Correlation of salivary immunoglobulin A against lipopolysaccharide of *Porphyromonas gingivalis* with clinical periodontal parameters

Pushpa S. Pudakalkatti, Abhinav S. Baheti

Abstract

Background: A major challenge in clinical periodontics is to find a reliable molecular marker of periodontal tissue destruction. **Aim:** The aim of the present study was to assess, whether any correlation exists between salivary immunoglobulin A (IgA) level against lipopolysaccharide of *Porphyromonas gingivalis* and clinical periodontal parameters (probing depth and clinical attachment loss). **Materials and Methods:** Totally, 30 patients with chronic periodontitis were included for the study based on clinical examination. Unstimulated saliva was collected from each study subject. Probing depth and clinical attachment loss were recorded in all selected subjects using University of North Carolina-15 periodontal probe. Extraction and purification of lipopolysaccharide were done from the standard strain of *P. gingivalis* (ATCC 33277). Enzyme linked immunosorbent assay (ELISA) was used to detect the level of IgA antibodies against lipopolysaccharide of *P. gingivalis* in the saliva of each subject by coating wells of ELISA kit with extracted lipopolysaccharide antigen. **Statistical Analysis:** The correlation between salivary IgA and clinical periodontal parameters was checked using Karl Pearson's correlation coefficient method and regression analysis. **Results:** The significant correlation was observed between salivary IgA level and clinical periodontal parameters in chronic periodontitis patients. **Conclusion:** A significant strong correlation was observed between salivary IgA against lipopolysaccharide of *P. gingivalis* and clinical periodontal parameters which suggest that salivary IgA level against lipopolysaccharide of *P. gingivalis* can be used to predict the severity of periodontal destruction in chronic periodontitis patients.

Keywords: Clinical attachment loss, clinical periodontal parameters, probing depth, salivary immunoglobulin A

Introduction

Porphyromonas gingivalis is considered as a major periodontal pathogen.[1] Lipopolysaccharide is a major immunodominant antigen of *P. gingivalis*.[2] Previous studies have shown increased the levels of salivary immunoglobulin A (IgA) against lipopolysaccharide of *P. gingivalis* in chronic periodontitis patients.[3]

The severity of periodontal disease refers to the amount of periodontal attachment that has been lost and termed as a clinical attachment loss. Most of the times increased probing depth leads to loss of clinical attachment and is also used to assess the severity of the periodontal destruction.[4]

This study was planned to assess, whether any correlation exists between clinical periodontal parameters (probing depth and clinical attachment loss) and salivary IgA levels against lipopolysaccharide of *P. gingivalis*.

Materials and Methods

Totally, 30 patients with chronic periodontitis were included for the study based on clinical examination. Inclusion criteria were presence of generalized clinical signs of gingival inflammation, the presence of generalized probing depth ≥5 mm and presence of generalized clinical attachment loss ≥3 mm.[4]

Exclusion criteria were the subjects below 30 years of age, subjects who have received periodontal therapy or antimicrobial therapy within last 3 months, patient with any systemic disease/condition, smokers, pregnant, and lactating women.

The study protocol was approved by ethical committee of the institution. The study protocol was explained to each participant and written informed consents were taken from all the subjects.

Unstimulated saliva was collected from all the study subjects. The spitting method was used to collect the saliva.[5]

Department of Periodontology, Maratha Mandal's Nathajirao G Halgekar Institute of Dental Science's and Research Center, Belgaum, Karnataka, India

Correspondence: Dr. Abhinav S. Baheti,
Dwarka Maternity Home, Junapress, Tal at Post
Shevgaon, Ahmednagar - 414 502, Maharashtra, India.
E-mail: abhinav1baheti@gmail.com

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Four-five milliliter (ml) of saliva was collected from each study subject. Then the saliva sample was sent to microbiology laboratory and was stored at −80°C.

Probing depth and clinical attachment loss were recorded in all selected subjects using University of North Carolina-15 periodontal probe. Probing depths for each tooth at six sites were measured and recorded to the nearest millimeter mark, as the distance between the gingival margin and the base of the pocket. Clinical attachment loss for each tooth at six sites were measured, and recorded to the nearest millimeter mark, as the distance between the cementoenamel junction and the base of the pocket. All the recordings were done by a single examiner.

Extraction and purification of lipopolysaccharide were done from the standard strain of *P. gingivalis* (ATCC 33277). Lipopolysaccharide was extracted by the hot phenol-water method as described previously with some modifications suggested by Westphal in 1965.[6]

Enzyme linked immunosorbent assay (ELISA) was used to detect the level of IgA antibodies against lipopolysaccharide of *P. gingivalis* in saliva sample of each individual with chronic periodontitis by coating wells of ELISA kit with extracted lipopolysaccharide antigen. LISA plus microplate reader was used to detect amount (μg/ml) of salivary IgA antibodies in each salivary sample.

Statistical analysis

The correlation was checked between salivary IgA level against lipopolysaccharide of *P. gingivalis* and clinical periodontal parameters (probing depth and clinical attachment loss) by Karl Pearson's correlation coefficient method and by regression analysis.

Results

Table 1 shows a summary of statistics for three variables salivary IgA (μg/ml) against lipopolysaccharide of *P. gingivalis*, probing depth (mm) and clinical attachment loss (mm). The mean salivary IgA level in chronic periodontitis patients was 43.86 μg/ml. Mean probing depth and mean clinical attachment loss were 4.79 mm and 4.86 mm, respectively.

Table 2 shows a correlation between salivary IgA (μg/ml) against lipopolysaccharide of *P. gingivalis* with probing depth (mm) and clinical attachment loss (mm) by Karl Pearson's correlation coefficient method. *P* value for correlation between salivary IgA (μg/ml) with probing depth (mm) was 0.0010, which indicates a significant correlation between these two variables. *P* value for correlation between salivary IgA (μg/ml) with the clinical attachment loss (mm) was 0.0009, which also indicates a significant positive correlation between these two variables.

Table 1: Summary statistics of three variables

Summary	Salivary IgA (μg/ml)	Probing depth (mm)	Clinical attachment loss (mm)
Minimum	24.60	3.64	3.50
Maximum	137.25	6.32	6.47
Mean	43.86	4.79	4.86
Median	42.93	4.89	5.01
Standard deviation	20.67	0.70	0.75
Standard error	3.77	0.13	0.14

Table 2: Correlation between salivary IgA (μg/ml) with probing depth (mm) and clinical attachment loss (mm) by Karl Pearson's correlation coefficient method

Variables	Correlation between salivary IgA (μg/ml) with			
	r	r^2	*t*	*P*
Probing depth (mm)	0.5716	0.3268	3.6866	0.0010*
Clinical attachment loss (mm)	0.5758	0.3316	3.7269	0.0009*

*$P<0.05$

Table 3 shows simple linear regression analysis of probing depth (mm) by salivary IgA against lipopolysaccharide of *P. gingivalis* (μg/ml). $P < 0.05$, which indicates that salivary IgA (μg/ml) can be used to predict mean probing depth value in chronic periodontitis patients.

Table 4 shows simple linear regression analysis of clinical attachment loss by salivary IgA against lipopolysaccharide of *P. gingivalis* (μg/ml). $P < 0.05$, which indicates that salivary IgA (μg/ml) can be used to predict mean clinical attachment loss values in chronic periodontitis patients.

Discussion

A major challenge in clinical periodontics is to find a reliable molecular marker of periodontal tissue destruction with high sensitivity, specificity, and utility.[7] In the present study, salivary IgA levels against lipopolysaccharide of *P. gingivalis* were correlated with clinical periodontal parameters (probing depth and clinical attachment loss), to assess ability of salivary IgA against lipopolysaccharide of *P. gingivalis* as a marker for severity of periodontal destruction in chronic periodontitis.

P. gingivalis is a Gram-negative, asaccharolytic, anaerobic bacterium which is considered as a major periodontal pathogen.[1] Krishnan *et al*. in their polymerase chain reaction study concluded that *P. gingivalis* is more prevalent in patients with chronic periodontitis compared to health and high odds ratio for *P. gingivalis* in their study suggested a strong association between *P. gingivalis* and chronic periodontitis.[8] According to van Winkelhoff *et al. P. gingivalis*

Table 3: Simple linear regression analysis of probing depth (mm) by salivary IgA (μg/ml)

Independent variable	Regression estimate	SE	*t*	*P*-level
Intercept	3.9312	0.2552	15.4031	0.00001*
Salivary IgA (μg/ml)	0.0195	0.0053	3.6866	0.0010*

*$P<0.05$. Regression equation, probing depth (mm)=3.9312+0.0195 (salivary IgA). SE: Standard error

Table 4: Simple linear regression analysis of clinical attachment loss by salivary IgA (μg/ml)

Independent variable	Regression estimate	SE	*t*	*P*-level
Intercept	3.9466	0.2700	14.6163	0.00001*
Salivary IgA (μg/ml)	0.0208	0.0056	3.7269	0.0009*

*$P<0.05$. Regression equation, clinical attachment loss (mm)=3.9466+0.0208 (salivary IgA). SE: Standard error

and *Bacteroides forsythus* are the strongest bacterial markers for periodontitis and are infrequently cultured from subjects without periodontal bone loss.[9] Byrne *et al*. stated that the odds of a site undergoing imminent periodontal disease progression increased with increasing levels of *P. gingivalis* and *Treponema denticola*.[10] Hence, *P. gingivalis* is considered as a major periodontal pathogen.[1]

Lipopolysaccharide is an essential macromolecule that comprises the outer surface of Gram-negative bacteria and recognized by the human host as a foreign molecule and elicits an immune response that is designed to eliminate the bacterial intruder.[1] Koga *et al*., studied the capacity of lipopolysaccharide from various bacteria to induce local Shwartzman reaction. They found that immune response, which is designed to eliminate the bacterial intruder, to *Bacteroides gingivalis* that is *P. gingivalis* was very weak, which disturbed oral immune homeostasis and thereby promoted periodontal disease.[11]

Several studies like the study by Eggert *et al.* have shown that saliva from treated periodontitis patients had higher total IgA antibody levels against *B. gingivalis* than did saliva from normal control subjects.[12] However, in these various studies levels of antibodies against whole cells or crude extracts of *P. gingivalis* were observed in periodontitis patients. Such preparations include a number of antigens, some of which may be associated with periodontitis and others that are unrelated to periodontal disease. Thus, in ELISA the binding of antibodies to disease associated antigens may be overshadowed by the binding of antibodies to antigens that are unrelated to disease.[3]

In a study by Guo *et al*., serum IgG antibody levels and avidities against extracted *P. gingivalis* whole cells were measured. Serum antibody levels against *P. gingivalis* lipopolysaccharide were also measured. They found that antibody levels against *P. gingivalis* lipopolysaccharide, highly correlated with that against *P. gingivalis* whole cells, indicating lipopolysaccharide is a major immunodominant antigen of *P. gingivalis*.[2] Therefore, in the present study, salivary IgA antibodies only against this immunodominant antigen were detected.

In the present study, saliva was used to assess IgA antibodies. Saliva is easy to collect and can be obtained in sufficient quantities compared to blood and gingival crevicular fluid.

In a study by Schenck specific serum IgG, IgA and IgM activities against lipopolysaccharide from *B. gingivalis* were measured by ELISA in serum samples from subjects with periodontal health and disease. They concluded that subjects with periodontitis had significantly higher levels of specific IgA compared to subjects with periodontal health.[3] Schenck *et al*. also concluded that the periodontal treatment showed a statistically significant mean reduction in specific antibody levels to LPS preparation. These studies indicate that salivary IgA level against lipopolysaccharide of *P. gingivalis* increases in subjects with periodontal destruction.[13]

The severity of periodontal destruction is assessed by the amount of periodontal attachment lost (clinical attachment loss). Most of the times increased probing depth leads to loss of clinical attachment and is also used to assess the severity of periodontal disease.[4] Clinical attachment loss and probing depth are conventionally used to assess the severity of periodontal destruction in periodontal disease. In the present study, significant strong correlation was observed between these clinical parameters and salivary IgA levels against lipopolysaccharide of *P. gingivalis*. It suggests that salivary IgA against lipopolysaccharide of *P. gingivalis* can be used to assess the severity of periodontal destruction in chronic periodontitis patients.

Wilton, *et al.* studied the salivary IgA responses to a number of plaque bacteria incriminated as periodontal pathogens in saliva from a population of rural Kenyan adolescents with gingivitis but no evidence of destructive periodontal disease. They also related immune response in the form of salivary IgA response to clinical status assessed by plaque index, gingival bleeding, and probing depth. They observed that antibody levels were negatively correlated with the average plaque index while the probing depth and bleeding index were positively correlated.[14] In the present study, strong positive correlation is observed between salivary IgA levels against lipopolysaccharide of *P. gingivalis* and individual periodontal clinical parameters (probing depth and clinical attachment loss).

Onoue, *et al.* compared the serum antibody titers against the lipopolysaccharide of *P. gingivalis* between periodontitis patients and healthy persons. They found that the IgG titers against the lipopolysaccharide of *P. gingivalis* were clearly higher in the patients than in the healthy persons. Their

results suggested that the antibody measurement of patients' sera against the lipopolysaccharide of periodontal bacteria can be applied for the diagnosis of periodontitis.[15] Results of the present study also support this statement.

Craig *et al*. suggested that elevated serum IgG antibody to *P. gingivalis* reflects destructive periodontal disease status, and may be considered as a risk factor for disease progression.[16]

In a study by Lakio *et al*., the number of deepened periodontal pockets (≥5 mm) correlated with IgG antibody levels against *P. gingivalis* in both plasma and saliva. Mean clinical attachment loss correlated with IgG and IgA antibody levels against *P. gingivalis*.[17] Furuichi *et al*. assessed 236 individuals for their periodontal conditions by use of the community periodontal index for treatment needs, and serum antibody titers for *P. gingivalis* fimbriae in their blood samples by ELISA. They demonstrated that the serum antibody titers against *P. gingivalis* fimbriae could be useful for screening individuals with moderate to severe periodontitis. Results of the present study are in line with these studies.[18]

Conclusion

Conventionally probing depth and clinical attachment loss are used to assess the severity of the periodontal destruction. In the present study, strong correlation was observed between salivary IgA level and probing depth, as well as between salivary IgA level and clinical attachment loss, in chronic periodontitis patients. This study suggests that salivary IgA level can be used to predict the severity of periodontal destruction in chronic periodontitis patients.

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