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Research Article

Total Salivary Immunoglobulin A Determination Before and After Non-Surgical Periodontal Treatment in Patients with Aggressive Periodontitis

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Abstract

Introduction

Aggressive periodontitis (AP) is an inflammatory disease with rapid periodontal attachment and bone loss in healthy individuals. It exists as Localized aggressive periodontitis (LAP) and Generalized aggressive periodontitis (GAP). They raise total salivary immunoglobulin A(IgA) levels. This study aimed at determining IgA in AP patients compared with controls before and after non-surgical debridement.

Materials and Methods

Consented 37 Ghanaians aged 20-50 years (19 cases and 18 controls), were recruited. Questionnaire was used and periodontal examination was done with Orthopantomogram taken. Unstimulated saliva was collected before and after non-surgical periodontal therapy (NSPT). Centrifuged Samples were stored at -800C. Human IgA ELISA kits were used to determine

levels.

Results

The mean concentration of total salivary IgA before treatment for the cases was 90.3 ± 33.1 (μg/ml) and controls was 92.0±29.4 (μg/ml). After treatment, the concentration of IgA among the cases was 90.5±31.7 (μg/ml) and controls was 84.6±31.7 (μg/ml). There was no significant difference in the mean IgA values between cases and controls as well as before and after treatment (P>0.05).

Conclusion

There was no significant difference in the total salivary IgA levels in participants with AP and the control group before and after treatment. But there was a significant drop in the total IgA levels for the LAP participants after treatment.

Keywords: Saliva; Salivary proteomic biomarker IgA; Localized aggressive periodontitis; Generalized aggressive periodontitis

1. Introduction

Aggressive periodontitis is an inflammatory disease characterized by rapid periodontal attachment loss and bone destruction in otherwise systemically healthy individuals [1]. Individuals with this disease have a rapid rate of disease onset and progression. These patients can be clinically differentiated by the extent of destruction and by age [2]. There are two clinical varieties of Aggressive periodontitis (AP), the Localized aggressive periodontitis (LAP) and the Generalized aggressive periodontitis (GAP). The LAP disease usually has an age onset at about puberty and is clinically characterized by interproximal attachment loss on at least two permanent teeth, one of which is a first molar, and involving no more than two other teeth, aside from the first molars and incisors [2,3]. The GAP disease usually affects individuals under the age of thirty, even though older patients may be affected [2]. Clinically, GAP is characterized by generalized interproximal attachment loss affecting at least three permanent teeth other than first molars and incisors [2]. *Aggregatibacter actinomycetemcomitans and Porphyromonas gingivalis* bacteria have been associated with both the localized and generalized aggressive forms of periodontitis in teenagers and young African adults [3,4]. It has been suggested that effective treatment of patients with AP requires non-surgical, surgical or both treatments with or without antibiotics. A study has shown that mechanical treatment combined with antibiotic treatment, provides better clinical outcomes in this group [5]. Arowojolu and Nwokorie [6] recorded an AP prevalence of 0.8-1.6% in Nigerian teenagers and young adults. Harley and Floyd [7] also reported a prevalence of 0.8% among Nigerian teenagers with AP. In the United States, a national survey of adolescents aged 14 to 17 years reported that 0.53% had LAP and 0.13% had GAP [8]. The report also revealed that blacks were at much higher risk than whites for both forms of the disease, and that male teenagers were more likely to have GAP than female adolescents.

Saliva, an intraoral fluid, is secreted by the three pairs of major salivary glands, the parotid, submandibular and sublingual and by numerous minor salivary glands situated under the tongue, buccal mucosa and the palate except the anterior part of the hard palate and gingivae. These minor glands are said to number between 450 and 750 [9]. Saliva has been used in the past few decades as a diagnostic fluid in the following; hereditary diseases, oral diseases including periodontal disease, autoimmune diseases, malignancies and in

infections, as well as in monitoring levels of hormones and drugs, bone turnover, biologic markers and in providing forensic evidence [10]. Saliva contains locally produced microbial and host response mediators, as well as systemic serum markers that could aid the diagnosis of periodontal and other diseases [11]. The pathogenesis of periodontitis is reasonably well established at the clinical and microscopic levels, but the details of the specific mechanisms are still being defined [12]. Bacterial plaque is essentially what triggers periodontitis and the clinical signs that follow are a result of the activated inflammatory and immune responses rather than from the direct effect of the bacteria themselves [12]. The relationship between saliva and the pathogenesis of periodontal disease is, therefore, indirect. Salivary markers that have been studied as potential diagnostic tests for periodontal disease include proteins of host origin such as enzymes and immunoglobulins, phenotypic markers, host cells, hormones (especially cortisol), bacteria and bacterial products, ions and volatile compounds [11]. Host-derived enzymes and other inflammatory mediators originating from gingival crevices appear to hold promise as salivary diagnostic aids in periodontal disease [11]. The use of proteomic biomarkers, especially IgA, have been considered to have good diagnostic promise through the determination of their levels in saliva especially in the presence of oral infections, when compared to other genomic and microbiological markers [5]. The various biomarkers which exist in saliva can be classified into: Proteomic, Genomic and Microbiological markers [13]. This study focuses on the use of the salivary biomarker, IgA to determine the presence or absence of LAP or GAP.

1.1 Aim and Objectives

The aim of this study was to determine the level of IgA, a salivary proteomic biomarker, in participants with AP and in a group of individuals without the disease, before and after non-surgical periodontal therapy. The following specific objectives were set for the study; to determine the level of IgA in participants with aggressive periodontitis as well as a control group, to determine the level of IgA in participants with AP before and after non-surgical periodontal therapy (NSPT) and finally to determine the level of this biomarker in the localized and generalized forms of aggressive periodontitis before and after treatment.

2. Methods

The study was an interventional prospective design. Ethical approval (CHS-Et/M.8-P 4.8/ 2015-2016) was obtained from the Ethical and Protocol Review Committee of the College of Health Sciences, University of Ghana. The sample size was estimated based on effect size in terms of standard deviation of the sample differences of 0.76 as reported in a study by Pagano and Ganvreau [14]. Using the formula for comparisons of two means at significant level of 0.05 and statistical power of 0.8, a minimum of thirty-seven (37) individuals were estimated but forty participants were recruited initially. Power analysis at the end of the study gave a value of 0.83. Three people did not have complete data therefore only 37 participants with complete data were included in the analysis. This was made up of 19 cases and 18 controls. Respondents aged 12-50 years who had LAP and GAP were recruited. A total of thirty-seven (37) individuals who consented and met the inclusion criteria according to Armitage[1] were selected. Participants with systemic disorders such as diabetes, hypertension, leukemia, genetic disorders, HIV/AIDS and patients with any past medical history of other

chronic diseases, smokers and those who drunk alcohol frequently were all excluded. The participants were selected consecutively from May 2016 - November 2016 at the Oral Diagnosis unit of the Dental Clinic of the University of Ghana Dental School, University of Ghana. Nineteen (19) participants were clinically and radiographically diagnosed as having AP and 18 participants who did not show evidence of the disease or other diseases served as controls. The sociodemographic data of all the participants were captured using a structured questionnaire. The cases had four (4) clinical visits whiles the controls had three (3). At the first visit, a comprehensive periodontal examination was done and an orthopanthomogram radiograph of each participant was taken. The clinical examination of all the cases and controls with AP was carried out using a periodontal chart. A protocol and guidelines for saliva collection by Wang et al.[9] was used. At the second visit, unstimulated whole saliva was expectorated into falcon tubes provided every minute under ice in a mug over a period of 15mins. At least 3-5mls of saliva was collected for each participant. The samples were kept at a temperature of -21^{0}C after which they were transported to the laboratory for centrifugation. A full mouth scaling and polishing for controls and root planing for cases was carried out. For the cases, 10mls of 0.2% Chlorhexidine gluconate mouthwash was used to irrigate all probing pocket depths (PPD) ≥ 4mm's and the solution gargled for one minute and expectorated. Oral hygiene instructions were given and the controls seen at four weeks (at the third visit) after which the saliva samples were collected again using the same protocol and the comprehensive periodontal examination repeated. The cases were seen twenty-four (24) hours for the second session of scaling and root planing (at the third visit) and then at four (4) weeks for re-evaluation fourth visit. Saliva samples were collected again at this time using the same protocol and a comprehensive periodontal examination done. The samples were transferred into a centrifuge by a laboratory technician and run at 2,500 rpm for 25min at 4°C to remove insoluble materials, cell and food debris. The supernatants were carefully decanted into four (4) cryotubes and the pellets placed into one tube. Pierce protease inhibitor cocktail EDTA-Free (5µl) was added to the supernatants and mixed. The samples were then transferred and stored in a −80 °C freezer after which laboratory analysis was carried out. *The materials and methods presented are similar to an article submitted pending publication in another journal with a different topic but based on the same population studied.*

2.1 Total Salivary IgA measurement by ELISA

The laboratory work for IgA determination was done by specialized laboratory technicians at the Noguchi Memorial Institute for Medical Research (NMIMR) of the College of Health Sciences, University of Ghana, Legon. Immunoglobulin A levels in the saliva samples were measured using an in-house optimized Sandwich ELISA procedure based on the Human IgA Ready-Set-Go kit eBioscience (Catlog number: 88-50600) and following the manufacturer's instructions. Analyses of the samples were done in two (2) days and the tests were done in duplicate. For colour development, 100 µl/well of a chromogenic substrate solution $3,3^{1},5,5^{1}$ tetramethylbenzidine (TMB) was added and incubated for fifteen (15) minutes in the dark. Only those wells that contained sIgA, biotinylated detection antibody and Avidin-HRP conjugate appeared blue in color. The enzyme-substrate reaction was terminated by the addition of sulfuric acid solution (0.2 N of H_2SO_4) in 100µl/well and the color turned yellow. The samples were transferred to an ELISA plate reader (BioTek, VT, USA) and optical density (OD) read at 450 nm.

Optical density data was converted to sIgA concentration using a 4-parameter logistic curve fit.

2.2 Data Analysis

Data was analyzed using Statistical Package of Social Sciences (SPSS version 22). Chi-square test was used to compare proportions of socio-demographic data. Mean sIgA levels were compared using T- test for two means (cases and controls) and ANOVA for more than two means (GAP, LAP and controls). Significance level was set at 0.05.

3. Results

The demographic characteristics of participants and the various subgroups are presented in table 1 below. The mean age of the GAP was 33.80 ± 8.93, LAP was 32.11 ± 8.07 and controls was 31.39 ± 8.96 years. There were no significant differences in the demographic characteristics between cases and controls.

	Participant category				
Demographics	**GAP n (%)**	**LAP n (%)**	**Controls n (%)**	**Total N (%)**	**P-Value**
Gender					
Male	4(40.0)	6(60.0)	10(55.6)	20(54.0)	0.06
Female	6 (60.0)	3(40.0)	8(44.4)	17(46.0)	
Total	10 (100.00)	9(100.00)	18(100.00)	37(100.00)	
Mean age ± Mean SD	10(33.80 ± 8.93)	9(32.11 ± 8.07)	18(31.39± 8.96)	37(32.22 ± 8.56)	0.78
Marital Status					
Never Married	6(60.0)	7(77.8)	12(66.7)	25(67.6)	0.84
Married	3(30.0)	2(22.2)	3(16.7)	8(21.6)	
Divorced	1(10.0)	0(0.0)	2(11.0)	33(8.1)	
Separated	0(0.0)	0(0.0)	1(5.6)	1(2.7)	
Widowed	0(0.0)	0(0.0)	0(0.0)	0(0.0)	
Tribe					
Ga/Dangme	3(30.0)	1(11.1)	3(16.7)	7(18.9)	0.57
Akan	5(50.0)	4(44.4)	12(66.7)	21(56.8)	
Ewe	1(10.0)	3(33.3)	2(11.0)	6(16.2)	
Northern	1(10.0)	1(11.1)	0(0.0)	2(5.4)	
Other	0(0.0)	0(0.0)	1(5.6)	1(2.7)	
Religion					
Christian	10(100)	8(88.9)	18(100.00)	36(97.3)	
Islam	0(0.0)	1(11.1)	0(0.0)	1(2.7)	0.2
Traditional	0(0.0)	0(0.0)	0(0.0)	0(0.0)	
Educational background					
No Formal Education					
Completed only Primary education	0(0.0)	0(0.0)	1(5.6)	1(2.7)	
Completed only Secondary education					0.25
Completed Tertiary education	2(20.0)	0(0.0)	1(5.6)	3(8.1)	

Total	7(70.0)	5(55.6)	7(38.8)	19(51.4)	
	1(10.0)	4(44.4)	9(50.0)	14(37.8)	
	10(100.00)	9(100.00)	18(100.00)	37(100.00)	

Table 1: Sociodemographic characteristics of all respondents

Control participants did not have any periodontal condition but came for routine dental checkups. All cases had aggressive periodontitis. The descriptive summary of the total IgA levels are reported in the table 2 below. There was no significant difference in the total IgA concentrations of the cases and controls before and after treatment with p=0.98 and 0.58 respectively. There was no significant difference in the total IgA concentration of the GAP group before and after treatment p = 0.08. There was a significant change in the total IgA concentration of the LAP group before and after treatment, p = 0.04.

		Concentrations of IgA (µg/ml)	
Participant category		**Before treatment**	**After treatment**
Cases (N=19)	Mean (SD)	90.32 ± 33.09	90.51± 31.69
Controls (N=18)	Mean (SD)	92.01 ± 29.37	84.56±31.69
	P-value	*0.98*	*0.58*
GAP (N=10)	Mean (SD)	74.70 ± 24.89	94.22 ± 27.96
	P-value		*0.08*
LAP (N= 9)	Mean (SD)	109.41 ± 32.88	85.98± 36.96
	P-value		**0.04**

Table 2: Summary of salivary IgA levels

4. Discussion

This study was conducted primarily to determine the levels of immunoglobulin A in saliva and to find out if there was any association between IgA levels in participants with AP at baseline and after non-surgical periodontal therapy. Not much reports have been published on AP in relation with IgA among the sub-Saharan African population. The present study was therefore regarded as part of an early effort to determine whether a link exists between AP and the concentration of IgA. It is important to note that the sample size used in this study compares with what some other investigators worked on. For example, in a study by Hagewald [15], there were thirty-eight (38) participants aged between twenty-five (25) and thirty-eight (38) years. Nineteen (19) of those participants had AP and an equal number nineteen (19) of periodontally healthy controls were included. The mean age of participants with AP was 33.3years while in the control group was 32.9 years. The average age of participants with GAP and LAP in this present study was 33.80 ± 8.93 years and 32.11 ± 8.07 years respectively, whilst that for the controls was 31.39± 8.96 years. In a study by Hua [16], the age of the participants with AP was also 33.5yrs, close to that in this study, even though the two separate groups of AP cases were not mentioned in Hua's study. The sex distribution of the participants was not indicated in

some of the studies reviewed for this paper. In the present study, this was taken into consideration. There were twenty (20) males and seventeen (17) females. Ten (52.6%) and nine (47.4%) of the males and females had AP. Four (4) males and six (6) females had the generalized type of AP whilst six (6) males and three (3) females had the LAP type (Table 1). As noted earlier, the results of the present study did not reveal significant differences in IgA levels based on sex or on marital status. Neither was there any evidence of association with tribe. IgA concentration depends primarily on the local secretion from the salivary glands and transepithelial transportation which is controlled by secretory immune mediators, expression of epithelial receptors, and intraepithelial cAMP [17]. The second source of IgA in saliva is the plasma IgA entering the oral cavity by gingival crevicular fluid [17]. Total saliva is suitable for evaluating the overall humoral immune response in the oral cavity in patients with chronic diseases such as AP especially the generalized disease type pattern [17]. The method developed for quantification of IgA was sensitive enough to measure IgA antibodies in the saliva samples. The concentration of IgA before treatment for the cases was slightly increased after treatment. For the controls there was a slight drop of IgA after treatment but in both groups the changes were not significant (Table 2). This implies that the periodontal condition did not show any significant association with the IgA levels in the saliva. The results obtained in this study are, to some degree, also similar to those in a study by other researchers like Henskens et al [18]. In their work, no significant changes were noted in the total IgA concentration nor in the *P. gingivalis*-reactive IgA group during mechanical treatment or after antibiotic treatment of the participants. In this study by Henskens et al. [18], IgA variables were independent of the clinical variables and they were of no diagnostic or prognostic value. Antibiotic treatment also did not affect the IgA levels. Studies by Blanchard et al. [19], Kinane et al. [20], and Guo et al. [21], showed variability in IgA proteins and specific antibody responses against *P. gingivalis* among periodontitis patients. Longitudinally, after scaling and root planing (SRP), the clinical variables further improved but were not correlated with IgA responses. These findings are similar to those obtained in the present study in both cases and controls. In a study by Hagewald et al in 2002 [15] on the salivary IgA subclasses and bacteria-reactive IgA in patients with AP, the levels of total salivary IgA differed widely from those of the controls. The total IgA concentration in the AP group was significantly reduced at baseline level when compared to the control group ($p < 0.01$). The mean concentrations recorded for the resting saliva for the cases and controls were 40.4 and 169.7µg/ml respectively. In this current study, there was no significant difference between the cases and controls. In an earlier study by Hagewald et al 2000 [17], the resting salivary IgA for the GAP group recorded was 121.2µg/ml (range 22.2-627.7µg/ml). The concentrations recorded for the GAP group before and after treatment in this study fell in the same range as was stated by Hagewald [17]. In the same study, the total IgA concentration in the GAP participants was lower when compared to the control group. In the current study, mean concentration of IgA before treatment among the LAP group was 109.41 µg/ml and decreased to 85.98µg/ml after treatment. The results obtained were statistically significant ($p=0.04$) (Table 2). Participants with periodontitis were found to have high salivary concentrations of IgA, IgG and IgM specific to periodontal pathogens compared with healthy patients in a study by Kathariya [13] and after treatment there was a reduction in the levels of these immunoglobulins [13]. Even though the specific group

of periodontitis patients were not mentioned in the study by Kathariya [13], the LAP group in this study produced similar findings. The significant drop in IgA levels in the LAP after treatment may be due to varied reasons. There are individual differences in host response to infective agents to the levels of IgA produced in the different participants and to the genetic makeup of the individual [15]. Oral hygiene as well as the nutritional status of the individual could possibly play a role in the immune responses to *P. gingivalis* and other putative microorganisms. Another report has a similar opinion that a rise in the humoral immune response to plaque-associated microorganisms is detectable in the presence of increasing extent of periodontitis [22]. Participants with acute disease had higher specific IgA titers in saliva compared to patients with chronic disease [23,24]. In this study, the initial total IgA concentration for the LAP cases was higher than that of the GAP group. When patients receive mechanical treatment, this should result in a reduction in the bacterial flora load in periodontal pockets before recolonization of bacteria. Although there had been no baseline data on antigen load, the result could be indicative of a dependency on antigen load and quantity of IgA protein present, as suggested by MacPherson [25]. Salivary immunoglobulins in patients with juvenile periodontitis and their healthy siblings were studied by Sandhlom et al. [22]. The concentrations of salivary immunoglobulin IgA were determined with a solid phase radioimmunoassay in the unstimulated whole saliva of twenty-one (21) patients with juvenile periodontitis (JP), twenty-seven (27) healthy siblings and seventeen (17) healthy age-matched controls. In the JP group, the concentrations of IgA were increased as compared to their healthy siblings in the controls. In this study, the analysis of total IgA concentration was done using an ELISA kit. The initial concentration of total IgA among the cases in my study was lower than that recorded in the controls. In the periodontal pocket, there are numerous antigens from oral microorganisms which can modulate the humoral host defense by many mediators [26,27]. These data pose a strong point to explain contradictory reports on the relationship periodontal disease and IgA antibodies in saliva [18,28]. Differences in antigen load might also help to explain immune tolerance [28,29] and failure of induction of significant levels of salivary antibodies after oral ingestion of an antigen [30], or after recolonization of the periodontal region [26]. To solicit immune response, a certain antigen load must be detected. The immune responses may differ due to the antigen load that may lead to the production of the IgA.

4.1 Recommendations

The study showed a significant drop in IgA among LAP as a response to treatment. Prompt intervention is recommended after LAP is detected to improve the overall outcome and better prognosis of treatment. Numerous markers in saliva have been used as prognostic, diagnostic and therapeutic monitoring indicators for periodontal disease with high specificity and sensitivity. Innovative techniques such as the lab-on-a chip microfluidic devices have the potential to determine the periodontal disease risk profile of patients, predict disease activity and response to therapeutic intervention. Although challenges remain, the use of saliva as a diagnostic prognostic fluid appears promising for future application to diagnose periodontal disease.

5. Conclusion

There was no significant difference in the total salivary IgA levels in participants with AP and the control group before and after treatment however, there was a significant drop in the total IgA levels for the LAP

participants after treatment. This was not the case in the GAP group.

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