Salivary IgA Depression in Drug-Influenced Gingival Enlargement among Hypertensive Patients

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Salivary IgA Depression in Drug-Influenced Gingival Enlargement among Hypertensive Patients

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ABSTRACT

Drug-influenced gingival enlargement (DIGE) among hypertensive patients is commonly associated with antihypertensive drugs such as calcium channel blockers (CCBs). Immune response alteration is one of the proposed mechanisms for DIGE. Immunoglobulin A (IgA) in saliva which involves in defense mechanism was shown to be affected in patients with DIGE. Objective: This study aimed to determine the association of salivary IgA level with DIGE. Methods: This cross-sectional study comprised 47 hypertensive patients who had consumed antihypertensive drugs for at least 3 months. Twenty-one (44.7%) males and 26 (55.3%) females had participated in this study. Data was analyzed using SPSS version 26.0. The p-value of less than 0.05 at a 95% confidence interval is considered statistically significant. Results: Eighty-three percent and 17.0% of hypertensive patients were on CCBs and non-CCBs respectively. Amlodipine was found to be the most common (55.3%) antihypertensive drug consumed. Twenty-one (44.7%) patients presented with DIGE. The salivary IgA level was significantly decreased (p=0.03) among hypertensive patients with DIGE [Median 4.9 ng/mL (IQR 5.268)] compared to those without DIGE [median 15.03 ng/mL (IQR 32.246)]. Conclusion: This data indicates the level of salivary IgA was significantly affected in patients with DIGE which may compromise the defense mechanism of saliva.

Key words: drug-influenced, gingival enlargement, calcium channel blocker, salivary IgA, anti-hypertensive drugs

INTRODUCTION

Hypertension is one of the most prevalent disorders worldwide; in which by year 2025, approximately 1.56 billion people will suffer from the disease.¹² Drug-influenced gingival enlargement (DIGE) is commonly associated with adverse effects of antihypertensive drugs. This unwanted effect is frequently influenced by calcium channel blockers such as nifedipine and amlodipine.³ The clinical features of DIGE include disfiguration of the gingiva usually affecting interdental papilla region which results in lobulated or nodular morphology. If not treated, the condition may lead to further deterioration of existing periodontal disease which includes attachment loss or tooth mobility.⁴ The aetiology of DIGE is not completely understood but it is thought to be multifactorial.⁶ The underlying pathogenesis of DIGE involves non-inflammatory and inflammatory pathways.⁷ In the non-inflammatory pathway, it is hypothesized that the decrease in cation influx of folic acid active transport within gingival fibroblasts leads to decreased cellular folate uptake, which in turn leads to changes in matrix metalloproteinases metabolism and failure to activate collagenase. Decreased availability of activated collagenase results in decreased degradation of accumulated connective tissue which presents as gingival enlargement.³
Immunoglobulin A (s-IgA) not only mediates the genitourinary tract, as well as prostate, colostrum, tears, secretions from the respiratory tract, mostly in mucosal secretions including saliva, milk, of the body’s total immunoglobulin. IgA is found isotype in the body, comprising almost 70 percent of the body’s total immunoglobulin. IgA is the most abundant antibody isotype in the body, comprising almost 70 percent of the body’s total immunoglobulin. IgA is found mostly in mucosal secretions including saliva, milk, colostrum, tears, secretions from the respiratory tract, genitourinary tract, as well as prostate. Salivary immunoglobulin A (s-IgA) not only mediates the humoral immune response to regulate caries activity but also interferes with the formation of caries causing microbial adhesion to the tooth surface and biofilm. It has been hypothesized that drug-influenced gingival enlargement altered the mechanisms of the host’s immune response causing an increase in gingival volume and its chronic use lead to a reduction in serum and salivary IgA, subsequently causing inflammation of the periodontal tissues.

Decrease in salivary IgA levels was accounted for the cause of gingival enlargement induced by phenytoin as well as alteration of subgingival microflora. This suggests that the phenytoin drug could be the cause for terminal differentiation failure of IgA bearing cells. However, limited data has been reported on immune changes in the patient taking calcium channel blockers (CCBs) influenced gingival enlargement. Therefore, the current study aimed to determine the association of salivary IgA level with the presence of DIGE. This relationship could provide additional information regarding s-IgA level in hypertensive patients and understanding its association with the mechanism of DIGE particularly in patients taking CCBs.

**METHODS**

This is a cross-sectional study which was conducted at the Medical Specialist Clinic and Dental Clinic, Hospital Universiti Sains Malaysia, Kelantan, Malaysia involving 47 hypertensive patients. Patients were selected by convenient sampling on a voluntary basis. All hypertensive patients aged 18 years old who were on regular doses of antihypertensive drugs for a minimum of 3 months prior to the study were included. Exclusion criteria include edentulism, pregnant mother, patients who had undergone periodontal treatment within 6 months prior to the study, patients who were on medications known to cause gingival enlargement such as phenytoin or having systemic disorders which known to affect the gingiva such as leukemia.

All patients were briefed regarding the study procedures and informed written consents were obtained. The demographic data, medical history, drug history were retrieved from medical record and oral clinical examination was performed.

**Saliva collection**

About 1.5 milliliters of unstimulated whole saliva samples were collected from all patients. The saliva samples were collected on one occasion under resting conditions during morning hours, between 9am to 12noon after cleaning their mouth. Patients were requested to keep their mouth closed for 5 minutes in order to pool the saliva, then spitted into a container. The saliva samples then were immediately sent to saliva samples were collected on one occasion under resting conditions during morning hours, between 9am to 12noon after cleaning their mouth. Patients were requested to keep their mouth closed for 5 minutes in order to pool the saliva, then spitted into a container. The saliva samples then were immediately sent to the laboratory and frozen at -80°C before analysis. Hypertensive patients who presented with DIGE,
periodontal disease or other dental problems were referred for further treatment. This study protocol was approved by Human Research Ethics Committee USM(USM/JEPeM/18090407).

Salivary IgA measurement

The s-IgA levels were determined using a commercial kit of Enzyme-linked Immunosorbent Assay (ELISA) test for IgA: Homo sapiens (Human) (Cloud-Clone Corporations, USA). The microplate provided has been pre-coated with an antibody specific to IgA. The samples were prepared in triplicates to avoid any inconsistency or variation of the test. Standard or samples were added into appropriate microplate wells with a biotin-conjugated antibody specific to IgA. Avidin conjugated to Horseradish Peroxidase (HRP) was added into each microplate well and incubated. After Thio- Methylene Blue substrate solution was added, only those wells that contain IgA, biotin-conjugated antibody and enzyme-conjugated Avidin showed a change in colour. The enzyme-substrate reaction was terminated by the addition of sulphuric acid solution. The colour change was equal to the concentration of IgA in the sample. The s- IgA concentration was measured spectrophotometrically at the wavelength of 450 ± 10nm. The IgA concentration of more than 0.07ng/mL was considered positive.

Data Analysis

Data were analyzed by using the Statistical Package for the Social Sciences (SPSS) version 26.0. Data checking and cleaning were performed before analysis. Descriptive analysis were presented as mean (SD) or median (interquartile range, IQR) for continuous variables, and frequency and percentage (%) for categorical variables. Mann-Whitney U test was used to compare the levels of s-IgA in hypertensive patients with and without DIGE. The data was not normally distributed based on Shapiro-Wilk Normality Test (p=0.001). Hence, the non-parametric test, Mann-Whitney U Test was employed. The level of s-IgA is expressed in median and interquartile range (IQR). The mean s-IgA levels in DIGE group was significantly low (4.90 ± 5.27 ng/mL) compared to non-DIGE group (15.03 ± 32.25 ng/mL), with p=0.033.

RESULTS

Forty-seven hypertensive patients with a mean age of 55.7 ± 10.0 years were included in this study. Majority of them were Malays (97.6%) comprised of 21 (44.7%) males and 26 (55.3%) females. Seventy percent of patients had consumed antihypertensive drugs for more than 5 years. Amlodipine was the commonest drug consumed (55.3%) followed by felodipine (25.5%) and nifedipine (2.1%). More than half (59.6%) of patients had other systemic diseases such as diabetes mellitus, dyslipidemia, ischemic heart disease, and lung disease which were under control and on regular follow up (Table 1).

Almost half of the patients (44.7%) presented with DIGE. Table 2 shows the comparison of the s-IgA levels between patients with and without GE. The data was not normally distributed based on Shapiro-Wilk Normality Test (p=0.001). Hence, the non-parametric test, Mann-Whitney U Test was employed. The level of s-IgA is expressed in median and interquartile range (IQR). The mean s-IgA levels in DIGE group was significantly low (4.90 ± 5.27 ng/mL) compared to non-DIGE group (15.03 ± 32.25 ng/mL), with p=0.033.

DISCUSSION

The precise mechanism by which DIGE occurs is not completely understood, although a number of hypotheses have been suggested. Three classes of drugs which are well-known to cause gingival enlargement in most cases, namely antihypertensive
drugs such as CCBs, immunosuppressant cyclosporine and anti-convulsant drug such as phenytoin. Drug-influenced gingival enlargement was first observed in patients taking phenytoin for epilepsy, with approximately 50.0% having gingival overgrowth. Cyclosporin is an immunosuppressant which has been reported to cause gingival enlargement in 25.0% to 80.0% of patients. Among the CCBs, the dihydropyridines such as nifedipine, felodipine, and amlodipine tend to be more commonly associated with gingival enlargement. The particular property that is common to these classes of drugs is that they directly affect the cellular calcium metabolism. Since cellular production of collagenase is modulated by calcium influx, fibroblasts from patients treated with these drugs may produce an inactive form of collagenase, leading to an increase in extracellular matrix deposition subsequently causing gingival enlargement.

The decrease in calcium influx in patients taking CCBs is due to alterations in calcium-sodium exchange, which causes a decrease in cellular folic acid uptake, thus, limiting the production of the collagenase-activating enzyme. Since the presence of inflammation secondary to dental plaque causes proliferative increase in connective tissue, the catabolic ability of collagenase is saturated, and the inhibited degradation of the extra cellular matrix causes a local accumulation of this matrix. Meanwhile, the inflammatory changes within the tissue may enhance the interaction of calcium and fibroblast cells. Oral microbes’ antigens are continuously being affected by the two major antibody classes in saliva; salivary IgA and IgG. There are two possible sequences of DIGE events. Firstly, in patients with existing gingival inflammation, consuming antihypertensive medication may further exacerbate the DIGE. It is now well established that gingival inflammation is an essential cofactor in the expression of DIGE. Secondly, there are occasions where patient with no existing gingival inflammation revealed DIGE after taking antihypertensive drugs. However, there are several studies that could not determine the true sequence of events with respect to the role of gingival inflammation in the development of DIGE.

The secretory immune system is referring to a complex regulation of immune cells in which it influences distinctly the activity of various cell types involved in s-IgA formation. Genco et al. has reported that immune complexes are formed during gingival inflammation. They suggested that plaque antigens entering gingival connective tissue combine with antibody forming antigen-antibody complexes and deposited in the tissue; or if they have an affinity for a tissue component, it will localize in this component. These antigen-antibody complexes were believed to enhance and block the local cellular immune reactions in gingiva during periodontal inflammation.

In this present study, s-IgA is significantly reduced in hypertensive patients with DIGE (p=0.033). A decrease in s-IgA antibody level was also reported by previous studies in patients taking phenytoin. As a consequence, patients taking this medication may be at greater risk for acquiring oral microbial infection. This finding is supported by another study which showed s-IgA deficiency in saliva and serum compared to IgM antibody, however, no statistically difference in the three types of tissue assayed; control, idiopathic gingival hyperplastic tissues and phenytoin influenced gingival enlargement.

In addition, the adaptive humoral immunity system at the mucosal surface is mediated by secretory IgA antibodies. On top of that, TGF-β, and interleukin-10 (IL-10) together with interleukin-4 (IL-4) have been shown to promote B cells to switch to IgA and differentiate into IgA-producing cells. It is believed that the decrease in salivary IgA in hypertensive patients with GE could be predominantly influenced by the diminution of TGF-β. Trackmann et al, reported that the effect of TGF-β, which has an unexpectedly less magnitude of regulation compared to other connective tissue cells which were performed under the same conditions of DIGE.

To understand the less magnitude of TGF-β on extracellular matrix synthesis in gingival fibroblasts, the researchers in recent studies have shifted and more focused on the presence and role of connective tissue growth factor (CTGF) as a possible matrix stimulatory factor regardless of TGF-β in gingival enlargement tissues. CTGF has been proposed to mediate the effects of TGF-β on extracellular matrix metabolism. Therefore, it can be proposed that the decrease in s-IgA levels among hypertensive patients with GE could be due to the effect of less magnitude TGF-β regulation, subsequently leads to reduce function of B cells in class-switching of IgA to IgG producing cells.

This study faced several limitations. First, the small sample size in this study was not enough to represent the entire antihypertensive drug-influenced GE population, so our findings cannot be generalized to any other groups. Another limitation was that the saliva samples were only collected after exposure of the antihypertensive drugs with various duration of the treatment. Hence, we could not identify the difference on each subject before and after exposure of antihypertensive drugs. We recommend to include larger sample size in future study to obtain more convincing findings. In addition, it is essential to determine the exact role of s-IgA in the pathogenesis of drug influenced gingival enlargement. CTGF and TGF-β also could be investigated and correlate with the quantification of s-IgA since both cytokines may have an effect towards the decrease in s-IgA level.
CONCLUSION
The s-IgA antibody levels were significantly reduced in hypertensive patients with DIGE which may compromise the defense mechanism of saliva. However, the s-IgA does not exclusively regulate the pathway of DIGE but could be served as a future potential biomarker in assessing gingival enlargement in hypertensive patients.

CONFLICT OF INTEREST
The authors declare no conflict of interest.

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