Salivary IgA, Interleukin-1β and MMP-8 as Salivary Biomarkers in Chronic Periodontitis Patients

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Objective: *To compare the clinical parameters and levels of salivary immunoglobulin A (IgA), Interleukin-1β (IL-1β) and matrix metalloproteinase-8 (MMP-8) in patients with moderate to severe chronic periodontitis and in individuals with healthy gingiva.*

Methods: *Levels of clinical parameters plaque index (PI), gingival index (GI), probing depth (PD), clinical attachment level (CAL) and bleeding on probing (BOP) along with salivary biomarkers salivary IgA, IL-1β and MMP-8 were recorded among 50 adults (30 test subjects with moderate to severe generalised chronic periodontitis, constituting group A, and 20 periodontally healthy controls – group B). Clinical evaluation was done before oral prophylaxis, and 6 weeks and 12 weeks after oral prophylaxis, and saliva samples were obtained before and 12 weeks after oral prophylaxis. Salivary IgA, IL-1β and MMP-8 levels in saliva were assessed using enzyme-linked immunosorbent assay.*

Results: *In group A, there were highly significant differences in terms of PI, GI, PD, CAL and BOP before oral prophylaxis, and 6 weeks and 12 weeks after oral prophylaxis when compared at these intervals. Differences in their levels in group B were non-significant at such intervals except PI. Mean levels of salivary IgA, IL-1β and MMP-8 in chronic periodontitis patients at baseline were significantly higher than in the periodontally healthy group. Their levels in group A decreased significantly 12 weeks after oral prophylaxis, but remained static in group B.*

Conclusion: *The levels of salivary IgA, IL-1β and MMP-8 showed significant reduction after oral prophylaxis, suggesting that these biomarkers could facilitate the screening, early diagnosis, and management of periodontal disease.*

Key words: *chronic periodontitis, saliva, biomarker, diagnosis, ELISA*

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Chronic periodontitis is a chronic microbial infection that triggers inflammation mediated loss of the periodontal ligament and alveolar bone that supports the teeth[1]. The current understanding of the pathogenesis of periodontal disease suggests that tissue destruction might result from the modulation of host defences by bacterial and host products, which stimulate the host inflammatory process[2].

Presently, the diagnosis of periodontitis relies almost entirely on the clinical parameters such as probing depth, attachment level, bleeding on probing, and radiographic assessment of alveolar bone loss, in some cases supplemented with microbial analysis[3]. Although these measurements are useful, they provide a measure of past destruction and are of limited use in early diagnosis[1]. They provide information on the severity of periodontitis, but they do not measure disease activity. As a result, screening and diagnostic modalities for the early identification of periodontitis initiation and progression, as well as objective measures for response to therapy, are being sought[4]. For this the medical researchers are devoted to finding the molecular disease biomarkers, as they might prove an efficient diagnostic tool[5].

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A biomarker is an objective measure that has been evaluated and confirmed either as an indicator of physiologic health, a pathogenic process or a pharmacologic response to a therapeutic intervention[1]. Biomarkers can be studied in various body fluids like serum, GCF, saliva, CSF, urine etc. There have been innumerable attempts over the years to establish methods of diagnosis or prognosis for oral disease by the analysis of saliva. Oral fluid/saliva, called the 'mirror of the body', is a perfect medium to be explored for health and disease surveillance[6].

A large number of diagnostic analytes have been shown to be present in saliva, including steroid hormones, salivary enzymes, growth factors, epithelial keratins, the HIV antibody etc. Various biomarkers in saliva for periodontitis include proteomic biomarkers, genomic biomarkers, microbial biomarkers, and others such as calcium and cortisol. These can be evaluated for various aspects of periodontitis: immunologic (salivary IgA), inflammatory (β-glucuronidase, C-reactive protein, IL-1β, IL-6, TNF-α), connective tissue degrading (α2-macroglobulin, matrix metalloproteinases, aminotransferases) and bone remodelling (alkaline phosphatase, osteoprotegerin etc.) [7,8].

Immunoglobulins (Ig) are important, specific defence factors of saliva. The predominant immunoglobulin in saliva is secretory IgA (sIgA), which is derived from plasma cells in salivary glands[9]. Salivary IgA influences the oral microbiota by interfering with the bacterial adherence or by inhibiting bacterial metabolism. It inhibits the mucosal penetration and acts as a first line of defence[3]. There is a positive correlation between the severity of inflammation and salivary IgA concentration. Also the concentration of salivary IgA depends on the presence of plaque[10].

Interleukin (IL)-1, a proinflammatory cytokine, plays a central role in immune regulation and a variety of inflammatory responses. It has been identified as a periodontal disease marker because of its function as not only an inflammatory mediator, but also as a modulator of extracellular matrix and bone. Although both isoforms of IL-1 (IL-1α and IL-1β) have similar biologic activities, IL- 1β is more potent in stimulating bone resorption and is the form that occurs more frequently in periodontitis[2].

Matrix metalloproteinases (MMPs, connective tissue destruction biomarkers) are the major players in collagen breakdown during periodontal tissue destruction. Gingival fibroblasts, keratinocytes, resident macrophages and PMNs are capable of expressing MMP-1, -2, -3, -8 and -9, inflammatory cytokines and growth factors that upregulate the transcription of MMPs. MMP-8 has the unique ability to break down type I and III collagen, which are major collagen species within the periodontium[11].

To date, some studies have reported the concentration of salivary IgA, IL-1β and MMP-8 in saliva of patients with chronic periodontitis. There is a paucity of reports about the effect of oral prophylaxis on salivary IgA, IL-1β and MMP-8 levels. Thus, the purpose of the present study was to estimate the levels (concentration) of salivary IgA, IL-1β and MMP-8, along with various clinical parameters in periodontal health and disease before and after oral prophylaxis.

Materials and methods

Patient population and study design

Patients aged 18 to 45 years visiting the Department of Periodontology and Oral Implantology, Luxmi Bai Institute of Dental Sciences and Hospital, Patiala (Punjab), were examined. The test group included 30 patients with pre-existing moderate-to-severe chronic periodontitis. The enrolment criteria for the cases were as follows:

- At least 20 teeth present.
- PD ≥ 5mm and loss of CAL ≥ 4mm in at least three teeth each in any two quadrants.
- Non-smokers.
- Individuals who have not undergone professional oral prophylaxis during the past 12 month and individuals who have not received any antibiotic, anti-inflammatory medication 6 months prior to the start of the study.

The control group included 20 individuals with clinically healthy periodontium. Exclusion criteria were:

- Patients with systemic diseases such as diabetes, arthritis, cancer, cardiovascular disorders, bleeding disorders, liver diseases, renal diseases etc, and immunocompromised patients (e.g. HIV positive, malnutrition etc.).
- Patients undergoing or who have undergone organ transplantation.
- Patients on corticosteroid medications or cytotoxic drugs.
- Pregnant and lactating patients.

This study was approved by the ethical committee of Luxmi Bai Institute of Dental Sciences and Hospital, Patiala (Punjab). Patients were given an explanation of the study and signed consent files were obtained.

Saliva sample collection

Method of collection of unstimulated whole saliva (Navazesh M and Kumar SKS 2008)[12]

The subjects were advised to refrain from intake of any food or beverage (except water) 1 hr before the test session. Chewing gum and coffee also were prohibited during this time. The subjects were advised to rinse their mouths with about 150 ml of distilled water and then to relax for five minutes. They were then told to make every effort to minimise movement, particularly mouth movement. The subjects were asked to swallow and then told to lean their heads forward over the plastic container, keeping their mouths slightly open and allow saliva to drool into the container. At the end of the collection period, the subjects were asked to collect any remaining saliva in the mouth and spit it into the test tube. This movement was allowed to be repeated in the same manner until 5 ml of saliva sample was collected. These samples were then immediately centrifuged at 5,000 rpm for 10 min and stored at -80°C for further analysis.

Clinical parameters

The full-mouth clinical periodontal measurements were recorded at four sites per tooth (mesio-buccal, mid-buccal, disto-buccal, and mid-lingual), including plaque index (PI) [13], gingival index (GI)[14], probing depth (PD), clinical attachment level (CAL) and bleeding on probing (BOP)[15]. A periodontal probe with Williams markings (Hu-Friedy, Chicago, IL) was used for periodontal measurements. The data were recorded after sample collection.

Oral prophylaxis

Oral prophylaxis consisted of full-mouth scaling, root planing and oral hygiene instructions. All the individuals received oral prophylaxis after sample collection and clinical measurements. No antibiotics were prescribed after the treatment. The patients were re-evaluated for clinical parameters 6 weeks and 12 weeks after completion of oral prophylaxis, and saliva samples were collected 12 weeks after completion of oral prophylaxis.

Salivary IgA, IL-1β and MMP-8 analysis in saliva using ELISA

Salivary concentrations of Salivary IgA, IL-1β and MMP-8 were determined for each subject using commercially available enzyme-linked immunosorbent assay kits, which were specific for these analytes individually (Human IgA ELISA Ready-Set-Go kit, Bender MedSystems, Vienna, Austria; Human IL-1β ELISA Ready-Set-Go kit eBioscience, San Diego, CA, USA; RayBio Human MMP-8 ELISA Kit from RayBiotech, Norcross, GA, USA).

The assay used the quantitative sandwich enzyme immunoassay technique. The ELISA plate was coated with 100 µl/well of capture antibody in coating buffer and was sealed and incubated overnight at 4°C. After coating, a total of 100 µl diluted standards with samples was dispensed, in duplicate, into the wells. The plate was incubated at room temperature for 1 hr, and the wells were washed three times with a wash solution. A total of 100 µl conjugate solution was added, and the plate was incubated at room temperature for 2 hrs. The wells were washed three times with a wash solution, followed by the addition of 100 µl substrate solution. The plate was incubated for 20 mins at room temperature. The addition of 50 µl stop solution was used to terminate colour development. Absorbance was determined by reading the plate in ELISA reader at 450 nm. The values obtained were multiplied by the dilution factor so as to obtain the actual concentration.

Statistical analysis

The collected data were analysed using a statistical package (SPSS software). Because the data were not normally distributed, statistical tests were performed using non-parametric techniques. The significance of statistical difference between levels of clinical parameters and biomarkers within the group before and after oral prophylaxis was tested using Student *t* test for two dependable means. P values < 0.05 were considered statistically significant.

Results

The present study was undertaken in 50 patients (30 showing the clinical evidence of generalised chronic periodontitis and 20 with the healthy periodontal condition). In the study, clinical parameters (plaque index, gingival index, probing depth, clinical attachment level and bleeding on probing) were recorded along with the levels of biomarkers in the saliva of the patients.

Table 1 shows the statistical comparison of values of clinical parameters of Group A at different time intervals. There were highly significant differences in terms of PI, GI, PD, CAL and BOP ($P < 0.001$) before oral prophylaxis, 6 weeks after oral prophylaxis and 12 weeks after oral prophylaxis when compared at these intervals. The statistical comparison of clinical

Table 1 Showing comparison of values of clinical parameters of Group A (N=30) at different time intervals.

Clinical parameter	Mean ± S.D.		*P*- value	Mean ± S.D.		*P*- value	Mean ± S.D.		*P*- value
	Before oral prophylaxis	6 weeks after oral prophylaxis		Before oral prophylaxis	12 weeks after oral prophylaxis		6 weeks after oral prophylaxis	12 weeks after oral prophylaxis	
Plaque Index	2.69 ± 0.51	1.46 ± 0.30	< 0.001*	2.69 ± 0.51	0.67 ± 0.32	< 0.001*	1.46 ± 0.30	0.67 ± 0.32	< 0.001*
Gingival Index	2.21 ± 0.38	1.29 ± 0.27	< 0.001*	2.21 ± 0.38	0.85 ± 0.41	< 0.001*	1.29 ± 0.27	0.85 ± 0.41	< 0.001*
Probing Depth	4.99 ± 0.98	3.93 ± 0.76	< 0.001*	4.99 ± 0.98	3.15 ± 0.55	< 0.001*	3.93 ± 0.76	3.15 ± 0.55	< 0.001*
CAL	6.71 ± 0.80	5.71 ± 0.73	< 0.001*	6.71 ± 0.80	4.90 ± 0.82	< 0.001*	5.71 ± 0.73	4.90 ± 0.82	< 0.001*
Bleeding on probing	2.46 ± 0.51	1.31 ± 0.44	< 0.001*	2.46 ± 0.51	0.98 ± 0.42	< 0.001*	1.31 ± 0.44	0.98 ± 0.42	< 0.001*

The significance of differences at different time intervals was determined using student *t* test for two dependable means
*Statistically highly significant
CAL, clinical attachment level

Table 2 Showing comparison of values of clinical parameters of Group B (N=20) at different time intervals.

Clinical Parameters	Mean ± S.D.		*P*- value	Mean ± S.D.		*P*- value	Mean ± S.D.		*P*- value
	Before oral prophylaxis	6 weeks after oral prophylaxis		Before oral prophylaxis	12 weeks after oral prophylaxis		6 weeks after oral prophylaxis	12 weeks after oral prophylaxis	
Plaque Index	0.25 ± 0.11	0.23 ± 0.11	0.027*	0.25 ± 0.11	0.22 ± 0.10	0.016*	0.23 ± 0.11	0.22 ± 0.10	0.329
Gingival Index	0.10 ± 0.13	0.097 ± 0.13	0.238	0.10 ± 0.13	0.095 ± 0.13	0.168	0.097 ± 0.13	0.095 ± 0.13	0.329
Probing Depth	1.87 ± 0.16	1.86 ± 0.15	0.061	1.87 ± 0.16	1.85 ± 0.15	0.219	1.86 ± 0.15	1.85 ± 0.15	0.727
CAL	3.12 ± 0.49	3.11 ± 0.49	0.186	3.12 ± 0.49	3.08 ± 0.51	0.109	3.11 ± 0.49	3.08 ± 0.51	0.303
Bleeding on probing	0.018 ± 0.03	0.017 ± 0.02	0.329	0.018 ± 0.03	0.015 ± 0.02	0.171	0.017 ± 0.02	0.015 ± 0.02	0.329

The significance of differences at different time intervals was determined using student *t* test for two dependable means
*Statistically significant
CAL, clinical attachment level

Table 3 Showing comparison of values of salivary biomarkers between Group A and Group B at two different time intervals.

Salivary biomarker	Salivary IgA (µg/ml)		IL-β (pg/ml)		MMP-8 (ng/ml)	
Group	Group A	Group B	Group A	Group B	Group A	Group B
Before oral prophylaxis	196.48 ± 54.61	81.23 ± 24.61[c]	530.76 ± 343.85	89.83 ± 25.48[c]	672.18 ± 411.0	57.95 ± 31.64[c]
12 weeks after oral prophylaxis	180.41 ± 47.68[a]	81.14 ± 24.54[c]	182.12 ± 105.69[a]	88.75 ± 24.62[c]	291.98 ± 187.39[a]	56.90 ± 32.30[c]

The significance of differences within a group was determined using student *t* test for two dependable means
The significance of differences between the groups was determined using student *t* test for two independent means
[a]Statistically highly significant, $P < 0.001$ (within group A)
[c]Statistically highly significant, $P < 0.001$ (between group A and group B)

parameters values of Group B at different time intervals is shown in Table 2. Differences in their levels were non-significant at such intervals except PI, which showed significant difference between 6 weeks after oral prophylaxis and before oral prophylaxis ($P = 0.027$), and between 12 weeks after oral prophylaxis and before oral prophylaxis ($P = 0.016$). Table 3 shows comparison of values of salivary biomarkers between group A and group B at two different time intervals. Mean salivary IgA, IL-1β and MMP-8 levels in patients with periodontitis before oral prophylaxis were significantly higher ($P < 0.001$) than in controls. The levels of these three biomarkers in patients with periodontitis reduced significantly ($P < 0.001$) after oral prophylaxis, but were still significantly higher ($P < 0.001$) than baseline values of controls. The reduction in the levels of biomarkers after oral prophylaxis in individuals with healthy periodontium was statistically non-significant ($P = 0.05$, 0.061 and 0.068 for salivary IgA, IL-1β and MMP-8 respectively). Figure 1 shows the comparison of levels of biomarkers in group A before and after oral prophylaxis. This comparison for group B is shown in Figure 2.

Discussion

Periodontal tissue destruction is assumed to result from the interaction of antigenic materials produced by dental plaque bacteria with the host's immune system. Endotoxins present in these bacteria are capable of inducing an inflammatory response by activating the immunologic effector system. The consequent release of biologically active mediators induces changes in vascular permeability, contraction of smooth muscles and chemotaxis of neutrophils. Edema and increased gingival tissue permeability may follow, allowing penetration of bacterial antigens with subsequent stimulation of the host's immune system. In addition, host neutrophils on phagocytising bacterial products may release damaging enzymes, which cause further tissue destruction.

In the continuous presence of these factors the condition becomes chronic[16].

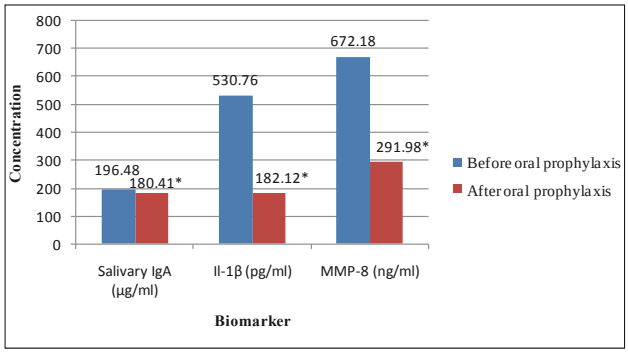

Fig 1 Showing comparison of mean levels of biomarkers in Group A before and after oral prophylaxis
*Statistically highly significant.

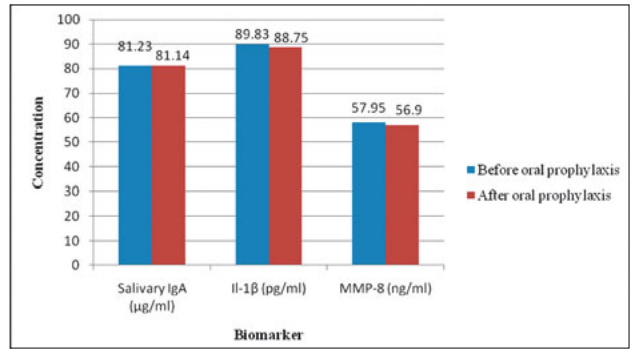

Fig 2 Showing comparison of mean levels of biomarkers in Group B before and after oral prophylaxis.

In the present study, biomarkers specific for three different aspects of periodontitis – immunologic phase (Salivary Immunoglobulin A), inflammatory phase (Interleukin-1β) and collagen degradation phase (Matrix Metalloproteinase-8) – were explored in saliva using enzyme linked immunosorbent assays.

These mediators of disease activity are studied in unstimulated saliva because of some of the problems inherent in serum and GCF sampling. Collection of blood involves potential risks to subjects, including transient discomfort, bruising, infection at the venipuncture site, and anaemia (if large volumes are required or if subjects are vulnerable). Blood collection is also less favoured in other research subjects for whom venous access is difficult (i.e. elderly or critically ill)[17]. Certain limitations are there in GCF sampling – GCF analysis is time consuming, procedure is labour-intensive, technically demanding, requiring equipment for calibrating and measuring fluid volumes, and miniscule amounts of fluid is involved which has an impact on laboratory analysis. Acquisition of saliva is easy, non-invasive, rapid, and requires less manpower and materials. Unstimulated whole saliva sample was collected as stimulated whole saliva is less suitable for diagnostic applications because the foreign substances used to stimulate saliva tend to modulate the fluid pH and generally stimulate the water phase of saliva secretion, resulting in a dilution in the concentration of analytes of interest[7]. The present study, designed taking these factors into consideration, deals with the levels of these markers in saliva of patients with chronic periodontitis and in healthy controls before and after oral prophylaxis.

In the present study, the mean levels of salivary IgA were initially 196.48 ± 54.61 µg/ml in chronic periodontitis patients. Due to quantitative dominance and superior stability IgA is a potentially important secretory immunoglobulin during periodontitis[18]. Structural studies have indicated that in secretory IgA, the conjugation of secretory piece to the dimer primarily induces inter-unit disulphide bonds, and that the quaternary structure thereafter is non-covalently stabilised. This protein conjugation may be of biological significance by rendering secretory-IgA antibodies relatively resistant to degradation[19]. Moreover, IgA is the most abundant immunoglobulin in secretions and in mucosal tissue[20].

Oral prophylaxis did elicit a decrease in IgA concentration to 180.41 ± 47.68 µg/ml when local antigenic stimuli could be reduced effectively by the clinician. This is in accordance with the results of the study conducted by Reiff[21]. The less severe the periodontal involvement, the more consistent the reduction in IgA concentration following oral prophylaxis, especially for unstimulated whole saliva. But because of the changing flora within each periodontal patient, the antigenic load presented to the periodontium will vary and the amount of salivary IgA is dependent upon the antigenic stimulus. Moreover, the more severely involved cases cannot have all local etiologic factors removed by oral prophylaxis only, so antigenic stimuli will remain[21].

Salivary IgA is mainly produced by plasma cells that are located adjacent to the duct and acini of salivary glands[9]. In a clinically healthy periodontium, antibodies to microorganisms are usually in low titers, suggesting the minimal antigenic stimulation by plaque during gingival health. The gingival tissues typically demonstrate some evidence of inflammation. Tissues are usually infiltrated with chronic inflammatory cells, generally lymphocytes. Neutrophils are also common within the junctional epithelium and in the gingival crevice. The infiltration of inflammatory cells is thought to be a response to bacterial plaque, and host defence mechanisms in a healthy individual are effective in managing the bacterial challenge[22]. The mean levels were found to be 81.23 ± 24.61 µg/ml, which were significantly lower than that of the diseased group. Such results are also found in the studies of Patil et al[3] and Butchibabu et al[23]. The levels of salivary IgA in the control group at two different time intervals are stable and the result is statistically non-significant.

In the present study, IL-1β could be detected in all the samples from patients with chronic periodontitis and healthy controls. This finding was similar to Miller et al[24] and Gursoy et al[25] who could detect IL-1β in all the saliva samples, including those of controls. The mean level of IL-1β in the saliva of the patients with generalised chronic periodontitis was found to be 530.76 ± 343.85 pg/ml in the present study.

IL-β is a prototype 'multifunctional' pro-inflammatory cytokine that plays a major role in acute and chronic inflammation[24]. Bacterial components capable of stimulating IL-1β synthesis are lipoteichoic acid, lipopolysaccharide, lipid A-associated proteins, peptidoglycan, capsular polysaccharides, toxins from both gram-positive and gram-negative bacteria etc[26].

IL-1β thus produced stimulate endothelial cells to express selectins that facilitate recruitment of leukocytes, cause activation of macrophage IL-1 production, stimulate production of inflammatory mediators (eg PGE2), induce MMPs expression, enhance osteoclast formation and activity, stimulate apoptosis of matrix-producing cells leading to inflammation, connective

tissue breakdown, bone loss and limited repair of periodontium[27].

After oral prophylaxis, the mean level reduced to 182.12 ± 105.69 pg/ml. The results are statistically highly significant and in accordance with the study of Sexton et al[28] and Kaushik et al[2]. But these values were still significantly well above those of controls and this finding is in accordance with the study of Kaushik et al[2]. In control group in the present study, the levels of IL-1β (89.83 ± 25.48 pg/ml) were lower than that found in the test group as identified in the previous studies[2,3,24]. Several mechanisms operate in the vicinity of the healthy teeth to fend off microbial infection and prevent the increase in concentration of inflammatory cytokines, including IL-1β. These mechanisms are the intact epithelial barrier of the gingiva, sulcular and junctional epithelium, salivary secretions with continuous flushing of the oral cavity and a continuing supply of agglutinins and specific antibodies, the gingival crevicular fluid with its continuous flushing of the sulcus or pocket and its serum components, like complement proteins and specific antibodies, a very high level of turnover of both the epithelium and the components of the extracellular matrix[29]. In addition to this, under conditions of health, the anti-inflammatory or protective mediators serve to control tissue destruction. If there are adequate levels of the anti-inflammatory or protective mediators to keep the host response to the bacterial challenge in check, the individual will be disease resistant[30]. The difference in the biomarker levels in control group at two different time intervals is statistically non-significant, as seen in the study of Syndergaard et al[31].

In the present study, elevated levels of MMP-8 in the saliva of patients with generalised chronic periodontitis were observed, i.e. 672.18 ± 411.0 ng/ml. Matrix metalloproteinases are members of a large subfamily (more than 25 members) of zinc- and calcium-dependent proteolytic enzymes (proteinases) responsible for remodelling and degradation of extracellular matrix components including collagens, elastin, gelatin, matrix glycoproteins, and proteoglycans[32].

These potent enzymes are made in a proenzyme (zymogen) form and activated extracellularly[32]. The latency of MMP precursors appears to be maintained, at least in part, by a coordinate bond which links an unpaired Cys residue in the propeptide to the active site Zn^{++}. Disruption of the Cys-Zn^{++} bond is a prerequisite to activation and may be achieved in a number of different ways, such as interaction or modification of the Cys residue by organomercurials, HOCl (chemical treatment), conformational change of the polypeptide backbone induced by certain chaotropic agents (KI, NaSCN) and detergents (SDS), (physical treatment), excision of a portion of the propeptide by proteolytic enzymes (trypsin, plasmin, chymotrypsin, neutrophil elastase, cathepsin B, and plasma kallikrein) (Enzymatic treatment) [33,34]. These proteolytic enzymes are obtained from tissue, plasma and bacteria and are raised in amount in inflammation[35]. In a second step, these active forms can be auto-catalytically cleaved by the activated metalloproteinase to remove the propeptide and confer permanent activity[34].

Once activated, these proteinases are involved in a number of physiological events, such as embryological development, tissue remodelling, wound healing, salivary gland morphogenesis and tooth eruption, in addition to various pathological processes, such as periodontal disease, arthritis, cancer, atherosclerosis, pulmonary emphysema and osteoporosis[32].

MMP-8 in periodontitis lesions is derived from a variety of host cells, such as polymorphonuclear leukocytes, macrophages, fibroblasts, bone, epithelial and endothelial cells[32]. Of all the MMPs of various groups, MMP-8 has the unique ability to break down type I and III collagens, which are the major collagen species within the periodontium (found in gingiva, periodontal ligament, and alveolar bone)[32,36].

So, in periodontal diseases, matrix metalloproteinases play key roles in the degradation of the extracellular matrix, basement membrane and protective serpins as well as in the modification of cytokine action and activation of osteoclasts[37].

The mean level of MMP-8 reduced to 291.98 ±187.39 ng/ml after oral prophylaxis. Similar results were found by Sexton et al[28] in their study in which they compared the levels of MMP-8 along with some other biomarkers in the saliva of chronic periodontitis patients before and after scaling and root planing. In control group in the present study, the levels of MMP-8 (57.95 ± 31.64 ng/ml) were found to be lower than that found in the test group, as identified in the studies of Miller et al[24] and Patil et al[3]. In periodontally healthy individuals the levels of MMP-8 are found within the normal limits and lesser than that found in the diseased individuals because of the lesser amount of ECM degrading components and more of the endogenous inhibitors of MMP-8 in such individuals. Studies have shown that high concentrations of the natural tissue inhibitor of MMPs (TIMPs) are found in the gingival tissues of healthy gingiva. Thus, the levels of MMP-8 are found to be low in such individuals[38]. The difference in the biomarkers levels in control group at two different time intervals is statistically non-significant as seen in the study of Syndergaard et al[31].

The levels of clinical parameters in generalised chronic periodontitis patients in the present study were higher than that of periodontally healthy controls. All parameters of periodontal health improved highly significantly in generalised chronic periodontitis patients after 6 weeks and after 12 weeks of oral prophylaxis compared with the levels before oral prophylaxis. The findings are in accordance with the previous studies[2,23,24,28].

The findings of markedly elevated biomarkers Salivary IgA, Interleukin-1β and MMP-8 levels and clinical parameters levels and their significant reduction post-therapy in patients with chronic periodontitis compared to healthy controls suggest a close association between these biomarkers and periodontal disease. The findings are in accordance with the studies conducted by various researchers[2,21,24,28,36,39]. Therefore, these three biomarkers might be potentially useful in distinguishing health from disease and monitoring periodontal disease activity and response to therapy.

Within the limitations of the study, like-elevated levels of these biomarkers may be obtained in some other unknown chronic diseases. Antigenic load presented to the periodontium may vary because of changing microflora within each periodontal patient. Moreover, with saliva as a source of biomarkers, whole saliva contains contributions from the GCF, oral bacteria, cells and other sources that make identification of the exact site of disease activity limited, and flow rate of saliva varies within and between individuals, thus affecting the concentrations of biomarkers. Other biomarkers associated with the onset and development of periodontal diseases not examined here might still be present in saliva at levels that could be used to discriminate between periodontal health and disease. Further investigation and more longitudinal studies with larger sample sizes and longer time duration with more intervals are needed to identify the set of biomarkers with the most favourable combination of sensitivity, specificity, and positive/negative predictive values.

In conclusion, the findings suggest that salivary IgA, Interleukin-1β and MMP-8 might be potentially useful in distinguishing health from disease with early diagnosis, monitoring periodontal disease activity, and response to therapy. Since in more severely involved cases it is difficult to get certain concentrations of salivary biomarkers to return to healthy levels by non-surgical therapy alone due to the presence of antigenic stimulus and continuous production of inflammatory mediators, it could be postulated that some additional therapies like local drug delivery, periodontal surgery etc. may be required to return a patient to biologic health.

Conflicts of interest

The authors reported no conflicts of interest related to the study.

Author contribution

Dr Vanik Rangbulla designed the study, analysed data and prepared manuscript; Dr Ashutosh Nirola collected data; Drs Madhu Gupta and Priyanka Batra designed the study, collected data, analysed data, and prepared manuscript; Dr Mili Gupta collected and analysed data.

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