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# A COMPARATIVE AND CORRELATIVE STUDY BETWEEN BLOOD AND SALIVARY GLUCOSE WITH BLOOD HbA1c IN TYPE 2 DIABETES

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**ABSTRACT:** Diabetes is a non-communicable disease characterized by increased blood glucose. As regular monitoring of glucose and glycated hemoglobin (HbA1c) levels in diabetic individual's blood causes psychological stress, development of a non-invasive technique to quantify glucose and HbA1c are essential. This study was carried out to compare and correlate the blood and salivary glucose and blood HbA1c levels in diabetic individuals. Results showed that mean blood and saliva glucose levels were higher in the uncontrolled diabetic group than controlled diabetic and control groups. Blood HbA1c level was also elevated in uncontrolled type-2 diabetes (T2D) group, followed by controlled diabetic and control groups. A significant difference was noticed between the blood and saliva glucose levels of control and uncontrolled T2D groups. Further, the difference in serum HbA1C levels between control, controlled T2D, and uncontrolled T2D groups was also significant. Taken together, these results explicated the use of saliva as a non-invasive, painless technique in the management of T2D patients.

**INTRODUCTION:** Diabetes mellitus (DM) is a major clinical syndrome and chronic metabolic disorder, which is characterized by high blood glucose. DM is either a result of a deficiency of insulin or inefficiency to use the insulin. Based on these, this oldest disease [1] is often characterized to either type 1 (T1D) or 2 (T2D). Development of T1D occurs due to the destruction of beta cells of the pancreas by antibodies, whereas the T2D was known to develop because of the insulin resistance in peripheral tissues [2].

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Prevalence of DM is increasing with a growing number of affected individuals in both low- and middle-income countries [3]. Therefore, DM is considered a major public health issue and demands significant research for its diagnosis and management. Approximately 90% of the total diabetic population belongs to T2D, with age, obesity, diet, ethnicity, genetics and family history is some of the common risk factors [3, 4].

The characteristic features of T2D include the increased blood glucose (hyperglycemia), a relative deficiency of insulin, and insulin resistance [5]. T2D is associated with many short-term and long-term complications, which is the primary cause of increased morbidity and mortality [1]. Cardiovascular diseases and stroke are the main predisposing factors of T2D and the reason for the higher mortality rate.

Therefore, T2D has become one of the epidemics in some part of the world. Also, this burden is going to be doubled in the next decade, since the total number of people with diabetes is expected to increase. Diabetes is primarily diagnosed by estimating the blood glucose level along with the symptoms of diabetes. In 2009, WHO and ADA recommended the use of glycated hemoglobin (HbA1c) in the diagnosis of diabetes [6]. HbA1c is known to provide the accurate measure of glycemic control of a diabetic individual during precursory 2 to 3 months [7, 8]. An earlier report has also established a clear relationship between average blood glucose and HbA1c, which can accurately convert the HbA1c values to average blood glucose [9]. Besides, HbA1c prognosticates the development of macro and microvascular complications, including cardiovascular diseases in both type 1 and type 2 diabetic patients [10, 11]. Thus, HbA1c is now widely used as an important factor in the diagnosis and management of diabetes, especially T2D [12].

Monitoring of glucose and HbA1c levels in patients' blood at regular intervals causes psychological trauma and discomfort due to repeated invasions [13, 14]. Hence, the development of a non-invasive technique to quantify glucose and HbA1c, which will help to reduce the anxiety of the patients, is very important [14]. A report has suggested a significant correlation between glucose and HbA1c levels in serum and saliva samples [15]. Thus, saliva can be a potential clinical tool for the diagnosis and management of diabetes.

Earlier from our laboratory, we have reported the correlation between salivary and serum lipid profile with the blood glucose level in T2D patients [16]. The present study aimed to evaluate the correlation between salivary and serum levels of glucose in non-diabetic and diabetic (controlled and uncontrolled) individuals. Further, the correlation of HbA1c in the serum of healthy and T2D patients was also studied. This study supports the use of saliva in the diagnosis and management of the T2D and its complications.

## MATERIALS AND METHODS:

**Subjects:** One hundred patients (both male and female) aged between 35 to 65 years were selected and classified into three groups. Control group consists of 50 healthy individuals (non-diabetic) with no history of T2D, whereas the second and third group includes 25 patients each with controlled and uncontrolled T2D, respectively. The patients with a fasting blood glucose level >110 mg/dl were considered as diabetic. The exclusion parameters for the selection of subjects are T1D patients, pregnant women, and patients who either smoked or consumed alcohol in the last 24 h. All the subjects were registered for the study on a convenient sampling basis. The approval of the Institutional Ethics Committee (IHEC number: MU-IHEC-2016-2) was obtained for this project.

**Sample Collection:** All individuals were informed clearly about the sampling methods. Saliva and blood samples from each subject of all the groups were collected after overnight fasting between 9 to 11 am. Subjects were requested not to drink (except water) or chew gum during the fasting period. Saliva was collected after rinsing the mouth with water to remove food particles. Standard spitting method was used to collect unstimulated saliva up to 5 min, which was then centrifuged for 15 min at 3000 rpm, before storing the supernatant at -20 °C. Serum was made ready from venous blood collected under aseptic condition. After allowing clotting for 30 min at room temperature, blood samples were centrifuged at 3000 rpm for 15 min. Serum was collected by aspirating the supernatant and stored at -20 °C until used [17].

**Measurement of Glucose:** Glucose was measured in both the saliva and serum samples of all subjects, on a semi-automated Biochemistry analyzer using the commercially available kit (Transasia Bio-Medicals Ltd, Germany). This quantitative analysis, which is based on the Trinder's method (GOD-POD) was analyzed using spectroscopy. Briefly, 1 ml of reagent containing glucose oxidase (20000 U/l), peroxidase (2000 µ/l), phenol (10 mM) and phosphate buffer (200 mM) was mixed with 10 µl of the sample and incubated at 37 °C for 10 min. The absorbance, which is equivalent to the concentration of glucose in sample [18, 19, 20] was then read at 505/670 nm.

**Estimation of HbA1c:** Quantification of HbA1c in blood samples of control, controlled T2D and uncontrolled T2D group subjects were done using an *in-vitro* diagnostic device, NycoCard® HbA1c.

Briefly, 5 µl of the sample (whole blood) was added to the test tube containing reagent 1 and mixed well. This not only lyses the blood cells and precipitate hemoglobin but also binds boronic acid conjugate to the cis-diols of glycated hemoglobin. After 2-3 min, 25 µl of the mixture was applied to a test device and was allowed to soak into the membrane. The test device was then washed by adding 25 µl of washing solution and read using NycoCard® Reader II (Alere, USA) within 5 min.

**Statistical Analysis:** Statistical analysis was performed using the SPSS software package (IBM SPSS Statics for Windows, Version 22.0, and NY: IBM Corp).

**RESULTS AND DISCUSSION:** Development of a non-invasive method for the regular monitoring of glucose and HbA1c levels are of primary importance as the conventional diagnosis and monitoring methods using venipuncture induces psychological stress in the patients [21]. Saliva is now being considered as one of the alternative samples for the blood since the collection of saliva is easier, non-invasive and economical [22]. Studies have shown the presence of glucose in both serum and saliva of diabetic individuals. As a result, saliva can be used as a sample for the diagnosis of diabetes [19]. In this study, 50 healthy, 25 controlled T2D and 25 uncontrolled T2D individuals were used. We evaluated the correlation between salivary and serum levels of glucose along with the serum HbA1c in control, controlled T2D and uncontrolled T2D groups. Mean sugar levels in both blood and saliva of all the three groups are represented in **Table 1**, and mean serum HbA1c levels are also tabulated **Table 2**.

**TABLE 1: MEAN SUGAR LEVEL IN BLOOD AND SALIVA OF NON-DIABETIC, CONTROLLED T2D AND UNCONTROLLED T2D GROUPS**

| | | n | Mean | SD | Min | Max | P |
|---|---|---|---|---|---|---|---|
| Blood glucose | Control | 50 | 91.88 | 10.67 | 72.00 | 126.00 | P<0.001 |
| | Controlled T2D | 25 | 103.00 | 17.18 | 73.00 | 144.00 | P<0.001 |
| | Uncontrolled T2D | 25 | 162.00 | 46.76 | 95.00 | 306.00 | P<0.001 |
| | Total | 100 | | | | | |
| Salivary glucose | Control | 50 | 4.27 | 0.48 | 3.09 | 5.45 | P<0.001 |
| | Controlled T2D | 25 | 4.75 | 0.74 | 3.71 | 6.01 | P<0.001 |
| | Uncontrolled T2D | 25 | 6.07 | 0.82 | 4.76 | 8.24 | P<0.001 |
| | Total | 100 | | | | | |

n, number of samples; SD, Standard deviation; Min, minimum; Max, maximum

**TABLE 2: MEAN HBA1C LEVEL IN NON-DIABETIC, CONTROLLED T2D AND UNCONTROLLED T2D GROUPS**

| | | n | Mean | SD | Min | Max | P |
|---|---|---|---|---|---|---|---|
| Blood HbA1c | Control | 50 | 5.26 | 0.60 | 4.00 | 6.30 | P<0.001 |
| | Controlled T2D | 25 | 5.96 | 0.48 | 4.60 | 6.70 | P<0.001 |
| | Uncontrolled T2D | 25 | 8.25 | 1.71 | 5.50 | 11.60 | P<0.001 |
| | Total | 100 | | | | | |

n, number of samples; SD, Standard deviation; Min, minimum; Max, maximum

The mean blood sugar levels of control, controlled T2D, and uncontrolled T2D groups were observed to be 91.88, 103.00 and 162.00 mg/dl, respectively. In case of saliva, the mean glucose level of the group with healthy individuals was 4.27 mg/dl, whereas the group with controlled and uncontrolled T2D patients showed mean glucose of 4.75 and 6.07 mg/dl, respectively.

The glucose levels of both blood and saliva are in agreement, with higher values in the uncontrolled diabetic group, followed by the group with diabetic and healthy individuals. This is consistent with the results obtained in earlier reports, wherein compared with control, the glucose levels were significantly higher in saliva samples of controlled and uncontrolled diabetics [23]. The main reason behind the presence of glucose in the saliva is due to its diffusion through semipermeable membrane [15]. Also, the glucose transportation to saliva after changes in the salivary gland basement membrane is also considered as the possible cause [24, 25].

Earlier studies had suggested that HbA1c is one of the markers to screen diabetes [26]. Hence, we evaluated the levels of HbA1c in all the three group subjects **Table 2**.

Results showed increased levels of HbA1c in uncontrolled T2D group (8.25%), compared to controlled T2D group (5.96%). The control group showed the lowest glycated hemoglobin level with a value of 5.26%. The increase in HbA1c is in agreement with the increased average plasma glucose of the respective group. This increased HbA1c is suggestive of complications of diabetes, including cardiovascular disease [9]. Furthermore, Currie and colleagues have earlier reported the increased risk of mortality both at high and low levels of HbA1c [27].

The mean difference between the glucose levels in serum and saliva, and HbA1c level in serum samples, in all the three groups, were measured **Table 3**. A significant difference was observed between the blood glucose levels of healthy and uncontrolled T2D groups (p=0.001), and between controlled and uncontrolled T2D groups (p=0.001). However, no significant difference was noticed between control and controlled T2D groups. But in saliva, there was a significant difference in the glucose levels between all the three groups (p=0.01 between control and controlled T2D; p=0.001 between controlled and uncontrolled T2D, and between control and uncontrolled T2D). In the case of serum HbA1c, there was a significant difference between controlled and uncontrolled T2D (p=0.001), control and uncontrolled T2D (p=0.001) and between control and controlled T2D (P=0.013) groups.

**TABLE 3: MEAN DIFFERENCE OF SERUM AND SALIVARY LEVELS OF SUGAR, AND HbA1c LEVEL IN SERUM**

| Dependent variable | Group (A) | Group (B) | Mean difference (A-B) | P |
|---|---|---|---|---|
| Blood glucose | Controlled T2D | Uncontrolled T2D | -59.00 | 0.001 |
| | | Control | 11.12 | 0.191 |
| | Uncontrolled T2D | Control | 70.12 | 0.001 |
| Salivary glucose | Controlled T2D | Uncontrolled T2D | -1.33 | 0.001 |
| | | Control | 0.47 | 0.010 |
| | Uncontrolled T2D | Control | 1.80 | 0.001 |
| Blood HbA1c | Controlled T2D | Uncontrolled T2D | -2.29 | 0.001 |
| | | Control | 0.70 | 0.013 |
| | Uncontrolled T2D | Control | 2.99 | 0.001 |

Further, the correlation of the blood and saliva glucose against HbA1c levels in serum was also analyzed in control **Table 4**, controlled T2D **Table 5** and uncontrolled T2D **Table 6** groups.

**TABLE 4: CORRELATION OF THE BLOOD AND SALIVARY GLUCOSE AGAINST SERUM HbA1c LEVELS IN CONTROL GROUP**

| | | HbA1c |
|---|---|---|
| Blood glucose | Pearson Correlation | 0.226 |
| | P | 0.115 |
| | n | 50 |
| Salivary glucose | Pearson Correlation | 0.054 |
| | P | 0.711 |
| | n | 50 |

**TABLE 5: CORRELATION OF THE BLOOD AND SALIVARY GLUCOSE AGAINST SERUM HbA1c LEVELS UNCONTROLLED T2D GROUP**

| | | HbA1c |
|---|---|---|
| Blood glucose | Pearson Correlation | 0.277 |
| | P | 0.180 |
| | n | 25 |
| Salivary glucose | Pearson Correlation | 0.277 |
| | P | 0.181 |
| | n | 25 |

In the healthy control group, a mild positive correlation was observed in both blood and saliva against HbA1c level. Nevertheless, the correlation was not very significant (P>0.05). This was in agreement with the controlled T2D group also **Table 6**. Interestingly, there is a highly significant positive correlation (r=0.779, p=0.001) between blood glucose and HbA1c levels in case of uncontrolled T2D group **Table 6**, with a moderate correlation between saliva glucose and serum HbA1c levels (P=0.001).

**TABLE 6: CORRELATION OF THE BLOOD AND SALIVARY GLUCOSE AGAINST SERUM HbA1c LEVELS IN UNCONTROLLED T2D GROUP**

| | | HbA1c |
|---|---|---|
| Blood glucose | Pearson Correlation | 0.779** |
| | P | 0.001 |
| | n | 25 |
| Salivary glucose | Pearson Correlation | 0.657** |
| | p | 0.001 |

**Correlation is significant

A strong correlation was observed between the fasting blood glucose levels and HbA1c in the earlier report [28]. We then analyzed the correlation

between the blood and salivary glucose levels in control, controlled T2D and uncontrolled T2D **Table 7** groups. Results showed a high positive correlation between blood and salivary glucose in all the groups. The difference between the groups was significant (p=0.001).

**TABLE 7: CORRELATION BETWEEN THE BLOOD AND SALIVARY GLUCOSE LEVELS IN CONTROL, CONTROLLED T2D AND UNCONTROLLED T2D**

| | | Salivary glucose | | |
|---|---|---|---|---|
| | | **Control** | **Controlled T2D** | **Uncontrolled T2D** |
| Blood glucose | Pearson Correlation | 0.841 | 0.748 | 0.917 |
| | p | <0.001 | <0.001 | <0.001 |
| | n | 50 | 25 | 25 |

In the present study, we have evaluated the possibility of using saliva as the potential sample for the screening and management of diabetes. Earlier reports also supported the use of saliva in estimating various factors such as hormones, infections and others [13, 29, 30]. Altogether, this study is important in the development of the non-invasive method for the diagnosis and monitoring of diabetes.

**CONCLUSION:** The present study was aimed at developing a non-invasive method to diagnose and manage diabetes. We found that mean blood and saliva glucose levels were higher in the uncontrolled diabetic group, followed by controlled diabetic and control groups. Results confirmed the increased levels of HbA1c in the uncontrolled T2D group, compared to the controlled T2D and control groups. A significant difference was observed between the blood and saliva glucose levels of control and uncontrolled T2D groups. In the case of serum HbA1C levels, we obtained a significant difference between controlled and uncontrolled T2D, control and uncontrolled T2D and between control and controlled T2D groups. In uncontrolled T2D group, there was a highly significant positive correlation between blood glucose and HbA1c levels. Also, we found a high positive correlation between blood and salivary glucose in all the groups. These, taken together confirmed that saliva could be used as a non-invasive, painless technique for the assessment of glucose during T2D.

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