Estimation and comparison of osteopontin levels in plasma in subjects with healthy periodontium and generalized chronic periodontitis and its assessment after scaling and root planing

Suruchi Hans, Amita M. Mali

Abstract:
Background: Osteopontin (OPN) is a bone matrix derivative, whose levels reflect active lesions of aggravated periodontal disease accompanied by alveolar bone resorption. OPN is also a component of human atherosclerotic plaque, suggesting a role of OPN in cardiovascular diseases. The present study was conducted to assess and compare plasma OPN levels in subjects with healthy periodontium and generalized chronic periodontitis and to evaluate the effect of scaling and root planing on Plasma OPN levels of generalized chronic periodontitis subjects.

Materials and Methods: 40 gender matched subjects were divided into two equal groups, Group I- Healthy and Group II- Generalized chronic periodontitis, based on the Periodontal Disease Index. Blood samples were collected from the subjects at the time of clinical examination (Group I, II) and two months after Scaling and Root planning of Group II. Plasma OPN level was determined using a OPN Enzyme Immunometric Assay Kit (Quantikine).

Results: The mean value of plasma OPN levels in subjects with generalized chronic periodontitis was higher (153.08 ng/ml) as compared to the subjects with Healthy periodontium (55.09 ng/ml). After treatment of generalized chronic periodontitis group, the level of plasma OPN decreased to 91.53 ng/ml.

Conclusion: The findings from the study suggest that Plasma OPN levels were highest in plasma from sites with periodontal destruction; however, scaling and root planing resulted in the reduction of OPN levels.

Key words: Osteopontin, periodontal disease, plasma

INTRODUCTION

Periodontal diseases comprise of a group of inflammatory diseases affecting the supporting tissues of the teeth resulting from a complex interplay between specific gram-negative microorganisms, their by products, and the host-tissue response.[1] This results in progressive destruction of the periodontal ligament and alveolar bone. Earlier, periodontitis had been considered as a disease confined to the oral cavity. However, in the past several years, substantial scientific data have emerged to indicate that the localized infections characteristic of periodontitis can have a significant effect on the systemic health. This increase in systemic inflammation has been implicated in having a modulating role in cardiovascular disease (CVD),[2] on an adverse pregnancy outcome,[3] and on diabetes mellitus[4] and in respiratory disease.[5] In recent years, the relationship between CVD and periodontal diseases has received considerable attention.

Evidence suggests that plasma osteopontin levels are associated with the presence and extent of CVD, an inflammatory mediator whose levels are also found to commensurate with the progression of periodontal disease in gingival crevicular fluid as well as in plasma. The concomitant increase of osteopontin in plasma is caused by spillage or overflow of osteopontin from the diseased periodontal tissues, or produced by circulating activated macrophages.[6] Osteopontin (OPN) is a non-collagenous, calcium binding, glycosylated phosphoprotien produced by osteoblasts.[7]

Studies have shown that OPN is a component of human atherosclerotic plaque and could be a mediator of arterial neointima formation. OPN is synthesized by resident macrophages, smooth muscle, and endothelial cells in primary and restenotic human coronary atherosclerotic plaques, which contribute to cellular accumulation and dystrophic calcification in atherosclerotic plaques.[8] OPN levels in blood serum also correlate positively with the extent
Osteopontin levels also reflect active lesions of aggravated periodontal disease accompanied by alveolar bone resorption. Thus by treating periodontal disease, we may lower the risk of future cardiovascular events by reducing OPN levels after periodontal therapy. This study is planned with an objective to provide a diagnostic tool which is expected to play an important role in the assessment of periodontal disease severity and it may also help in prevention and control of systemic diseases such as CVD, inflammatory kidney disease, diabetes mellitus, and respiratory disease etc.

**Objectives**

The study was conducted with the following aims:

1. To estimate and compare the levels of OPN in plasma of subjects with healthy periodontium and generalized chronic periodontitis.
2. To estimate OPN levels in plasma of generalized chronic periodontitis subjects 2 months after scaling and root planing.
3. To compare OPN levels in plasma of generalized chronic periodontitis subjects before and after two months after scaling and root planing.
4. To correlate OPN with Periodontal disease index before and two months after scaling and root planing.
5. Subjects who are on Antiresorptive drugs such as Bisphosphonates (eg, Alendronate)
6. Pregnant or lactating females
7. Smokers.

Informed consent was obtained from those subjects who agreed to participate voluntarily in this study after institutional ethical clearance was obtained. Based upon the periodontal disease index scores, the subjects were divided into two groups:

- Group I- 20 subjects with healthy periodontium.
- Group II- 20 subjects with generalized chronic periodontitis.

For OPN assessment, non-fasting, venous blood samples were collected from the subjects at the time of clinical examination (Group I, II) and two months after scaling and root planing in Group II.

Blood was withdrawn by venepuncture from the anterior cubital vein using a sterile syringe and needle at the Pathology Laboratory at the Bharati Hospital. Five ml of blood sample was transferred to the vials containing anticoagulant and transferred immediately to the laboratory at Interactive Research School of Health Affairs (IRSHA). The stored plasma was used for estimation of OPN levels at a later date. Plasma OPN level was determined using an Osteopontin Enzyme Immunoassay Assay Kit (Quantikine).

Thereafter, scaling and root planing was carried out for subjects with generalized chronic periodontitis and oral hygiene instructions were given to the subjects. Periodontal disease index was assessed after two months of scaling and root planing and plasma samples were also collected to estimate levels of OPN.

**Materials and Methods**

**Source of data**

In the present study, 40 subjects were selected from the outpatient department of Periodontology (Post-graduate Section) of Bharati Vidyapeeth University Dental College and Hospital, Pune.

Screening examination included: (1) Medical history (2) Dental history, and (3) Periodontal disease index (Ramfjord).

**Criteria for selection**

**Inclusion criteria**

1. Systemically healthy patients
2. Patients in age group of 20-45 years
3. Random selection of male and female patients
4. Twenty subjects with healthy periodontium
5. Twenty subjects with generalized chronic periodontitis.

**Exclusion criteria**

1. History of systemic diseases (e.g., Diabetes Mellitus, Ischemic heart disease, other CVDs contributing to Arthrosclerosis, Stroke, Hypertension
2. History of any Bone Disorders
3. Subjects who had undergone periodontal treatment in the last six months
4. Subjects who had taken antibiotics, anti-inflammatory drugs, steroids and Contraceptives in the last six months
5. Subjects who are on Antiresorptive drugs such as Bisphosphonates (eg, Alendronate)
6. Pregnant or lactating females
7. Smokers.

All the assayed samples of plasma showed the presence of OPN. The mean concentration of OPN in plasma was observed to be higher in the Generalized chronic periodontitis group (153.08 ng/ml) as compared with the subjects with Healthy periodontium (55.09 ng/ml) [Table 1]. The difference in the mean of OPN levels in Group I and II was 98.00 ng/ml; with 't' value as 9.249 and P value=0.00 which is statistically significant [Table 1]. The OPN level in plasma of generalized chronic periodontitis subjects was found to be significantly higher than that of healthy subject.

OPN levels decreased to 91.52 ng/ml in Group II, two months after scaling and root planing. The difference in mean of OPN levels was 61.5566 ng/ml with 't' value as 6.843 and P value as 0.00 which is statistically significant [Table 2]. The mean OPN level in plasma of generalized chronic periodontitis group was 5.1186±0.469, which is significantly higher as compared to Group I.
### Table 1: OPN levels in plasma of group I and II subjects at baseline

<table>
<thead>
<tr>
<th>Groups</th>
<th>Mean OPN levels (ng/ml)</th>
<th>Standard deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>55.09041</td>
<td>20.645815</td>
</tr>
<tr>
<td>Group II</td>
<td>153.08569</td>
<td>42.646530</td>
</tr>
</tbody>
</table>

OPN – Osteopontin

### Table 2: Comparison of OPN levels in plasma of group I and II subjects at baseline

<table>
<thead>
<tr>
<th>Groups</th>
<th>Difference of mean OPN levels (ng/ml) 't' value 'P' value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I and II</td>
<td>98.00                                                   9.249 0.00</td>
</tr>
</tbody>
</table>

OPN – Osteopontin

### Table 3: Comparison of OPN levels in plasma of group II at baseline and two months after scaling and root planing

<table>
<thead>
<tr>
<th>Groups</th>
<th>Mean OPN levels (ng/ml) at baseline</th>
<th>Mean OPN levels (ng/ml) after 2 months</th>
<th>Difference of mean OPN levels (ng/ml) 't' value 'P' value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group II</td>
<td>153.08569</td>
<td>91.52909</td>
<td>61.5566 6.843 0.00</td>
</tr>
</tbody>
</table>

OPN – Osteopontin

whereas the mean PDI score in healthy subjects was 1.00±0.245 [Table 4]. A significant difference (P<0.05) was found in the mean PDI scores between the healthy and generalized chronic periodontitis group. The mean PDI score in the generalized chronic periodontitis group i.e. Group II was 5.11865±0.469 which reduced to 3.68±1.126 as assessed two months after scaling and root planing [Table 5]. Statistically significant difference (P<0.05) in the PDI scores between the healthy and generalized chronic periodontitis group with the P=0.00 with higher values in the subjects with generalized chronic periodontitis.

The OPN levels in plasma were correlated with the PDI scores using Pearson’s correlation coefficient. When all the subjects (Group I and II) were considered together at Baseline, the Pearson’s correlation coefficient was 0.893 which is statistically significant (P<0.05). When only the generalized chronic periodontitis group i.e. Group II (at Baseline) was considered, the Pearson’s correlation coefficient was 0.731 which is also statistically significant (P<0.05). This suggests that OPN levels in plasma show a positive correlation with the severity of the periodontal disease.

When the generalized chronic periodontitis group (Group II) was considered two months after scaling and root planing, the Pearson’s correlation coefficient was 0.181 which is statistically not significant (P>0.05) after two months of scaling and root planing [Table 6]. This result indicates that the rate of change of PDI is not similar to the rate of change of OPN. Both the parameters show improvement after scaling and root planing, but correlation could not be established which can be attributed to the other factors such as hosts response or patients compliance in the resolution of periodontitis after treatment of the same.

### DISCUSSION

The results of this study demonstrated the elevated OPN levels in plasma in subjects with generalized chronic periodontitis as compared with subjects with healthy periodontium. The results of the study are consistent with outcomes of recent investigations which reported which reported an elevation of OPN in periodontitis patients.[6,11,12]

The results of our study are similar to a study by Sharma and Pradeep.[6] They found a highly significant elevation of OPN plasma in subjects with chronic periodontitis group as compared with the healthy group. The assessment of OPN concentration was determined using a sandwich type human Osteopontin Enzyme Immunoesssay kit (Titerzyme) which is different from our study where Osteopontin Enzyme Immuno metric Assay Kit (Quantikine) was used to assess OPN levels. The highest mean plasma OPN concentrations observed in the periodontitis group in their study were 1273.21 ng/ml, and in the healthy group was 476.35 ng/ml. This was statistically significant (P =< 0.05). In addition they also assessed and correlated OPN levels in plasma with OPN levels in gingival crevicular fluid (GCF). The highest mean gingival crevicular fluid concentrations were observed in the periodontitis group 1575.01 ng/ml and the lowest in the healthy group 1194.80 ng/ml. They postulated that the concomitant increase of OPN in plasma may be caused by the spillage or overflow of OPN from the diseased periodontal tissues, or produced by the circulating activated macrophages. Subjects in the periodontitis group were treated by scaling and root planing, and strict oral hygiene measures were instituted. The mean concentration of OPN in the plasma in the generalized chronic periodontitis group decreased from 153.09 ng/ml to 91.52 ng/ml, which showed a statistically significant relation. The findings of our study are also in agreement with Sharma and Pradeep.[6] who found that the level of OPN decreased from 1273.21 ng/ml to 1051.68 ng/ml in plasma of chronic periodontitis patients after treatment i.e. two months after scaling and root planing. These results also showed a positive correlation with OPN levels in GCF which were significantly reduced from 1575.01 ng/ml to

### Table 4: PDI scores in group I and II subjects at the baseline

<table>
<thead>
<tr>
<th>Groups</th>
<th>Mean PDI score (ng/ml)</th>
<th>Standard deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>1.00</td>
<td>0.245</td>
</tr>
<tr>
<td>Group II</td>
<td>5.11865</td>
<td>0.469</td>
</tr>
</tbody>
</table>

PDI – Periodontal disease index

### Table 5: PDI scores in group II at baseline and two months after scaling and root planing

<table>
<thead>
<tr>
<th>Groups</th>
<th>Mean PDI score at baseline</th>
<th>Mean PDI score after 2 months</th>
<th>Difference of Mean PDI score 't' value 'P' value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group II</td>
<td>5.11865</td>
<td>3.6765</td>
<td>1.4418 8.052 0.00</td>
</tr>
</tbody>
</table>

PDI – Periodontal disease index

### Table 6: Correlation between osteopontin levels in plasma and periodontal disease index

<table>
<thead>
<tr>
<th>Group</th>
<th>Pearson’s correlation coefficient 'P' value</th>
</tr>
</thead>
<tbody>
<tr>
<td>All cases together group I and II (at baseline)</td>
<td>0.893 0.00</td>
</tr>
<tr>
<td>Group II (at baseline)</td>
<td>0.731 0.00</td>
</tr>
<tr>
<td>Group II (after 2 months)</td>
<td>0.181 0.446</td>
</tr>
</tbody>
</table>
Hans and Mali: Plasma osteopontin levels in healthy and chronic periodontitis subjects

1194.80 ng/ml after treatment of subjects with chronic periodontitis.

Also the mean PDI score of generalized periodontitis group decreased from 5.19 to 3.68 as assessed two months after scaling and root planing. Statistically significant positive correlation between OPN in plasma and PDI was found at the baseline in subjects with generalized chronic periodontitis. However, weak positive correlation was observed between OPN and PDI two months after scaling and root planing in subjects with generalized chronic periodontitis.

The result of this study indicates a significant association between inflammatory marker OPN and periodontal disease and a tendency for significant reduction of plasma OPN levels after treatment of periodontitis. However, a weak correlation was observed between OPN and PDI in generalized chronic periodontitis subjects after scaling and root planing.

As the correlation between OPN and cardiovascular disease has already been established and the OPN levels also increase periodontal diseases which is evident from our results; this may indicate a strong correlation between periodontitis and CVD with one of the mediator as OPN. Thus, it is clear that the risk of CVD can be reduced in otherwise healthy individuals with prevention/treatment of periodontitis at an early stage. Scaling and root planing may also help in reducing the severity of pre-existing CVD. This implies that the assessment of periodontal status at the time of cardiovascular examination should also be incorporated in routine practice.

CONCLUSION

From the above observation, it can be concluded that elevated levels of OPN were seen in subjects with periodontitis as compared with healthy individuals. It was also found that the OPN levels significantly decreases two months after periodontal therapy. A positive correlation was observed between OPN and PDI in chronic periodontitis group.

As the correlation between OPN and cardiovascular disease has already been established and the OPN levels also increases in periodontal diseases which is evident from our results; this may indicate a strong correlation between periodontitis and CVD with one of the mediator as OPN. Thus, it is clear that the risk of CVD can be reduced in otherwise healthy individuals with prevention/treatment of periodontitis at an early stage. Periodontal therapy may also help in reducing the severity of pre-existing CVD. This implies that the assessment of periodontal status at the time of cardiovascular examination should also be incorporated in routine practice.

For clinical application of this approach, a long term study of large sample size is required with relevant laboratory investigations to evaluate cardiovascular status. Studies could also be carried out in relation to the subjects with periodontitis and pre-existing CVD to evaluate the effect of periodontal therapy on severity of CVD.

ACKNOWLEDGMENTS

I am grateful to Bharati Vidyapeeth Dental College and Hospital, Pune, Maharashtra, India, and Interactive Research School of Health Affairs (IRSHA), Pune, Maharashtra, India, for allowing me to conduct the study.

REFERENCES


Source of Support: Nil, Conflict of Interest: None declared.