IgA levels in smokers could prove to be of therapeutic use for the control and prevention of oral diseases.

Key Words: Antibodies, immunoglobulin A, saliva, smoking, tobacco

Introduction
Cigarette smoking is a social epidemic worldwide and is one of the prime reasons of preventable death and morbidity. Many of the adverse effects of smoking might result from cigarette smoke-induced impairment of the immune system. Increased vulnerability of cigarette smokers to infections reflects a suppression of their humoral and cellular immune responses in the form of decreased response to antigens, diminished immunoglobulin (Ig) levels and reduced chemotactic and phagocytic activity of neutrophils.

Smoking has been associated chronic respiratory diseases, heart diseases, and an increased risk of malignancy. It also disturbs the ecology of the oral environment paving way for periodontal diseases, caries, oral precancer and cancer.

IgA is the most ambiguous of all Igs. It is one of the most predominant Ig, being present in the blood plasma at concentrations approaching 2-3 mg/ml, and is dominant in secretions where its output approximates 5-8 g/day in adults.

Serum IgA has been known to activate the complement system and trigger cell-mediated events. Secretory IgA is the first line of defense against pathogens which invade through the mucosal surfaces primarily the eye, oral cavity, gastrointestinal tract, and respiratory system. Salivary IgA antibodies are produced locally by the plasma cells in salivary glands and transverse the mucous membrane. Antibodies in the oral cavity are primarily of the IgA, IgG, and IgM subtypes. Normal levels of IgA, IgG, and IgM in the saliva are 19.4, 5.37 mg/dl and 1.44, 0.9 mg/dl, respectively.

These contribute to oral immunity by intercepting adherence of microbes, neutralizing enzymes, and toxins or by acting synergistically with lysozymes and lactoferrin.

Many studies have been carried out to estimate IgA levels in smokers, smoking cessation, periodontal diseases, dental caries, aphthous ulcers, etc.

Most of these studies hint at the fact that serum and salivary IgA have a role in disease processes and their levels vary with disease progression and severity.
Hence, these above findings have led us to investigate whether serum and salivary IgA levels varied among smokers and nonsmokers and to what extent.

Materials and Methods
The study was conducted in the Department of Oral Pathology, Faculty of Dental Sciences. The study group comprised 60 smokers (study group) and 60 nonsmokers (control group) between the age group 18 and 45 years who visited the outpatient department of our hospital. Smoking status was determined according to the smokerlyzer breath test. Smoking status was determined according to the smokerlyzer breath test. Smoking history was assessed in terms of consumption (number of cigarettes per day) and duration (in years). Smokers were defined as those who smoked a minimum of 10 cigarettes/day for a minimum period of 6-month. Smoking status was determined according to the smokerlyzer breath test. Smoking status was determined according to the smokerlyzer breath test. Following this, patients were classified as either smokers or nonsmokers. The patients with oral lesions and those suffering from systemic disorders, especially immunodeficiency disorders were excluded from the study. The control group comprised 60 age-matched healthy individuals who did not have any deleterious habits.

About 2 ml of venous blood was drawn by vein puncture of the median cubital vein under aseptic precautions. Blood was allowed to clot for 1 h and then centrifuged at 1000 rpm for 10 min to provide serum. 1 ml of unstimulated saliva was collected by asking the subjects to dribble into a sterile glass centrifuge tube. All saliva samples were centrifuged at 12,000 rpm for 10 min, and the supernatant fluid was stored at −20°C until used for the IgA assay. Salivary and serum IgA were detected using the turbidimetric IgA immunoassay (Quantia IgA kit). The presence of IgA in the test specimen resulted in the formation of an insoluble complex producing a turbidity, which was measured at a wavelength of 340 nm using an ultraviolet spectrophotometer. IgA concentration for all serum and saliva samples was calculated as per the manufacturer’s guidelines.

Data were analyzed using Statistical Package for Social Science, Version 10.0.5 package. Normality of data was tested using Shapiro–Wilk test. Proportions were compared using Chi-square test of significance. $P < 0.05$ was accepted as indicating statistical significance.

Results
The mean age of the 60 nonsmokers who participated in the study was 35.4 years and that of 60 smokers was 37.1 years. 46 males and 14 females comprised the control group and 57 males and 3 females formed the study group (Table 1).

In smokers, a mean value of the serum IgA levels was 180.7 mg/dl and that of the non-smokers was 248.4 mg/dl (Table 2). Serum IgA levels were found to be significantly higher in nonsmokers as compared to smokers.

Mean value of the salivary IgA level in smokers was found to be 3.86 mg/dl compared to the nonsmokers where the mean IgA level was 14.74 mg/dl. Salivary IgA levels were found to be significantly higher in nonsmokers as compared to smokers (Table 2).

Discussion
The discovery of the gamma A (γA) class of IgA can be credited to Gugler, Heremans, and coworkers who demonstrated that not all myeloma proteins were considered for by the 7S (low carbohydrate content) and the 16S (high carbohydrate) classes. They characterized an antigenically distinct Ig with high carbohydrate content and called it γA because it migrates in the γ-globulin region during electrophoresis. This class of IgA was later called IgA and was found to be predominant in exocrine secretions and played a fundamental role in humoral mucosal immunity. Although not all the locally produced antibodies are of the IgA class, a distinct immune response at the mucosal

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<th>Table 1: Comparison of mean age and gender distribution among the control and study group.</th>
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<tr>
<td>Control group (non smoker)</td>
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<td>Study group (Smoker)</td>
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<th>Table 2: Comparison of serum IgA and salivary IgA levels among the control and study group.</th>
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surfaces and a particular Ig that predominated among the mucosal antibodies suggested the role of a local immune system which is structurally and functionally distinct from the systemic. It has been demonstrated that mucosal immunity is depressed among tobacco users (tobacco chewers and tobacco smokers). Studies demonstrate that smoking alters both the systemic and mucosal immunity with changes reciprocated in mucosal and systemic antibody production.\(^5\)\(^6\) Ig levels have so far not been extensively studied in oromucosal lesions.\(^7\) We have, therefore, tried to correlate the effect of smoking on systemic and mucosal immunity using estimating the levels of IgA in serum and saliva.

In our study, we found that the serum IgA levels in smokers were significantly less as compared to nonsmokers which are in accordance to similar studies.\(^8\)\(^9\)\(^10\) Smoking decreases the proliferative capacity of T-cells and T-cell-dependent antibody responses which affect B-cell function and antibody production resulting in reduced production of serum antibodies. It has also been seen that alveolar macrophages in smokers show reduced expression of antigen-presenting cells. This may subsequently lead to a suppression in the humoral immune response to antigens.\(^19\)

Our results differed from Gonzalez-Quintela et al.\(^3\) where serum IgA did not show statistically significant results between smokers and nonsmokers. On the contrary, Arinola and Obikoya\(^20\) reported increased levels of serum IgA in smokers and tobacco users as compared to healthy non-smokers. Mean IgA levels were highest in alcoholics and smokers compared with only alcoholics, only smokers, and controls. This has been explained by the fact that cigarette smoke and alcohol tends to irritate the respiratory and gastrointestinal mucosa triggering the immune system. In an attempt to protect the seromucous membranes by preventing adherence of antigens to the mucosal cells, IgA production is increased.\(^20\)

In our study, salivary IgA levels were also significantly reduced in smokers as compared to non-smokers. Many studies have shown similar results.\(^1\)\(^2\)\(^9\)\(^10\)\(^21\) Smokers have reduced polymorphonuclear and natural killer cell activity, a decrease in the T-helper/suppressor cell ratio, augmented formation of proinflammatory cytokines such as tumour necrosis factor-alpha, interleukin-1 (IL-1), IL-6, IL-8, granulocyte-macrophage colony-stimulating factor and decreased levels of anti-inflammatory cytokines such as IL-10, all leading to decreased IgA levels.\(^21\)

Conclusion

By understanding how cigarette smoke causes reduced salivary Ig levels, increasing the levels of these salivary IgS may be therapeutic in the control and prevention of oral diseases.\(^1\) Further research is required to explain the molecular mechanism by which smoking affects the immune system. Additional longitudinal studies are needed to assess the significance of serum and salivary antibodies as potential markers for therapeutic use.\(^4\)\(^24\)

The functions of different IgA forms range from neutralization to active immune suppression and pro-inflammatory responses. These can be put to use for designing IgA monoclonal antibodies with specific and desired therapeutic functional activity. In the near future IgA, monoclonal antibodies could prove to be a potent therapeutic modality and IgA levels could be used as potential markers to indicate disease progression.

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Salivary IgA and serum IgA ... Agarwal A et al


