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Research Article

STUDY OF GINGIVAL CREVICULAR FLUID LEVEL OF SOLUBLE TRIGGERING RECEPTOR EXPRESSED ON MYELOID CELLS-1 AS PER PERIODONTAL HEALTH STATUS

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ABSTRACT

Background: There is a need for the development of new diagnostic tests that can detect the presence of active periodontal disease, predict future disease progression and evaluate the response to periodontal therapy.
Aim: To study gingival crevicular fluid level of soluble triggering receptor expressed on myeloid cells-1 as per periodontal health status.
Methods: Thirty subjects were divided in three groups as per periodontal health status: healthy (Group A), gingivitis (Group B) or chronic periodontitis (Group C). Plaque Index (PI), Gingival Index (GI), Probing pocket depth (PPD) and Clinical attachment level (CAL) were recorded
Results: At baseline, the mean sTREM-1 levels in group A, group B and group C were 68.1±2.2 pg/ml, 263.14±80.3 pg/ml and 3664.3 ±13135.4 pg/ml respectively. A reduction was seen from baseline to 8 weeks in group B and group C, with mean sTREM-1 level in group B and group C of 115.98±36.04 pg/ml and 228.07±102.76 pg/ml respectively. A positive correlation between GCF levels of sTREM-1 and Plaque Index (PI), Gingival Index (GI), Probing pocket depth (PPD) and Clinical attachment level (CAL) was also observed.
Conclusion: sTREM-1 were observed in periodontitis patients. It may act as inflammatory biomarker of periodontal disease and can be useful both as a diagnostic biomarker and predictor of future disease initiation and progression.

INTRODUCTION

Periodontal diseases present as inflammation, connective tissue disruption and alveolar bone destruction and are important polymicrobial infections in human beings. Symptoms are usually mild during the early phase of disease, and subjects tend to ignore the condition until more severe symptoms appear.[1] There is a need for the development of new diagnostic tests that can detect the presence of active disease, predict future disease progression and evaluate the response to periodontal therapy.

The Triggering Receptor Expressed on Myeloid cells 1 (TREM-1) is a cell-surface receptor which propagates the inflammatory process to bacteria. Triggering Receptor Expressed On Myeloid cells-1 (TREM-1) has been identified in gingival crevicular fluid (GCF) and associated with periodontal disease pathogenesis. The engagement of TREM-1 in in vivo and in vitro models by agonist monoclonal antibodies further stimulates the production of pro-inflammatory cytokines.[2,3] Even though the systemic involvement of sTREM-1 has been demonstrated in a number of infections, little is known about its association with periodontal disease. Gingival crevicular fluid (GCF) is an inflammatory exudate that reflects ongoing events in the periodontal tissues that produce it, may act as potential diagnostic or prognostic markers for the progression of the disease.[4]

Analysis of GCF levels of sTREM-1 may provide useful clue into the pathogenesis. It may also provide a lead to newer set of tools to diagnose and manage the patients with advanced periodontitis. Thus, this study was planned with an objective to analyze GCF levels of sTREM-1 in periodontally healthy, gingivitis and chronic periodontitis subjects and evaluate the

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effect of non- surgical periodontal therapy in the form of scaling and/or root planing on the same.

METHODS

The study was conducted at the department of Periodontics of tertiary care dental teaching hospital of northern India. Subjects were selected from both genders, and preferably in the age group of 20-50 years, and having at least 20 teeth. Thirty subjects were divided in three groups as per periodontal health status: healthy (Group A), gingivitis (Group B) or chronic periodontitis (Group C).

Group A- Absence of clinical signs of gingival inflammation (GI= 0), probing depth ≤ 3mm, absence of loss of clinical attachment.
Group B- Presence of clinical signs of gingival in- flammation (GI >1), probing depth ≤ 3mm, absence of loss of clinical attachment.
Group C- Presence of at least four teeth in each jaw with probing pocket depth of ≥ 5mm along with clinical signs of gingival inflammation, clinical attachment loss of ≥ 3mm, radiographic evidence of alveolar bone loss.

***Exclusion Criteria*:** 1) those with systemic diseases like diabetes, hypertension, heart disease and rheumatoid arthritis that could alter the course of periodontal disease 2) who have taken antibiotic/ anti-inflammatory/ immuno- suppressive medication in the previous 6 months of periodontal therapy 3) who have undergone periodontal therapy in the previous 6 months of the study 4) Pregnant women and lactating mothers 5) Smokers and subjects using smokeless tobacco.

The assessment of the samples for the detection of sTREM-1 was carried out in the Microbiology laboratory. Plaque Index (PI), Gingival Index (GI)[6], Probing pocket depth (PPD) and Clinical attachment level (CAL) were recorded at baseline for all the three groups and at follow-up visits scheduled at 8 weeks following SRP in group B and group C. PPD and CAL were recorded using a constant pressure probe. Orthopantomogram (OPG) for every patient showing clinical signs of attachment loss was taken at baseline. Radiographic bone loss was recorded dichotomously to differentiate subjects with chronic periodontitis from other groups. GCF was collected from all the three groups at baseline and 8 weeks following SRP in groups B and C. GCF was collected by placing a microcapillary pipette at the entrance of the gingival sulcus, gently touching the marginal gingiva. 1µl was collected with an extracrevicular approach from test site.

Levels of sTREM-1 in GCF was estimated by ELISA by ELISA kit. Samples were incubated with a monoclonal antibody specific for TREM-1 pre-coated onto the wells of a micro plate. Following a wash, to eliminate the unbound substances, an enzyme-linked polyclonal antibody specific for TREM-1 was added to the wells. After washing away the unbound conjugate, a substrate solution was added to the wells. Color development was stopped, and optical density of each well was determined within 30 minutes using a micro plate reader set to 450 nm, with a wave- length correction set to 540 nm. All measurements were performed in duplicate and the sTREM-1 concentration was expressed in picogram/mililiter (pg/ml). The mean absorbance for duplicate standards, controls and samples was calculated, and the average zero standard optical density was subtracted from it.

Written and informed consent was obtained from study subjects. Permission of ethical committee was obtained from the Institutional Ethics Committee. All the questionnaires were manually checked and edited for completeness and consistency and were then coded for computer entry. After compilation of collected data, analysis was done using Statistical Package for Social Sciences (SPSS), version 21 (IBM, Chicago, USA). The results were expressed using appropriate statistical variables.

RESULTS

Mean age of study participants were; Group A- 23. 65±2.5 years, Group B- 32.73±5.8 years, Group C- 41.02±7.5 years. Table 1 shows the Probing pocket depth, Clinical attachment loss, Plaque index and Bleeding on probing among the three groups.

Table 1 Periodontal measurements (mean ± standard deviations) of the three study groups

Variables	Group A	Group B	Group C
Probing pocket depth (mm)	1.80 ± 0.26	4.95 ± 0.37	5.32 ± 0.77
Clinical attachment loss (mm)	1.83 ± 0.28	5.56 ± 0.67	6.02 ± 1.07
Plaque index	0.85 ± 0.39	2.35 ± 0.48	2.09 ± 0.44
Bleeding on probing (%)	25 ± 15	74 ± 41	66 ± 21

Following levels of sTREM-1 were detected in GCF samples of all the groups. At baseline, the mean sTREM-1 levels in group A, group B and group C were 68.1±2.2 pg/ml, 263.14±80.3 pg/ml and 3664.3 ±13135.4 pg/ml respectively; this difference between the groups were statistically significant ($p<0.05$) (Table 2).

Table 2 Levels of sTREM-1 were detected in GCF samples of all the groups at baseline

Variables	Group A	Group B	Group C
sTREM-1 levels (pg/ml)	68.1±2.2	263.14±80.3	3664.3 ±13135.4
Level of significance		p<0.05	

A reduction was seen from baseline to 8 weeks in group B and group C, with mean sTREM-1 level in group B and group C of 115.98±36.04 pg/ml and 228.07±102.76 pg/ml respectively.
The potential correlation between GCF levels of sTREM-1 and Plaque Index (PI), Gingival Index (GI), Probing pocket depth (PPD) and Clinical attachment level (CAL) was also investigated, a positive correlation was observed. ($r = 0.45$, $p < 0.0001$).

DISCUSSION

TREM-1 is a cell-surface receptor of the immunoglobulin family, expressed on the surface of human polymorphonuclear neutrophils (PMNs) and monocytes. It gets activated upon bacterial recognition by host cells via toll like receptors and triggers a number of intracellular signalling events that result in enhanced pro-inflammatory cytokine production. Various infections can cause up-regulation of membrane-bound TREM-1 as well as release its soluble form (sTREM- 1), rendering it a useful early inflammatory biomarker for systemic infections. TREM-1 amplifies the inflammatory response induced by pathogenic bacteria, it may serve as a useful marker for detecting the progression of periodontal lesions.

We obsrved that, at baseline, the mean sTREM-1 levels in group A, group B and group C were 68.1±2.2 pg/ml, 263.14±80.3 pg/ml and 3664.3 ±13135.4 pg/ml respectively. A reduction was seen from baseline to 8 weeks in group B and group C, with mean sTREM-1 level in group II and group III of 115.98±36.04 pg/ml and 228.07±102.76 pg/ml respectively. Another study from France[9] found that mean sTREM-1 level in collected fluid was significantly higher in pathological sites than in healthy sites from either periodontal or control patients: 353.9 pg/ml, 50.2 pg/ml and 25.4 pg/ml respectively. Soluble TREM-1 concentration was significantly correlated with PPD. The sTREM-1 levels increased with the augmentation of the PlI and GI scores and levelled off at score 2 for both indexes.

In GCF analysis using sandwich ELISA techniques, sTREM-1 levels are significantly increased in pathologic sites of periodontitis when compared to healthy samples or control sites of healthy gingiva in patients with periodontal disease. There was a significant increase of sTREM-1 from pockets with PPD 0–3mm to PPD 5–7mm, but not again for pockets with a PPD of 8+mm. sTREM-1 levels varied between participants and between sites in the same participant and were higher in mobile teeth; smoking affected sTREM-1 levels, but the impact did not reach significance.[10]

The lack of measurable sTREM-1 elevation as periodontitis advances clinically could be due in part to bacterial proteolysis of sTREM-1 or the progression of the disease from an acute to chronic state. In another study evaluating GCF samples of 62 participants (20 controls, 22 with CP, and 20 with GAP each with at least 20 teeth).[11]

In GCF samples from elderly patients, there was sTREM-1 expression in all samples with no significant differences between healthy participants, or participants with gingivitis or periodontitis. The researchers posit this elevation is due to a dysregulated immune response in elderly populations due to compromised function of monocytes and macrophages (e.g. reduced chemotaxis, phagocytosis, production of reactive oxygen, and chemokine response).[12,13]

In this study, The potential correlation between GCF levels of sTREM-1 and Plaque Index (PI), Gingival Index (GI)6, Probing pocket depth (PPD) and Clinical attachment level (CAL) was also investigated, a positive correlation was obsrved. This finding supports the role of sTREM-1 in the propagation of inflammation in response to microbial infections. Further evidence to support this comes from the positive correlation of sTREM-1 levels with bleeding on probing (BOP), a finding backed up by the earlier demonstration of a positive correlation with gingival index scores.[14,15]

CONCLUSION

This study observed that the role of TREM-1 in periodontal tissue destruction as high GCF levels of sTREM-1 were observed in periodontitis patients. It may act as inflammatory biomarker of periodontal disease and can be useful both as a diagnostic biomarker and predictor of future disease initiation and progression. Further studies are warranted to determine the molecular role of sTREM-1 in bone destruction.

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