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Quantitative Determination of Serum Soluble E-Selectin in Periodontal Health and Disease

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Abstract:

Back ground: Inflammatory periodontal disease has been associated with an increased risk of cardiovascular events. Emerging evidence shows an association of periodontal disease with systemic inflammation and endothelial dysfunction, while the underlying mechanism remains unclear. Circulating cell adhesion molecule sE-selectin has been suggested as a potential candidate marker of endothelial dysfunction, which contributes to the pathogenesis of cardiovascular diseases. **Aim:** To determine the serum sE-selectin level in generalised severe chronic periodontitis and periodontally healthy subjects. **Materials and methods**: Eighty subjects in the age group of 35-55 years were included in the study. Forty subjects with generalised severe chronic periodontitis as study group and 40 healthy subjects without periodontal disease as control group were selected. The blood samples were collected and the serum was analysed for sE-selectin level by ELISA. **Results:** sE-selectin expression was found in serum of both the groups. Mean serum sE-selectin levels were 37.05ng/mL in the study group and 30.78ng/mL in control group. Statistically significant increase was not found in serum sE-selectin levels between the groups. **Conclusion:** sE-selectin is expressed in serum of both the study and control groups with a wide range of distribution. No significant association was observed between sE-selectin level and periodontal inflammation. Therefore, circulating sE-selectin may not be used as a reliable biomarker of endothelial function measure in periodontal disease.

Key words: Chronic periodontitis, periodontal disease, selectin, E-selectin, endothelial dysfunction

Introduction:

Periodontal disease, a low grade chronic inflammation is associated with an increased risk for cardiovascular diseases due to periodic transient bacteremia leading to invasion of vascular endothelial cells and increased levels of cytokines that accelerate the atherogenic process.[1]

E-selectin (ELAM-1 or CD62E) is a cell surface glycoprotein adhesion molecule of 115 kDa, expressed only on endothelial cells after activation by inflammatory cytokines (IL-1β or TNF-α) or endotoxins. Expression of E-selectin is transitory, reaching maximum within about 6 hours of stimulation and then declining with shedding of soluble E-selectin which can be easily detected in serum.[2] sE-selectin (soluble E-selectin) is also found in the blood of healthy individuals.[3,4] Endothelial dysfunction, considered as a preclinical stage of atherosclerosis, is associated with an increased level of glycoprotein adhesion molecules of the selectin group, such as E-selectin.[5, 6, 7]

E-selectin is probably the most specific marker for endothelial cell activation and its level increases in association with cardiovascular risk factors and has been associated with structural and functional measures of atherosclerotic diseases.[8,9]

Periodontitis is associated with a chronic systemic status of endothelial cell activation that perhaps might represent one of the mechanisms responsible for moderate increase of cardiovascular risk in periodontitis patients.[10] Hence, the present study was undertaken to determine the circulating serum concentration of sE-selectin in generalised severe chronic periodontitis subjects compared to periodontally healthy subjects and to correlate with periodontal inflammation.

Materials and Methods:

The study was approved by the Institutional Ethical Committee. About 80 subjects in the age group of 35-55 years who attended the outpatient department of Periodontics, Tamil Nadu Government Dental College, Chennai participated in the study. The patients with minimum of twenty teeth were divided into 2 groups, study group- 40 subjects with generalised severe chronic periodontitis and control group- 40 subjects with healthy periodontium were included in the study.

Following selection of subjects, written informed consent was obtained after explaining the study procedure. Examination was preceded by a thorough medical and dental history of the subjects. Intra-oral examination was done using mouth mirror and William's Periodontal Probe. Periodontal evaluation was done by measuring the Gingival Bleeding Index, Plaque Index, Probing Pocket Depth (PPD), Clinical Attachment Level (CAL) and alveolar bone loss with Orthopantomogram (OPG).

Exclusion criteria:
1. History of smoking or use of tobacco in any forms.
2. History of any systemic diseases (Diabetes Mellitus, Hypertension, Cardiovascular diseases, Kidney, Liver or Lung diseases).
3. History of systemic antibiotics or anti-inflammatory drugs in previous 6 months.
4. History of periodontal therapy in previous 6 months.
5. History or presence of any other chronic infectious diseases.
6. Pregnancy or Lactation.

The selected subjects were divided into two groups (Study and Control groups) based on the following criteria:

Control group:

40 subjects exhibiting the following features were included:
- Sites with probing depth measurement < 3 mm
- < 20 % of sites exhibiting gingival bleeding
- Absence of Clinical Attachment Loss as determined by CAL
- (Clinical Attachment Level) measurements i.e. CAL=0.
- Good oral hygiene status with plaque index score less than 1.

Study group:
Forty subjects exhibiting generalized chronic periodontitis with the following features were included:
- Bleeding on Probing in >30% of sites
- $\geq$ 2 teeth per quadrant, excluding third molars with Probing Pocket Depth $\geq$ 5 mm
- Presence of Clinical Attachment Loss as determined by CAL measurements $\geq$ 5mm[11]
- Radiographic evidence of alveolar bone loss
- Poor oral hygiene status with plaque index score of 2.0 to 3.0

Study protocol:
1. Institutional Ethical Committee approval
2. Medical History and Informed Consent
3. Periodontal Examination using clinical parameters namely, Gingival Bleeding Index, Plaque Index, Probing Pocket Depth and Clinical Attachment Level
4. Orthopantomogram (OPG) for radiographic evaluation of generalized chronic periodontitis
5. Collection of blood samples
6. Estimation of soluble E- selectin level in serum by ELISA method

Blood sample collection and storage:

After skin preparation, 5 ml of fasting venous blood sample was obtained from each patient by using disposable hypodermic syringe with 23 gauge needle by venipuncture without stasis from ante-cubital fossa and transported using standardised aseptic techniques. The blood sample was allowed to clot for 30 minutes in plain vacutainer tube, and then centrifuged for 15 minutes at 3000 rpm to separate the serum. Then 500µL of serum sample was divided in aliquots and stored at -20ºC until analysis.

Procedure:

I. ELISA METHOD:

The circulating soluble E-selectin arises from shedding or proteolytic cleavage of the cell surface-expressed E-selectin and is measurable in serum by ELISA. In this study, Quantikine Human sE-Selectin/ CD62E, R&D Systems U.S.A. ELISA Kit, was used.

Assay procedure summary

Reagents, samples and standards were prepared as instructed
↓
100µL Assay Diluent RD1W was added to each well
↓
100µL of sample was added to each well Incubated for 2 hours at room temperature
↓
Aspirated and washed four times
↓
200µL Conjugate was added to each well Incubated for 2 hours at room temperature
↓
Aspirated and washed four times
↓
200 µL of Substrate Solution was added to each well
Incubated for 30 minutes at room temperature and was protected from light
↓
50 µL Stop Solution added to each well
↓
Absorbance read at 450 nm within 30 minutes

Calculation of results:

The optical density of each sample was plotted against its concentration and a curve was drawn through the points. Because the samples were diluted, the concentrations read from the standard curve must be multiplied by the dilution factor.

Results:

The mean age in the study group was 42 years and 44 years in the control group respectively. The males constituted 42.5% while females constituted 57.5% in the study group. Both males and females constituted about 50% each respectively in the control group. The mean Plaque Index score in the study group was 2.494±0.147 and 0.587 ±0.079 in the control group, which was statistically highly significant (p=0.000). The mean % of sites with Bleeding on Probing was 90.407 ±5.385 in the study group and4.563±1.289 in the control group, which was statistically highly significant (p=0.000). The mean PPD was 5.565±0.443mm in the study group and 1.680±0.142mm in the control group, which was statistically highly significant (p=0.000).The mean CAL was 6.14±0.69 mm in the study group, which was statistically highly significant (p=0.000).

The mean sE-selectin levels in serum were 37.05±16.76ng/mL in the study group and 30.78±11.76 ng/mL in the control Group (**Figure I**). There was no statistical significant difference in serum sE-selectin levels between the study and the control group. The mean sE-selectin levels in serum in males were 38.117±17.186 ng/mL in the study group and 30.250±13.583ng/mL in the control group (**Figure II**). The mean sE-selectin levels in females were 36.260±16.774ng/mL in the study group and 31.300±9.947 ng/mL in the control group (**Figure III**). There was
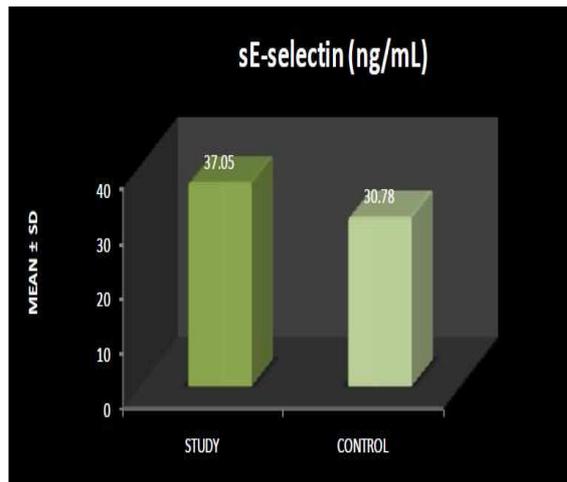
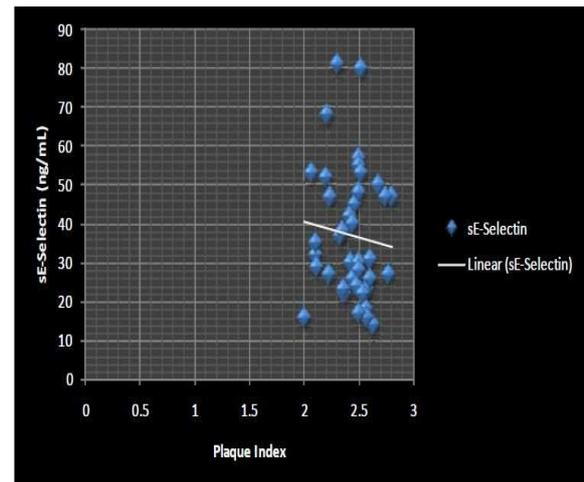**Figure I: Comparison of mean sE-selectin levels between study and control group**

Figure II: Comparison of mean sE-selectin levels in male between study and control group
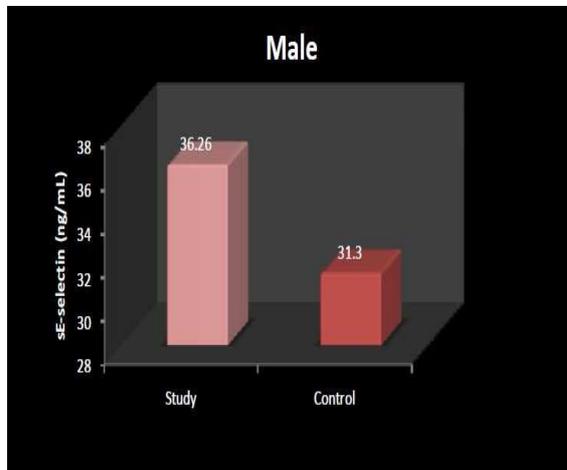

Figure III: Comparison of mean sE-selectin levels in female between study and control group

Figure IV: Correlation of Plaque Index and sE-selectin level in study group

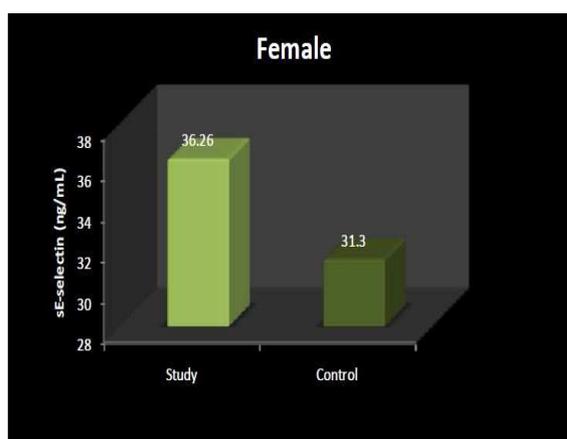**Figure V: Correlation of Plaque Index and sE-selectin level in control group**

no statistical significant difference in serum sE-selectin levels between the study and the control group both in male and females.

A linear negative correlation was found between Plaque Index and sE-selectin level in the study group, which was statistically significant (p=0.011) (**Figure IV**). No significant correlation was found between Plaque Index and sE-selectin level in the control group (**Figure V**). No significant correlation was found between the clinical parameters % of sites with BOP and PPD with sE-selectin level in the study and control group. There was no statistical significant correlation between

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CAL and sE-selectin level in the study group.

Statistical analysis:

The statistical analysis was done using the computer software program SPSS (Statistical Package for Social Sciences) for Windows version 17. Mean and Standard Deviation were estimated for different variables in each group. Mean values were compared between the two groups by using Student's Independent t-test. Pearson's Chi-square test was done to compare the gender distribution between the two groups. Kendall's tau-b rank correlation coefficient test was used to analyze the correlation between the clinical parameters and sE-selectin level. In the present study, P-value <0.05 was considered as the level of significance.

Discussion:

Inflammation in the periodontal tissues results in ulceration of the sulcular epithelium which leads to dissemination of oral bacteria and their products into the systemic circulation which triggers the production and release of various pro-inflammatory mediators including IL-1β , IL-6, CRP and TNF-α. Periodontal disease once established provides a biological burden of endotoxin (LPS) and inflammatory cytokines which serves to initiate and exacerbate atherogenesis and thromboembolic events[12]. The vascular endothelium, a thin monocellular layer, is a likely target for circulating cytokines and oral pathogens which plays a central role in the regulation of vascular homeostasis.

E-selectin (CD62E) is cytokine-inducible endothelial cell adhesion molecule that tethers polymorphonuclear neutrophils and supports PMNs rolling under conditions of flow. E-selectin is constitutively expressed only on activated endothelium in contrast to other circulating adhesion molecules that have a wider tissue distribution, but it is difficult to quantify E-selectin in vivo

due to its transient nature of expression. sE-selectin arises from shedding or proteolytic cleavage of the extracellular portion of the surface expressed molecule which can be easily detected in systemic circulation. The level of soluble adhesion molecules correlates with the concentration of adhesion molecules expressed on the endothelial cells.[13,14] sE-selectin has the ability to activate neutrophils and act as chemoattractant.[15] sE-selectin actually exerts a pro-inflammatory effects upon neutrophil function at site of inflammation, thereby exacerbating the disease process.[16] The circulating concentrations of sE-selectin may serve as a potential biomarker of endothelial dysfunction and may be useful for monitoring the process of inflammation of the vessel wall.[17]

Biomarker is a substance that is measured objectively and evaluated as an indicator of normal biologic processes, pathogenic processes or pharmacologic responses to a therapeutic intervention. Hence in the present study, serum sE-selectin level was estimated in order to assess the effect of generalized severe chronic periodontitis on vascular endothelium and to determine the relationship of sE-selectin level with periodontal inflammation.

Epidemiological studies suggest a link between severe periodontal disease and atherosclerosis whereas there is no association with milder periodontal disease. The advanced periodontal disease exhibits endothelial dysfunction and evidence of systemic inflammation, possibly placing them at 25% to 90% increase in increase in risk for cardiovascular diseases.[18]E-selectin and myeloperoxidase levels were significantly increased in plasma of untreated patients with moderate to severe chronic periodontitis suggesting association of periodontitis with systemic inflammation that could increase the risk of cardiovascular events.[19]Hence in the present study, subjects with generalized severe chronic periodontitis were only

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included. Elevated levels of sE-selectin in serum have been reported in variety of pathological conditions such as hyperlipidemia,[20] bronchial asthma,[21] acute myocardial infarction,[22] atopic dermatitis,[23] diabetes,[24] hypertension.[25] Hence the subjects with the above mentioned systemic disease were excluded from the study.

A hallmark of infection with *P.gingivalis*, a Gram-negative bacterium strongly associated with chronic periodontitis is the induction of chronic inflammatory response. *P.gingivalis* LPS is a key inflammatory mediator with low biological reactivity that alerts the host of potential bacterial infection, also evokes a highly unusual host cell response due to its structural heterogeneity of lipid A. The tetra-acylated lipid A structures are potent antagonist for E-selectin expression, while penta-acylated lipid A structures facilitate E-selectin expression.[26] *P.gingivalis* LPS inhibits the expression of E-selectin by human endothelial cells, thereby hindering extravasation of leukocytes, which contributes to the proposed bacterial bloom that occurs in periodontal disease. *P. gingivalis* LPS was also shown to inhibit the ability of other bacterias normally found in supragingival plaque (*Tannerella forsythia*, *Fusobacterium nucleatum* and *Eikenella corrodens*) to stimulate E-selectin expression by endothelial cell. *P. gingivalis* LPS is also a poor activator of IL-1β and TNF-α from monocytes which are indirect activators of E-selectin expression.[27] *P.gingivalis* LPS may selectively modify host response by altering the relative amount of lipid A structures, as a means to facilitate persistent colonization of host tissues contributing to destructive inflammatory periodontal disease. Periodontitis causes serum amyloid A stimulation, an acute phase protein which results in upregulation of adhesion molecules ICAM, VCAM, and E-selectin in human aortic endothelial cells via TLR-2.[28] The lack of significant increase in sE-selectin level in chronic periodontitis subjects observed in the present study might be due to the inhibition of E-selectin expression by *P.gingivalis* LPS in spite of the inflammatory component being present in the periodontal tissues. This might have contributed for the significant negative correlation between the Plaque Index and sE-selectin level observed in the study group (**Figure IV**).

A few limitations of the study requires special considerations, the most significant being relatively low sample size. There might be discrepancies in concentrations between the cell membrane expressed E-selectin and their circulating counterpart sE-selectin which has to be elucidated. The contributing factors for sampling error could be the sharp downregulation of E-selectin gene transcriptions within 6 to 9 hours after induction, the short half life of E-selectin mRNA and rapid internalisation and degradation of E-selectin in lysosomes.[29,30,31]

In this study, the results revealed that there was expression of sE-selectin in serum of both the study and control groups. The expression of sE-selectin in the serum of control group infers that E-selectin is synthesized and released into blood stream even in the absence of overt inflammatory processes. The bacterial products present along gingival margin activates keratinocytes to release IL-1 followed by expression of ELAM-1 on endothelial cell.[32] Despite lack of clinical signs of inflammation, this activation could be responsible for infiltration of inflammatory cells found in clinically healthy gingiva.

In the present study, the chronic periodontitis subjects accomplished serum sE-selectin level that was in the range of periodontally healthy subjects.[33,34] The wide range of value exhibited in periodontally healthy individuals warrants the caution interpretation between the study and control groups.

The single measurement of sE-selectin gives only a snapshot and limited information because of the technical and

intrinsic biological variability of the endothelial function measure. There was an improvement in endothelial function and reduced disease severity indices, six months after intensive periodontal therapy; hence the benefits in oral health were associated with improvement in endothelial function. Therefore, interventional studies have to be undertaken in order to assess the effect of periodontal disease status on E-selectin expression.

Conclusions:

The main value of the study of endothelial function may be to detect earlier in the disease course while in advanced disease, a Pandora's Box of mechanisms would have been opened, and outcome may depend on many factors, not all of which can be identified and modified.[35] In periodontal health, the innate immune defense system is in active state and immune mediators such as E-selectin are expressed in a carefully orchesterated manner. Within the limitations of the present study the following conclusions were drawn. The serum sE-selectin expression was found in both chronic periodontitis and periodontally healthy subjects with wide range of distribution and no statistical significance.

The effect of periodontal disease status on systemic levels of sE-selectin is highly unpredictable and no significant association was observed with periodontal inflammation. The circulating serum sE-selectin, therefore may not be used as a reliable biomarker of endothelial function measure in periodontal disease. Further longitudinal studies incorporating a larger sample size including further variables are required before drawing more definitive conclusions.

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