"STUDY OF SERUM AND SALIVARY GLUCOSE LEVELS IN TYPE 2 DIABETIC PATIENTS"

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by

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BACKGROUND OF THE PROPOSED RESEARCH

The hormone insulin is secreted by B cells of pancreas. The physiologic effects of insulin are both far reaching and complex. Though the hormone has rapid, intermediate and delayed actions, the best known is the hypoglycemic effect. Apart from its additional effects on amino acid and electrolyte transport, action on many enzymes and growth, the net effect of the hormone is storage of carbohydrate, protein and fat. The anabolic nature of hormone which increases the storage of glucose, fatty acids and amino acids has lead to the hormone being called as "the hormone of abundance". The constellation of abnormalities caused by insulin deficiency is called "diabetes mellitus".[1] Diabetes is characterized by polyurea, polydypsia and weight loss in spite of polyphagia, hyperglycemia, glucosuria, ketosis, acidosis and coma.[1]

Interest in monitoring the glucose concentrations of diabetic patients has increased since the publication of the diabetes control and complications trials report showing that tight control of blood glucose concentrations, by frequent testing and concomitant adjustment of insulin doses, reduces the long term complications resulting from diabetes.

A computer simulation based on the Diabetes control and complication trials results estimates an additional 5 years of life, 8 years of sight, 6 years of free - from kidney disease, and 6 years free – from- amputations for a diabetic following the tight control using the standard regimen.[2]

Despite the tremendous value of self monitoring of blood glucose for the treatment of diabetes, many patients find the testing onerous and some refuse to perform the measurement. These complaints are largely justified because self monitoring of blood glucose is painful, inconvenient, messy, embarrassing and above all expensive.[3] Most

patients consider the finger lancing necessary for obtaining blood for self monitoring of glucose to be the most painful part of diabetes therapy. The direct pain of the lancet is several folds greater than that of an insulin syringe because of the greater lancet thickness, the site of lancing (the finger tip usually used for blood glucose monitoring has many more pain fibers than does the thigh often used for insulin injections) and other factors. In addition, patients frequently complain of residual pain at the site of lancing that may last several hours and be especially distressing during important tasks, such as opening a bottle or typing.

Non invasive (NI) monitoring of glucose has been of particular interest because of the pain associated with invasive self monitoring. Ease of use and reduction of pain can encourage more frequent testing and hence tighter control of the glucose concentration. Patient care need and the commercial importance of NI glucose monitoring has led to a flurry of "research" by entrepreneurial and commercial concerns that have been published mainly in patent literature. However a large number of NI glucose patents lack scientific rigor and some may be based on wrong or unproved assumptions.[4] Despite few limitations, the use of saliva for diagnostic purposes is increasing in popularity due to its many potential advantages. It provides an attractive alternative to more invasive, time consuming, complicated glucose monitoring tests as saliva can be collected in a non invasive manner by individuals with modest training including patients.[5]

Saliva has been used reliably for reflecting and monitoring the blood glucose concentration in the patients of diabetes mellitus.[6,7,8]

Hence the present study was undertaken to quantitatively estimate the amount of salivary glucose levels in type-2 diabetic patients and explore the possibility of using saliva to reflect the glucose concentration in blood, thereby making assessment of glucose levels less invasive.

AIM AND OBJECTIVES:

AIM :

The present study was undertaken to quantitatively estimate the amount of salivary glucose levels in type-2 diabetic patients and explore the possibility of using saliva to reflect the glucose concentration in blood, thereby making measurement of glucose non invasive.

OBJECTIVES :

- To determine the presence or absence of glucose in saliva of diabetic and non diabetic individuals

- To estimate the level of glucose in serum in both the groups

- To estimate the level of glucose in saliva in both the groups

- To determine degree of correlation between the salivary glucose and serum glucose levels

MATERIALS AND METHODS

Study type: observational study

Study design: Cross sectional study

Study duration: Aug 2007- March 2011

Source of Data collection: KLE City Polyclinic and Diagnostic center, Samadevi Galli, Belgaum, Karnataka State, India.

Sample size: Total 400 (200 diabetic patients and 200 non diabetic individuals)

Calculation of sample size:

Sample size for screening test was assessed, based on the formula

$$n= \frac{4\, Z_\alpha^2\, pq}{d^2}$$

P : sensitivity of screening test (p value assumed to be 80%)

q: 100- p%   q=10%

d: Error (10% of relative error)

Z_α**=1.**96   for 95% confidence.

$$N= \frac{4 \times 2^2 \times 80 \times 20}{8^2} =400.$$

400 = 200 individuals with disease (diabetic),

 200 individuals without disease (non diabetic).

Sampling design: Non probability sampling

Sampling method: Consecutive sampling (those met the inclusion criteria).

Study participants: Subjects who reported to the laboratory for blood glucose analysis, during study period as per inclusion and exclusion criteria.

Instruments: Micropipettes, Semi automated machine (Erba CHEM − 5 Plus V2), reagent Erba Glucose Kit, Trinders method, end point.

Materials used: Structured proforma, consent form, Glucose reagent kit (Liquixx company)

Inclusion criteria for diabetic patients: Patients with confirmed diabetes.

Exclusion criteria: Presence of any obvious oral lesions.

Patients treated for any salivary gland disorders.

Patients on medication for any other local or systemic disease other than diabetes mellitus and hypertension.

Procedure: After obtaining Institutional ethical clearance the study was carried out prospectively between 2007 August -2011 Feb in K.L.E'S Polyclinic and Diagnostic Centre, Samadevi Galli, Belgaum. A total of, 200 type 2 diabetic patients and 200 healthy subjects were included in the study. All subjects were from same geographic area. A written informed consent was obtained from all the subjects.

Estimation of fasting and post prandial serum and salivary glucose levels were carried out for these subjects.

Case history and consent form: A detailed case history was recorded as per the proforma attached. Patient's history regarding duration of the disease, type of glycemic control, family history and personal history were recorded. Patients were briefed regarding the study and their enrolment, for which a written consent was obtained. Patients blood & saliva were collected in fasting and postprandial state.
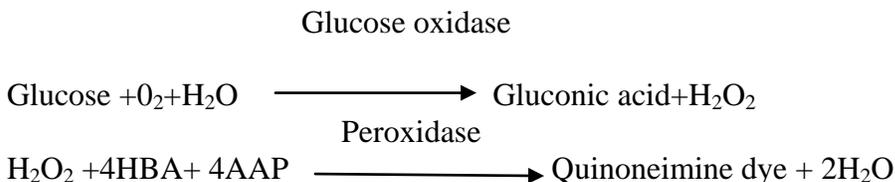
For fasting sample: 10-12 hours following fast.

For post prandial sample : 2 hours after meal.

Collection of blood: Under aseptic conditions using a sterile disposable 25 gauge needle, intravenous blood was collected from the median vein. The blood was allowed to clot in the test tube, centrifuged at 3000 rpm for 10 minutes and then serum was separated.

Collection of Saliva: The patients were asked to rinse their mouth thoroughly with water. Unstimulated whole saliva was collected in a sterile container by asking the patient to expectorate into it gradually over a period of 5-10 minutes till approximately 1 ml of saliva was collected. Sample was transferred to a disposable test tube and centrifuged at 2000 rpm for 2-3 minutes and supernatant was separated.

After addition of reagent the sample was mixed well and incubated at 37^0c for 5 minutes. The absorbance of standard and test against blank was noted at 505-670 nm on semi automated machine.

Assay principle : Glucose Oxidase oxidize glucose to gluconic acid and hydrogen peroxide. In presence of enzyme peroxidase, released hydrogen peroxide is coupled with Phenol and 4-Aminoantipyrine (4-AAP) to form coloured Quinoneimine dye. Absorbance of coloured dye is measured at 505 nm and is directly proportional to glucose concentration in the sample.

$$Glucose + 0_2 + H_2O \xrightarrow{\text{Glucose oxidase}} Gluconic\ acid + H_2O_2$$

$$H_2O_2 + 4HBA + 4AAP \xrightarrow{\text{Peroxidase}} Quinoneimine\ dye + 2H_2O$$

4AAP : 4 - amino antipyrine

4HBA : 4 – Hydroxy benzoic acid

The obtained results were tabulated separately for diabetic and non diabetic group.

Statistical Analysis : The data was entered separately for diabetic and non diabetic individuals on Microsoft excel spread sheet, tabulated and subjected to statistical analysis by using SPSS software. The mean, Standard deviation, degree of freedom (DF) and P value were assessed for Age, Blood glucose levels in fasting and post prandial state as well as salivary glucose levels in fasting and post prandial state.

The sensitivity, specificity, negative predictive value and positive predictive value were analyzed at different salivary glucose levels. This was done in order to determine the use of salivary glucose levels as screening mode in post prandial state and in fasting state.

RESULTS

Sex distribution for diabetic and non diabetic individuals:

Among the diabetic individuals 55%(110) were males and 45%(90) were females. Among the non diabetic individuals 49.5% (99) were males and 50.5% (101) were females.

Age Distribution for diabetic and non diabetic individuals :

The age range of all diabetic and non diabetic individuals which was 20-81 years. Among the diabetic patients 67% (134) of patients were in the age range 41-60 followed by 26.5%(53) between 61-80,6% (12) between 20-40 years and among the non diabetic group of individuals 68.5% (137)of them were between 41-60 years, 16.5% (33) between 6-80 years, 14% (28) of them between 20-40 years ,1% (2) of them were above 8 years. In both the groups maximum of them belonged to 41-60 years of age.

Fasting Blood sugar level for diabetic patients:

It was seen that fasting blood sugar levels in diabetics ranged between 70-600 mg/dl. 83.5% (167) of these patients had their fasting blood sugar levels between 70-200. 13.5 % (27) between 20-400, 3%(6) between 40-600.

Fasting salivary levels in diabetic patients:

The salivary glucose level in diabetic patients ranged between 0-31mg/dl. Maximum patients i.e. 86% (172) had their levels between 0-10, followed by 10% (20) between 10.1-20 mg/dl and 4% (8) between 20.1-3 mg/dl.

Distribution of patients who received medication for diabetes :

It was noted that 68% (136) of them were on medication and 32% (64) were unaware of disease status.

Fasting Blood sugar level for Non-diabetic patients:

Fasting blood sugar ranged between 60-135 mg/dl. 51.5% (103) of them between 81-100 mg/dl followed by 40.5% (81) between 01-135 mg/dl and 8%(16) between 60-80 mg/dl.

Fasting Saliva level for Non-diabetic patients:

It ranged between 0-4 mg/dl.88% (176) of the individuals had their salivary levels between 0-4%, 9.5% (19)between 4.-8mg/dl and 2.5%(5) between 8.1-14 mg/dl

Post Prandial Serum glucose levels for diabetic patients:

It was seen that the post prandial serum glucose ranged between 90-600 mg/dl. Maximum i.e. 73% (146) between 201-400, 26.5% (53) between 90-200 and 0.5% (1) had their levels between 401-600 mg/dl.

Post Prandial Salivary Glucose Levels (PPSGL) in diabetic patients:

The post prandial salivary glucose levels ranged between 0-40 mg/dl. 80.5% (161) had their salivary glucose levels between 0-10, 14% (28) between 10.1-20 and 5.5%(11) between 20.1-40 mg/dl.

Post Prandial Serum Glucose Levels (PPSGL) for Non- diabetic individuals:

In the non diabetic individuals 56.5% (113) of patients had postprandial serum glucose levels between 80-140 mg/dl, 35.5% (71) of patients between 41-200mg/dl and 8% (16) of them 201-250 mg/dl.

Post Prandial Salivary Glucose Levels in Non-diabetic individuals:

In non diabetic individuals the post prandial salivary glucose levels ranged between 0-11 mg/dl.90% (180) of them between 0-4, 6.5%(13) of them between 4.1-8 mg/dl and 3.5%(7) between 8.1-11 mg/dl.

Comparison of mean glucose levels in serum and saliva of diabetic and non diabetic individuals:

SD and mean (x) between diabetic patients and non diabetic individuals was calculated. Among the diabetic patients the mean age was 55.2 years (SD-9.26) and among non diabetic individuals it was 54.3 years. The mean fasting blood sugar levels mean in diabetic was 162.2 (SD 7.47) & 99.2 (SD 12.98) in non diabetic group. The mean post prandial blood sugar level mean in diabetic was 240.9 (SD 6.95) & 144.3 (SD 32.18) in non diabetic group. The mean fasting salivary sugar level in diabetics was 5.1 (SD 5.60) and 2.2 (SD 1.81) in non diabetic group. The mean post prandial salivary sugar levels in diabetics was 8(SD 6.54) & 2.7 (SD 1.69) in non diabetic group.

Comparison of saliva and serum glucose levels in diabetic group of patients those who were on medication and those who were not on medication:

The mean of serum and salivary sugar levels among diabetic patients with or without medication was analyzed. There was no significant difference in serum and salivary sugar levels between patients with medication and without medication.

Comparison of serum and salivary glucose levels between males and females (both diabetic and non-diabetic individuals:

When serum and salivary sugar levels of males and females were compared only the post prandial salivary sugar level showed significant difference between males and females.

The sensitivity, Specificity, Positive predictive value and negative predictive value were analyzed at different salivary glucose levels.

Fasting salivary glucose level being 1mg/dl:

The comparison between fasting salivary glucose levels with fasting blood sugar levels was done when salivary glucose levels are 1 mg/dl, it was observed that Sensitivity = 97.9%, Specificity=9.1%,Positive predictive value = 38.2% and Negative Predictive value=88.5%.

Salivary glucose levels being 1.5 mg/dl:

When salivary glucose levels are 1.5mg/dl. Sensitivity= 86.3%, Specificity=43.7%, Positive Predictive value=43.7% and Negative Predictive Value=84.7% was observed.

Salivary glucose levels being 2 mg/dl:

When salivary glucose levels ware 2mg/dl. Sensitivity= 73.3%, Specificity=60.2%, Positive Predictive value=51.4% and Negative Predictive Value=79.7% was obtained.

Salivary glucose levels being 2.5 mg/dl:

When salivary glucose levels ware 2.5 mg/dl. Sensitivity= 59.6%, Specificity=70.8%, Positive Predictive value=54% and Negative Predictive Value=75.3% was seen.

Salivary glucose levels being 3 mg/dl:

When salivary glucose levels was 3 mg/dl. Sensitivity= 49.3%, Specificity=77.9%. Positive Predictive value=56.2% and Negative Predictive Value=72.29% was observed.

As per the above observations the best cut off point for Fasting saliva would be 2 mg/dl.

Post Prandial Saliva as screening procedure (PPS) against Post Prandial Blood sugar (PPBS)

Salivary glucose levels being 1.5 mg/dl :

When salivary glucose levels were 1.5 mg/dl. Sensitivity = 97.1%, Specificity=6.9%, Positive Predictive value=43.6% and Negative Predictive Value=76.2% was observed.

Salivary glucose levels being 2 mg/dl :

When salivary glucose levels were 2 mg/dl. Sensitivity= 95.3%, Specificity=36.7%, Positive Predictive value=52.9% and Negative Predictive Value=91.3% was noticed.

Salivary glucose levels being 2.5 mg/dl

When salivary glucose levels were 2.5 mg/dl, Sensitivity= 89.5%, Specificity=62.9%, positive Predictive value=62.9% Negative Predictive Value=88.5% was observed.

Salivary glucose levels being 3 mg/dl:

When salivary glucose levels were 3 mg/dl, Sensitivity= 84.8%, Specificity=67.7%, Positive Predictive value=66.2% and Negative Predictive Value=85.6% was observed.

Salivary glucose levels being 3.5 mg/dl:

When salivary glucose levels were 3.5 mg/dl. Sensitivity= 80.1%, Specificity=76.8%, Positive Predictive value=72.1% and Negative Predictive Value=83.8% was seen.

Salivary glucose levels being 4 mg/dl:

In post prandial state when salivary glucose levels were 4 mg/dl, Sensitivity= 78.9%, Specificity=78.6%, Positive Predictive value=73.3% and Negative Predictive Value=85.7% was observed.

Salivary glucose levels being 4.5 mg/dl:

When salivary glucose levels were 4 .5mg/dl. Sensitivity= 73.7%, Specificity=81.6% Positive Predictive value=75% and Negative Predictive Value=80.6% was seen.

Salivary glucose levels being 5 mg/dl:

In post prandial state when salivary glucose levels were 5mg/dl, Sensitivity= 67.8%, Specificity=83.8%, Positive Predictive value=75.8%. Negative Predictive Value=77.7% was seen.

By above observations it was seen that the best cut off points in post Prandial salivary level would be either 3.5 or 4 mg/dl

DISCUSSION

Diabetes mellitus is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both.[1] Type 2 diabetes accounts for ~90–95% of those with diabetes, previously referred to as non-insulin-dependent diabetes. Impaired insulin secretion is found uniformly in type 2 diabetic patients in all ethnic populations. The β-cells are unable to read the severity of insulin resistance and fail to adjust their secretion of insulin to maintain normal glucose tolerance. In these patients, the fasting plasma insulin concentration is normal or increased and basal insulin secretion is elevated.[9] The chronic hyperglycemia of diabetes is associated with long-term damage, dysfunction, and failure of various organs, especially the eyes, kidneys, nerves, heart, and blood vessels.[1]

Undiagnosed diabetes can cause progressive microvascular damage. At the time of diagnosis, approximately 20 % of newly diagnosed patients with type 2 diabetes have diabetic retinopathy and 10 % have nephropathy.Delayed diagnosis translates into more organ damage since effective therapy cannot be applied. The Diabetes Control and Complications Trial (DCCT) demonstrated that interventions that improve glycemic control in patients with type 1 diabetes reduce the risk of development and slow the progression of diabetic micro vascular disease. The United Kingdom Prospective Diabetes Study (UKPDS) has shown that strict glycemic control has a similar benefit in patients with type 2 diabetes. The treatment outcome depends largely on strict glycemic control.[10]

There is no known cure for diabetes and patient relies on constant monitoring to maintain acceptable blood glucose levels. Blood glucose monitoring by the patient and the physician is an important aspect in management of diabetes in order to control the devastating complications of the disease. Conventional monitoring techniques require blood

sample and the invasive procedures often cause pain and discomfort which may limit frequent testing.

With ever improving advances in diagnostic pathology, the race for the next generation of bloodless, painless and accurate glucose instruments has began. Most commonly used laboratory diagnostic procedures involve the analysis of blood, but other biological fluids like urine, sweat and saliva are also being utilized for the diagnosis of other diseases like salivary gland disorders, viral infections etc. Of these, saliva offers distinctive advantages because it can be collected non invasively by individuals with modest training. Furthermore saliva may provide a cost effective approach for the screening of large populations.

Hence the present study was undertaken to quantitatively estimate the amount of salivary glucose levels in type-2 diabetic patients and explore the possibility of using saliva to reflect the glucose concentration in blood, thereby making measurement of glucose non invasive.

A total of 200 diabetic patients and 200 age and sex matched non diabetic individuals were included in the study. An informed consent was obtained from all. Assessment of serum and salivary glucose levels in fasting state (Fasting is defined as no caloric intake for at least 8 hours) and post prandial state (2 hours following diet intake) was carried out. Glucose estimation was carried out immediately by GOD-POD, end point kinetic assay using semi automated machine.

The age range of the patients in our study was 21-81 years. With maximum individuals being between 41-60 years of age. Among the diabetic patients maximum i.e. 134 (67%) number of patients were in 41-60 years group followed by 53(26.5%) in 61-80

years and 12(6%) in 20-40 years of age group. The mean age of affected patients was 55.2years with SD 9.26. Among the non diabetic group of individuals 137(68.5%) of them were in 41-60 years, 33(16.5%) in 61-80 years, 28(14%) in 20-40 years and 2(1%) above 80 years of age. It is known that type 2 diabetes is more frequently a disease of old age. However the mean age affected in the study group in the present study was 54.3 and SD 11.33. This supports the recent study of CURES.   The CURES (Chennai Urban Rural Epidemiology Study) have reported a temporal shift in the age at diagnosis to a younger group.[11]

The earlier age of onset combined with increasing prevalence of diabetes could have adverse effects on nation's health and economy. This is of great concern because if the epidemic shifts to younger age it could have serious consequences on the health of the nation.[11]

Early identification of at-risk individuals using simple screening tools like the Indian Diabetes Risk Score (IDRS) and appropriate lifestyle intervention would greatly help in preventing or postponing the onset of diabetes and thus reducing the burden on the community and the nation as a whole. [11]

On eliciting personal history of our patients it was noticed that most of the patients in present study were of middle class. One point worth emphasizing is that diabetes can no longer be considered as a disease of the rich. The prevalence of diabetes is now rapidly increasing among the poor in the urban slum dwellers, the middle class and even in the rural areas. This is due to rapid changes in physical activity and dietary habits even among the poorer sections of the society. Unfortunately the poor diabetic subjects delay taking treatment leading to increased risk of complications.[12] Moreover, as the epidemic matures and reaches

the next stage of transition, the rich and affluent will rapidly change their activity patterns and start making healthier food choices and ultimately the diabetes and heart disease will decrease in this section of the society.

Standards of medical care in diabetes—2010 by American Diabetes Association has established certain criteria for diagnosis of Diabetes.According to this diagnostic cut point of ≥126 mg/dl (7.0 mmol/l) for FPG (Fasting Plasma Glucose) and confirmed the long-standing diagnostic 2-h PG(plasma Glucose) value of ≥200 mg/dl (11.1mmol/l). ADA has not previously recommended the use of A1C for diagnosing diabetes, in part due to lack of standardization of the assay. However, A1C assays are now highly standardized, and their results can be uniformly applied both temporally and across populations. [13]

Based on these criteria, diabetic status of known 200 diabetic patients and 200 non diabetic individuals was studied. Among the diabetic patients (167)83.5% of these patients had their fasting blood sugar levels between 70-200. (27)13.5% between 201-400 mg/dl and (6)3% of them between 400-600mg/dl. The mean serum glucose levels in diabetic group were 162.2 mg/dl with SD being 7.47. These levels were comparably higher in this group as compared to non diabetic group of individuals where the levels ranged between 60-135 mg/dl with maximum 103(51.5%) individuals between 81-100 mg/dl and 81(40.5%) of them between 100-135mg/dl.

In the present study, post prandial serum glucose levels in diabetic patients ranged from 90-600 mg/dl. The mean value was 240.9 mg/dl with SD 6.95. These values were much higher as compared to the post prandial serum glucose levels in non –diabetic patients where highest value was 250 mg/dl. Maximum individuals 113-(56.5%) in non diabetic

group had their serum glucose level between 80-140 mg/dl and 71(35.5%) between 141-200 mg/dl. Mean serum glucose level in non diabetic was 144.3 mg/dl with SD 32.18.

The variation in fasting plasma glucose levels and post prandial glucose levels in diabetic patients could be because 68% of these patients were on medication which ranged from few months to years.

Blood glucose levels are signs of disease status of a person, or how well the disease is in control. Glycemic control is fundamental to the management of diabetes. In type 2 diabetes, the Kumamoto study [14]and the UKPDS [15] demonstrated significant reductions in micro vascular and neuropathic complications with intensive therapy.   In the large randomized prospective clinical trials, treatment regimens that reduced average A1C to 7% (1% above the upper limits of normal) were associated with fewer markers of long-term micro vascular complications. The UKPDS trial of type 2 diabetes observed a 16% reduction in cardiovascular complications (combined fatal or nonfatal MI and sudden death) in the intensive glycemic control arm, although this difference was not statistically significant ($P = 0.052$).

The implementation of the standards of care for diabetes has been suboptimal in most clinical settings. A recent report indicated that only 57.1% of adults with diagnosed diabetes achieved an A1C of $\leq$ 7%, only 45.5% had a blood pressure $\leq$ 130/80 mmHg, and just 46.5% had a total cholesterol $\leq$ 200 mg/dl. Most distressing was that only 12.2% of people with diabetes achieved all treatment goals. While numerous interventions to improve adherence to the recommended standards have been implemented, the challenge of providing uniformly effective diabetes care has thus far defied a simple solution.[13]

Despite the tremendous value of monitoring of blood glucose for the treatment of diabetes, many patients find the testing onerous and some refuse to perform the measurement. These complaints are largely justified because self monitoring of blood glucose is painful, inconvenient, messy, embarrassing and above all expensive.

Non invasive monitoring of glucose has been of particular interest because of the pain associated with invasive monitoring. Ease of use and reduction of pain can encourage more frequent testing and hence tighter control of the glucose concentration.

Thus, several non invasive devices have currently been researched to provide diabetics an alternative. These include shining a beam of light onto the skin or through body tissue, measuring the energy waves (infrared, radiation) emitted by the body, applying radio waves to the fingertip, using ultrasound, checking the thickness (viscosity) of fluids in tissue underneath the skin.[16]

Diagnostic tests based on fluid generally use blood and urine and less frequently the esoteric fluids such as saliva, sweat and tears.[17]The assay of saliva is an increasing area of research with implications for basic clinical purpose. Recently the use of saliva has provided a substantial addition to the diagnostic armamentarium as an investigative tool for disease process and disorders.   The changes brought about by some systemic diseases directly or indirectly influence the saliva. These characteristic changes may contribute to the diagnosis and early detection of these diseases. Saliva has already been used in: sjogren's syndrome, dental caries, carcinomas, endocrine disorders, pregnancy, steroid and protein analysis, HCV, HIV, Hepatitis B antibodies, Cystatin A in GCF (Gingival Crevicular Fluid) in Periodontitis . In addition to its oral indications, the analysis of saliva provides important information about the functioning of various organs in the body, [13] Saliva offers distinctive advantages over

serum because it can be collected non invasively by individuals with modest training. Furthermore saliva may provide a cost effective approach for the screening of large populations.

In the present study glucose concentration in the saliva of diabetic and non diabetic individuals was analyzed.

The analysis of salivary glucose was done in same manner as that of serum by GOD-POD method. Salivary glucose levels in diabetic patients and in non diabetic individuals in both fasting and postprandial state were assessed.

Among the diabetic patients in the fasting state it ranged from 0-31. 86 % (172) of these patients had their salivary levels between 0-10mg/dl, 10%(20) of the patients in the range of 10.1-20 mg/dl, 4%(8) of patients had salivary glucose levels between 20.1-30 mg/dl. Mean salivary glucose levels in fasting state was 5.1 mg/dl with SD 5.60. Thus salivary glucose levels in diabetic patients were much higher as compared to salivary glucose levels in non diabetic individuals in their fasting state. 88% (176)of them had their levels between 0-4% and 9.5% (19)of them between 4.1-8 mg/dl and 2.5%(5) of the individuals between 8.1-14 mg/dl. The mean level was 2.2 with SD 1.81.This was statistically significant (p=0.000). Maximum salivary glucose level did not exceed 14 mg/dl which was much lesser than least values of diabetic patients.

The analysis of saliva in post prandial state of diabetic patients ranged between 0-40 mg/dl. 80.5 % (161)of them had between 0-10 mg/dl followed by 14 %(280 of them between 10.1-20 mg/dl and only 5.5%(11)of them between 20.1 – 40 mg/dl. Mean was 8 mg/dl with SD 6.54.

Among the non diabetic individuals it ranged between 0-11 mg/dl. 90% (180) of them had their values between 0-4mg/dl, followed by 6.5%(13) between 4.1-8 mg/dl and 3.5% between 8.1-11 mg/dl. The mean salivary glucose level in post prandial state in non diabetic individuals was 2.7 with SD 1.69.This was statistically significant (p=0.000).

It is also worth mentioning that both in diabetic and non diabetic individuals there was a corresponding increase in salivary glucose levels as compared to serum in both fasting and post prandial state. This was also found to be statistically significant (p=0.000).

Our study is in association with Study by Ana Carolina Vasconcelos, et al who found that the concentration of salivary glucose in diabetic patients was significantly higher than in non diabetic individuals.[18] Study by Shehla Amer, et al on 25 age and sex matched diabetic and non diabetic subjects has shown that glucose was found only in salivary sample of patients with diabetes mellitus, while the salivary sample of non-diabetic subjects did not show the presence of glucose. They further concluded that salivary glucose concentrations in patients with diabetes mellitus – could be a minimally invasive technique for monitoring blood glucose levels.[19]Study by Beker and Kesterman on 23 diabetics has revealed that 13 of 23 patients had detectable glucose levels, where in it ranged from 10 to 34 mgm%. Mehrotra et al in their study on 50 diabetics and 50 non diabetics reported higher levels of glucose in diabetics. Darwazeh AMG et al have demonstrated significantly higher glucose levels out of 41diabetics and 34 controls they have studied.[20]

An association between diabetes mellitus and alterations in the oral mucosa has been observed in experimental studies and clinical practice, and includes changes in the healing process of lesions as well as the triggering of infectious process in the oral mucosa lining. It has been hypothesized that the increased presence of certain inorganic and organic

constituents in saliva of diabetic patients could be attributed to this. A study by Murrah, Crusson and Sauk in 1985 have shown changes associated with basement membrane of parotid gland of diabetic patients.[21] The elevated glucose levels in saliva also confirms the effect of diabetic membranopathy, which leads to increased percolation of glucose from blood to saliva, thus affecting the salivary composition in these patients.

The test, developed by the research team led by Paturi V. Rao, is based on chemical recognition of biomarkers in the patients' saliva. This proteomic analysis of the human saliva in type 2 diabetes provides the first global view of potential mechanisms altered in diabetic saliva and their utility in detection and monitoring of diabetes. Further characterization of these markers in additional groups of subjects may provide the basis for new, non-invasive tests for diabetes screening, detection and monitoring.[22]

The normal levels do not significantly affect oral health or support the growth of micro organisms. However higher salivary glucose levels favor the proliferation of micro organisms and enhance their colonization on teeth and oral mucous membranes.

In the present study analysis of sensitivity, specificity, negative and positive predictive values at various concentrations of sugar in saliva was carried out.

It was observed that on analysis of sensitivity and specificity in fasting state best cut off point would be "2mg/dl". Because the sensitivity at this point was 73.3% and specificity was 60.2%. The positive predictive value was 51.4% and negative predictive value was 79.7%. The remaining higher sugar concentrations till 3 mg/dl also did not give expected sensitivity, specificity, positive predictive value and negative predictive values which were seen at 2 mg/dl.

The present study revealed that the best cut off value of salivary sugar level in post prandial state would be either 3.5mg/dl where sensitivity was 80.1%, specificity was 76.8% Positive predictive value was 72.% and negative predictive value was 83.8% or 4 mg/dl where we observed a sensitivity of 78.9%, Specificity of 78.6%, positive predictive value 73.3% and negative predictive value 85.7%.

Thus it can be inferred that glucose was detectable in saliva in both diabetic and non diabetic individuals. The glucose was demonstrable in both fasting and postprandial state. However, the likelihood ratio of positive predictive value was 3.45 of post prandial state as compared to 1.84 for fasting salivary glucose levels which suggested better results with post prandial salivary glucose levels than the fasting salivary glucose levels.

The identification of biomarkers to noninvasively detect prediabetes/diabetes will facilitate interventions designed to prevent or delay progression to frank diabetes and its attendant complications.

CONCLUSION

Interest has been increasing recently in non invasive diagnostic testing, some of this storms from the AIDS epidemic in the west, which has provided a new rationale for haemophilia, while other factors include new development in home based diagnostic tests and a demand of samples collected in the home or work place. Diagnostic tests based on fluid generally use blood and urine and less frequently the esoteric fluids such as saliva, sweat and tears.[17]

The present study explored the possibility of using saliva to reflect the glucose concentration in blood, thereby making self measurement of glucose less invasive.
From the study it was inferred that: Glucose was detectable in saliva of both diabetic and non diabetic individuals. A significant positive correlation was established between blood glucose and salivary glucose levels.

Among diabetic patients the fasting salivary levels ranged between 0-31 mg/dl. Among the diabetic patients, the postprandial salivary levels ranged between 0-40 mg/dl. The best cut off value at fasting state for saliva would be 2mg/dl with sensitivity of 73.3% and specificity was 60.2%. The positive predictive value was 51.4% and negative predictive value was 79.7%.

The best cut off point at post prandial state would be either 3.5mg/dl with sensitivity of 80.1% and specificity of 76.8% Positive predictive value was 72% and negative predictive value was 83.8% or 4 mg/dl where we observed a sensitivity of 78.9%, Specificity of 78.6%, positive predictive value 73.3% and negative predictive value 85.7%.

It is becoming increasingly apparent to investigators and clinicians in a variety of disciplines that saliva has many diagnostic uses and is especially valuable in the young, the

old and infirm and in large scale screening and epidemiological studies. The highly sensitive test procedures that are now available make it practical to quantitative, despite very low concentrations of a number of hormones and drugs in saliva. Indeed all steroids of diagnostic significance in routine clinical endocrinology can now be readily measured in saliva. Tests based on saliva have already made substantial inroads into diagnosis and hence could be considered in diagnosis of diabetes.

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