

Salivary immunoglobulin classes in Nigerian smokers with periodontitis

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Author contributions: Arinola OG designed and supervised the study; Joseph IO diagnosed the subjects with periodontitis and ruled out oral diseases in the control subjects; Olayanju OA recruited the subjects and did the laboratory analyses with Rahamon SK; Rahamon SK and Arinola OG wrote the manuscript.

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mine significant differences between the means. Values of $P < 0.05$ were regarded as significant.

RESULTS: No significant differences were observed in the mean salivary levels of the immunoglobulin classes (IgG, IgA, IgM and IgE) when S+P was compared with S-P. Mean salivary levels of IgA (520.0 ± 155.1 ng/mL vs 670.0 ± 110 ng/mL, $P = 0.000$) and IgM (644.5 ± 160.0 ng/mL vs 791.4 ± 43.7 ng/mL, $P = 0.000$) were significantly lower in the S+P compared with NS+P group. Salivary IgA (570.4 ± 145.6 ng/mL vs 670.0 ± 110 ng/mL, $P = 0.008$) and IgM (703.1 ± 169.3 ng/mL vs 791.4 ± 43.7 ng/mL, $P = 0.012$) levels were significantly lower in the S-P compared with NS+P group. Only one (5%) periodontal patient had detectable levels of salivary IgE (0.20 IU/mL). Similarly, only one smoker (4.17%) had detectable levels of salivary IgE (0.04 IU/mL) and two non-smokers (9.52%) had detectable levels of IgE (0.24 IU/mL).

CONCLUSION: Our study suggests that reduced salivary IgA and IgM levels in smokers with periodontitis could enhance increased susceptibility to periodontitis.

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Key words: Cigarette smoke; Immunoglobulin; Periodontitis; Saliva; Smokers

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Abstract

AIM: To determine the levels of salivary immunoglobulin classes in Nigerian smokers and non-smokers with periodontitis.

METHODS: Sixty-nine individuals were recruited into this study after obtaining informed consent. They were subdivided into three groups that consisted of 20 (aged 46 ± 11 years) cigarette smokers with periodontitis (S+P); 24 (40 ± 12 years) smokers without periodontitis (S-P); and 25 (53 ± 11 years) non-smokers with periodontitis (NS+P). An oral and maxillofacial surgeon used radiographs for periodontal probing for the diagnosis of periodontitis. The smokers included subjects who smoked at least six cigarettes per day and all the periodontitis patients were newly diagnosed. About 5 mL of unstimulated saliva was expectorated by each subject into plain sample bottles. Salivary immunoglobulin levels were estimated using enzyme linked immunosorbent assay. Student's t test was used to deter-

INTRODUCTION

Tobacco smoking has consistently been identified as an important risk factor for the development of periodontal disease^[1]. Cross-sectional studies have demonstrated that smoking leads to epidemiologically and biologically enhanced risk of periodontal disease^[2]. The role of cigarette smoke in periodontal disease development was further established by a United States National Health Survey that showed that about 42% cases of periodontal diseases were associated with cigarette smoking^[3].

Heat from cigarette smoke increases calculus formation. Nicotine deposition from cigarette smoking is associated with local effects such as attachment loss, recession on the lingual surfaces of maxillary teeth and mandibular incisors, plaque retention, reduced collagen synthesis, fibroblast growth inhibition, and damage to cell membranes^[4,5]. The cellular and humoral immune systems are both affected by smoking. It exerts effects throughout the cytokine network and suppresses both chemotactic and phagocytic functions of polymorphonuclear leukocytes in saliva and tissues^[6,7]. Suppression of immune responses, impaired stromal cell function, and amplified inflammatory responses have been reported in smokers. Changes in molecular and cellular mechanisms at both the local and systemic levels explain the strong association between tobacco smoke and the severity of periodontal disease^[1]. Smokers with periodontal disease typically have less clinical inflammation and gingival bleeding than non-smokers. This has been attributed to nicotine with a vasoconstrictive effect that culminates in reduced blood flow, edema, and clinical signs of inflammation, which mask serious periodontal disease in smokers^[1].

Smoking decreases levels of salivary IgA and serum IgG to *Prevotella intermedia* and *Fusobacterium nucleatum*. It also creates an imbalance between T-lymphocyte subset ratios, hindering T-helper-cell function and compromising antibody production. A study among Nigerian cigarette smokers has reported increased levels of serum IgG and IgM^[1,8]. Smoking suppresses the production of IgG2, which is predominantly required for the protection against *Aggregatibacter actinomycetemcomitans* and *Porphyromonas gingivalis*. This suggests that suppression of IgG2 antibodies may be the main mechanism inducing severe periodontitis^[9].

Humoral immune response has been reported to have a protective role in periodontal disease pathogenesis^[10]. Alterations in specific IgG and IgA responses both locally at inflamed sites and systemically have relevance in disease progression^[11,12].

Saliva contains immunoglobulins that might have originated from the salivary glands and serum^[13] but previous studies on salivary immune parameters are scarce, and to the best of our knowledge, no such study has been carried out among Nigerians. The present study assessed the levels of salivary immunoglobulin classes in cigarette smokers with or without periodontitis. This would probably, explain the contributory role of cigarette smoke in the development of periodontitis.

MATERIALS AND METHODS

Study subjects

Sixty-nine individuals were recruited for this study after obtaining informed consent and ethical approval from the University of Ibadan/University College Hospital Joint Ethics Review Committee. They were subdivided into various groups that consisted of 20 (aged 46 ± 11 years) cigarette smokers with periodontitis (S+P); 24 (40 ± 12 years) smokers without periodontitis (S-P); and 25 (53 ± 11 years) non-smokers with periodontitis (NS+P). An oral and maxillofacial surgeon used radiographs for periodontal probing for the diagnosis of periodontitis. The smokers smoked at least six cigarettes per day and all the periodontitis patients were newly diagnosed. Pregnant women and patients with diabetes or human immunodeficiency virus infection were excluded from the study. Patients with other forms of oral disease were also excluded from the test group. A short structured questionnaire was administered to each subject to obtain information on age, sex, occupation, cigarette smoking, and drug consumption.

Spitting was used for saliva collection. About 5 mL of unstimulated saliva was expectorated by each subject into plain sample bottles. The samples were collected between 09:00 and 11:00 h, 1 h after eating or mouth washing. The samples were centrifuged at $3000 \times g$ for 5 min and the clear supernatant was gently pipetted into another clean plain bottle and stored at -20°C until analyzed.

Immunoglobulin levels were estimated using enzyme linked immunosorbent assay (ELISA) (Immunology Consultant Laboratory, Portland, OR, United States). The IgE kit was supplied by Leinco Technologies (St Louis, MO, United States). The assay was carried out following the manufacturer's instructions.

Statistical analysis

The data were presented as mean and standard deviation. Student's *t* test (unpaired) was used to determine significant differences between the means. Values of $P < 0.05$ were regarded as statistically significant.

RESULTS

The mean levels of salivary immunoglobulin classes were lower in the S+P group compared with the S-P group, although the differences were not significant (Table 1). Mean salivary levels of IgA and IgM were significantly lower in the S+P group when compared with the NS+P group ($P = 0.000$, $P = 0.000$, respectively) (Table 2). No significant differences were observed in the mean levels of IgG and IgE. In Table 2, salivary IgA and IgM levels were significantly lower in the S-P group when compared with the NS+P group. IgG and IgE levels were not significantly different.

Only one (5%) periodontitis patient had detectable levels of salivary IgE (0.20 IU/mL). Similarly, only one smoker (4.17%) had detectable levels of salivary IgE (0.04 IU/mL) and two non-smokers (9.52%) had detectable

Table 1 Levels of salivary immunoglobulin classes in smokers with periodontitis and smokers without periodontitis

	S+P (n = 20)	S-P (n = 24)	P values
IgG (ng/L)	349.4 ± 91.5	355.7 ± 40.8	0.734
IgA (ng/L)	520.0 ± 155.1	570.4 ± 145.6	0.203
IgM (ng/L)	644.5 ± 160	703.1 ± 169.3	0.111
IgE (IU/mL)	0.2 ± 0.9	0.04 ± 0.2	0.519

S+P: Smokers with periodontitis; S-P: Smokers without periodontitis.

Table 2 Levels of salivary immunoglobulin classes in non-smokers with periodontitis, smokers with periodontitis, and smokers without periodontitis

	NS+P (n = 25)	S+P (n = 20)	S-P (n = 24)	P ¹ value	P ² value
IgG (ng/L)	370.0 ± 60.9	349.4 ± 91.5	355.7 ± 40.8	0.268	0.42
IgA (ng/L)	670.0 ± 110	520.0 ± 155.1	570.4 ± 145.6	0.000	0.008
IgM (ng/L)	791.4 ± 43.7	644.5 ± 160.0	703.1 ± 169.3	0.000	0.012
IgE (IU/mL)	0.2 ± 1.0	0.2 ± 0.9	0.04 ± 0.2	1.000	0.494

¹NS+P vs S+P; ²NS+P vs S-P. S+P: Smokers with periodontitis; S-P: Smokers without periodontitis; NS+P: Non-smokers with periodontitis.

levels of IgE (0.24 IU/mL). This could be an indication that the level of IgE was low in the saliva of smokers and periodontitis patients, and therefore immeasurable by ELISA.

DISCUSSION

Periodontal diseases are infectious diseases caused by anaerobic Gram-negative bacteria^[14]. Cigarette smoking is a significant risk factor for the initiation and progression of periodontal disease. Studies have reported altered inflammatory cytokine levels in serum and gingival crevicular fluid in smokers^[15]. Nicotine in cigarette smoke affects the host inflammatory response to oral pathogens by upregulating release of prostaglandin and interleukin-2 leading to accelerated periodontal tissue destruction^[1].

Reduced, but nonsignificant levels of immunoglobulin classes were observed when we compared S+P with S-P groups. This observation suggests that cigarette smoking might not have a profound effect on periodontitis at the early stage because all our patients were newly diagnosed. However, the interaction between cigarette smoke and periodontitis was reflected in the lower levels of salivary IgA and IgM in smokers with periodontitis (S+P) when compared with periodontitis patients who were non-smokers (NS+P). Al-Ghamdi and Sukumaran^[15] have reported reduced IgA in the serum of smokers with periodontitis. Our observation corroborates earlier reports^[16,17] that cigarette smoking is associated with suppression of B-cell function and immunoglobulin production. This further explains the potential mechanism by which cigarette smoking exacerbates periodontal disease.

In order to understand the independent effects of smoking and periodontitis on the levels of salivary im-

munoglobulin classes, the S-P group was compared with the NS+P group. It was observed that smokers without periodontitis had significantly lower levels of IgA and IgM when compared with non-smokers with periodontitis. This indicates that cigarette smoke could be involved in suppression of oral immunity, thus making smokers more prone to Gram-negative bacterial infections that cause periodontal diseases. This observation could partly explain the mechanism through which smoking enhances increased susceptibility to periodontitis and its subsequent poorer response to treatment.

The small sample size was a major limitation of this study. Therefore, further research with a larger sample is required to confirm the differences observed in this study.

In conclusion, our study suggests that cigarette smoking could enhance initiation and progression of periodontitis due to its associated immunosuppression. Mechanisms to increase the levels of these salivary immunoglobulin classes may be therapeutic in the control and prevention of oral diseases.

COMMENTS

Background

Studies on salivary immune parameters have been scarce and no such study has been carried out among Nigerians.

Research frontiers

This study assessed the levels of salivary immunoglobulin classes in cigarette smokers with or without periodontitis. This could explain the contributory role of cigarette smoke to the development of periodontitis.

Innovations and breakthroughs

It was observed that salivary IgA and IgM were decreased in smokers with periodontitis compared with those who did not smoke.

Applications

By understanding how cigarette smoke causes reduced salivary immunoglobulin levels, increasing the levels of these salivary immunoglobulins may be therapeutic in the control and prevention of periodontitis.

Peer review

This work is interesting and merits publication. It is clearly written and the study was adequately carried out.

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