Original Article

CORRELATION OF SERUM AND SALIVARY GLUCOSE LEVELS IN DIABETES MELLITUS PATIENTS

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INTRODUCTION

Diabetes mellitus (DM) is a metabolic disorder; it requires constant monitoring of glucose levels. Currently, the diagnosis of diabetes is achieved only by analysing blood glucose levels, which is an invasive method and is physically and psychologically traumatic to the patient. This study is done to ascertain whether glucose present in saliva can be used to monitor the diabetes patients. Aim: This study is done to estimate and correlate the glucose levels in blood and saliva of diabetic patients, to assess if salivary glucose can be used as an alternative non-invasive tool for diagnosing and monitoring diabetes. Materials & methods: 200 diabetic patients were chosen for the study (100 controlled & 100 uncontrolled). 50 control subjects were age and gender matched. Quantitative estimation of glucose in the blood and saliva was performed by Hexokinase method. Glycosylated haemoglobin (HbA1c) levels were also estimated to confirm the diabetic status of the patient. Tabulation and Statistical analysis of the data was done. Results & Conclusion: Positive significant correlation between plasma and salivary glucose levels (<0.01). Salivary glucose was significantly higher in diabetics (<0.01). Significant positive correlation between glycosylated haemoglobin and salivary glucose levels (<0.01). The results showed that salivary glucose concentration can be used as a non-invasive tool for diagnosing and monitoring diabetic patients.
Sample collection: The methods of serum and saliva collection were clearly explained to the patients prior to the collection of sample.

Serum - 3ml of intravenous blood was obtained from median cubital vein of forearm with a 5ml syringe for the estimation of fasting blood sugar from the study population under aseptic conditions. Each sample was centrifuged at 2000 rpm for 5 minutes and the serum was separated and stored at 20°C. The serum thus obtained was used for further analysis.

Saliva: Patients were advised to avoid food and fluid intake at least 2 hours prior to the sample collection. After rinsing the mouth with distilled water, 3ml of unstimulated whole saliva was collected in resting position. The patient was instructed to spit into the sterile plastic container over a period of 5 minutes. The sample then can be sent to the laboratory immediately or stored at 20°C.

Procedure:

Estimation of Glucose: Glucose levels in serum and saliva was estimated by using Hexokinase method. The serum sample was centrifuged and then given for autoanalyzer. Hexokinase catalyses the phosphorylation of glucose in the presence of adenosine 5 triphosphate and magnesium to form glucose 6 phosphate and ADP. G6P is then oxidised by glucose 6 phosphate dehydrogenase in the presence of NAD to produce 6 phosphogluconate and NADH. It is an end point technique[6].

Estimation of Glycosylated haemoglobin (Hba1c):

This was done by High performance liquid chromatography method. The blood sample was introduced into a EDTA vacutainer and mixed well. Then it was analysed by BIORAD D10 analyser. It utilizes the principle of ion exchange high performance liquid chromatography. The samples are automatically diluted on the D-10 and injected into the analyser cartridge. The d10 delivers a programmed buffer gradient of increasing ionic strength to the cartridge, where the hemoglobins are separated based on their ionic interactions with the cartridge material. The separated hemoglobins then pass through the flow cell of the filter photometer, where the changes in absorbance are measured[9,10,11].

STATISTICAL ANALYSIS was performed by using Statistical Package for Social Sciences Software Version 21 (SPSS 21). To find the correlation between Serum glucose, Saliva glucose & Glycosylated hemoglobin in controlled and uncontrolled diabetics, Karl-Pearson’s Coefficient of correlation was used. For the comparison of mean value of Serum glucose, Saliva glucose & Glycosylated hemoglobin between controlled & uncontrolled diabetics, Mann-Whitney test was used.

RESULTS:

The total number of subjects were 250, divided into two groups. Group-1 (study group) consists of 200 Diabetes Mellitus patients (100 controlled – 50 males, 50 females & 100 uncontrolled diabetes – 50 males, 50 females). Group-2 consists of 50 non-diabetic healthy individuals as controls (25 males, 25 females). Male to female ratio is 1:1 for both diabetic and non-diabetics.

The age range of the DM patients was 40-60 years with a mean age of (50.52 ± 7.02years) for controlled diabetics and (49.4 ± 9.39years) for uncontrolled diabetics (Table 1).

In controlled diabetics, the mean value of serum glucose was found to be 159.37 ± 23.7 mg/dl (Table 4).

In uncontrolled diabetics the mean value of serum glucose was found to be 297.45 ± 68 mg/dl (Table 4).

In controlled diabetics the mean value of salivary glucose was 5.46 ± 1.99 mg/dl (Table 4).

In uncontrolled diabetics the mean value of salivary glucose was 11.70 ± 3.9 mg/dl (Table 4).

In control group, the mean value of serum glucose was 89.65 ± 7.89 mg/dl (Table 5).

In control group, the mean value of salivary glucose was 8.59 mg/dl ± 0.59 mg/dl (Table 5).

In controlled diabetics, the mean value of Glycosylated hemoglobin (Hb1AC) level was 6.89 ± 0.60. In uncontrolled diabetics, it was 10.42 ± 2.29 (Table 4).

It was <6 in control group.

On statistical analysis, there was a significant positive correlation between serum and salivary glucose levels (<0.01), salivary glucose was significantly higher in diabetics (<0.01) than non-diabetics. There was a significant positive correlation between glycosylated hemoglobin and salivary glucose levels (<0.01) also (Table 2,3). The Karl Pearson’s coefficient correlation was found to be significant (p<0.01) for serum and salivary glucose levels in both the groups (Table 2,3). All the results obtained are hereby explained in the form of tables and graphs below.

Table 1: Age distribution between controlled and uncontrolled diabetic patients

<table>
<thead>
<tr>
<th>Age</th>
<th>Controlled diabetes</th>
<th>Uncontrolled diabetes</th>
</tr>
</thead>
<tbody>
<tr>
<td>50.52 ± 7.02</td>
<td>49.4 ± 9.38</td>
<td></td>
</tr>
</tbody>
</table>

Karl-Pearson’s Coefficient of correlation

Table 2: Inter relation between Serum glucose, Saliva glucose and Glycosylated hemoglobin in Controlled diabetics

<table>
<thead>
<tr>
<th>Group</th>
<th>Variables</th>
<th>t-value</th>
<th>p-value</th>
<th>Inference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controlled diabetics</td>
<td>Serum glucose</td>
<td>0.56</td>
<td>&lt;0.01</td>
<td>HS</td>
</tr>
<tr>
<td></td>
<td>Saliva glucose</td>
<td>0.47</td>
<td>&lt;0.01</td>
<td>HS</td>
</tr>
<tr>
<td></td>
<td>Glycated hemoglobin</td>
<td>0.36</td>
<td>&lt;0.01</td>
<td>HS</td>
</tr>
</tbody>
</table>

Table 3: Inter relation between Serum glucose, Saliva glucose and Glycosylated hemoglobin in Uncontrolled diabetics

<table>
<thead>
<tr>
<th>Group</th>
<th>Variables</th>
<th>t-value</th>
<th>p-value</th>
<th>Inference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uncontrolled diabetics</td>
<td>Serum glucose</td>
<td>0.63</td>
<td>&lt;0.01</td>
<td>HS</td>
</tr>
<tr>
<td></td>
<td>Saliva glucose</td>
<td>0.74</td>
<td>&lt;0.01</td>
<td>HS</td>
</tr>
<tr>
<td></td>
<td>Glycated hemoglobin</td>
<td>0.54</td>
<td>&lt;0.01</td>
<td>HS</td>
</tr>
</tbody>
</table>
**Mann-Whitney U test**

**Table 4: Comparison of mean values of Serum glucose, Saliva glucose & Glycosylated hemoglobin between Controlled & uncontrolled diabetics**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Variables</th>
<th>Mean</th>
<th>SD</th>
<th>Z-value</th>
<th>P-value</th>
<th>Inference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum glucose</td>
<td>Controlled</td>
<td>159.37</td>
<td>23.7</td>
<td>-11.45</td>
<td>&lt;0.01</td>
<td>HS</td>
</tr>
<tr>
<td></td>
<td>Uncontrolled</td>
<td>297.45</td>
<td>68</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saliva glucose</td>
<td>Controlled</td>
<td>5.46</td>
<td>1.99</td>
<td>-10.03</td>
<td>&lt;0.01</td>
<td>HS</td>
</tr>
<tr>
<td></td>
<td>Uncontrolled</td>
<td>11.70</td>
<td>3.9</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glycated hemoglobin</td>
<td>Controlled</td>
<td>6.88</td>
<td>0.60</td>
<td>-8</td>
<td>&lt;0.01</td>
<td>HS</td>
</tr>
<tr>
<td></td>
<td>Uncontrolled</td>
<td>10.42</td>
<td>2.29</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Table 5: Comparison of mean values of Serum glucose & Saliva glucose between Control subjects & diabetics**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Variables</th>
<th>Mean</th>
<th>SD</th>
<th>Z-value</th>
<th>P-value</th>
<th>Inference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum glucose</td>
<td>Control</td>
<td>89.65</td>
<td>7.89</td>
<td>-7.33</td>
<td>&lt;0.01</td>
<td>HS</td>
</tr>
<tr>
<td></td>
<td>Diabetic</td>
<td>228.41</td>
<td>85.58</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saliva glucose</td>
<td>Control</td>
<td>8.59</td>
<td>0.49</td>
<td>-7.1</td>
<td>&lt;0.01</td>
<td>HS</td>
</tr>
<tr>
<td></td>
<td>Diabetic</td>
<td>2.5</td>
<td>4.34</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Graph 1:** Distribution of Serum glucose, Saliva glucose and Glycosylated hemoglobin in controlled & uncontrolled diabetics

**Graph 2:** Distribution of Serum glucose & saliva glucose in control subjects & diabetics patients

**Graph 3:** Relation between Serum glucose, Saliva glucose and Glycosylated hemoglobin in controlled diabetics

**Graph 4:** Relation between Serum glucose, Saliva glucose and Glycosylated hemoglobin in uncontrolled diabetics

**DISCUSSION:**

Diabetes mellitus is a multi-systemic metabolic disorder characterized by a relative or absolute deficiency of insulin secretion and/or concomitant resistance to the metabolic action of insulin on target tissues[1]. In 2000, the incidence of diabetes mellitus in India was 31.7 million (highest), followed by China and United States with 20.8 million, 17.7 million in second and third positions respectively. The prevalence was predicted to be double globally from 171 million in 2000 to 366 million in 2030 with a maximum increase in India up to 79.4 million[12,13].

Based on etiology Diabetes mellitus is classified as Type 1, Type 2, other specific types, and gestational diabetes. Type 1 diabetes, also termed as immune mediated, insulin-dependant, juvenile-onset diabetes. It accounts for 5–10%, occurs mainly due to cellular-mediated autoimmune destruction of β-cells of pancreas, thereby leading to absolute insulin deficiency. Type 2 diabetes is also termed as non-insulin dependent diabetes, adult-onset diabetes. It accounts for 90–95%, occurs predominately due to insulin resistance with relative insulin deficiency. These individuals do not need insulin treatment to survive. Gestational diabetes mellitus is defined as a degree of glucose intolerance with onset or first recognition during pregnancy. This condition resolves with delivery in most of the cases, but sometimes may persist after pregnancy. Other specific types of diabetes are: diabetes due to Genetic defects of the β-cell, Genetic defects in insulin action, Diseases of exocrine pancreas, Endocrinopathies, Drug or chemical-induced diabetes, Infections, syndrome associated[14]. The complications of this disease are mainly due to abnormalities in carbohydrate, lipid and protein metabolism. They can be either microvascular & macrovascular. Microvascular
complications are neuropathy (nervous system damage), nephropathy (renal system damage) and retinopathy (eye damage). Macrovacular complications are cardiovascular disease, stroke, and peripheral vascular disease leading to bruises, non-healing injuries, gangrene.

From the past two decades, a lot of research work has been carried on different constituents of saliva in order to find the alterations in their levels with respect to blood so as to use saliva as an alternative diagnostic tool. Arati S, conducted a study in 2010, and concluded by saying that there was a positive correlation between serum and salivary glucose levels. In the studies conducted by Satish Kumar S in 2014, Amit Ladgotta et al in 2016, there was a positive correlation between serum and salivary glucose. Indira M et al in 2015, Anjali Gupta in 2015, in their studies found no significant correlation between serum and salivary glucose. PVSD Lakshmi in 2015, concluded in her study by saying that there was only a weak positive correlation between serum and salivary glucose levels in diabetic children. There are some more research papers in literature in which some of them stating a positive correlation between serum and saliva glucose and some stating no significant correlation. As there is a lot of debate going on whether glucose levels in saliva can be used to monitor diabetes, we conducted this study, to ascertain whether saliva can be used as an alternative tool for diagnosis, as it has certain distinct advantages like it can be collected non-invasively, individuals need limited training, no need of special equipment for sample collection. It is potentially valuable for children and older adults, since the collection of blood sample is associated with fewer compliance problems.

CONCLUSION:
Unlike the previous studies with a very limited sample size, we have put some efforts in terms of increasing the sample size with respect to controlled and uncontrolled DM, males and females in equal ratio, choosing the study population suitable to the earlier prescribed inclusion and exclusion criteria, estimation of glycosylated haemoglobin, following standardized methods. There was a high statistically significant positive correlation between serum and salivary glucose levels in DM patients and Non-diabetes control group. There was a significant positive correlation of Glycosylated haemoglobin values between controlled, uncontrolled DM and healthy control subjects. However further studies should be carried out on a much larger population in different geographical areas in order to establish the “Estimation of salivary glucose” as a valuable tool for diagnosing and monitoring diabetes mellitus.

Acknowledgements:
Conflicts of interest: Nil

REFERENCES