Evaluation of serum and salivary sialic acid and nitric oxide levels in chronic periodontitis patients

Shiny Inasu, Biju Thomas, Suchetha Kumari, Amitha Ramesh, Amita Rao

Abstract
Background: Chronic gingivitis and periodontitis are inflammatory diseases. Sialic acid occupies the interface between the host and pathogenic microorganisms. Nitric oxide has been linked to the etiopathogenesis of periodontal disease and is expressed in salivary glands as well as in their product. Hence this study has been designed to compare the evaluation of serum and salivary nitric oxide and sialic acid in chronic periodontitis patients.

Aim: To evaluate the serum sialic acid and nitric oxide level in periodontally healthy and chronic periodontitis subjects.

To evaluate the salivary sialic acid and nitric oxide level in periodontally healthy and chronic periodontitis subjects.

To correlate the levels of serum and salivary sialic acid and nitric oxide with disease severity.

Material and Methods: 40 subjects will be selected and will be divided into two groups.

Group I – 20 subjects who are systemically healthy with healthy gingiva (Control group).

Group II – 20 subjects who are systemically healthy with chronic periodontitis. (Periodontitis group).

Clinical examination was done and the following parameters were assessed: Gingival index, Clinical Attachment Loss.

Keywords: Evaluation, serum, salivary sialic acid, nitric oxide, chronic periodontitis patients

Introduction
Chronic periodontitis is an inflammatory disease that affects the supporting tissues of the teeth. It is initiated by specific bacteria within the plaque biofilm and progresses due to an abnormal inflammatory-immune response to those bacteria, involving the release of excess proteolytic enzymes and reactive oxygen species [1].

Sialic acids are a family of nine carbon acidic monosaccharides that occur naturally at the end of sugar chains attached to the surface of cells and soluble proteins. Sialic acid occupies the interface between the host and pathogenic microorganisms. An important function of host sialic acid is to regulate innate immunity [2].

Currently, the clinical diagnosis of periodontal diseases involves primarily the assessment of clinical measures of tissue destruction and signs of tissue inflammation [3]. Increased SA concentrations have been reported during inflammatory processes, probably resulting from increased levels of richly sialylated acute-phase glycoproteins [4].

Nitric oxide, Sialic acid and Alkaline phosphatase have been proved to be potential inflammatory markers. These biomarkers of host response can be found in gingival crevicular fluid (GCF), saliva and serum samples and can potentially be used as diagnostic markers [3].

Saliva could be used as a noninvasive diagnostic fluid to measure biomarkers released during disease initiation and progression such as periodontal infections.

Till date, there is very little data regarding simultaneous estimation and comparison of total SA content in saliva and serum of chronic periodontitis patients [3].

Hence this study has been designed to compare the evaluation of serum and salivary nitric oxide and sialic acid levels in chronic periodontitis patients and also to correlate the levels of serum and salivary sialic acid and nitric oxide with disease severity.
**Source of data**
- 40 subjects were selected and divided into two groups:
  - Group I – 20 subjects who are systemically healthy with healthy gingiva (Control group).
  - Group II – 20 subjects who are systemically healthy with chronic periodontitis. (Periodontitis group).

**Method of collection of data**
- The method of collection of saliva.
  - For each patient, testing sessions were held between 8.00 and 10.15 in the morning. Subjects will be asked not to eat or drink at least 3 hour before collection of the sample.
  - Prior to the collection procedure, the subjects began by rinsing their mouth, thoroughly several times with water and then resting quietly for 3 min. Unstimulated whole saliva (3 ml) was collected by means of the “spitting method” according to the directions given by Narazesh (1993).
  - Saliva was collected for 4 min. The collected was centrifuged at 3000 G for 15 min at 4 degree. The supernatant fraction was then aliquoted into storage vials and kept at -70 degree until required for analysis.
- The method of collection of serum:
  - 5ml of venous blood is collected in sterile tubes and kept at 4 degree for 30 min before centrifugation for 10 min at room temperature.

**Criteria for Selection**

**Inclusion Criteria**
1. Subjects aged between 22 to 55 years.
2. Subjects with a minimum complement of 20 teeth.
3. Subjects with healthy gingiva (Group I).
4. Subjects who are diagnosed with moderate form of chronic periodontitis (Group II) showing the presence of more than 30% of sites with clinical attachment loss >3mm.

**Exclusion Criteria**
1. Subjects with any known systemic disease or conditions.
2. Patients who have undergone any periodontal treatment in the last 6 months.
3. History of any antibiotic / analgesic for three months prior to study.
4. Subjects with history of smoking, tobacco chewing, alcohol consumption.
5. Pregnant and lactating women

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>Mean</th>
<th>Std. Deviation</th>
<th>t</th>
<th>df</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum Sialic Acid</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>20</td>
<td>59.40065</td>
<td>7.026437</td>
<td>-7.055</td>
<td>24.375</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Chronic Periodontitis</td>
<td>20</td>
<td>90.605</td>
<td>18.49121</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Salivary Sialic Acid</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>20</td>
<td>37.189</td>
<td>4.739013</td>
<td>0.497</td>
<td>38</td>
<td>0.622</td>
</tr>
<tr>
<td>Chronic Periodontitis</td>
<td>20</td>
<td>36.3</td>
<td>6.440252</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serum Nitric Oxide</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>20</td>
<td>50.2035</td>
<td>6.181005</td>
<td>-4.123</td>
<td>38</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Chronic Periodontitis</td>
<td>20</td>
<td>65.34604</td>
<td>15.21614</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Salivary Nitric Oxide</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>20</td>
<td>10.09605</td>
<td>2.685565</td>
<td>-13.62</td>
<td>38</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Chronic Periodontitis</td>
<td>20</td>
<td>25.98068</td>
<td>4.471083</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Discussion**
On comparison, group I who are systemically healthy with healthy gingiva (Control) showed better oral hygiene than group II (Periodontitis) who are systemically healthy with chronic periodontitis. According to the above results, there was a correlation between the severity of disease and the salivary and serum sialic acid concentration. The salivary and serum sialic acid concentration in the affected group was significantly higher than that in the healthy group. These results suggest that the total salivary sialic acid concentration is a useful parameter for determining the severity and course of periodontal diseases. Although clinical parameters such as probing depth, clinical attachment loss, bleeding on probing, plaque index and radiographic assessment of alveolar bone loss provide information on the severity of periodontitis, they do not measure disease activity. Shinohara and co-workers, studied the relationship between...
the salivary sialic acid concentrations in rats with naturally occurring gingivitis. Their results suggested that the amount of sialic acid elevated in saliva can be a useful index of the severity of periodontal disease.

Elevated levels of Total Sialic Acid in saliva and serum may have a protective role in periodontitis as the elevated levels of total sialic acid might be considered as a defense molecule against the increased oxidative stress in inflammatory diseases including periodontitis. Sialic acid levels are markedly increased in those with periodontal diseases, confirming that this saccharide plays an important role in the immune system. Therefore, the present data indicates that salivary and serum TSA levels have a significant association with periodontal health and disease.

The results of this study showed significantly increased concentrations of nitrite in the patients with periodontitis as compared to those in the healthy control group. The significantly higher levels of NO in this study group had contributed to the development of the frequently found clinical symptoms of periodontitis.

The increased tendency of the soft tissue to bleed on gentle probing may be due to the inhibitory effect of NO on platelet aggregation and the adhesion-inhibitory effect of NO. Though many studies have revealed the alterations in the salivary NO concentrations, only few studies have reported on the serum NO levels. Hence, an attempt was made to compare the levels of salivary NO with respect to the serum NO concentrations.

Advances in diagnostic research are moving towards methods whereby the periodontal risk can be identified and quantified by objective measures using biomarkers. Patients with periodontitis may have elevated circulating levels of specific inflammatory markers that can be correlated to the severity of the disease. In the present study, an attempt was made to evaluate the roles of both the serum and the salivary biochemical parameters as inflammatory markers in periodontitis.

Various studies have reported the significance of NO as an inflammatory marker, but the present study has correlated both the serum and the salivary elevated levels in chronic periodontitis.

Conclusion
In the present study, we found significantly higher levels of salivary free sialic acid in chronic periodontitis compared to healthy controls. This may be due to the release of different lysosomal exoglycosidases during the progression of periodontal disease and there was a correlation between the severity of disease and the salivary and serum sialic acid concentration.

This study was also conducted to focus on the relationship between the salivary and the serum levels of NO in generalized chronic periodontitis patients. The results of this study indicated that there was a direct positive correlation between the salivary and the serum nitric oxide levels.

References