

DETERMINATION OF TOTAL SALIVARY IMMUNOGLOBULIN A AND SALIVARY LACTOFERRIN LEVELS BEFORE AND AFTER NON-SURGICAL PERIODONTAL TREATMENT IN PATIENTS WITH AGGRESSIVE PERIODONTITIS.

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INTRODUCTION

Aggressive periodontitis (AP) is an inflammatory disease condition of the periodontium, characterized by rapid periodontal tissue attachment loss and alveolar bone destruction in otherwise systemically healthy individuals. ¹There are two clinical varieties of AP: the Localized aggressive periodontitis (LAP) and the Generalized aggressive periodontitis (GAP) forms.

LAP usually has an age onset at about puberty and is clinically characterized by interproximal tissue attachment loss on at least two permanent teeth, one of which is a first molar, and involving no more than two teeth other than the first molars and incisors". AGAP usually affects individuals under age thirty, but older patients may be affected. Clinically, GAP is characterized by "generalized interproximal tissue attachment loss affecting at least three permanent teeth other than the first molars and incisors." The bacteria Aggregatibacter actinomycetemcomitans and Porphyromonas gingivalis have been associated with LAP and GAP in teenagers and young African adults. 3.4

Arowojolu and Nwokorie⁵ recorded a prevalence of 0.8-1.6% in Nigerian teenagers and young adults with aggressive periodontitis. Harley and Floyd⁶ also reported a prevalence of 0.8% among Nigerian teenagers with aggressive periodontitis. In the United States, a national survey of adolescents aged 14 to 17years reported that 0.53% had LAP⁷ and 0.13% had GAP⁷. In addition, blacks were at much higher risk than whites for all forms of aggressive periodontitis, and male teenagers were more likely to have GAP than female adolescents.

Immunoglobulin A and Salivary lactoferrin levels are raised in patients with aggressive periodontitis **9

Saliva is secreted mainly by three pairs of major salivary glands (parotid, submandibular and sublingual) and numerous minor salivary glands (450-750). ¹⁰ Immunoglobulin A and lactoferrin are biomarkers secreted in saliva. Saliva can be used to monitor the levels of hormones, drugs and medications, bone turnover, biologic markers, forensic evidence and oral diseases which include caries and periodontal disease. ¹¹ Human saliva is a plasma ultra-filtrate and contains proteins either synthesized in situ in the salivary glands or derived from blood and contains biomarkers derived from serum, gingival crevicular fluid, and mucosal transudate. ¹²

The use of proteomic biomarkers such as IgA and sLf including other biomarkers have been found to have diagnostic and prognostic value with promising outcomes in the near future. The aim of the present study was to determine and compare levels of IgA and sLf in individuals with aggressive periodontitis and control participants. The objective of this study includes determination of the level of IgA and sLf in the cases and controls before and after non-surgical periodontal therapy.

AIM

The aim of this study was to determine the salivary levels of IgA and lactoferrin in participants with aggressive periodontitis and compare the levels in individuals with this disease and controls (those without the disease) before and after non-surgical mechanical debridement.

OBJECTIVES

- 1. To determine Immunoglobulin A and salivary lactoferrin levels in aggressive periodontitis participants before treatment.
- 2. To determine levels of Immunoglobulin A and salivary lactoferrin in control participants who present at the dental clinic before treatment.
- 3. To determine the level of Immunoglobulin A and salivary lactoferrin in participants with aggressive periodontitis after treatment.
- ${\bf 4.} \, {\bf To} \, {\bf determine} \, {\bf the} \, {\bf level} \, {\bf of} \, {\bf these} \, {\bf biomarkers} \, {\bf in} \, {\bf control} \, {\bf participants} \, {\bf after} \, {\bf treatment}.$

METHODOLOGY

The study was an interventional and prospective design. Ethical approval was obtained from the Ethical and Protocol Review Committee of the College of Health Sciences, University of Ghana. Respondents aged 12-50 years who had LAP and GAP were recruited.

A total of 37 individuals who consented and met the inclusion criteria according to Armitage were selected.

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 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 clinical visits whiles the controls had three. 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 controls and cases with aggressive periodontitis was carried out using a periodontal chart. A form was designed for entry of details of saliva collection and a protocol and guidelines for saliva collection by Wang et al was provided and explained. 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 $^{\circ\text{c}}$ 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 $controls\, seen\, at four\, weeks\, (third\, visit)\, after\, which\, the\, saliva\, samples\, were\, collected\, again\, using\, the saliva\, samples\, were\, collected\, again\, using\, the\, saliva\, samples\, the\, saliva\, samples\, were\, collected\, again\, using\, the\, saliva\, samples\, the\, saliv$ the same protocol and a comprehensive periodontal examination repeated. The cases were seen twenty-four hours for the second session of scaling and root planing (third visit) and then at four 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 cryo tubes and the pellets placed into one tube. Pierce protease inhibitor cocktail EDTA-Free (5 μ l) was added to the supernatants and mixed. The samples were then transferred and stored in a -80 °C freezer awaiting laboratory analysis.

The laboratory work for IgA and salivary lactoferrin 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.

Total Salivary IgA measurement by ELISA

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 days and the tests were done in duplicate.

For colour development, 100 μ l/well of a chromogenic substrate solution 3,3¹,5,5¹ tetramethylbenzidine (TMB) was added and incubated for 15 minutes in the dark. Only those wells that contained slgA, 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 H₂SO₄) in 100 μ l/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.

Salivary Lactoferrin measurement by ELISA

Salivary lactoferrin was quantified using the Human Lactoferrin ELISA kit (ab108882- Lot: GR232543-29 Lactoferrin (HLF2) based on the manufacturer's instructions. Saliva samples and lactoferrin standards were added to the plate in duplicate. For colour development, 50 μ l/well of chromogen (3,31,5,51-tetramethylbenzidine (TMB) was added with the samples turning blue

and the plate incubated in the dark for 15 mins. The colour reaction was subsequently stopped by the addition of 50 µl/well of stop solution (0.2 N H₂SO₄) with the colour of samples turning 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 lactoferrin concentration using a 4-parameter logistic curve fit.

Data Analysis

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

RESULTS

Table 1: Sociodemographic characteristics of all respondents

	Participant category					
Demographics	GAP	LAP	Controls	Total	P-Value	
	n (%)	n (%)	n (%)	N (%)		
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 ±SD	$10(33.80 \pm 8.93)$	$9(32.11 \pm 8.07)$	18(31.39± 8.96)	$37(32.22 \pm 8.56)$	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)		
Total	10(100.00)	9(100.00)	18(100.00)	37(100.00)		
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)		
Total	10(100.00)	9(100.00)	18(100.00)	37(100.00)		
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)	0.20	
Traditional	0(0.0)	0(0.0)	0(0.0)	0(0.0)		
Total	10(100.00)	9(100.00)	18(100.00)	37(100.00)		
Educational						
background						
No Formal	0(0.0)	0(0.0)	1(5.6)	1(2.7)		
Education					0.25	
Completed only	2(20.0)	0(0.0)	1(5.6)	3(8.1)		
Primary						
education						
Completed only	7(70.0)	5(55.6)	7(38.8)	19(51.4)		
Secondary						
education						
Completed	1(10.0)	4(44.4)	9(50.0)	14(37.8)		
Tertiary						
education						
Total	10(100.00)	9(100.00)	18(100.00)	37(100.00)		

Table 2: Concentration of Total IgA before and after treatment among all participant categories

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

Table 3: Comparison of concentration of Salivary Lactoferrin levels before and after treatment between cases and controls

	Means Concentrations of sLf			
	sLf conc(μg/ml)	sLf conc (μg/ml)		
Participant category	Before treatment	After treatment		
Cases (n=19)	70.92 ± 26.84	68.57 ± 25.34		
Controls (n=18)	48.39 ± 28.56	43.42 ± 21.54		
Total (n=37)	59.96 ± 29.59	56.33 ± 26.51		
P-value	0.018	0.003		

DISCUSSION

This study was conducted primarily to determine the levels of immunoglobulin A and lactoferrin in saliva and to find out if there was any association between IgA and sLf levels in participants with aggressive periodontitis at baseline and after non-surgical periodontal therapy. Not much reports have been published on aggressive periodontitis in relation with IgA and sLf among the African population. The present study was therefore regarded as part of an early effort to determine whether a link exists between aggressive periodontitis and the concentration of IgA and sLf.

Total Immunoglobulin a levels in saliva of Aggressive periodontitis patients

The mean concentration values for the cases were close to those of the controls (Table 2). One possible explanation for this may be that, whole salivary IgA was considered which evaluates the overall immune response in the oral cavity in a generalized pattern. The results obtained in this study are, to some degree, also similar to those in a study by other researchers like Henskens and Van Den Keijbus. 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 respondents.

Lactoferrin levels in saliva of Aggressive periodontitis patients

The normal Lactoferrin concentration in saliva is 0.008 mg/ml. Among salivary proteins, Lf is the most important factor of natural immunity. Its concentration were 8.96 and 7.11 mg/l in unstimulated and stimulated saliva, respectively. No

baseline levels of Lactoferrin in healthy patients and individuals with aggressive periodontitis were found in the literature.

Unique to this work was the introduction of GAP cases which most studies in the literature did not report on. Re-evaluation after the initial periodontal therapy in one month was considered in this study which was also not indicated in the earlier reports.

A study by Berlutti et al 14 confirmed an increase in lactoferrin levels from 20 to 60 μ g/ml during infection and inflammatory processes and this was attributed to the recruitment of neutrophils which increases saliva Lf concentration.

The overall increase and decrease in salivary lactoferrin concentration obtained in this study among the aggressive periodontitis participants before and after periodontal therapy respectively are consistent with some studies with designated increased levels of this biomarker when compared to control participants without periodontal disease.

RECOMMENDATIONS

More modern methods 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. These techniques must be implemented where adequate funds are available to assist in the dental needs of patients. Although challenges remain, the use of saliva as a prognostic and diagnostic fluid appears promising for future application.

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.

At baseline, sLf levels were significantly different for the cases and controls. The significant difference persisted even after treatment between the cases and controls.

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