Original Article

Levels of Salivary Immunoglobulin A and Immunoglobulin G in Type 2 Diabetic Patients

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Abstract

Background: Diabetes mellitus is a chronic disorder of glucose metabolism and it is associated with a compromised oral immunity. Salivary immunoglobulins offer a comprehensive protection for the oral cavity; however, there is insufficient data regarding their levels in type 2 diabetic patients. This study aimed to measure salivary Immunoglobulin G (IgG) and Immunoglobulin A (IgA) in diabetic patients in comparison to healthy nondiabetic controls. **Methods:** Diabetic patients from the outpatient clinic and nondiabetic healthy members of staff, were recruited for this study. Unstimulated saliva samples were collected from all participants and levels of immunoglobulins A and G were determined using enzyme-linked immunosorbent assay techniques; the values were compared between the two groups. **Results:** A total of 167 participants were recruited for this study, 95 (56.9%) of them were diabetic patients, while the remaining 72 (43.1%) were healthy nondiabetic controls. The median salivary IgA was 12.57 (Interquartile range [IQR] 11.05–13.67) g/ml in the diabetics and 11.94 (IQR 10.41–13.65) µg/ml in the control group; $P = 0.31$ while the median salivary IgG was 32.27 (IQR 25.26–38.33) µg/ml in the diabetics and 26.26 (22.48–31.29) µg/ml in the control group; $P < 0.001$. **Conclusion:** Salivary IgG was significantly elevated in the diabetic patients, in spite of a higher prevalence of oral infections, this calls for a more stringent attention to oral hygiene in diabetic patients.

Keywords: Diabetes, IgA, IgG, oral cavity, oral infection, saliva

Introduction

Immunoglobulins are found in body fluids where they protect against infections. The blood, tears, saliva, mucosa secretions, synovial fluids, etc., have all provided useful information when evaluated for different types of immunoglobulins in different disease conditions.[1] Salivary immunoglobulins for instance, have been studied in periodontitis, autoimmune diseases, chronic renal failure, and other systemic diseases.[2,3] In the diabetics, salivary levels of several biochemical and immunological parameters have been evaluated, but some of the findings were equivocal.[4-6] Immunoglobulin A (IgA), clearly the most commonly studied was elevated, reduced and indifferent, in several saliva studies, generating controversies regarding the relationship between diabetes and salivary immunoglobulins.[7-9] Although it was not clear if there were confounders in the studies, what is generally apparent is the impaired protective functionality as evidenced by increased oral infections in the diabetics.[10,11]

Periodontal infections, tooth loss, and oral cancers all have increased prevalence in the diabetics;[12,13] burden and severity of these diseases have also been reported to be associated with the level of glucose control.[14-16] A cardinal factor identified for this manifestation in chronic hyperglycemia is the dysfunctional immune system characterized by defective phagocytosis and chemotaxis by innate immune cells, resulting in unfettered proliferation of microbes in the oral cavity.[17,18] The adaptive immune system also is not spared in diabetes; there are reports of B-cells dysfunction resulting in proliferation of sepsis and increased mortality in diabetic patients.[19,20] Although most of the studies interrogating the relationship between infection and diabetes were done in blood samples reflecting an equally predominant systemic infections, salivary studies have also

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provided a good insight into how chronic hyperglycemia predisposes to increased susceptibility to oral infections.[10,11]

Serum Immunoglobulin G (IgG), IgA, and Immunoglobulin M (IgM) were reportedly reduced in patients with type 2 diabetes mellitus, and this was said to be implicated in the recurrent systemic infection in diabetic patients.[21,22] In another study, while serum IgG and IgM were both reportedly reduced in Type 2 diabetic patients, serum IgA was elevated, unlike in the previous study.[23] Other studies have reported various conflicting levels of these immunoglobulins, and they suggested different reasons for their findings, essentially highlighting generalized immune dysfunction.[24,25] Given the positive correlation between the salivary and serum immunoglobulins as reported by yet other studies,[4,9] it is not surprising that the saliva which baths the oral cavity has shown similar trends with several studies reporting divergent opinions about the status of salivary immunoglobulins following chronic hyperglycemia in diabetic patients.[9,26,27] This study is designed to determine the level of salivary IgA and IgG in Type 2 diabetic patients to establish how chronic hyperglycemia relates to oral cavity immune status.

Methods

Participants

This was a hospital-based cross-sectional study conducted on Type 2 diabetic patients attending the outpatient clinic; healthy nondiabetic members of staff served as the control group. Patients were systematically selected based on monthly clinic attendance data, and the study lasted about three months. Biodata and clinical information were collected from each participant using a structured questionnaire after obtaining informed consent from them.

Ethical consent

Approval was duly obtained from the University of Ibadan/University College Hospital Ibadan Research Ethics Committee.

Sample collection and processing

Saliva samples were collected from participants in the morning after rinsing their mouths with clean water. They were asked to sit, bend their heads, open their mouth and passively drool saliva into a clean universal bottle. About 3 ml of saliva was collected, centrifuged at 3000 radian/min for 15 min, and the clear supernatant dispensed into a clean Eppendorf bottle for storage at-20 degrees centigrade until analysis was done; the study lasted about three months. Salivary IgG and IgA were analysed using enzyme-linked immunosorbent assay technique according to the manufacturer's instruction (Melsins Medicals, Changchun, China).

Statistical analysis

Qualitative variables were reported as number and percentages, quantitative variables as mean ± standard deviation (SD) if normally distributed and as median ± interquartile range (IQR) if not. Independent samples *t*-test was used to determine the statistical difference between the mean ± SD of normally distributed data, while Mann–Whitney *U*-test was used to determine statistical difference between data that were not normally distributed, between diabetic subjects and the healthy controls. Significance was set at $P \leq 0.05$, an analysis was done using Statistical Package for Social Science (SPSS) version 26.0 (IBM Inc., Armonk, NY, USA).

Results

A total of 167 participants were recruited for this study and 82 (49.1%) of them were males. Ninety-five (56.9%) participants were type 2 diabetic patients, while the remaining 72 (43.1%) were healthy nondiabetic control group. There was no significant difference between the ages ($P = 0.08$) and weights ($P = 0.43$) of the two groups. However, the healthy controls were taller ($P \leq 0.001$) and had a significantly lower body mass index [$P = 0.002$; Table 1]. Co-morbid conditions were compared between the two groups and there were more cases of hypertension (25 vs. 3), visual impairment (37 vs. 9), oral infections (9 vs. 2), and skin diseases (5 vs. 3) in the diabetic participants compared to the healthy controls. The salivary immunoglobulins were higher in the diabetic participants with salivary IgA measuring a median 12.57 (IQR 11.05–13.67) µg/ml compared to 11.94 (IQR 10.41–13.65) µg/ml in the healthy controls although the difference was not statistically significant ($P = 0.31$) [Figure 1]. The salivary IgG on the other hand was significantly elevated ($P < 0.001$) in the diabetic participants with a median of 32.27 (IQR 25.26—38.33) µg/ml [Figure 1] versus 26.26 (IQR 22.48–31.29) µg/ml in the healthy controls. Salivary IgA is significantly correlated positively with the Salivary IgG with a correlation coefficient of 0.372 and a $P < 0.001$. Salivary IgA and IgG were both positively correlated with the duration of diabetes treatment; correlation coefficients were 0.006 and 0.0068, respectively [Figure 2]. The correlation between the comorbidities and salivary IgA and IgG are presented in Table 2.

Discussion

The increased prevalence of oral cavity infections in diabetic patients was suggestive of a characteristic oral immunosuppression;[28,29] this hypothesis was tested in this study by evaluating a major component of the oral immunity in the diabetics. Although the saliva is composed of a myriad of

Table 1: Demographic characteristics of diabetic patients and nondiabetic control

Variables	Patients ($n=95$)	Control ($n=72$)	*P*
Age (years)†	58.9±13.8	55.7±9.2	0.08
Weight (kg)‡	74.0 (63.3-83.0)	72.0 (61.0-81.0)	0.43
Height (m)‡	1.60 (1.54-1.65)	1.68 (1.60-1.74)	<0.001
BMI (kg/m^2)‡	29.0 (24.5-33.1)	25.9 (22.5-28.7)	0.002

†Age was reported as mean±SD, ‡Weight, height and BMI were reported as median (IQR). BMI: Body mass index, SD: Standard deviation, IQR: Interquartile range

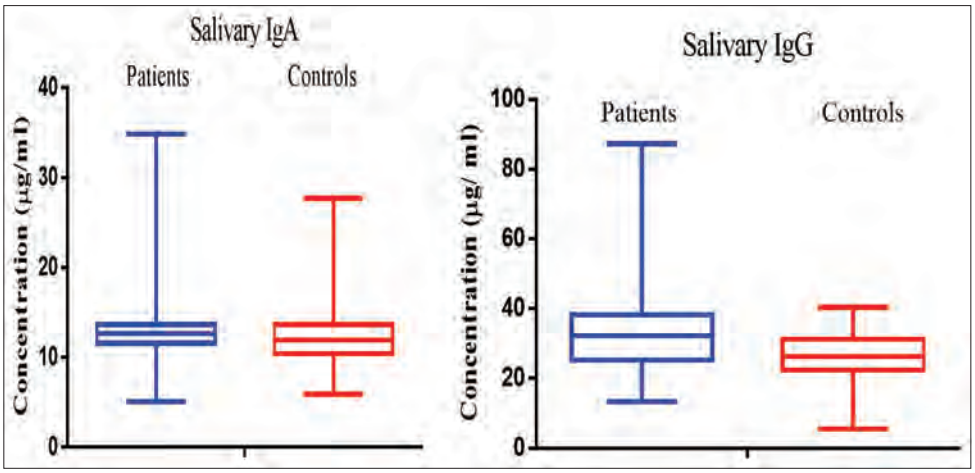

Figure 1: Graphical presentation of salivary proteins comparison between patients and controls, showing the median values; error bars indicate the minimum and maximum values

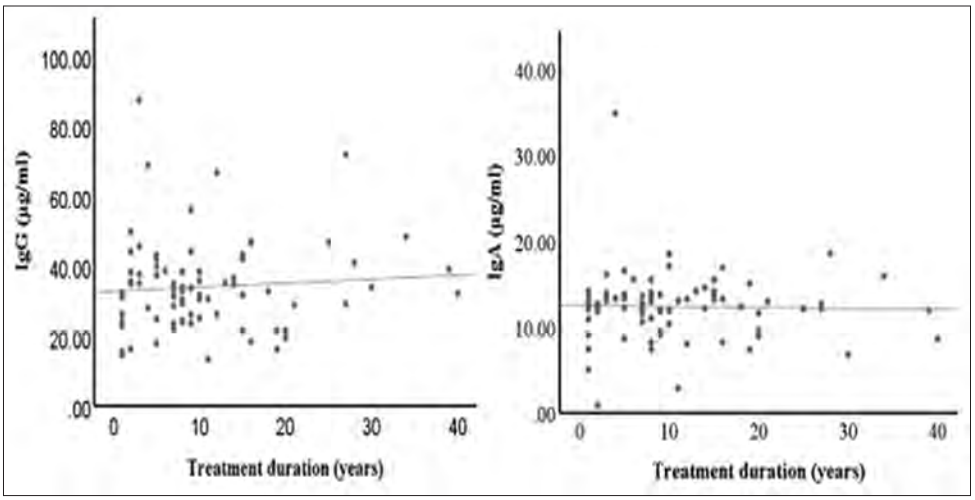

Figure 2: Scatterplot showing the correlation between salivary immunoglobulin G and immunoglobulin A versus treatment duration in diabetic participants

Table 2: Correlations between the co-morbid conditions in the diabetic patients and salivary immunoglobulin A and immunoglobulin G

Co-morbid conditions	IgA		IgG	
	Correlation coefficient	*P*	Correlation coefficient	*P*
Skin diseases	0.06	0.54	0.04	0.67
Visual Impairment	0.13	0.22	0.17	0.1
Oral infection	0.07	0.5	0.01	0.99
Hypertension	0.16	0.12	0.13	0.22

IgA: Immunoglobulin A, IgG: Immunoglobulin G

protective proteins,[30] the immunoglobulins play a major role in safeguarding the integrity of the oral mucosa; thus, any alteration in secretion or functional impairment may be contributory to the high oral infection rate in the diabetics. In this study, we found elevated levels of salivary IgG in Type 2 diabetic patients compared to those in healthy nondiabetic control group. This finding was common in most of similar studies earlier reported, either in the blood or saliva,[4,23] and it was suggested that immune response to advanced glycosylation end product, a common sequela of chronic hyperglycemia, might be the trigger.[23] Other studies, highlighting the protective function of the salivary immunoglobulins, arrogated the finding to increased immunoglobulins secretion against specific bacterial and fungal agents which occur more frequently in the diabetics.[4,5,7]

The justification for elevated salivary immunoglobulins levels in the diabetics, as stated above, would suffice if they were effective and provide commensurate protection for the oral cavity. However, in spite of the increased immunoglobulins secretion, diabetics still present with more cases of oral infection compared to the nondiabetic population,[13] this calls for a critical questioning of their functionality. A study in this regard indicated that chronicity of hyperglycemia, duration of treatment and genetic background of patients play significant roles.[31] These may be explored in elucidating the contradiction that has characterised the findings in similar studies, given that the vast majority of the studies did not account for the duration of disease exposure, with or without treatment, during

recruitment and data analysis.[9,32] Time factor may suggest a gradual but progressive deterioration in functionality and quantity of salivary immunoglobulins, until a certain period when the drop becomes apparently measurable; a prospective study would be necessary to establish this consideration.

Given that the innate and the adaptive immune system are intricately intertwined, the crosstalk between the two systems provides an efficient protection within the oral cavity.[33] However, the widespread dysfunction of the innate immune system which primarily offers the first line of defence against invading microbes, disrupts the synergy that exists between the two systems and the triggers necessary for effective immunoglobulin stimulation in the diabetics. The complement system, cytokine production and cellular activations which play important roles in immunoglobulins stimulation are also significantly dysfunctional.[34] This may explain why salivary immunoglobulins are not produced in adequate amount, or fail to secure the oral cavity even when present in sufficient quantity in the diabetics.[35,36] The initial drive executed by the innate immune system, is apparently lost or mostly ineffective, leading to a suboptimal adaptive immune response in the oral cavity.

This study showed a significant positive correlation between salivary IgA and IgG, indicating that protective proteins in the saliva move in the same direction in diabetic patients. This is not unexpected as both immunoglobulins are expected to respond to the increased susceptibility of diabetic patients to oral infection. A similar pattern has been described in patients with oral submucous fibrosis.[26] This study also showed that salivary immunoglobulins are positively correlated with the duration of diabetic treatment suggesting that immunoglobulin secretion increases over time in diabetic patients possibly to circumvent increased susceptibility to infections.

There are a few limitations to this study. Only Type 2 diabetic patients were recruited for this study; inclusion of Type 1 diabetic patients may have been more inclusive and provided a broader outcome, but the role of immune dysregulation in the pathogenesis of Type 1 diabetes could contribute a confounder.[37,38] Also, this is a cross sectional study in which no recourse was given to duration of exposure to diabetes, a longitudinal study where changes may be observed in the immunoglobulin level over a long period of time may add more flavour to the study.

Conclusion

Salivary immunoglobulin levels are higher in diabetic patients, even though prevalence of oral infection still surpasses that in the general population. This calls for a more stringent approach to management and oral hygiene in diabetic patients to ameliorate morbidity.

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Conflicts of interest

There are no conflicts of interest.

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