



## Salivary Immunoglobulin Classes in Nigerians with Periodontitis

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### ABSTRACT

**Aim:** To provide information on the usefulness of salivary immunoglobulin classes in the diagnosis of periodontitis.

**Materials and methods:** About 5 ml of unstimulated saliva was collected from 25 newly diagnosed subjects with periodontitis and 21 sex/age-matched apparently healthy individuals into plain sample bottles. The samples were collected between 9 am and 11 am at least, 1 hour after eating or washing of mouth and levels of salivary immunoglobulin classes (IgA, IgG, IgE and IgM) were determined using enzyme-linked immunosorbent assay (ELISA).

**Results:** Only the mean level of IgA was significantly raised ( $p = 0.05$ ) in the saliva of periodontitis patients compared with controls. The mean levels of IgG, IgM and IgE were not significantly elevated in patients with periodontitis, when compared with controls ( $p > 0.05$ ).

**Conclusion:** This study showed that elevated salivary levels of IgA could be used as a screening tool for periodontitis.

**Clinical significance:** Identification of patients at risk and the diagnosis of active phases of periodontal disease remains a challenge due to lack of laboratory test routinely employed in the diagnosis and monitoring of patients with periodontal disease. This study showed that elevated salivary levels of immunoglobulin classes especially, IgA could be used as a screening tool for periodontitis.

**Keywords:** Periodontitis, Immunoglobulin, Saliva, Laboratory research.

**How to cite this article:** Olayanju OA, Rahamon SK, Joseph IO, Arinola OG. Salivary Immunoglobulin Classes in Nigerians with Periodontitis. *J Contemp Dent Pract* 2012;13(2):163-166.

**Source of support:** Nil

**Conflict of interest:** None declared

### INTRODUCTION

Periodontitis can be defined as the presence of gingival inflammation at sites, where there has been a pathological detachment of collagen fibers from the cementum and the

junctional epithelium has migrated apically.<sup>1</sup> Chronic periodontitis results from inflammation within the supporting tissues of the teeth in response to the dental plaque biofilm. This can eventually lead to a progressive loss of attachment of the periodontal tissues and loss of the supporting alveolar bone. Inflammatory events associated with connective tissue attachment loss also lead to the resorption of coronal portions of tooth supporting alveolar bone.<sup>2</sup>

Periodontitis is characterized by pocket formation and/or gingival recession.<sup>3</sup> It is generally accepted that the primary etiological factor of periodontal disease is the dental plaque biofilm,<sup>4</sup> but the host response is considered to be an important disease modifying factor.<sup>5,6</sup> Environmental and systemic risk factors, such as smoking and diabetes as well as some drug therapies, are also known to modulate periodontal progression and severity.<sup>7-9</sup>

Identification of patients at risk and the diagnosis of active phases of periodontal disease remains a challenge.<sup>10</sup> Clinical parameters, such as probing depth, attachment level, bleeding on probing (BOP), plaque index (PI) and radiographic loss of alveolar bone, are used to assess disease severity.<sup>11</sup> Occasionally, monitoring of the microbial infection<sup>12,13</sup> and analysis of the host response in gingival crevicular fluid (GCF) are utilized in an attempt to identify individuals at risk for future breakdown.<sup>14-16</sup> Recently, genetic analysis has also been suggested as a means to identify individuals who are at increased risk for more severe periodontitis.<sup>17</sup> Although, clinical and radiographic assessment of periodontal disease remains the basis for patient evaluation, no clinical or laboratory test is presently, routinely employed in the monitoring of patients with periodontal disease. This gave rise to considerable research activities on possible salivary markers for periodontal diagnosis. These markers include enzymes, immunoglobulins, proteins, ions and hormones.<sup>18</sup>

Saliva contains locally-derived and systemically-derived markers of periodontal disease.<sup>10</sup> It has been used as a diagnostic fluid in medicine.<sup>19,20</sup> Human saliva contains several factors with protective or antibacterial properties in the oral cavity. These factors could be immune or nonimmune agents. IgA and IgG are examples of local immune response, CRP and haptoglobin take part in inflammatory defence.<sup>21</sup>

Levels of salivary immunoglobulin classes A, G and M have not been widely studied in subjects with periodontal diseases. The predominant immunoglobulin in saliva is secretory IgA (sIgA) which is derived from plasma cells in the salivary glands. Although the minor salivary glands play an important role in sIgA mediated immunity of the oral cavity, cells in the parotid gland are responsible for the majority of the IgA found in saliva.<sup>22,23</sup> The sIgA constitutes the main specific immune defense mechanism in saliva and may be important in maintaining homeostasis in the oral cavity. sIgA may control the oral microbiota by reducing the adherence of bacterial cells to the oral mucosa and teeth.<sup>24</sup> There are two subclasses of IgA: IgA1 and IgA2. IgA1 predominates in serum while IgA2 is found in higher concentrations in external secretions (i.e. tears, saliva and milk).<sup>25</sup> sIgA levels, which are undetectable in newborns, increase progressively and reach adult values in stimulated saliva by 2 to 4 years of age and, in unstimulated saliva, by 6 to 8 years of age.<sup>26</sup> In contrast, salivary IgG is primarily derived from serum via gingival crevicular fluid (GCF) and is present in low concentrations. Consequently, the IgG concentration in saliva should increase as inflammation of the periodontal tissues becomes more severe and vascular permeability increases, resulting in increased flow of GCF.<sup>27,28</sup>

There have been conflicting reports on levels of salivary immunoglobulin classes in periodontal disease patients. Guven et al<sup>29</sup> and Napimoga et al<sup>30</sup> reported elevated concentration of salivary IgA in patients with gingivitis and periodontitis. Positive correlation was also observed between severity of inflammation and salivary IgA concentration. Similarly, Sandholm et al<sup>31</sup> reported elevated concentration of salivary IgA, IgG and IgM in juvenile periodontitis subjects. In contrast, Harding et al<sup>32</sup> and Bokor<sup>33</sup> reported low levels of salivary IgA and IgG in patients with acute necrotizing ulcerative gingivitis and subjects with gingival inflammation.

To the knowledge of the authors, there has been no study on the levels of immunoglobulin classes in Nigerians with periodontitis, therefore, to provide information on the oral mucosa immune status in Nigerian patients with periodontitis, this study estimated salivary immunoglobulin classes (IgG, IgA, IgM and IgE).

## MATERIALS AND METHODS

Forty-six subjects were recruited for this study after obtaining informed consent from each subject and an ethical approval from the University of Ibadan/University College Hospital (UI/UCH) Joint Ethics Review Committee. The test group consisted of 25 newly diagnosed subjects with periodontitis, while the control group was made up of 21 sex and age-matched subjects who were apparently healthy. All the subjects were nonsmokers and had no previous treatment for periodontal disease. Periodontitis was diagnosed by a dentist using periodontal probing radiographs. Those excluded from the study were individuals with pregnancy, diabetes and human immunodeficiency virus (HIV) infection. Subjects with other forms of oral disease were also excluded from the test group. A short-structured questionnaire was administered on each subject to obtain information on age, sex, occupation, cigarette smoking and drug consumption.

About 5 ml of unstimulated saliva was collected from each subject into plain sample bottles. The samples were collected between 9 and 11 am at least, 1 hour after eating or washing of mouth. The samples were centrifuged at 3000 g for 5 minutes and the clear supernatant gently pipetted out into another clean plain bottle and stored at  $-20^{\circ}\text{C}$  until analyzed.

Immunoglobulin levels were estimated using enzyme linked immunosorbent Assay (ELISA) supplied by Immunology Consultant Laboratory, USA. IgE kit was supplied by Leinco Technologies, USA. The assay was carried out by following manufacturer's instructions.

## STATISTICAL ANALYSIS

The data were presented as mean and standard deviation. Student's t-test (unpaired) was used to determine significant difference between the means. Values of  $p \leq 0.05$  were regarded as statistically significant.

## RESULTS

The mean ages of periodontal subjects (PS) and controls were  $53 \pm 11$  and  $52 \pm 11$  years respectively. No significant differences were observed in the mean salivary levels of IgG, IgM and IgE but significant increase was observed in the level of IgA in periodontitis patients compared with the controls ( $p = 0.05$ ). Only one (5%) periodontal subject had detectable level of salivary IgE (0.20 IU/ml) while two (9.52%) control subjects had detectable levels of IgE (0.24 IU/ml). Also, the proportion of periodontal subject with detectable level of salivary IgE was lower compared with controls (Table 1).

**Table 1:** Level of salivary immunoglobulin in active smokers and nonsmokers

	PS (n = 25)	Control (n = 21)	p-value
IgG (ng/l)	370 ± 60.9	369.6 ± 44.6	0.98
IgA (ng/l)	670 ± 110.0	596.9 ± 103.6	*0.05
IgM (ng/l)	791.4 ± 43.7	779 ± 49.5	0.72
IgE (IU/ml)	0.20 ± 1.0	0.24 ± 0.89	0.87

\*Significant at  $p \leq 0.05$ ; PS: Periodontal subject

## DISCUSSION

Periodontal disease is defined as inflammatory destruction of periodontal tissue and alveolar bone supporting the teeth. Severe and prolonged periodontal inflammation causes loss of teeth, thereby affecting oral functions (mastication speech and facial esthetics). The process of periodontal disease is such that a microbial challenge induces a host response which results in connective tissue and alveolar bone destruction.<sup>34</sup> Immunological, microbial, environmental, genetic factors, age, sex and race are risk factors with complex interactions which determine the severity and progression of the disease.<sup>35</sup>

The local host response to periodontal diseases includes recruitment of leucocytes and subsequent release of inflammatory mediator and proinflammatory cytokines. Cytokines, such as interleukin (IL)-1 $\beta$  and tumor necrosis factor- $\alpha$  induce and enhance the production of prostaglandin E2 (PGE2) and matrix metalloproteinases (MMPs). These molecules mediate destruction of the extracellular matrix of gingiva and periodontal ligament as well as resorption of alveolar bone.<sup>36</sup>

Salivary levels of IgG, IgM (nonsignificantly) and IgA (significantly) were higher in periodontitis subjects, when compared with controls. The observed elevated levels of salivary IgG and IgM corroborate the reports of Guven et al,<sup>29</sup> Sandholm et al<sup>31</sup> and Hagewald (2002), while the elevated salivary IgA level supports the report of Sandholm et al<sup>31</sup> and Napimoga et al.<sup>30</sup>

Secretory IgA inhibits microorganisms attaching to oral epithelium or teeth.<sup>37,38</sup> The elevated level of salivary IgA observed in periodontitis patients to prevent further attachment or entrance of already attached particles into the gums. Our observation could also be due to accumulation of dental plaque which may stimulate IgA production by increasing the amounts of swallowed bacteria that activate B-cells. Upregulation of IL-21 and IL-10 and downregulation of IL-4 in periodontitis tissues were reported in periodontitis patients. These cytokines play role in inducing IgA production by B-cells in the humoral immune response (Napimoga et al).<sup>30</sup> Thus, observed elevated levels of IgA in our periodontitis patients.

The reports of Wilton et al<sup>27</sup>, Shapiro et al<sup>28</sup> and Patidar et al<sup>39</sup> showed that salivary IgG concentration depends on serum concentration of IgG and that salivary IgG concentration increases as inflammation of the periodontal tissues becomes more severe and vascular permeability increases, resulting in increased flow of GCF. Periodontitis patients considered for the present study were newly diagnosed and none had any pathological symptom, this might explain our observed elevated, but nonsignificant levels of salivary IgG and IgM.

Only one (5%) periodontal patient had detectable level of salivary IgE (0.20 IU/ml), while two (9.52%) control subjects had detectable levels of IgE (0.24 IU/ml). This might be an indication that the level of IgE is very low in the saliva of healthy and diseased subjects, therefore, unmeasurable by this method (ELISA).

## CONCLUSION

Our study showed that there were elevated levels of salivary immunoglobulin classes in subjects with periodontal disease, and that salivary IgA could serve as a screening tool for people at risk of developing the disease.

## REFERENCES

1. Savage A, Eaton KA, Moles DR, Needleman I. A systematic review of definitions of periodontitis and methods that have been used to identify this disease. *J Clin Periodontol* 2009;36: 458-67.
2. Armitage GC. Periodontal diseases: Diagnosis. *Ann Periodontol* 1996;1:37-215.
3. Armitage GC. Development of a classification system for periodontal diseases and conditions. *Annals of Periodontology/The American Academy of Periodontology* 1999;4:1-6.
4. Kinane DF. Periodontitis modified by systemic factors. *Annals of Periodontology/The American Academy of Periodontology* 1999;4:54-64.
5. Page RC, Offenbacher S, Schroeder HE, Seymour GJ, Kornman KS. Advances in the pathogenesis of periodontitis: Summary of developments, clinical implications and future directions. *Periodontology* 2000, 1997;216-48.
6. Silva N, Dutzan N, Hernandez M, Dezerega A, Rivera O, Aguillon JC, et al. Characterization of progressive periodontal lesions in chronic periodontitis patients: Levels of chemokines, cytokines, matrix metalloproteinase-13, periodontal pathogens and inflammatory cells. *Journal of Clinical Periodontology* 2008;35:206-14.
7. Johnson ML, Keeton LG, Zhu ZB, Volanakis JE, Cooper MD, Schroeder HW Jr. Age-related changes in serum immunoglobulins in patients with familial IgA deficiency and common variable immunodeficiency (CVID). *Clinical and Experimental Immunology* 1997;108:477-83.
8. Albandar JM. Global risk factors and risk indicators for periodontal diseases. *Periodontology* 2000, 2002;29:177-206.
9. Mealey BL, Ocampo GL. Diabetes mellitus and periodontal disease. *Periodontology* 2000, 2007;44:127-53.



10. Kaufman E, Lamster IB. Analysis of saliva for periodontal diagnosis. A review. *J Clin Periodontol* 2000;27:453-65.
11. Polson AM, Goodson JM. Periodontal diagnosis-current status and future need. *Journal of Periodontology* 1985;56:25-34.
12. Listgarten MA. Microbiological testing in the diagnosis of periodontal disease. *Journal of Periodontology* 1992;63:332-37.
13. Greenstein G. Microbiologic assessments to enhance periodontal diagnosis. *Journal of Periodontology* 1988;59: 508-15.
14. Curtis MA, Gillett IR, Griffiths GS, Maiden MF, Sterne JA, Wilson DT, et al. Detection of high-risk groups and individuals for periodontal diseases: Laboratory markers from analysis of gingival crevicular fluid. *Journal of Clinical Periodontology* 1989;16:1-11.
15. Lamster IB, Novak MJ. Host mediators in gingival crevicular fluid: Implications for pathogenesis of periodontal disease. *Critical Reviews in Oral Biology and Medicine* 1992;3:31-60.
16. Lamster IB. Evaluation of components of gingival crevicular fluid as diagnostic test. *Annals of Periodontology* 1997;2:123-37.
17. Kornman KS, Crane A, Wang HY, Di Giovine FS, Newman MG, Pirk FW, et al. The interleukin-1 genotype as a severity factor in adult periodontal disease. *Journal of Clinical Periodontology* 1997;24:72-77.
18. Mandel ID. Markers of periodontal disease susceptibility and activity derived from saliva. In: Johnson NW (Ed). *Risk markers for oral diseases. Periodontal diseases: Markers of disease susceptibility and activity.* 1991;3:228-53. Cambridge University Press.
19. Mandel ID. The diagnostic uses of saliva. *Journal of Oral Pathology and Medicine* 1990;19:119-25.
20. Ferguson DB. Current diagnostic uses of saliva. *Journal of Dental Research* 1987;66:420-24.
21. Krasteva A, Perenovska P, Ivanova A, Altankova I, Bocheva T, Kisselova A. Alteration in salivary components of children with allergic asthma. *Biotechnol and Biotechnol. Eq* 2010;24(2): 1866-69.
22. Bienenstock J, Befus AK, McDermott M. Mucosal immunity. *Monographs in allergy* 1980;16:1-18.
23. Nair PNR, Schroeder HE. Duct-associated lymphoid tissue (DALY) of minor salivary glands and mucosal immunity. *Immunology* 1986;57:171-80.
24. Marcotte H, Lavoie MC. Oral microbial ecology and the role of salivary immunoglobulin A. *Microbiology and Molecular Biology Reviews* 1998;62:71-109.
25. Delacroix DL, Dive C, Rambaud JC, Vaerman JP. IgA subclasses in various secretions and in serum. *Immunology* 1982; 47:383-85.
26. Burgio GR, Lanzavecchia A, Plebani A, Jayakar S, Ugazio AG. Ontogeny of secretory immunity: Levels of secretory IgA and natural antibodies in saliva. *Paediatric Research* 1980;14: 1111-14.
27. Wilton JMA, Curtis MA, Gillett IR, Griffiths GS, Maiden MFJ, Sterne JAC, et al. Detection of high-risk groups and individuals for periodontal diseases: Laboratory markers from analysis of saliva. *Journal of Clinical Periodontology* 1989;16:475-83.
28. Shapiro L, Goldman H, Bloom A. Sulcular exudate flow in gingival inflammation. *Journal of Periodontology* 1979;50: 301-04.
29. Guven O, De Visscher JG. Salivary IgA in periodontal disease. *Journal of Periodontology* 1982;53:334-35.
30. Napimoga MH, Nunes LHAC, Maciel AAB, Demasi APD, Benatti BB, Santos VR, et al. Possible involvement of IL-21 and IL-10 on salivary IgA levels in chronic periodontitis subjects. *Scandinavian Journal of Immunology* 2011;74:596-602.
31. Sandholm L, Gronblad E. Salivary immunoglobulins in patients with juvenile periodontitis and their healthy siblings. *Journal of Periodontology* 1984;55:9-12.
32. Harding J, Berry WC Jr, Marsh C, Jolliff CR. Salivary antibodies in acute gingivitis. *Journal of Periodontology* 1980;51:63-69.
33. Bokor M. Immunoglobulin A levels in the saliva in patients with periodontal disease. *Medicinski Pregled* 1997;50:9-11.
34. Ojima M, Hanioka T. Destructive effects of smoking on molecular and genetic factors of periodontal disease. *Tobacco Induced Diseases* 2010;8:4.
35. Nunn ME. Understanding the etiology of periodontitis: An overview of periodontal risk factors. *Periodontol* 2000, 2003; 32:11-23.
36. Madianos PN, Bobetsis YA, Kinane DF. Generation of inflammatory stimuli: How bacteria set up inflammatory responses in the gingiva. *Journal of Clinical Periodontology* 2005;32(Suppl 6):57-71.
37. Hagewald S, Bernimoulin JP, Kottgen E, Kage A. Salivary IgA subclasses and bacteria-reactive IgA in patients with aggressive periodontitis. *Journal of Periodontal Research* 2002;37:333-39.
38. Vudichamnong K, Walker DM, Ryley HC. The effect of secretory immunoglobulin A on the in vitro adherence of the yeast *Candida albicans* to human oral epithelial cells. *Arch Oral Biol* 1982;27:617-21.
39. Patidar KA, Parwani RN, Wanjari SP. Correlation of salivary and serum IgG, IgA levels with total protein in oral submucous fibrosis. *Journal of Oral Science* 2011;53(1):97-102.

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