Original Article

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Dentistry Section

Comparison of Serum Levels of Endothelin-1 in Chronic Periodontitis Patients Before and After Treatment

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ABSTRACT

Introduction: Endothelin-1 (ET-1) is a potent vasoconstrictive peptide with multi functional activity in various systemic diseases. Previous studies indicate the detection of ET-1 in gingival tissues and gingival crevicular fluid.

Aim: The aim of this study was to estimate the serum ET-1 levels in clinically healthy subjects and subjects with chronic periodontitis, before and after treatment, and correlate it with the clinical parameters.

Materials and Methods: A total of 44 patients were included in the study. Group I comprised of 20 subjects with clinically healthy periodontium. Group II comprised of 24 subjects with chronic periodontitis. Group III comprised of same Group II subjects following periodontal management. Serum samples were collected from the subjects and an Enzyme Linked Immunosorbent Assay (ELISA) was done to estimate the ET-1 levels. The ET-1 levels were then correlated among the three groups with the clinical parameters namely, Plaque Index (PI), Sulcus Bleeding Index (SBI), probing pocket depth, clinical attachment loss and Periodontally Inflamed Surface Area (PISA). The independent t-test and paired t-test were used for comparison of clinical parameters and Pearson's correlation coefficient test was used for correlating the ET-1 levels.

Results: ET-1 levels in chronic periodontitis subjects were significantly higher compared to healthy subjects ($p<0.001$). However, the clinical parameters did not statistically correlate with the ET-1 levels. There was a significant decrease in ET-1 levels following treatment ($p<0.001$).

Conclusion: Serum ET-1 is increased in chronic periodontitis and reduces after periodontal therapy. Further studies are required to establish ET-1 as a biomarker for periodontal disease.

Keywords: Periodontally inflammed surface area, Periodontal therapy, Systemic diseases

INTRODUCTION

ET-1 is one of three sub types namely ET-1, ET-2 and ET-3, which was identified in 1988 as a potent vasoconstrictor [1]. It is a 21 amino acid peptide, which acts via its receptors, ET A or ET B, to express its vasoactive properties [2]. It is produced in vascular endothelial cells from a prepropeptide called big ET-1, via Endothelin Converting Enzyme (ECE) [3]. It is also secreted by fibroblasts, epithelial and smooth muscle cells.

ET-1 plays a role in pathogenesis of various systemic diseases. It is found to contribute to the development of vascular diseases such as hypertension [4], and atherosclerosis [5] through the activation of ETA receptors. It is also found to play a role in development of pulmonary hypertension by regulating the bronchial tone and proliferation of pulmonary airway blood vessels [6]. It is also found to play a role in respiratory inflammation [7], liver disease [8], gastric ulcer [9], bone metabolism [10] and diabetes [11].

Chronic periodontitis is a host mediated inflammatory disease which is provoked by pathogenic microorganism and is characterized by elevated levels of various cytokines and inflammatory mediators [12].

ET-1 has also been identified in periodontal diseases such as chronic periodontitis [13-18] and drug-induced gingival overgrowth [19-22] and has found to play a role in the pathogenesis [14,23]. Out of two studies which estimated ET-1 levels in Gingival Crevicular Fluid (GCF), one showed elevated levels of ET-1 [16] whereas, the other did not detect ET-1 [24]. Also, an animal study which was done on rats shows a 2.2 fold increase in levels of ET-1 in aortic samples in ligature induced periodontitis [25].

The available literature suggests ET-1 may play a link between the prolonged chronicity of periodontal disease and its association with systemic and cardiovascular diseases.

Hence, this study was aimed to estimate the serum ET-1 levels in clinically healthy subjects and subjects with chronic periodontitis, and also to compare the serum ET-1 levels before and after treatment in chronic periodontitis patients, and correlate it with the clinical parameters.

The null hypothesis for the primary outcome of the study was to prove that there is no significant increase in serum levels of ET-1 in subjects with chronic periodontitis compared to healthy subjects, and it does correlate with the clinical parameters. The null hypothesis for the secondary outcome of the study was to prove that there is no significant difference in serum levels of ET-1 in patients with chronic periodontitis, before and after treatment, and it does not correlate with the clinical parameters.

MATERIALS AND METHODS

The study had two parts, a cross-sectional and longitudinal part. The study population included 44 subjects (20 females, 24 males, age range 30-50 years) attending the outpatient clinic of the Department of Periodontics, Saveetha Dental College and Hospital, Chennai , India. Written informed consent was obtained from those who agreed to participate voluntarily. The study was approved by the scientific review board, and ethical clearance was obtained from the ethical committee of Saveetha University, Chennai.

Sample Size Calculation

Sample size calculation was done based on the results of the study by Fujioka D et al., [16]. At a power of 90% and confidence interval

of 95%, the required sample size was calculated to be 18 per group. The present study included minimum of 20 subjects in each group.

Inclusion and Exclusion Criteria

The study comprised of three groups. Patients were chosen based on clinical and radiographical examination. These patients were diagnosed on the basis of American Association of Periodontology (AAP) criteria of 1999 [26]. Group I consisted of 20 patients with clinically healthy periodontium and probing pocket depth of <3 mm and clinical attachment loss of 0. Group II consisted of 24 subjects who showed clinical signs of inflammation, a pocket probing depth of ≥5 mm and clinical attachment loss of ≥4 mm for atleast 10 sites, with radiographic evidence of bone loss. Group III consisted of group II patients three months following treatment. The Group II patients were treated either by non-surgical procedures or by periodontal surgery, based on the pocket depth. Shallow pockets were treated by non-surgical procedures whereas, deep pockets were treated by periodontal flap surgeries with resective or regenerative osseous surgeries [27] wherever indicated.

Subjects with a history of diabetes, hypertension, sclerotic diseases, hepatic cirrhosis, coronary heart disease, chronic renal failure, gross oral pathology, habits of smoking, betel nut/areca nut chewing or alcoholism, taking anti-inflammatory drugs, antibiotics, H2 blockers and/or immunosuppressive drugs or who had received periodontal therapy in the preceding six months were excluded from the study, as these factors could influence the expression of ET-1 in the serum.

Measurement of Outcome Parameters

Each subject underwent a full mouth periodontal probing with a UNC-15 periodontal probe and the clinical parameters assessed were PI [28], SBI [29], probing pocket depth, clinical attachment loss and PISA score [30]. All the measurements were done by a single examiner.

The primary outcome of the study was to compare the serum levels of ET-1 in healthy subjects and subjects with chronic periodontitis, and correlate with clinical parameters. The secondary outcome of the study was to evaluate and compare the serum levels of ET-1, before and after treatment, and correlate with the clinical parameters.

Serum Collection

The patients did not receive any initial periodontal therapy before serum collection. Almost 2 ml blood was withdrawn from the antecubital vein under aseptic conditions. Blood was then collected in a sterile test tube and allowed to clot for 30 minutes at room temperature before centrifugation for 15 minutes at 1000 rpm. Serum was then separated and stored at ≤ -70 °C. For Group III, serum collection was done after one month after periodontal therapy, during the review appointment.

ELISA

ELISA procedure was carried out for the stored samples by using a commercially available ELISA kit (R&D SYSTEMS, QUANTIKINE ELISA endothelin-1 kit, and catalog number DET-100) at the central research facility Regenix Super Specialty Laboratories Pvt. Ltd. Chennai, India.

The assay employs the quantitative sandwich enzyme immunoassay technique, where a monoclonal antibody specific for ET-1 is pre-coated onto the microplate. Standards (concentrations 25pg/ml, 12.5 pg/ml, 6.25 pg/ml, 3.13 pg/ml, 1.56 pg/ml, 0.78 pg/ml, 0.39 pg/ml and 0 pg/ml) and samples were then pipetted into the wells and any ET-1 present is bound by the immobilized antibody. The ELISA test was performed, according to the manufacturer's instructions manual. A standard was computed by plotting the optical density reading of the diluted standard. After plotting the standard curve, the optical density was extrapolated on the Y-axis and concentration in pg/ml was measured on the X-axis. The duplicate readings for each standard and control were averaged to give the mean concentration.

STATISTICAL ANALYSIS

SPSS 17.0 software was used for statistical analysis. The cross-sectional part of the study included the comparison of clinical parameters between Group I and Group II which were done by independent t-test. The longitudinal part of the study included the comparison between Group II and Group III by paired t-test. The correlation of serum concentration of ET-1 with clinical parameters was done using Pearson's correlation coefficient test.

RESULTS

Comparison between Groups

A significant difference (p<0.001) was seen on comparing the mean serum ET-1 levels in Group I (1.02±0.35 pg/ml) and Group II (2.52±0.52 pg/ml). Also, a significant reduction (p<0.001) in Group III (1.53±0.67 pg/ml) was seen, when compared to the chronic periodontitis group [Table/Fig-1].

Significantly elevated clinical parameters were noticed in Group II compared to Group I. These parameters were significantly reduced in Group III (p<0.001) [Table/Fig-1].

Correlation of Clinical Parameters with Serum ET-1 Levels

A statistically insignificant correlation between the clinical parameters and serum ET-1 is seen [Table/Fig-2].

DISCUSSION

Periodontitis is an inflammatory disorder characterised by the interaction between pathogenic bacteria and the immune and inflammatory responses of the host. A complex network of cytokines is involved in the inflammatory and immune responses in the inflamed

Parameters	Group I: Healthy	Group II: Chronic Periodontitis	Group III: Treatment	Independent t-test value (Grp.I VS Grp.II)	p-value (Grp.I VS Grp.II)	Paired t-test value (Grp.II VS Grp.III)	p-value (Grp.II VS Grp.III)
Plaque Index (Mean±SD)	0.64±0.12	1.89±0.37	0.37±0.10	14.27	<0.001*	19.71	<0.001*
Bleeding Index (Mean±SD)	0.53±0.09	2.70±0.34	0.36±0.14	28.10	<0.001*	32.36	<0.001*
Probing Depth (Mean ±SD)	0.79±0.23	3.99±0.64	1.06±0.43	20.87	<0.001*	21.01	<0.001*
Clinical Attachment Level (Mean±SD)	NA	5.08±0.99	1.10±0.72	NA	NA	28.29	<0.001*
PISA Score (Mean ±SD)	298.22 ±62.22	1895.97 ±500.94	126.48 ±81.3	14.08	<0.001*	17.41	<0.001*
Serum ET-1 Levels (pg/ml) (Mean±SD)	1.02±0.35	2.52±0.52	1.53±0.67	10.80	<0.001*	8.6	<0.001*

[Table/Fig-1]: Comparison of clinical parameters between the groups.
NA = Not Applicable *= Statistically Significant p≤ 0.001

Parameters	Pearson's Correlation Coefficient	Group I: Healthy	Group II: Chronic Periodontitis	Group III: Treatment
Plaque Index	Correlation Coefficient	0.243	0.116	-0.410
	Significance	0.303	0.588	0.047
Bleeding Index	Correlation Coefficient	0.297	0.151	-0.228
	Significance	0.204	0.481	0.284
Probing Depth	Correlation Coefficient	-0.099	0.070	0.171
	Significance	0.677	0.745	0.425
Clinical Attachment Loss	Correlation Coefficient	NA	-0.214	-0.077
	Significance	NA	0.315	0.721
PISA Score	Correlation Coefficient	0.025	0.054	-0.055
	Significance	0.915	0.804	0.797

[Table/Fig-2]: Correlation of clinical parameters with serum ET-1 levels.
NA = Not Applicable Pearson's correlation test applied

periodontal tissues during the progression of periodontal disease [31,32]. To this long list of cytokines and inflammatory mediators, one of the newest additions is ET-1, a vasoactive substance that might play a role in inflammatory process. It has been established from various studies that ET-1 and its receptors ET-A and ET-B are produced and secreted by various gingival and periodontal ligament cells such as endothelial cells, human gingival fibroblast, human periodontal ligament cells and human gingival keratinocytes and is found to be elevated in gingival crevicular fluid in periodontitis [15,16].

Mean serum ET-1 concentration in the periodontitis group (2.52± 0.52 pg/ml) was significantly higher than the healthy group (1.02± 0.35 pg/ml). Also, a significant reduction in the mean serum ET-1 concentration is seen in the treatment group (1.53±0.67 pg/ml) compared to the chronic periodontitis group. Previous studies have reported an upregulation of ET-1 in inflamed gingival tissues and periodontal tissues. Chen S et al., found an increased level of ET-1 in inflamed gingiva (0.856±0.788 pg/ml) when compared to healthy tissue (0.139±.810 pg/ml) [18]. These results were in concurrence with a study by Ansai T et al., where gingival tissues from adult periodontitis subjects, showed increased expression of ET-1 (9 pg/ml) compared with tissue from normal healthy donors (4 pg/ml) [17]. Yamamoto E et al., also had similar findings that showed increased concentration of ET-1 in chronic periodontitis group (6 pg/ml) when compared with the periodontally healthy group (1 pg/ml) [15]. Tamil selvan T et al., compared the ET-1 levels in three different groups, namely healthy, chronic periodontitis and cyclosporine induced gingival overgrowth. The results revealed an elevated ET-1 level in the gingival tissue samples of chronic periodontitis patients (373.6 pg/mg) than the healthy controls (84.8 pg/mg) [20]. Two studies assessed the GCF levels of ET-1. Fujioka D et al., found a higher concentration of ET-1 in chronic periodontitis (388.6 pg/ml) than periodontally healthy subjects (46.8 pg/ml) [16]. But, the study by Pradeep AR et al., did not detect ET-1 in GCF [24].

The present study found a significant reduction in the ET-1 levels following treatment. Since there is no previous literature available on comparison of ET-1 levels before and after treatment in chronic periodontitis, a direct comparison of our results is not possible. Nevertheless, a study by Thomas Biekler et al., evaluated the gene expression profiles of various cytokines following non-surgical therapy, and found that ET-1 gene expression was in the least 5% expressed [33].

In the present study, we also correlated the clinical parameters with serum ET-1 levels. The Pearson's rank correlation between clinical parameters and serum ET-1 shows no statistical significance in all three groups when assessed. The possible reason for this could be that within the group, the range of the individual clinical parameters was very small to be correlated. The increased serum level of ET-1 in periodontitis patients which was observed in our study could be due to the local production of ET-1 from periodontal cells influenced by periodontal pathogens and cytokines. *P.gingivalis* stimulates ET-1 expression with up-regulation of inflammatory cytokines and intercellular adhesion molecules in epithelial cell line, and may also stimulate the induction of ET-1 in oral epithelial cell line [15, 17]. This stimulation may contribute to chronic inflammatory reaction in periodontitis.

Studies have shown that proinflammatory cytokines like interleukin-1β (IL-1β) and Tumour Necrosis Factor- α (TNF-α) are responsible for the upregulation of ET-1 in gingival and periodontal tissues [16]. From these studies it is clear that periodontal organisms and cytokines influence the production of ET-1.

It has been suggested that ET-1 and pro-inflammatory cytokines may establish an inflammatory loop, which can become independent of the original stimulus and contribute to long-term inflammatory changes in bronchial epithelium [7,34]. In a similar model in periodontitis, ET-1 may induce the production of pro-inflammatory cytokines in human periodontal ligament cells. Additionally, the secreted pro-inflammatory cytokines may simultaneously stimulate the expression of ET-1 in periodontal tissues [23]. In the inflamed gingival tissue the continuous relapsing inflammations could further stimulate the endothelial cells and promote ET-1 expression that would cause increased vessel constriction and vascular injury independent of the original stimulus [18]. This suggests that the increased expression of ET-1 in gingival epithelial cells and periodontal ligament cells might be related to the inflammatory events of periodontitis. It has been found in the study by Lerman A et al., a 2.28 fold increase in ET-1 concentration in atherosclerosis patients compared to healthy subjects [35]. A study in a ligature induced periodontitis rat model by Ekuni D et al., there was a 2.2 fold increase in levels of ET-1 mRNA expression compared to control groups in descending aorta samples [25]. Collectively, the data suggests that ET-1 can be an additional link by which periodontitis leads to the development of cardiovascular complications.

LIMITATION

A limitation of our study is the relatively smaller sample size. As a result, a statistically significant correlation between the ET-1 levels and clinical parameters could not be established. The study had three groups namely, healthy, chronic periodontitis and treatment groups. Further studies can address this limitation by adding more groups with varying degrees of periodontal diseases such as gingivitis and aggressive periodontitis. This can help to establish concentrations of ET-1 for every stage of periodontal disease and establish ET-1 as a diagnostic biomarker for periodontal disease.

In our study, we found that there is almost a 2.5 fold increase in the serum ET-1 levels in chronic periodontitis group compared to the healthy group. We also found there is a significant reduction of serum ET-1 levels in treatment group (1.53±0.67 pg/ml), which is almost similar to the healthy group levels (1.02±0.35 pg/ml). According to Cullinan MP et al., one of the mechanisms associating periodontitis and systemic diseases is inflammation [36]. Increased ET-1 levels might have a role in increasing the inflammatory burden, thereby increasing the risk of cardiovascular disease risk. Studies by Armitage GC and Yin Ouyang et al., have reported a beneficial effect of periodontal treatment on pregnancy outcomes [37] and on cardiovascular disease risk [38] respectively. From our results, we can hypothesize that the reduced ET-1 levels observed in our study indirectly will have a beneficial effect on cardiovascular system and other systemic diseases. Further studies are needed to prove this hypothesis.

CONCLUSION

From the present study it is evident that serum ET-1 is upregulated in chronic periodontitis subjects than healthy subjects and there is a significant reduction following treatment. Further longitudinal studies are required to establish an exact role of ET-1 in the pathogenesis of periodontal disease and its influence on systemic diseases such as cardiovascular diseases. Further interventional studies with a larger sample size are needed to establish its role as a diagnostic and prognostic marker.

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