**Role of endothelin-1 in periodontal diseases: A structured review**

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**ABSTRACT**

**Background and Objective:** Endothelin-1 (ET-1) is a 21-amino acid peptide and is a potent vasoconstrictor produced by endothelial cells. It plays a role in the development of diseases such as hypertension and atherosclerosis. Previous studies have identified ET-1 in gingival tissues obtained from patients affected by chronic periodontitis or gingival overgrowth. Thus, there is a need to appraise the role of ET-1 in periodontal disease.

**Materials and Methods:** The electronic search strategy included the databases such as PubMed, PubMed Central, LILACS, MEDLINE, ScienceDirect, MedSH, Cochrane database of systematic reviews, and EMBASE databases. Hand search of relevant journals was also carried out until September 2013. The included studies were both cross-sectional and longitudinal performed *in vivo*/*in vitro*, which measures the expression of ET-1 from various cells of the periodontium and in periodontal disease. Further, studies assessing the factors which influence ET-1 expression were included in the study.

**Results:** A total of 15 articles were found relevant and fulfilled the inclusion criteria posed in this review. Ten studies discussed the concentration of ET-1 in periodontal disease, whereas eight studies investigated the cells expressing ET-1. Nine studies assessed the factors influencing ET-1 expression and two studies evaluated the influence of ET-1 on inflammatory mediators and other cytokines. The results suggested that ET-1 is elevated in periodontal diseases and is influenced by inflammatory cytokines and periodontal pathogens.

**Conclusion:** ET-1 was found to have a role in periodontal disease, but further research will be required to substantiate its use as a biomarker.

**Key words:** Chronic periodontitis, cytokines, gingival overgrowth, endothelin

Endothelins were originally identified by Yangisawa in 1988. It is of three different subtypes which include endothelin-1 (ET-1), ET-2, and ET-3. ET-1 is the most common type seen in humans. It is composed of 21 amino acid residues and is a potent vasoconstrictor.

It is mostly secreted by endothelial cells, epithelial cells, macrophages, smooth muscle cells, and fibroblasts. It is involved in a wide array of disease states such as atherosclerosis, hypertension, and inflammatory and sclerotic diseases.

Chronic periodontitis is a host-mediated inflammatory disease which is provoked by pathogenic microorganisms, and it is characterized by elevated levels of various cytokines and inflammatory mediators. Drug-induced gingival overgrowth can be encountered as a side effect in patients under long-term usage of drugs such as cyclosporine, phenytoin, and nifedipine. Studies have reported an expression of ET-1 in these periodontal conditions. Thus, there is a strong need to probe into the role of ET-1 in various periodontal diseases.

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This structured review aims to evaluate the role of ET-1 in periodontal disease by addressing the following questions:

- What are the cells which express ET-1 in the periodontium?
- What is the influence of ET-1 on other cytokines and other inflammatory mediators in periodontal tissues?
- What are the levels of ET-1 in periodontal health and disease? With increasing severity of the disease, is there a concomitant increase in ET-1 levels?
- What are the factors influencing ET-1 expression in periodontal disease?

MATERIALS AND METHODS

Search strategy

A comprehensive literature search for the structured question was performed using the keywords and MeSH terms provided in Tables 1 and 2 in the following electronic databases until September 2013. No limits and language restriction were applied during the electronic search to obtain all the relevant articles pertaining to the review topic. In addition, a manual hand search for articles was carried out. The entire search strategy has been represented in Flowchart 1.

Table 1: Electronic search strategy for PubMed

| Search Query | Items found |
|--------------|-------------|
| #27 Search (#26) AND #10 | 74 |
| #26 Search endothelin system OR “Endothelins” [MeSH] OR “Receptors, Endothelin”[MeSH] OR “Receptor, Endothelin A” [MeSH] OR “Receptor, Endothelin B” [Mesh] OR “Endothelin-3” [MeSH] OR “Endothelin-2” [MeSH] OR “Endothelin-1” [MeSH] OR “endothelin-converting enzyme” [Supplementary Concept] OR “ambrisentan” [supplementary concept] OR “4-hydroxymethyl ambrisentan” [supplementary concept] OR “bosentan” [supplementary concept] OR “sitaxsentan” [supplementary concept] OR vasoconstrictor peptide OR endothelial cell-derived vasoconstrictor | 115336 |
| #25 Search endothelial cell-derived vasoconstrictor | 757 |
| #24 Search vasoconstrictor peptide | 97979 |
| #23 Search “sitaxsentan” [supplementary concept] | 126 |
| #22 Search “bosentan” [supplementary concept] | 1322 |
| #21 Search “4-hydroxymethyl ambrisentan” [supplementary concept] | 3 |
| #20 Search “ambrisentan” [supplementary concept] | 110 |
| #19 Search “endothelin-converting enzyme” [supplementary concept] | 829 |
| #18 Search “Endothelin-1” [MeSH] | 10138 |
| #17 Search “Endothelin-2” [MeSH] | 200 |
| #16 Search “Endothelin-3” [MeSH] | 480 |
| #15 Search “Receptor, Endothelin B” [MeSH] | 2054 |
| #14 Search “Receptor, Endothelin A” [MeSH] | 2766 |
| #13 Search “Receptors, Endothelin” [MeSH] | 7225 |
| #12 Search “Endothelins” [MeSH] | 18167 |
| #11 Search endothelin system | 4305 |
| #10 Search “Periodontitis” [MeSH] OR “Chronic Periodontitis” [MeSH] OR “Aggressive Periodontitis” [MeSH] OR “Gingival Diseases” [MeSH] OR “Periodontal Diseases” [MeSH] OR gingival fibroblasts OR gingival epithelial cells OR gingival keratinocytes OR “Periodontium” [MeSH] | 90663 |
| #9 Search “Periodontium” [MeSH] | 34458 |
| #8 Search gingival keratinocytes | 437 |
| #7 Search gingival epithelial cells | 2072 |
| #6 Search gingival fibroblasts | 2898 |
| #5 Search “Periodontal Diseases” [MeSH] | 67875 |
| #4 Search “Gingival Diseases” [MeSH] | 21810 |
| #3 Search “Aggressive Periodontitis” [MeSH] | 1458 |
| #2 Search “Chronic Periodontitis” [MeSH] | 1163 |
| #1 Search “Periodontitis” [MeSH] | 22109 |

Databases

1. PubMed
2. PubMed Central
3. MeSH
4. LILACS
5. MEDLINE
6. ScienceDirect
7. EMBASE

Inclusion criteria

Cross-sectional and longitudinal studies which associated ET-1 and different types of periodontal diseases or disease severity were included in the study.

Clinical trials which correlate the levels of ET-1 before and after periodontal therapy were included in the study.

In vitro and in vivo studies (both human and animal) which evaluated or estimated the ET-1 expression in periodontal cells and periodontal disease were included in the study.

Exclusion criteria

Studies which evaluated only endothelin-converting enzyme or endothelin receptor expression without measuring ET-1
levels were excluded from the study. Further, case reports were not included in this review.

**Search results**
The systematic search narrowed down to 25 articles from all the electronic databases based on the relevance of the title and abstract to the topic of interest. After assessing the full text, a total of 15 articles relevant to this review and satisfying the inclusion criteria were subjected to data extraction.
**Data extraction**

Data extraction was performed by two independent review authors. If there was disagreement on the inclusion of certain articles, a discussion was held to resolve it. In cases where a study did not report raw data but included precise graphical representations, the data were extracted. The articles were classified based on the levels of evidence given by the center of evidence-based medicine (available online at http://www.cebm.net/oxford-centre-evidence-based-medicine-levels-evidence-march-2009/).

**RESULTS**

A total of 15 articles were selected based on the inclusion and exclusion criteria. The general information regarding the articles is given in Table 3.

The level of evidence of the articles is given in Table 4.

The excluded articles are given in Table 5.

**Cells which express endothelin-1 in the periodontium**

Eight histochemistry-based studies evaluated the constituent cells of the periodontium for ET-1 expression. The ET-1 expression from fibroblasts was the most observed, with six studies showing expression by gingival and periodontal fibroblasts. Other cell types which express ET-1 include periodontal ligament cells, vascular endothelial cells, and human gingival keratinocytes. Human gingival keratinocytes showed the strongest expression for ET-1 compared to fibroblasts and periodontal ligament cells.

**Influence of endothelin-1 on inflammatory mediators and other cytokines**

Two studies have evaluated the influence of ET-1 on inflammatory mediators and other cytokines. One in vitro study has evaluated the relationship between ET-1 expression and proinflammatory cytokines such as interleukin-1β (IL-1β), tumor necrosis factor-α (TNF-α), and IL-6, where ET-1 increases the expression of the above-mentioned cytokines. Another PCR-based study has shown that ET-1 expression can upregulate the IL-1β mRNA and protein expression.

**Endothelin-1 levels in periodontal disease**

The concentration of ET-1 has been estimated in both human and animal studies. A total of ten studies have evaluated the levels of ET-1 in chronic periodontitis and drug-induced gingival overgrowth. Out of these, six human studies have shown increased levels of ET-1 in chronic periodontitis. Two studies showed increased ET-1 protein expression, and four studies showed increased protein as well as mRNA expression.

Studies by Yamamoto et al. and Ansai et al. assessed RNA and protein expression in the gingival samples of chronic periodontitis and healthy controls and found the elevated expression in the former. Studies by Lester et al. and Rikimaru et al. showed that in gingival samples of severely diseased periodontitis patients, the ET-1 levels were significantly higher than the slightly diseased or healthy groups. A similar study was conducted by Fujioka et al. where ET-1 protein expression were assessed in the gingival crevicular fluid (GCF) and arrived at similar results.

In one animal study, ligature-induced periodontitis model was found to show increased levels of ET-1 mRNA expression. The study in a ligature-induced periodontitis rat model by Ekuni et al. revealed a 2.2-fold increase in levels of endothelin-1 mRNA expression compared to healthy control groups in descending aorta samples.

One human study and one animal study showed that ET-1 protein and mRNA expression was upregulated in subjects with drug-induced gingival overgrowth. Tamilselvam et al. found that ET-1 mRNA expression was significantly higher in gingival samples of patients with cyclosporine-induced gingival overgrowth than in patients with periodontitis and healthy controls, whereas Chin et al. arrived at similar results in established edentulous ridges of rats treated with cyclosporine than in controls.

One study reported that ET-1 was not detected in GCF samples in chronic periodontitis patients when compared with healthy controls.

**Factors which may influence endothelin-1 expression in periodontal disease**

A total of nine studies have studied about various factors that influence the ET-1 expression in periodontal disease condition. Porphyromonas gingivalis stimulates the production of ET-1 levels as seen in two in vitro studies. Two human studies and one animal study reveal increased ET-1 mRNA concentration in cyclosporine-induced gingival overgrowth. One animal study reveals that ET-1 is upregulated in response to phenytoin and nifedipine.

One in vitro study has evaluated the effect of proinflammatory cytokines on ET-1 production which shows that IL-1β increases expression of ET-1 mRNA up to twice the control group in human gingival keratinocytes. TNF-α increases level of ET-1 mRNA expression at 1 h. Both cytokines increased ET-1 production in a time-dependent manner. The effect of mechanical stress on gingival fibroblasts proliferation has been evaluated in one human study which shows increased ET-1 production from gingival fibroblasts in response to mechanical stress. The effect of nonsurgical treatment has also been evaluated.
**Table 3: General information of selected articles**

| Author          | Study design         | Groups and methodology used                                                                 | Statistical analysis                         | Results                                                                 |
|-----------------|----------------------|-----------------------------------------------------------------------------------------------|----------------------------------------------|------------------------------------------------------------------------|
| Liang et al., 2014[5] | In vitro study      | Group 1: Healthy controls \( n=4 \) Group 2: Chronic periodontitis \( n=4 \) H-hPDL cells and P-hPDL cells were obtained from the respective groups and treated with different concentrations of ET-1 Cytokines mRNA expression were evaluated by RT-PCR and cytokine protein secretion was measured by ELISA | ANOVA tests were done                         | 12 h exposure to ET-1 at different concentrations, dose-dependently induced the secretion of TNF-\( \alpha \), IL-1\( \beta \), and IL-6 in H-hPDL and P-hPDL cells, with 100 nM ET-1 stimulation showing maximal induction ET-1-induced TNF-\( \alpha \), IL-1\( \beta \), and IL-6 mRNA expression in a dose-dependent manner |
| Guo et al., 2011[5]    | In vitro study      | HGF from three donors were used, and cultures were prepared. HDF culture was purchased. Both cultures were subjected to 10% uniaxial cyclic strain at 0.5 Hz for up to 72 h The cultures were treated with or without TGF-\( \beta \)1 and then RT-PCR, ELISA and western blot analysis was done for analysis | One-way ANOVA and Tukey's post hoc test was done | Both HGF and HDF cultures showed expression of ET-1 at an mRNA (\( P<0.005 \)) and protein level (\( P<0.001 \)) under mechanical strain HDF showed significantly higher levels of ET-1 compared to HGF group |
| Ekuni et al., 2010[5]    | Experimental animal study | Group 1: Control: 12 male Zucker fatty rats Group 2: Periodontitis: 12 male Zucker fatty rats with ligature-induced periodontitis Immunohistochemical, microarray analysis and RT-PCR was done on aorta samples | Mann-Whitney U-test was used for pairwise comparisons between the control and periodontitis groups | RT-PCR shows a 2.2 fold increase in ET-1 levels in periodontitis group compared to control group |
| Lester et al., 2009[5] | Cross-sectional study | Gingiva sample taken from 110 Hispanic subjects Group 1: Normal \( n=12 \) Group 2: DS \( n=32 \) Group 3: DM \( n=40 \) Group 4: DSev \( n=26 \) ELISA test was done on gingival samples taken | Data were compared by factorial analysis of variance ANOVA, post hoc Tukey’s test and the Pearson correlation test | Gingival concentrations of ET-1 were significantly higher in DSev and DM than in normal and DS groups. (\( P<0.001 \)) Gingival concentrations of ET-1 were significantly greater in DSev than in DM tissue (\( P<0.001 \)) Significant positive correlations among sulcular depths, IL-1\( \beta \), II-6, TNF-\( \alpha \), VEGF, and negative correlations among VCAM-1, Ang-1, and ET-1 is seen (\( P<0.001 \)) |
| Rikimaru et al., 2009[5] | Cross-sectional study Part 2: In vitro study | Cross-sectional: 26 gingival samples taken from 21 adult patients with adult periodontitis and ELISA test was done for evaluation In vitro: Cell cultures using HPdLF, NHDF, and human oral epithelial cell (KB cell line) lines were exposed to increasing concentrations of ET-1 and RT-PCR was done for evaluation | ANOVA and post hoc tests were done Correlation of ET-1 and IL-1\( \beta \) were evaluated using Spearman’s correlation | Cross-sectional: Concentrations of ET-1 and IL-1\( \beta \) in gingival tissues were significantly correlated.(Spearman’s rank correlation \( r=0.653, P=0.001 \)) In vitro: ET-1 tended to stimulate IL-1\( \beta \) mRNA expression in a dose-dependent manner Compared with levels in cells without ET-1 treatment, IL-1\( \beta \) mRNA levels were significantly higher in HPdLF cells treated with 10 nM and 100 nM of ET-1 |
| Chin et al., 2009[5]   | Experimental animal study Part 2: In vitro study | Maxillary edentulous ridges established in 32 5-week-old Sprague-Dawley rats Group 1: CsA treated Group 2: Control group Specimens were then sent for RT-PCR and immunohistochemical analysis Gingival samples taken for human gingival fibroblasts culture and treated with CsA and RT-PCR and ELISA was done | Student’s t-test was used to evaluate differences in expression of mRNA for ET-1 One-way ANOVA was selected to evaluate effect of CsA dose on ET-1 mRNA expression and protein release Duncan’s test was used for post hoc analysis | Student’s t-test was used to evaluate differences in expression of mRNA for ET-1 One-way ANOVA was selected to evaluate effect of CsA dose on ET-1 mRNA expression and protein release Duncan’s test was used for post hoc analysis |

Contd...
### Table 3: Contd...

| Author | Study design | Groups and methodology used | Statistical analysis | Results |
|--------|--------------|------------------------------|----------------------|---------|
| Pradeep et al., 2008<sup>[11]</sup> | Part 1: Cross-sectional study  
Part 2: Longitudinal study (clinical trial for 1 group) | 60 subjects were divided into three groups  
Group I: Healthy n=20  
Group II: Gingivitis n=20  
Group III: Chronic periodontitis n=20  
Group IV: 20 subjects from Group III, 6-8 weeks after treatment (scaling and root planing)  
GCF samples collected for quantification using ELISA  
In vitro: Cell cultures prepared from KB cell line were used and infected with *P. gingivalis*. RT-PCR and ELISA test were used for quantification  
Cross-sectional: Group 1: Diseased tissue samples n=6  
Group 2: Healthy control samples n=3  
Gingival samples were taken for ELISA test and immunohistochemistry analysis | No statistical analysis was applicable | ET-1 was not detected in any sample from any of the study groups |
| Beikler et al., 2008<sup>[12]</sup> | Longitudinal study over a period of 8 weeks | Group 1: Severe chronic periodontitis n=12  
Group 2: Healthy controls n=11  
Gingival samples were taken 6-8 weeks following nonsurgical periodontal therapy for RT-PCR was done to assess 196 gene expressions | Average expression values from treated periodontitis biopsies were divided by expression values from healthy gingival tissues. Mann–Whitney was performed to determine differences between treated periodontitis sites and healthy controls | The 5% least strongly expressed genes included ET-1  
The results suggest that decreased expression following periodontal therapy may indicate a normal inflammatory status that is reached following the periodontal therapy |
| Tamilselvan et al., 2007<sup>[13]</sup> | Part 1: Cross-sectional study  
Part 2: *In vitro* study | Cross-sectional: Group 1: Patients with chronic periodontitis n=8  
Group 2: Patients with DIGO n=8  
Group 3: Healthy patients n=8  
Gingival samples collected for quantification using RT-PCR and ELISA  
*In vitro*: HGF culture prepared from three biopsies of normal human gingival tissue samples and exposed to CsA treatment using RT-PCR and ELISA | Difference in levels of ET-1 gene expression following periodontal therapy | Cross-sectional: ET-1 mRNA expression was significantly higher in patients with CsA-induced gingival overgrowth (678.0 pg/mg) than in patients with periodontitis (367.3 pg/mg) and healthy controls (84.8 pg/mg)  
*In vitro*: ET-1 expression was increased with CsA incorporation compared to controls the results suggest that CsA may modulate the expression of ET-1 on HGFs, and ET-1 generated by the cells can contribute to the development of gingival overgrowth |
| Büchler et al., 2004<sup>[14]</sup> | Longitudinal study over a period of 4 h | 11 renal transplant patients including 4 gingival hypertrophy were selected  
Plasma ET-1 concentrations in 26 normal blood donors were taken as controls  
CsA was given and CsA and ET-1 blood concentrations were assessed just before (t<sub>0</sub>), and 1 (t<sub>1</sub>), 2 (t<sub>2</sub>), and 4 (t<sub>4</sub>) h after CsA dosing  
Blood samples were taken and immunoassay tests were done | Friedman repeated measures test was done  
Difference in quantitative parameters was analyzed by Mann-Whitney U-test  
Spearman rank tests were used to analyze nonparametric linear regression between quantitative parameters  
Unpaired t-test was used for analysis of expression of ET-1 mRNA from KB cells and concentrations of ET-1 | ET-1 plasma concentrations was (2.44-4.09) 3.69 pg/ml and higher than in controls (2.50-4.30) 3.10 pg/ml  
Patients with gingival hypertrophy had higher ET-1 concentrations with median values of 4.04 pg/ml versus 3.53 pg/ml |
| Yamamoto et al., 2003<sup>[15]</sup> | Part 1: *In vitro* study  
Part 2: Cross-sectional study | *In vitro*: Cell cultures prepared from KB cell line was used and infected with *P. gingivalis*. RT-PCR and ELISA test were used for quantification  
Cross-sectional: Group 1: Diseased tissue samples n=6  
Group 2: Healthy control samples n=3  
Gingival samples were taken for ELISA test and immunohistochemistry analysis | In vitro: *P. gingivalis* upregulated ET-1 mRNA expression (3 fold higher compared to control group)  
*P. gingivalis* induced 5 fold increase in production of ET-1 compared to controls  
Cross-sectional: ET-1 expression was detected in both oral and pocket epithelial cells and ET-1 mRNA was expressed in inflamed and weakly in uninflamed gingival tissues | Contd... |
Study design called asPgPepO was similar to statistical testing. Authors Table 3: Contd...

Fujioka et al., 2003[4] Part 1: Cross-sectional study Part 2: In vitro study

- **Cross-sectional:** Group 1: Patients with chronic periodontitis \( n=10 \)
- **Group 2:** Healthy control samples \( n=10 \)

GCF samples were taken for ELISA test analysis.

*In vitro:* HGK and HGF prepared from healthy gingival samples, and HPL samples from healthy premolar roots were obtained. These cultures were then exposed to cytokines IL-1β and TNF-α. Northern blotting analysis was done to evaluate mRNA expression.

**Results:** Cross-sectional: ET-1 level in GCF from periodontitis patients was 388.6 pg/ml and from healthy was 46.8 pg/ml.

ET-1 was expressed in all examined HGK, HGF, and HPL cells, particularly HGK.

*In vitro:* IL-1β increased expression of ET-1 mRNA up to twice the control group in HGK.

MTN-α increased levels of ET-1 mRNA expression at 1 h.

Both cytokines increased ET-1 production in a time-dependent manner.

**DISCUSSION**

Endothelin-1 in periodontal health and disease

Increased ET-1 levels play a pivotal role in the pathogenesis of various diseases. It contributes to the development of vascular diseases such as hypertension and atherosclerosis through the activation of ETA receptors.

The concentration of ET-1 has been found to be elevated in both human and animal studies. The reasons for the elevated ET levels in diseased groups than the healthy groups can be attributed to various factors.

One among these factors could be due to *P. gingivalis*, a key pathogen causing chronic periodontitis. In the study by Awano et al., it has been found that a novel endopeptidase gene from *P. gingivalis* called as PgPepO was similar in structure and function with endothelin-converting enzyme-1 (ECE-1).

The endothelin precursors or...
Elevated expression of proinflammatory cytokines in periodontitis may play a role in the expression of ET-1. Fujioka et al. found that the exposure of the cell lines with IL-1β and TNF-α enhanced the expression of ET-1 and its receptors. The results were concurrent with the study by Rikimaru et al., where a positive correlation existed between the concentrations of ET-1 and IL-1β in gingival tissues. The findings by Lester et al. also revealed a positive correlation between ET-1 and IL-6, IL-1β, TNF-α. Another study by Endo et al. found an upregulation of ET-1 as a result of IL-1, -6, and -8 in cultured porcine respiratory epithelial cells.

It has also been reported that certain anti-inflammatory cytokines play a role in the inhibition of ET-1 expression. The study by Lester et al. revealed a negative correlation of angiotensin-1 (Ang-1) with ET-1 and gingival inflammation. A study by McCarter suggested that the anti-inflammatory action of Ang-1 was key to the inhibition of ET-1 secretion, which dampened the proinflammatory cytokine synthesis.

Another anti-inflammatory cytokine which can play a role in the expression of ET-1 is nitric oxide (NO). In blood dynamics, the balance between NO and ET system is an important factor. NO has biologic function to inhibit ET levels. During periodontitis, it is seen that NO is relevantly increased; however, due to development of reiterative infection, the levels are much lower than ET levels and this leads to pathological changes. In the study by Chen et al., it was found that the expression of ET-1 was increased significantly in the gingiva of chronic periodontitis compared with healthy gingiva. This could be due to continuous relapsing inflammation which could stimulate endothelial cells to synthesize and release ET, causing a vessel constriction and injuring endothelial cells, causing a further increase in release of ET. This suggests the role ET plays in amplifying inflammation in chronic periodontitis.

Another factor influences that the expression of ET-1 is mechanical strain. In the study by Guo et al., mechanical strain was able to upregulate ET-1 expression in gingival fibroblasts which increased its proliferative capacity. They further assessed the synthesis of ET-1 between dermal and gingival fibroblasts. They concluded that the ET-1 synthesis was much lower in gingival compared to dermal fibroblasts, which explains the absence of scar formation in the oral cavity.

Out of all the studies included in this review, only one study showed no detection of ET-1 in GCF samples in healthy, gingivitis, and chronic periodontitis groups. The inability to detect ET-1 could be due to the rapid degradation by microbial- and host-derived proteases, short plasma half-life of 1.5 min, and a heterogeneous study population.

Preproendothelins are cleaved by endopeptidases to form the biologically inactive intermediates termed as big ETs. ECE-1 is responsible for conversion of big ET-1, -2, and -3 to ET-1, -2, and -3, respectively. Similarly, an in vitro study by Ansai et al. showed upregulated mRNA expression of ET-1 on exposure to P. gingivalis strains. Cross-sectional studies by Yamamoto et al., Ansai et al., and Fujioka et al. also revealed an elevated ET-1 expression in diseased condition compared to healthy controls.
reason was the absence of free form in the GCF due to the binding of ET-1 to its receptors in the gingival tissues.\textsuperscript{[11]}

Among the articles reviewed, two studies estimated the influence of ET-1 on inflammatory mediators and other cytokines. The study conducted by Rikimaru et al.\textsuperscript{[9]} demonstrated that the ET-1 in human periodontal ligament fibroblasts and human oral epithelial cell lines (KB cells) stimulated the IL-1β mRNA expression and protein release in a dose-dependent manner. Chronic periodontitis reflects an underlying constant inflammatory condition which is established by an ET-1-IL-1β inflammatory loop which is independent of the original stimulus. A similar model was suggested by Mullol et al. in nasal sinusitis, where an inflammatory loop was established, by interplay of ET-1 and IL-1β.\textsuperscript{[34]} Another study by Li Liang et al.\textsuperscript{[5]} demonstrated that ET-1-induced proinflammatory cytokine such as TNF-α, IL-1β, and IL-6 expression acted via mitogen-activated protein kinase (MAPK) pathway signaling pathways. Extracellular signal-regulated protein kinase 1/2 inhibitor showed the pronounced reducing effects on TNF-α, IL-1β, and IL-6 expression, c-Jun N-terminal kinase inhibitor demonstrated the decreasing effect on TNF-α and IL-1β expression, whereas p38 inhibitor showed the reducing effects on IL-1β and IL-6 expression. They collectively concluded that each cytokine acted via a specific variant of MAPK pathway.

In this structured review, one study assessed the influence of treatment on secretion of ET-1. Thomas Beiker et al.\textsuperscript{[12]} found that following periodontal therapy the proinflammatory cytokine profile fell within a normal healthy range, which led to a decreased expression of ET-1.

**Endothelin-1 in drug-induced gingival overgrowth**

Gingival overgrowth is observed in patients receiving cyclosporine, phenytoin, and nifedipine. It has been found that these drugs also play a role in elevating ET-1 levels during the pathogenesis of drug-induced gingival overgrowth. The structured review included four articles that showed elevated levels of ET-1 in drug-induced gingival overgrowth.

The effects of phenytoin and nifedipine have been studied in the in vitro study by Ohuchi et al.\textsuperscript{[18]} It has been found that above drugs can induce Ang-II and ET-1 secretion in cultured porcine gingival fibroblasts. Phenytoin and nifedipine prevent calcium accumulation in juxtaglomerular cells which in turn increase renin secretion. Renin mediates the conversion of angiotensinogen to Ang-I which in turn is converted to Ang-II by the action of angiotensin-converting enzyme. Ang-II acts as a potent hypertrophic agent and is known to stimulate ET-1 secretion from endothelial cells.\textsuperscript{[9,30]} This mechanism was substantiated by the results of latter part of Nozomi’s study where they found an increased cell proliferation in fibroblast cultures when treated with Ang-II and ET-1. It has been shown by Yangisawa et al.\textsuperscript{[1]} that cyclosporine indirectly stimulated renin-angiotensin and thereby lead to an increase in ET-1 levels. It is also found that cyclosporine can indirectly induce the ET-1 stimulation by inducing the synthesis of Ang-II. This could be the direct mechanism by which ET-1 plays a role in the pathogenesis of drug-induced gingival overgrowth.

The second possible mechanism is through mast cell chymase as suggested by Toyoda et al.\textsuperscript{[37]} Mast cell chymase are elevated in drug-induced gingival overgrowth and tends to replace endothelin-converting enzyme which converts big ET to ET-1.

An indirect mechanism by which ET-1 is expressed in drug-induced gingival overgrowth is by the upregulation of proinflammatory cytokines as a result of *P. gingivalis* infection.

In the study by Chin et al.,\textsuperscript{[10]} it was suggested that the mRNA expression of ET-1 presented a biphasic nature where increasing concentrations of cyclosporine, induced increased ET-1 expression up to a point after which it started to decrease. However, a clear reasoning for this effect has not been discussed.

**Limitations and future implications**

The major limitations posed in our structured review were that the unpublished research was not procured. Moreover, only published data from the articles were taken for analysis. Conversely, a more detailed understanding could have been possible had raw data been available. Finally, the search strategies did not extend to other databases which would have hampered the thoroughness of the review.

The available literature on ET-1 in periodontal disease is limited to *in vitro* and cross-sectional studies which are of lower levels of evidence. More number of long-term association and interventional studies is required to establish it as a diagnostic and prognostic marker for periodontal disease.

The present state and future implications of ET-1 in periodontal disease are given in Table 6.

**CONCLUSION**

From this structured review, we can conclude that ET-1 plays a role in the pathogenesis of chronic periodontitis and drug-induced gingival overgrowth. However, further utilization of ET-1 as a marker requires additional interventional and longitudinal studies.

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Nil.
Table 6: Present state and future considerations of endothelin-1

Present status

ET-1 is found to play a role in the pathogenesis of periodontal disease, by influencing the cytokines and inflammatory mediators in periodontal tissues.

ET-1 is found to be elevated in periodontal diseases such as chronic periodontitis and drug-induced gingival overgrowth, when compared to periodontal health.

Various factors such as Porphyromonas gingivalis, proinflammatory cytokines, drugs such as cyclosporine, nifedipine, phenytoin influence ET-1 expression in periodontal disease.

The level of evidence of available literature is low, consisting mainly of in vitro, experimental animal and cross sectional studies. Heterogeneous populations have been studied in the available literature, and techniques and samples used for measurement varied

Future consideration

Further research can be done to target the progression of periodontal diseases due to ET-1

Further research is required to estimate baseline values of ET-1 based on health and disease severity, to establish it as a biomarker for periodontal disease.

Further research can be done to target these factors to inhibit ET-1 expression and treatment of periodontal disease.

More long term association studies are required to establish ET-1 as a diagnostic and prognostic marker.

Studies can be done using homogenous population, and a common standard and sensitive technique and sample should be developed for the assessment of ET-1.

ET-1=Endothelin-1, P. gingivalis=Porphyromonas gingivalis

Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Yanagisawa M, Kurihara H, Kimura S, Tomobe Y, Kobayashi M, Mitsui Y, et al. A novel potent vasoconstrictor peptide produced by vascular endothelial cells. Nature 1988;332:411-5.

2. Inoue A, Yanagisawa M, Kimura S, Kasuya Y, Miyauchi T, Goto K, et al. The human endothelin family: Three structurally and pharmacologically distinct isopeptides predicted by three separate genes. Proc Natl Acad Sci U S A 1989;86:2863-7.

3. Socransky SS, Haffajee AD, Cugini MA, Smith C, Kent RL Jr. Microbial complexes in subgingival plaque. J Clin Periodontol 1998;25:134-44.

4. Offenbacher S. Periodontal diseases: Pathogenesis. Ann Periodontol 1996;1:821-78.

5. Liang L, Yu J, Zhou W, Liu N, EI LL, Wang DS, et al. Endothelin-1 stimulates proinflammatory cytokine expression in human periodontal ligament cells via mitogen-activated protein kinase pathway. J Periodontol 2014;85:618-26.

6. Guo F, Carter DE, Leask A. Mechanical tension increases CCN2/CTGF expression and proliferation in gingival fibroblasts via a TGFβ-dependent mechanism. PLoS One 2011;6:e19756.

7. Ekuni D, Tomofuji T, Irie K, Kasuyama K, Umakoshi M, Azuma T, et al. Effects of periodontal therapy on aortic insulin resistance in an obese rat model. Lab Invest 2010;90:348-59.

8. Lester SR, Bain JL, Serio FG, Harrellson BD, Johnson RB. Relationship between gingival angiopoietin-1 concentrations and depth of the adjacent gingival sulcus. J Periodontol 2009;80:1447-53.

9. Rikimaruo T, Awanuo S, Mineoaka T, Yoshida A, Ansai T, Takehara T. Relationship between endothelin-1 and interleukin-1β in inflamed periodontal tissues. Biomed Res 2009;30:349-55.

10. Chin YT, Tu HP, Chen YT, Dai NT, Shen EC, Chiang CY, et al. Expression and bioactivities of endothelin-1 in gingiva during cyclosporine A treatment. J Periodontal Res 2009;44:25-42.

11. Pradeep AR, Gunuprasad CN, Swati P, Shikha C. Crevicular fluid endothelin-1 levels in periodontal health and disease. J Periodontal Res 2008;43:275-8.

12. Beikler T, Peters U, Prior K, Eisenach M, Fleming TF. Gene Expression in periodontal tissues following treatment. BMC Med Genomics 2008;1:30. doi: 10.1186/1755-8794-1-30.

13. Tamilselvan S, Raju SN, Loganathan D, Kamatchiammal S, Abraham G, Suresh R. Endothelin-1 and its receptors ET (A) and ET (B) in drug-induced gingival overgrowth. J Periodontal Res 2007;42:290-5.

14. Büchner M, Leibenguth P, Le Guellec C, Carayon A, Watier H, Otoud F, et al. Relationship between calcineurin inhibition and plasma endothelin concentrations in cyclosporine-A-treated kidney transplant patients. Eur J Clin Pharmacol 2004;60:703-8.

15. Yamamoto E, Awanuo S, Koseki T, Ansai T, Takehara T. Expression of endothelin-1 in gingival epithelial cells. J Periodontal Res 2003;38:417-21.

16. Fujioka D, Nakamura S, Yoshino H, Shinohara H, Shiba H, Mizuno N, et al. Expression of endothelins and their receptors in tissues from human periodontal tissues. J Periodontal Res 2003;38:269-75.

17. Ansai T, Yamamoto E, Awanuo S, Yu W, Turner AJ, Takehara T. Effects of periodontopathic bacteria on the expression of endothelin-1 in gingival epithelial cells in adult periodontitis. Clin Sci (Lond) 2002;103 Suppl 48:327S-315.

18. Ohuchi N, Koike K, Sano M, Kusama T, Kizawa Y, Hayashi K, et al. Proliferative effects of angiotensin II and endothelin-1 on guinea pig gingival fibroblast cells in culture. Comp Biochem Physiol C Toxicol Pharmacol 2002;132:451-60.

19. Chen S, Wu J, Song Z, Zhang J. An investigation of immunocompetence substances in normal gingival and periodontitis tissue. Chin Med J (Engl) 2000;113:844-7.

20. Hollá LI, Fassmann A, Vasku A, Znojil V, Vanek J, Vácha J. Interactions of lymphotixin alpha (TNF-beta), angiotensin-converting enzyme (ACE), and endothelin-1 (ET-1) gene polymorphisms in adult periodontitis. J Periodontol 2001;72:85-9.

21. Bain JL, Lester SR, Henry WD, Naftel JP, Johnson RB. Effects of induced periapical abscesses on rat pregnancy outcomes. Arch Oral Biol 2009;54:162-71.

22. Kinoshita N, Awanuo S, Yoshida A, Soh I, Ansai T. Periodontal disease and gene-expression levels of metalloendopeptidases in human buccal mucosal epithelium. J Periodontal Res 2013;48:606-14.

23. Ghorbani B, Holmstrup P, Edvinsson L, Kristiansen KA, Sheykhzade M. LPS from Porphyromonas gingivalis increases the sensitivity of contractile response mediated by endothelin-B (ET (B)) receptors in cultured endothelium-intact rat coronary arteries. Vasc Pharmacol 2010;53:250-7.

24. Awanuo S, Ansai T, Mochizuki H, Yu W, Tannawa K, Turner AJ, et al. Sequencing, expression and biochemical characterization of the Porphyromonas gingivalis pepO gene encoding a protein homologous to human endothelin-converting enzyme. FEBS Lett 1999;460:139-44.

25. Shimo T, NishiyamaA, Kobota S, Kusum O, Okui T, Katase N, et al. Novel pathogenic role of fibrin as revealed by a case study on ligneous gingivitis. Oral Sci Int 2011;8:44-9.

26. Ohuchi N, Hayashi K, Iwamoto K, Koike K, Kizawa Y, Nakama T, et al. LPS from Porphyromonas gingivalis increases the sensitivity of contractile response mediated by endothelin-B (ET (B)) receptors in cultured endothelium-intact rat coronary arteries. Vasc Pharmacol 2010;53:250-7.
29. Guo F, Carter DE, Mukhopadhyay A, Leask A. Gingival fibroblasts display reduced adhesion and spreading on extracellular matrix: A possible basis for scarless tissue repair? PLoS One 2011;6:E27097.
30. Schiffrin EL. State-of-the-art lecture. Role of endothelin-1 in hypertension. Hypertension 1999;34(4 Pt 2):876-81.
31. Barton M. Endothelial dysfunction and atherosclerosis: Endothelin receptor antagonists as novel therapeutics. Curr Hypertens Rep 2000;2:84-91.
32. Endo T, Uchida Y, Matsumoto H, Suzuki N, Nomura A, Hirata F, et al. Regulation of endothelin-1 synthesis in cultured guinea pig airway epithelial cells by various cytokines. Biochem Biophys Res Commun 1992;186:1594-9.
33. McCarter SD, Lai PF, Suen RS, Stewart DJ. Regulation of endothelin-1 by angiopoietin-1: Implications for inflammation. Exp Biol Med (Maywood) 2006;231:985-91.
34. Mullol J, Picado C. Endothelin in nasal mucosa: Role in nasal function and inflammation. Clin Exp Allergy 2000;30:172-7.
35. Imai T, Hirata Y, Emori T, Yanagisawa M, Masaki T, Marumo F. Induction of endothelin-1 gene by angiotensin and vasopressin in endothelial cells. Hypertension 1992;19(6 Pt 2):753-7.
36. Dohi Y, Hahn AW, Boulanger CM, Bühler FR, Lüscher TF. Endothelin stimulated by angiotensin II augments contractility of spontaneously hypertensive rat resistance arteries. Hypertension 1992;19:131-7.
37. Toyoda M, Morohashi M. Morphological assessment of the effects of cyclosporin A on mast cell – Nerve relationship in atopic dermatitis. Acta Derm Venereol 1998;78:321-5.