Potential of Salivary Protein as a Biomarker in prognosis of Diabetes mellitus.

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ABSTRACT

Assessment of blood sugar control is vital in managing diabetes. To assess the glyceremic level of the body, blood sugar estimation and glycosylated hemoglobin (HbA1c) are done routinely all over the world. Diabetes is known to influence salivary composition and function. A total of 60 patients, comprising 30 diabetic patients and 25 healthy controls were selected. Salivary investigations were performed using unstimulated whole Saliva. A significant correlation was found between salivary and blood concentrations in the diabetes. Mean salivary glucose levels were found to be significantly elevated in uncontrolled diabetics when compared to healthy non-diabetics. There were significant Increase in mean salivary amylase, Protein & Potassium in diabetic patients when compared to healthy non-diabetics. Furthermore, in this study the protein profiles of whole saliva of diabetic and healthy non-diabetic were compared using SDS-PAGE considerable variations between individuals in the protein profiles were observed. The saliva from diabetic patients appeared to have more of proline-rich protein bands. This findings suggests that saliva can be used reliably for reflecting and monitoring the blood glucose concentration in the patients of diabetes mellitus.

Key words: Whole Saliva, Uncontrolled Diabetics, Polyacrylamide gel electrophoresis (PAGE), Proline-rich proteins.

Introduction

Saliva is the watery and frothy substance produced in the mouths of humans and most other animals. [1] Human saliva contains informative components that can be used as diagnostic markers for human disease. [2] Biomarkers detected in saliva can be valuable in a wide range of clinical pathology, forensic medicine and sport medicine [3].

Knowledge of the effects of diabetes on salivary composition and function remains equivocal. Basement membrane permeability of the parotid gland is reported to be higher in diabetes mellitus, and this result in raised percolation of components such as glucose, amylase and protein from blood, thus raising their levels in saliva [4-7].

The proline rich proteins (PRPs) constitute about 70% of human salivary proteins and unusual in their Amino acid compositions, with Proline comprising 25%-45% of the Amino acid residues [8].

If the glycation of salivary proteins is linked with glycated proteins in blood and blood glucose, it can be used to detect diabetes at an early stage. Hence the aims of this study were as follows; First to estimate the constituents of saliva (glucose, amylase, total protein, sodium and potassium) in order to aid in reaching firm conclusions about their alterations in diabetics as compared to healthy non-diabetics; second to compare and correlate these parameters in uncontrolled and controlled diabetics, finally, to assess the significant variations of protein profiles in diabetic and healthy non-diabetics by SDS-PAGE[9] using coomassie-blue R 250 staining were studied and compared.

Subjects and Method:
The study population was composed of 25 healthy patients (non diabetic and without oral pathology), aged 20-60 years and 60 diabetic individuals from Mothers care, Vellore. Based on their blood sugar level, they were divided into 5 groups.

Group 1: Consist of 25 individuals who are clinically healthy their clinical characteristics matched the patient groups.

Group 2: Consist of 15 patients whose blood sugar level was below 100 mg/dl

Group 3: Consist of 15 patients whose blood sugar level was below 100-150 mg/dl

Group 4: Consist of 15 patients whose blood sugar level was below 150-250 mg/dl

Group 5: Consist of 15 patients whose blood sugar level was above 250mg/dl

Collection of sample:

Saliva:
Unstimulated saliva (5 ml) from the diabetic and control groups was collected [10]. Salivary sample collection was performed in the morning, at 8 o’clock in an ice-chilled sterile container bearing the appropriate preservatives. Once the saliva was collected, it was centrifuged at 3000 rpm for 20 min to remove any particulate material. The clean supernatants were processed immediately for estimation of glucose, amylase, total protein, sodium & potassium [11-13].

Serum:
10 ml of venous blood was drawn and the serum was separated by centrifugation, supernatant were aspirated.

Measurement methods:

Glucose Estimation:
Serum and salivary glucose estimation was performed using the glucose oxidase end-point method [14, 15] at the wavelength of 505 nm.

Serum Amylase Estimation:
Salivary á-amylase estimation was performed using the direct substrate kinetic enzymatic method [15, 16].

Total Protein Estimation:
Serum salivary protein estimation was performed usingBradford method [17] using bovine serum albumin as a standard at the wavelength of 595 nm.

SDS PAGE:
SDS PAGE was carried out according to the method of Laemli et al [18].

Sodium & Potassium estimation by Flame Photometry:
Salivary sodium and potassium was estimated by flame photometry [10].

Data and Statistical Analysis:
All data were analyzed with SPSS program ver.10.0[19] comparison between groups were made by analysis of variance(ANOVA) test. The statistically significant level was set to p<0.05. Pearson’s correction coefficients were used to examine the relation between the variables.

RESULTS:
Considering the prevalence of diabetes mellitus and its oral manifestations, it has become of paramount importance to study the levels of some crucial parameters in diabetic saliva. The demographic characteristics of the subjects are shown in Table 1.

Table 1: Distribution of the groups studied according to sex and age of the patients

<table>
<thead>
<tr>
<th>Experimental group</th>
<th>Control group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>30</td>
</tr>
<tr>
<td>Female</td>
<td>15</td>
</tr>
<tr>
<td>Female</td>
<td>30</td>
</tr>
<tr>
<td>Age</td>
<td>56.5±14.3</td>
</tr>
</tbody>
</table>

Levels of significance was p<0.005

Salivary protein:
Mean salivary protein were higher in uncontrolled diabetics when compared to controlled diabetics and healthy non-diabetics.

SDS-PAGE also revealed the presence of total protein content to be higher in uncontrolled diabetics and was found to be consistent with the extent of diabetes mellitus.

Salivary potassium were not significantly different when diabetics and healthy non-diabetics were compared. But there increased significant variation in salivary sodium content with the extent of disease.

DISCUSSION:
The present study was undertaken with the aim of suggesting the possibility of using salivary protein as a biomarker for assessing the control of blood sugar.

The salivary protein concentrations of the non-diabetic control subjects did not show the presence of glucose in higher concentrations, while the samples obtained from the diabetics showed significant concentrations of glucose in the saliva along with their serum glucose concentration and is found to correlate [20, 21]. Salivary glucose, amylase and total protein values in diabetic patients and control groups are shown in Table 2 and salivary sodium and potassium levels in Table 3.

Table 2: Intergroup comparisons of mean of salivary parameters in the diabetic group and healthy non-diabetic groups

<table>
<thead>
<tr>
<th>Blood sugar level (mg/dl)</th>
<th>Salivary glucose (mg/dl)</th>
<th>Mean ± SD</th>
<th>Salivary amylase (U/ml)</th>
<th>Mean ± SD</th>
<th>Salivary total protein (mg/dl)</th>
<th>Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;100</td>
<td>7.30 ± 5.84</td>
<td>102.32 ± 67.64</td>
<td>87.51 ± 40.26</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;150-250</td>
<td>7.04 ± 6.64</td>
<td>108.65 ± 60.77</td>
<td>89.31 ± 49.71</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;250</td>
<td>7.09 ± 7.17</td>
<td>111.12 ± 59.14</td>
<td>105.28 ± 45.11</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>5.91 ± 2.19</td>
<td>96.72 ± 50.70</td>
<td>98.25 ± 40.59</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Levels of significance was p<0.005

Salivary glucose:
Mean salivary glucose levels were higher in the uncontrolled and controlled diabetic groups than in the healthy non-diabetic group and the differences were highly significant (Table 2). Uncontrolled diabetics had higher mean salivary glucose levels than controlled diabetics.

Salivary amylase:
The salivary amylase levels were significantly higher in controlled diabetics when compared with healthy non-diabetics.

A dramatic important of salivary amylase activity has been found in diabetic patients compared to healthy controls. The present study has shown that the alpha-amylase levels in unstimulated saliva from type 2 diabetic patients are higher than in control groups. There is considerable disagreement in the literature about salivary amylase activity in diabetic patients; different rescales have reported that salivary amylase concentrations from diabetics are higher [22], lower [15] or the same [23]. With regard to salivary total protein, the present study results are consistent with most previous studies, higher [15] lower or the same. A significantly positive correlation was observed between total protein levels of uncontrolled diabetic group and controlled diabetic group [14].

SDS PAGE RESULT:
Also the protein bands appeared during SDS PAGE was found to correlate with the extent of diabetes mellitus and there was a significant correlation between the healthy non-diabetic patients and diabetic patients.

REFERENCES:

The observations derived from this study require more comprehensive evaluation with emphasis on broader representation.

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