Elevated Levels Of Salivary Sialic Acid In Periodontitis Disease

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Abstract
Sugars are found as a free form present only in traces in fasting saliva. Most of the carbohydrate content of saliva is in bound form. The carbohydrate part of glycoproteins is readily split off from the proteins by ill defined bacteria. In saliva liberating reducing sugars.

A total of 50 volunteers suffering from a case studied periodontitis, they were treated medically, their total sialic acid and lipid associated sialic acid were investigated.

A comparison study was adopted using 50 samples of healthy subjects as controls.

Significant elevated values of total sialic acid (75.43±0.1mg/dl) and lipid associated sialic acid (45.04±3.15mg/dl) were found in the cases studied compared with normal levels in controls (TSA= 60.13±5.06mg/dl, and LASA= 20.41±6.05 mg/dl).

A colorimetric determination was applied to follow the determinations.

Keywords: Total sialic acid, lipid associated sialic acid, periodontitis saliva.
Introduction

Periodontitis is a case found to affect the gum of human being leading to an inflammation that results from bacterial infections [1]. Periodontitis is a common disease due to bad oral hygiene, smoking and systemic disease, affecting 15% of adults between 21-50 years old and 30% of adults over 50 years have this disease. A plaque (sticky, bacterial biofilm) and tartar (calcified plaque) build up along the gingival and causes inflammation [2,3]. Pockets form between teeth and gum, making anaerobic region where the presence of bacteria cause the destruction of tissue and bone that supporting teeth. Among the 500 species of oral bacteria, *F. nucleatum* is the dominant species, which is responsible for tooth and gum decay through tissue irritation that inhibit fibroblast cell division and the wound healing process.

It has been suggested that the enzyme neuraminidase, responsible for sialic acid synthesis (N-acetyl neuraminic acid), may play a role in plaque formation and periodontitis disease [4].

Sialic acid occurs in body tissues and fluids as structural units of oligosaccharides, glycolipids and glycoproteins [5], where they are bound in glycosidic linkage, usually to D-galactose or N-acetyl-D-galactosamine [6,7]. Sialic acid function in the salivary secretions have always created much interest because of its possible relevance to oral health, thus it was found that monitoring of sialic acid activity was of importance to extend previous morphological description of the alterations in submandibular tissue structure [2,8] which represent the aim of the recent study.

Materials and Methods

Chemicals : Butyl acetate, hydrochloric acid, chloroform were the products of BDH. Methanol, phosphotungstic acid, phosphoric acid, perchloric acid, used were analar grade. Resorcinol, cupper sulfate, from Aldrich chemical company.

Sampling :

Patients: salivary samples used in this study were collected from persons attending the outpatient clinics of the maxillofacial surgery
department, college of dentistry, sulaimani university. They were represented 50 patients suffering from periodontitis disease and they formerly treated.

Controls: they were consisted of student and staff volunteers (50 Samples), they were healthy with no systemic disease.

**Sampling technique:**
Flow stimulated whole saliva samples were collected around 9-10 AM, (after chewing unsweetened gum by the volunteers), two hours after the variability in salivary flow and composition be minimized due to diurnal variation. Subjects were rinse their mouth with distilled water thoroughly to remove any food debris, and then after 10 minutes directed to spit into a sterile glass beaker. Three ml of saliva was centrifuged for 10 minutes at 800 g to obtain a clear supernatant fluid, which was assayed immediately (5).

**Methods:**
(a) Assay of sialic acid: the principle of measurement depends on the Formation of chromagen complex resulted from the addition of Resorcinol reagent to the mixture samples. The chromagen formed was Extracted by butyl acetate / methanol reagent and measured at 580nm $^9$.

\[
5 = \text{conc. of standard solution.}
\]
\[
0.0188 = \text{dilution factor.}
\]

(b) Assay of lipid associated sialic acid : the procedure was adopted of That of Katopodis etal $^{10}$. The principle depends on the formation of a colored complex resulted from the reaction of phosphotungstic acid With the separated layer of the lipid using a mixture of chloroform / Methanol (2:1v/v).

**Results and Discussion**
For assaying sialic acid activity a colorimetric determination using the resorcinol method was found to be the most suitable, being subject to the least interference and relatively precise $^9$. It was found that monitoring of sialic acid activity was of important to
extend previous morphological descriptions of the alterations in submandibular tissue structure [8]. The data presented in this study was found to be in consistence with already published researches. For example, Shinohara and co-workers [11], 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. Same results were found by Kumar and Pattabirman [5], whom they were suggested that protein bound sialic acid level per se was found to be decreased in periodontitis. Their data indicated the increased in sialidase activity could be the cause for the sialic acid elevation. As seen in table (1), the results obtained reflects such significant increases in the levels of both total sialic acid and lipid associated sialic acid in the salivary patients with periodontitis disease. The data obtained reflects a significant (p<0.005) elevation in investigated salivary sialic acid as compared with that in controls (fig -1). An explanation also can be made for such elevation in sialic acid to be due to the bacterial infection in case of periodontitis disease [12]. Their study was suggested that elevation in sialic acid concentration could be resulted from the destruction of connective tissues and bone near the pocket, which formed as a result of large term accumulation of plaque and tartar between teeth and gum continuously[13].

Researchs have been focused on the increasing level of lipid associated sialic acid (LASA). In a study of Reinlgin [14], LASA level have been reported to be a useful in monitoring patients with inflammatory diseases. The elevations were observed with progression of the disease. Jawazaly [13], in a study of monitoring periodontitis disease, found that LASA levels were also elevated as the inflammation of the gum was advanced. Figure (2), Shows such type of elevation in salivary LASA concentration of periodontitis compared with that of control, so that it can be used as a useful parameter in distinguishing between healthy individuals and patients. Such elevation in LASA concentration cab be explained to be due to the damage occurs in collagen contents [15].
Table-1: Levels of TSA and LASA in control and periodontitis.

<table>
<thead>
<tr>
<th>Case</th>
<th>Parameter(mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TSA</td>
</tr>
<tr>
<td>Control</td>
<td>60.13 ± 5.06</td>
</tr>
<tr>
<td>Periodontitis</td>
<td>75.43 ± 0.1</td>
</tr>
</tbody>
</table>

Readings made of triplicate.
P < 0.005 value was selected as significant.

Fig-1 Levels of TSA in control and periodontitis saliva.

Fig-2: Level of LASA in control and periodontitis saliva.
References

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12. Romppanen, J. (2003), (Serum sialic acid in clinical diagnostics), Medical Sciences, 309-Kuopio University, Puplications.