

# Evaluation of salivary immunoglobulin A levels in tobacco smokers and patients with recurrent aphthous ulcers

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## Abstract

**Objectives:** The aim of the present study was to analyze the influence of smoking on the salivary immunoglobulin response in smokers and to evaluate the salivary immunoglobulin A in patients with recurrent aphthous ulcers. **Materials and Methods:** The study included total of 80 subjects, of whom 40 were having history of chronic smoking habit, 20 were clinically diagnosed cases of recurrent aphthous ulcer and 20 were in the control group. Sample of unstimulated saliva was collected, centrifuged and analyzed for the level of salivary immunoglobulin A with turbidimetric immunoassay. For all the tests, a P- value of < 0.05 was considered for statistical significance. **Results:** The mean salivary immunoglobulin A level in control group was 0.20 Grams/litre and in smokers the mean salivary immunoglobulin A level was 0.13 Grams / Litre. In patients with recurrent aphthous ulcers mean salivary immunoglobulin A level was 0.31 Grams / Litre. The mean salivary immunoglobulin A levels showed a decreasing trend from controls to smokers. These results were highly significant for values between control groups to smokers. **Conclusion:** The mean salivary immunoglobulin A levels demonstrated a progressive decrease from controls to smokers. This investigative procedure although non-specific, can be used as a diagnostic marker in smokers and patients with recurrent aphthous ulcers.

**Key words:** Controls, recurrent aphthous ulcers, salivary immunoglobulin A, smokers

## INTRODUCTION

The indigenous microbiota plays an important role in health and diseases of the humans and animals. It contributes to the development of the immune system and provides resistance to colonization by allochthonous or pathogenic microorganisms. It also constitutes a reservoir of potentially pathogenic bacteria that may infect host tissues. Oral diseases seem to appear after an imbalance among the indigenous microbiota, leading to the emergence of potentially pathogenic bacteria.<sup>[1]</sup>

Immunoglobulins are proteins of the animal origin endowed with known antibody activity and for certain other proteins related to them by chemical structure. Immunoglobulins are synthesized by plasma cells and to some extent by lymphocytes also. All antibodies are immunoglobulins, but all immunoglobulins may not be antibodies. Immunoglobulins constitute 20–25% of the total serum proteins.<sup>[2]</sup>

Five distinct classes of immunoglobulin molecules are recognized in higher mammals, namely IgG, IgA, IgM, IgD, and IgE.<sup>[3]</sup>

The immunoglobulins responsible for the protection are IgA and IgG.<sup>[4]</sup> Secretory IgA (SIgA) constitutes the predominant immunoglobulin isotype in secretions, including saliva. It is considered to be the first line of defense of the host against pathogens, which colonize or invade surfaces bathed by external secretions.<sup>[5]</sup>

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IgA is the primary protective antibody at mucosal surfaces. IgA is produced by plasma cells in the minor salivary glands. Secretory IgA can traverse mucosal membranes; in this way, it helps to prevent the entry of infectious microorganisms.<sup>[4]</sup>

Several factors may influence the IgA levels in serum as well as in secretions. The secretion rate is an important factor. An increase in the secretion rate is accompanied by a decrease in concentration of SIgA in saliva. Another factor is cigarette smoking, which has been reported to decrease SIgA concentrations.<sup>[6]</sup> Smoking has been shown to affect T-cell subsets, natural killer cells, and serum immunoglobulin concentrations.<sup>[7]</sup> Decrease of S-IgA may be due to an influence on the salivary gland cells responsible for the completion of the S-IgA or on the cells of immunologic system involved in the production of the IgA molecules.<sup>[8]</sup>

Many studies were performed relating the physiology of IgA secretion occurrence of recurrent aphthous stomatitis (RAS).<sup>[9]</sup>

RAS is one of the most common oral lesions seen by dentists.<sup>[10]</sup> RAS affects approximately 20% of general population, but when specific ethnic or socioeconomic groups are studied, the incidence ranges from 5% to 50%.<sup>[11]</sup> The etiology of RAS may be immunological one. Increased circulating levels of antibody against oral mucous membrane may be found in affected individuals. These increased antibody levels may be due to an immunologic cross-reaction between oral epithelium and indigenous microorganisms or the exposure, by ulceration, of previously sequestered, hidden antigens setting up cycles of recurrent disease. Thus, there will be increased IgA levels in RAS patients.<sup>[12]</sup>

This study was carried out to find if patients with recurrent aphthous ulcers show any abnormality in the immunoglobulin levels and also evaluation of immunoglobulin levels in tobacco smokers.

## MATERIALS AND METHODS

The study and control population of approximately 80 subjects were considered for this study which were drawn from the outpatient department. The work was approved by an Ethical Committee. Twenty subjects with a provisional diagnosis of RAS was made on the basis of clinical examination described by Stanley<sup>[13]</sup> and 40 subjects with chronic smoking habits were included in the study group. Age and sex matched apparently healthy 20 subjects who had no recent history of systemic conditions were included in the control group. A written consent was obtained from

those involved in the study. The patient's personal history was recorded with tobacco smoking habits, frequency, and duration of smoking.

In each case approximately 1.0 ml of unstimulated saliva was collected from each subject by drawing the saliva with a disposable syringe and later passing it into a sterile glass centrifuge tube. The samples were immediately frozen at  $-20^{\circ}\text{C}$ , and maintained at that temperature until shortly before assay. Assay samples were thawed at  $37^{\circ}\text{C}$ , and then immediately centrifuged at 12,000 rpm for 10 min and the supernatant fluid was used for assay of IgA using Quantia-IgA turbidimetric immunoassay.

### Statistical analysis

The IgA levels are expressed as mean  $\pm$  SD and range values with coefficient of variation (CV %). Since normality was not presumed in SIgA levels, a nonparametric method, the Mann–Whitney test was used to compare between the groups. ANOVA was used for the multiple group comparisons. For all the tests, a *P*-value of  $<0.05$  was considered statistically significant.

## RESULTS

This study comprised of 80 subjects which include 20 controls in Group I, 40 smokers in group II, and 20 cases of recurrent aphthous ulcers in Group III. Table 1 shows age and sex distribution in Groups I, II, and III.

### Comparison of SIgA levels between the control and the study group

The value of SIgA in controls ranged from 0.07 to 0.31 g/l with a mean of 0.20 g/l and a SD of 0.07 [Table 2].

The SIgA levels in patients with recurrent aphthous ulcers ranged from 0.03 to 1.02 g/l with a mean SD of  $0.31 \pm 0.36$  g/l [Figure 1].

### SIgA levels in relation to smoking frequency

Among the 40 patients with chronic smoking habits, 19 individuals had the habit of smoking 1–9 cigarettes per

**Table 1: Age and sex distribution in Groups I, II, and III**

	I Controls	II Smokers	III Recurrent aphthous ulcer patients
No. of subjects	20	40	20
Mean $\pm$ SD	36.9 $\pm$ 13.2	41.5 $\pm$ 13.4	26.9 $\pm$ 7.4
Range	22–65	20–65	20–45
Sex			
Male	16	39	14
Female	4	1	6

**Table 2: Comparison of SIgA levels between the control group and study group**

Groups	Number	SIgA levels (g/l)	
		Range	Mean $\pm$ SD
I Controls	20	0.07–0.31	0.20 $\pm$ 0.07
II Smokers	40	0.02–1.04	0.13 $\pm$ 0.15
III Aphthous ulcer patients	20	0.03–1.02	0.31 $\pm$ 0.36

**Table 3: Difference between SIgA levels in the control group and the study group**

Groups compared	Mean difference	P value
I–II	0.07	<0.01, S
I–III	0.11	0.42, NS
II–III	0.18	0.06, NS

S, Significant; NS, N ot significant

day and had a mean SIgA level of 0.11 g/l with a SD of 0.05 g/l, 12 individuals had the habit of smoking 10–19 cigarettes per day and had a mean SIgA level of 0.20 g/l with a SD of 0.27 g/l, and 9 individuals had the habit of smoking 20 or more cigarettes per day and had a mean SIgA level of 0.09 g/l with a SD of 0.05 g/l [Figure 2].

The ANOVA test for the smoking frequency and SIgA levels was of a *P*-value of 0.23. However, correlation between SIgA levels and smoking frequency was not statistically significant.

### SIgA levels in relation to duration of smoking

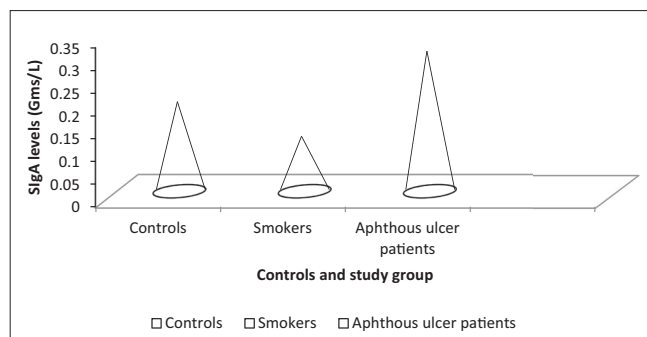
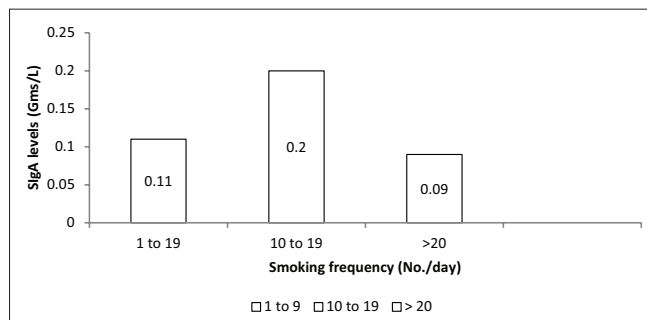
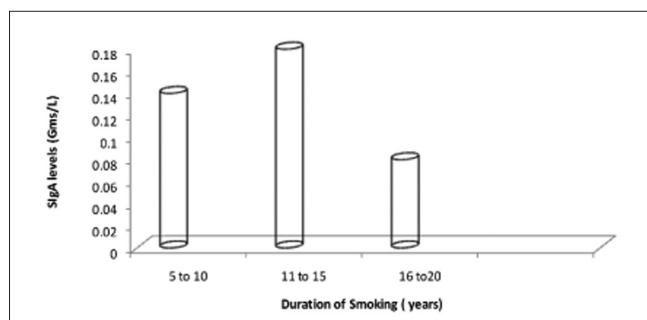
Among the 40 patients with chronic smoking habits, 12 individuals had 5–10 years, 13 individuals with 11–15 years, and 15 individuals with 16–20 years of duration of smoking had mean SIgA levels of 0.14 g/l, 0.18 g/l, 0.08 g/l with a SD of 0.04 g/l, 0.26 g/l, and 0.04 g/l, respectively [Figure 3]. However, there was no significant correlation between SIgA levels and duration of smoking.

### Difference between SIgA levels in the control group and the study group.

The mean SIgA level in Groups I and II was 0.07 g/l, and there was a significant difference of (*P* < 0.01) between Groups I and II. The mean SIgA levels in Groups I and III was 0.11 g/l and mean SIgA levels in Groups II and III were 0.18 g/l. However, there was no significant difference between Groups I and III, and II and III [Table 3].

## DISCUSSION

Salivary immunoglobulin A (SIgA) is produced by local plasma cells situated in the mucosa and salivary glands and transport protein by ductal epithelial cells. Two or three IgA molecules are united with transport protein to form the composite molecule which is secreted as salivary IgA.<sup>[14]</sup>

**Figure 1: SIgA levels in the control and study group****Figure 2: SIgA levels in relation to smoking frequency****Figure 3: SIgA levels in relation to duration of smoking**

Tobacco use is the single biggest contributor to ill health, and is the most important preventable cause of death.<sup>[15]</sup> The local influence of tobacco smoking can alter the immunoglobulin levels in saliva.<sup>[16]</sup> The ill effects of tobacco include cardiovascular disorders, respiratory disorders, and lung cancer. Smoking also has a profound effect on the oral tissues. In addition, the risk of oral cancer and potentially malignant lesions is higher among smokers compared with those who have ever smoked.<sup>[15]</sup>

Much work has been conducted on systemic immune status but, surprisingly, the influence of smoking on mucosal immunity has been relatively neglected.<sup>[17]</sup>

Cigarette smoking alters the immunoglobulin profile of saliva. Smoking impairs T-cell immunoregulation of B-cell differentiation and maturation thus leading to a decrease in

SIgA levels. Smokers have increased polymorphonuclear neutrophil counts, decreased natural killer cell activity, an increased total T-cell numbers with a decrease in the T helper/suppressor cell ratio in heavy smokers leading to decreased immunoglobulin A levels. Low immunoglobulin levels are important predisposing factor in the development of infections associated with smoking, such as chronic bronchitis.<sup>[18]</sup>

Immunoglobulin levels have so far not been studied comprehensively in oromucosal lesions. However, some authors say that salivary IgA has no definitive role in the pathogenesis of aphthous ulceration.<sup>[19]</sup>

The mean SIgA level in Group I was 0.20 g/l and a SD of 0.07 g/l. These values were close to values established by Ben-Aryeh *et al.*<sup>[9]</sup> (2–19 mg/100 ml) Bennet and Reade<sup>[8]</sup> (0.05–0.26 g/l). These values were higher compared to that of Lehner<sup>[14]</sup> (13 mg/100 ml), Olson *et al.*<sup>[20]</sup> ( $6.2 \pm 0.4$  mg/100 ml), Baron *et al.*<sup>[18]</sup> in Edinburg subjects (92–145 µg/ml) and in Cairo workers (120–200 µg/ml), Norhagen Engstrom<sup>[6]</sup> (151.8 mg/l), and Griesel and Germishuys<sup>[4]</sup> ( $11.0 \pm 3.1$  mg/dl).

Further, there was no significant correlation between age and SIgA levels in controls. The exact reason for the variations in the levels in controls is not clear, and it can be due to variation in the methodology and sampling.

The mean SIgA levels in Group II were 0.13 g/l with a SD of 0.15 g/l. These values were close to the values established by Bennet and Reade<sup>[8]</sup> (0.05–0.18 g/l). These values were lesser compared to that of Hersey *et al.*<sup>[21]</sup> ( $1.38 \pm 0.45$  g/l), Norhagen Engstrom<sup>[6]</sup> (240 mg/l). These values were higher compared to that of Olson *et al.*<sup>[20]</sup> ( $6.7 \pm 0.3$  mg/100 ml), Barton *et al.*<sup>[18]</sup> in Edinburg subjects (41–44 µg/ml), in Cairo workers (80–180 µg/ml), and Griesel and Germishuys<sup>[4]</sup> ( $10.4 \pm 2.9$  mg/dl).

Variation in the level of immunoglobulin may be due to 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. Smoking also impairs T-cell immunoregulation of B-cell differentiation and maturation leading to decreased immunoglobulin A levels.<sup>[18]</sup>

The mean SIgA level in Group III was 0.31 g/l with a SD of 0.30 g/l. These values were higher compared to that of Lehner<sup>[14]</sup> (15 mg/100 ml), Ben-Aryeh *et al.*<sup>[9]</sup> in the dormant phase 3–16 mg/100 ml and in the acute phase 1.5–15 mg/100 ml, Bennet and Reade<sup>[8]</sup> (0.06–0.26 g/l), Sistig *et al.*<sup>[19]</sup> ( $166.3 \pm 113.4$  mg/l).

The mean SIgA levels in Groups I and II was 0.07 g/l, and there was a significant difference ( $P < 0.01$ ) between Groups I and II. These findings are similar to Bennet and Reade<sup>[8]</sup> Hersey *et al.*,<sup>[21]</sup> and Barton *et al.*<sup>[18]</sup> who demonstrated decreased immunoglobulin A levels. Smoking impairs T-cell immunoregulation of B-cell differentiation and maturation leading to decreased SIgA levels.<sup>[18]</sup>

The mean SIgA levels in Groups I and III was 0.11 g/l. However, there was no significant difference between Groups I and III. These findings are similar to Lehner,<sup>[14]</sup> Ben-Aryeh *et al.*,<sup>[9]</sup> and Bennet and Reade.<sup>[8]</sup>

## CONCLUSION

Although being non-specific, estimation of SIgA levels can contribute to diagnosis of RAS and can be used as a diagnostic marker in RAS and can also be used to analyse the influence of smoking on immunoglobulins.

However, the findings of this study needs to be carefully interpreted because of the small sample size and to the best of our knowledge, lack of studies involving the estimation of SIgA levels in patients with RAS and smokers. Further research involving larger samples and estimation of SIgA levels during the course of disease and treatment is suggested along with extensive work involving specificity of estimation techniques before a definite statement on decreased SIgA levels and their clinical applications can be made.

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