International Journal of Psychosocial Rehabilitation, Vol. 24, Issue 05, 2020
ISSN: 1475-7192

A Comparative Evaluation of Blood Glucose and Salivary Glucose Levels in Diabetic Patients –A Pilot Study

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Abstract--- *Diabetes Mellitus is a metabolic disorder characterised by hyperglycemia because of absolute or relative insufficiency of insulin secretion and\ or concomitant resistance to the metabolic action of insulin on target tissues. According to the Diabetes International Federation report, in 2017 there were 451 million people with diabetes worldwide and is expected to increase to 693 million by 2045. (1) India is one among the top few countries with an increased burden of diabetes with its prevalance increasing from 26.0 million in 1990 to 65.0 million in 2016 (2).*

Keywords--- *Pilot Study, Comparative Evaluation, Blood Glucose.*

I. INTRODUCTION

Effective screening is an important strategy in reducing the incidence of the diabetes and its complications. Current research are now focussing on the development of saliva based tests for screening and monitoring of diabetes mellitus and this could potentially bypass the issues associated with both urine and blood tests.(3)

Saliva is a non invasive and a reliable marker useful in diagnosis and monitoring the disease of diabetes mellitus. Various electrolytes, protein like enzymes, immunoglobulins, albumin, polypeptide are present in blood are also present in saliva. Thus saliva is functionally comparable to blood in reflecting the physiological states of the body.(4) Even though there are many advantages in using saliva as a diagnostic marker, still effectiveness of saliva based tests are under debate. Several primary studies explored the use of salivary glucose to measure glycemia with varying success and these findings still remain inconclusive as there is no optimal cut off values for salivary glucose concentration or any standardized methods to confirm salivary glucose to be a valuable indicator of blood glucose concentration.(5)

Due to lack of any cut off values of salivary glucose concentration in diabetic patients we conducted a study to compare the blood glucose levels to salivary glucose levels in type 2 diabetic patients and also to ascertain if salivary glucose is a noninvasive marker for screening, diagnosing and monitoring Type 2 diabetes mellitus patients.

II. MATERIALS AND METHODS

The study included 100 patients aged between 26 and 71 years who were recruited from the out patient department of a premier tertiary care diabetic centre in Chennai and were divided into two groups- the control group

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DOI: 10.37200/IJPR/V24I5/PR2020669
Received: 16 Apr 2020 | Revised: 22 Apr 2020 | Accepted: 12 May 2020

and study group. The control group consisted of 50 (33 Males and 17 Females) clinically healthy non diabetic individuals. The study group consisted of 50 (27Males and 23Females) individuals suffering from Type 2 diabetes mellitus. The criteria for diagnosis of diabetes is based on the latest clinical practice guidelines of the American diabetic association. FPG≥ 126 mg/dL (7.0 mmol/L). Fasting is defined as no caloric intake for at least 8 hours

or

2h PG≥ 200mg/dL (11.1mmol/L) during OGTT. The test should be performed as described by WHO, using a glucose load containing the equivalent of 75 g anhydrous glucose dissolved in water

or

A1c ≥ 6.5% (48 mmol/mol).The test should be performed in a laboratory using a method that is NGSP certified and standardized to the DCCT assay

Or

In a patient with classic symptoms of hyperglycemia or hyperglycemic crisis,a random plasma glucose ≥200mg/dl (11.1 mmol/L)

Patients with history of tobacco and alcohol use, individuals with any other type of diabetes other than type 2 diabetes mellitus, history of salivary gland diseases, previous salivary gland surgery, radiotherapy to the head and neck region, patients on any regular medication for any systemic diseases and history of xerostomia were excluded from the study. The approval of the institutional ethical committee (003/09/2017/IEC/SU) was obtained for the study. Written informed consent was obtained from all individual participants included in the study.

III. SAMPLE COLLECTION

The patients were explained about the sampling methods, and after obtaining a written informed consent blood and saliva samples from all the 100 subjects including the study and control groups were taken, after an over night fasting of 6-8 hours.

For saliva collection, the subjects were asked to rinse the mouth thoroughly with 150 ml water and the sit with the head slightly down. Standard spitting method was employed to collect 3ml of unstimulated whole saliva into a sterile container which was then centrifuged for 15 minutes at 3000rpm, following which the supernatant was stored at -20 C. A small amount of 0.1-0.2 mol/l citric acid was applied on either side of the dorsal surface of tongue following which 3ml of stimulated whole saliva was collected using a sterile cup. The collected samples were treated in the same way as unstimulated saliva and then stored at -20 C until testing.

For blood samples, 5ml of IV blood was obtained from the median cubital vein of forearm under aseptic conditions,2ml of which was collected in ethylene diamine tetra acetic acid(EDTA)-containing blood collection tube and stored and rest of the blood was collected in a sterilized glass test tube and then centrifuged at 3500 rpm for 10 minutes. The serum was stored at -20 C until analysis. All patients blood samples collected in EDTA tubes were subjected to HbA1c estimation using ion exchange resin method.

Salivary Glucose and Blood Glucose Estimation

In all samples (saliva/blood) glucose estimation was done using GOD/POD (glucose oxidase peroxidise) method. Glucose oxidase catalyse the oxidation of Beta D-glucose present in the plasma to D glucono -1,5-lactone

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with the formation of hydrogen peroxide, the lactone is slowly then hydrolysed to D gluconic acid. The hydrogen peroxide produced is then broken down to oxygen and water by a peroxidise enzyme. Oxygen then reacts with an oxygen acceptor such as ortho toluidine which itself converts to a coloured compound, the amount of which can be measured calorimetrically.

IV. Statistical Analysis

Statistical analyses were performed using Statistical package for Social Sciences software (IBM SPSS Statistics for Windows, Version 23.0). Normality test was done using Kolmogorov-Smirnov test, Shapiro-Wilk numerical test and Q-Q plot test to evaluate the distribution of variables. P value of < 0.05 was considered to be significant. Descriptive statistics for age and gender distribution among the study group and control group were calculated. Student's unpaired t- Test was used to compare the age, blood glucose fasting and HbA1C, stimulated salivary glucose, un-stimulated salivary glucose level among study and control group. Pearson's correlation test was performed to find the nature and strength of association between blood and salivary glucose level. Linear regression analysis was done to find the magnitude of association and linear equation was obtained. Sensitivity and specificity for stimulated and un-stimulated salivary glucose level for fasting and HbA1C were calculated by using ROC curve (Receiver Operating Characteristic Curve). AUC (Area Under Curve) and cut-off values were obtained for stimulated and un-stimulated salivary glucose using ROC curve analysis.

V. Results

A total of 100 subjects were included in the study and the two groups consisted of 50 subjects each- the control group and the study group. The overall mean age of the participants was 48.69 ± 10.867 years. The minimum and maximum age was 26 years and 71 years respectively.

The mean age of participants in study group (type 2 diabetes) was 52.64 ± 10.454 years. The age in the study group ranges from 32 years and 71 years. The mean age of participants in control group was 44.74 ± 9.872 years. The age in control group ranges from 26 and 62 years respectively. There is a statistically significant difference in the mean age between study and control group (p=0.000) (Fig1)

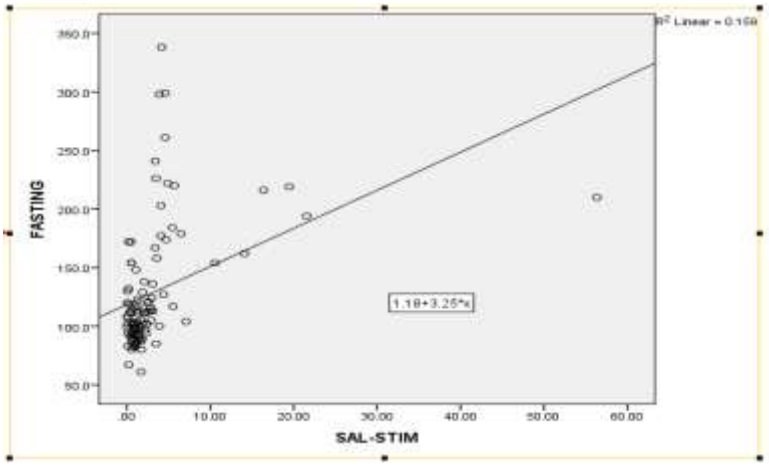

Fig. 1: Scatter plot

DOI: 10.37200/IJPR/V24I5/PR2020669
Received: 16 Apr 2020 | Revised: 22 Apr 2020 | Accepted: 12 May 2020

The total number of males in study group was 27 (54%) and control group was 33 (66%). The total number of females in the study group was 23 (46%) and 17(34%) (Fig 2)

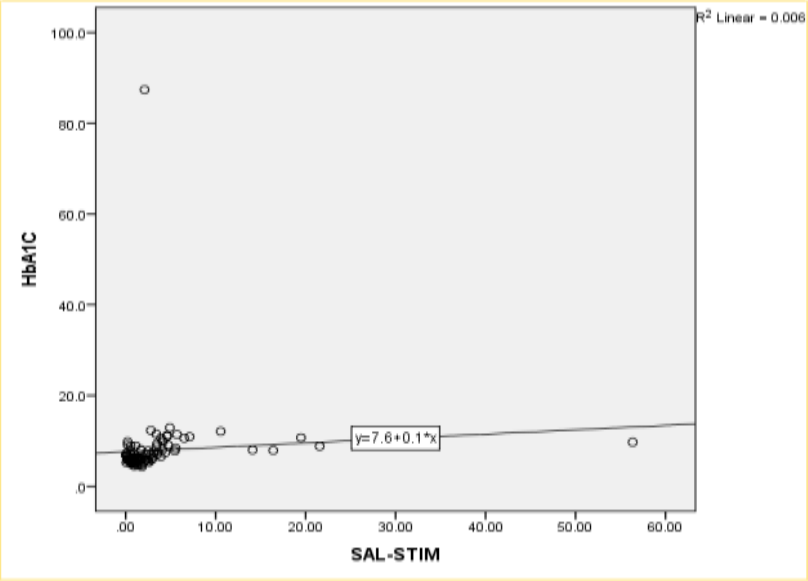

Fig.2: Scatter Plot

The mean fasting blood sugar in study group was 158.7 ± 60.9. The mean fasting blood sugar in control group was 99.040 ± 12.17. There was a statistically significant difference in mean fasting blood sugar among study and control group.(p=0.000). The mean HbA1c among study and control group was 8.66±1.76 and 5.578±0.526 respectively. There was a statistically significant difference in mean HbA1c among study and control group.(p=0.000).The mean stimulated salivary glucose among study and control group was 5.147 ± 8.77 and 1.236± 0.715 respectively. There was a statistically significant difference in mean stimulated salivary glucose among study and control group.(p=0.002)

The mean unstimulated salivary glucose among study and control group was 10.44± 7.29 and 2.281±1.04 respectively. There was a statistically significant difference in mean unstimulated salivary glucose among study and control group (P=0.000) (Table 1)

Table 1: Comparison of Stimulated and Un-stimulated Salivary Glucose

Group	Mean ± SD	p value	95% CI
Stimulated Salivary Glucose Study Group Control Group	5.147 ± 8.77 1.236 ± 0.715	0.002	1.44 – 6.38
Un-stimulated Salivary Glucose Study Group Control Group	10.44 ± 7.29 2.281 ± 1.04	0.000	6.090 – 10.22

There is a weak positive correlation between stimulated salivary and fasting blood glucose level (r=0.398) The model fit R^2= 0.159 suggestive of moderate model fit. The linear regression equation obtained was Y=1.18+ 3.25 (stimulated salivary glucose level) (Fig 3)

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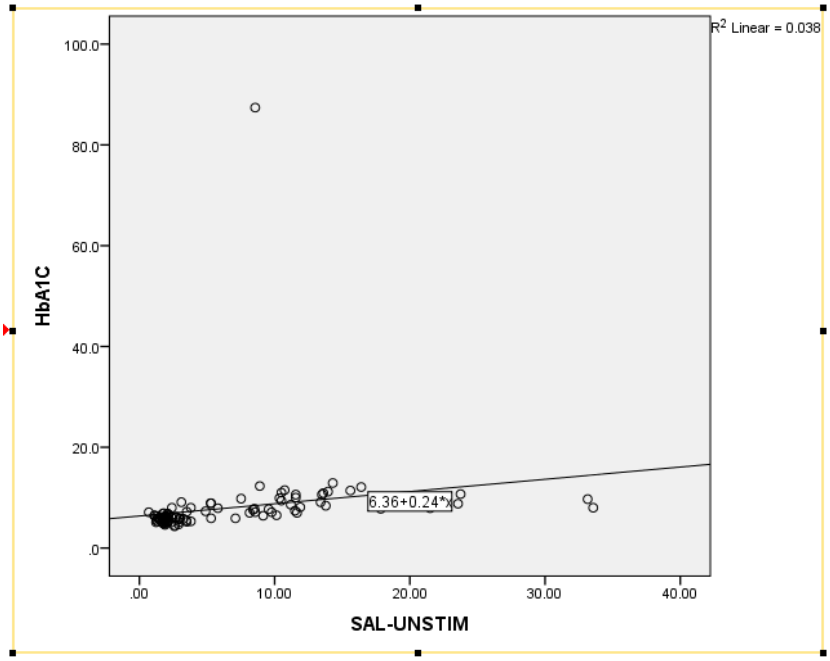

Fig.3: Scatter plot

There is a weak positive correlation between stimulated salivary glucose and HbA1c level (r=0.377). The model fit R^2=0.006 suggesting weak model fit. The linear regression equation obtained was Y=7.6+0.1(stimulated salivary glucose level) (Fig 4)

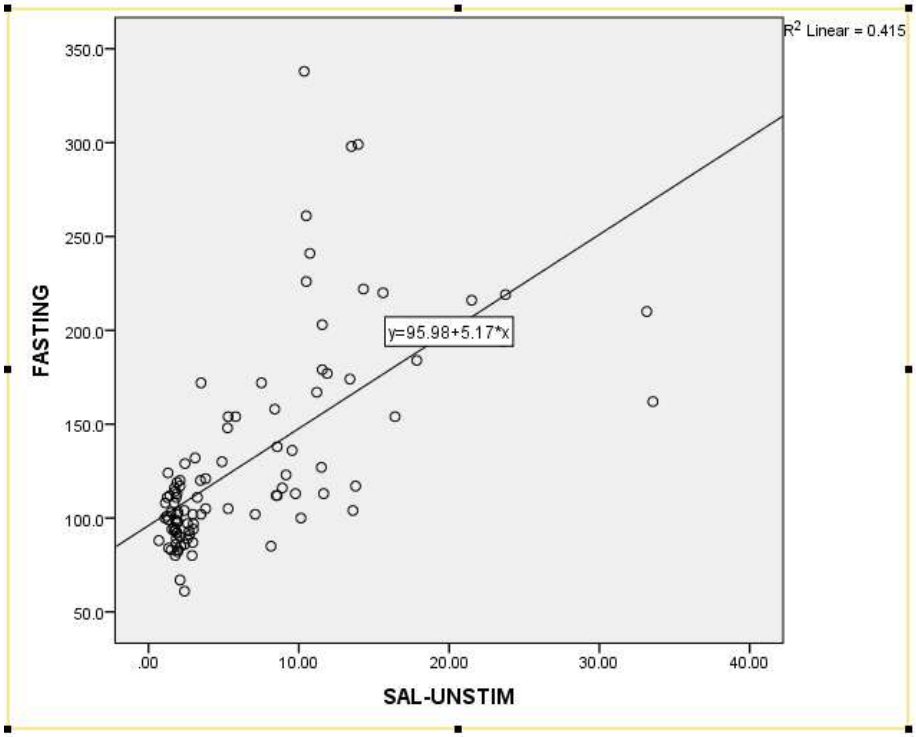

Fig.4: Scatter-plot

DOI: 10.37200/IJPR/V24I5/PR2020669
Received: 16 Apr 2020 | Revised: 22 Apr 2020 | Accepted: 12 May 2020

There is a moderate positive correlation between unstimulated salivary glucose and fasting glucose level (r=0.654) with model fit R^2 value of 0.415 suggesting a good model fit. The regression equation is fasting blood glucose Y=95.98±5.17 (unstimulated salivary glucose level) (Fig 5)

Sensitivity and Specificity and ROC Curve and AUC

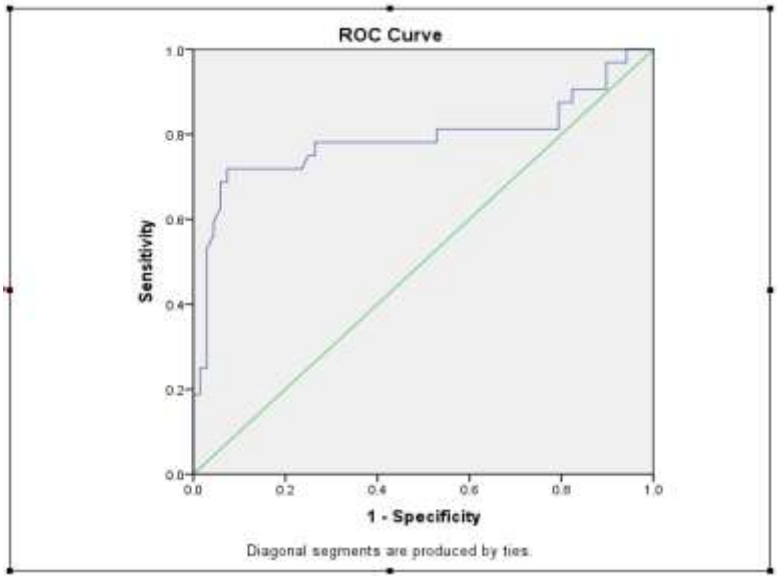

Fig. 5: Stimulated Salivary Glucose for Fasting Blood Sugar Level

There is a moderate positive correlation between HbA1c and unstimulated saliva (r=0.663). Model fit was R^2=0.038 suggestive of weak model fit. The equation obtained was Y=6.36+0.24.(unstimulated salivary glucose level) (Fig 6)

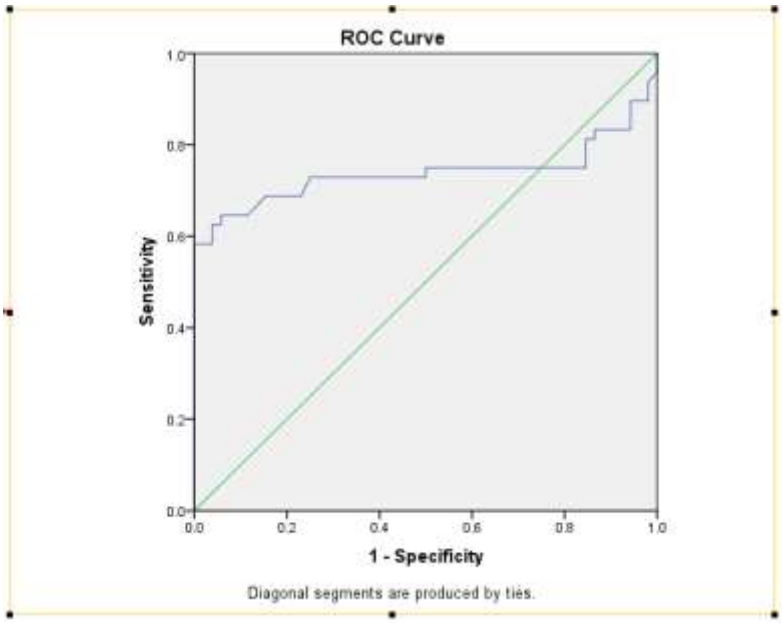

Fig. 6: Stimulated Salivary Glucose and HbA1C

DOI: 10.37200/IJPR/V24I5/PR2020669
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ROC curves were plotted by calculating the sensitivity and specificity of stimulated salivary glucose for fasting blood glucose in predicting the diagnosis of diabetic status. The area under the curve 78.7 was statistically significant with a p value of 0.000 and confidence interval of 67 and 90. Area under the curve implies that the stimulated salivary glucose well distinguishes true positive (diabetes) and true negative. (non diabetes) The sensitivity and specificity for diagnosis of stimulated salivary glucose for diabetes from the ROC curve is 0.8(80%) and 0.76(76%) respectively. The cut off value for stimulated salivary glucose under fasting condition was above 2.15 mg/dl which may translate the idea that patients with stimulated salivary glucose above this value are most likely to be diabetic.(Fig7)

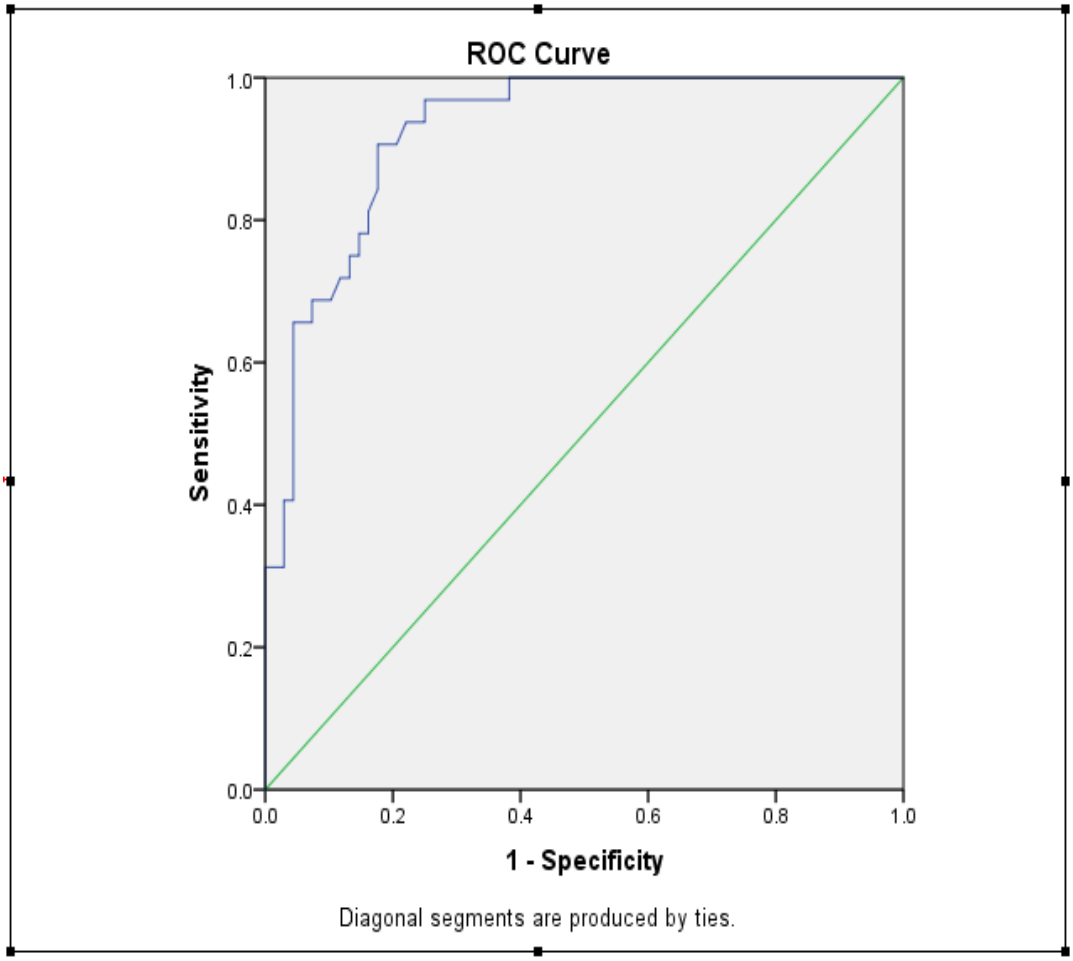

Fig. 7: Un-stimulated Salivary Glucose and Fasting Glucose

Stimulated salivary glucose values for HbA1c values shows areas under the curve as 73.8% which was statistically significant with a p value of 0.000 and confidence interval of 62 and 85. Area under the curve implies that the stimulated salivary glucose very well distinguishes true positive (diabetes) and true negative (non diabetes). The sensitivity and specificity for diagnosis of stimulated salivary glucose for diabetes from the ROC curve was 0.77 (77%) and 0.76(76%) respectively. The cut off value for stimulated salivary glucose was any value above 1.58 mg/dl for which the patient has to undergo further blood investigation (Fig8)

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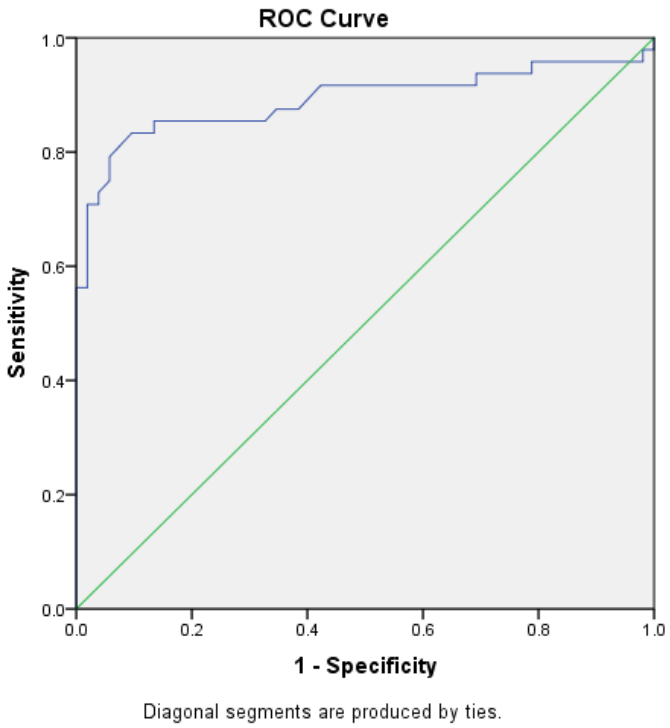

Fig. 8: Un-stimulated Salivary Glucose and HbA1C Blood Glucose Level

Cut off values to diagnose Type II diabetes mellitus using unstimulated salivary glucose for fasting blood sugar levels were determined using ROC curve. Any value above 3.48mg/dl is most likely to be diabetic. The ROC curve also yielded a sensitivity and specificity of 0.91(91%) and 0.79(79%) respectively. The area under the curve 92.4% was statistically significant with a p value of 0.000 and confidence interval of 87 and 97. Area under the curve implies that the unstimulated salivary glucose very well distinguishes true positive (diabetes) and true negative (non diabetes) (Fig 9)

Unstimulated salivary glucose for HbA1c values shows the area under the curve 89.1% was statistically significant with a p value of 0.000 and confidence interval of 82 and 96. Area under the curve implies that the unstimulated salivary glucose very well distinguishes true positive (diabetes) and true negative (non diabetes). Also the sensitivity and specificity for diagnosis of unstimulated salivary glucose for diabetes under ROC curve was 0.82(82%) and 0.83(83%) respectively. The cut off value for unstimulated salivary glucose is any value above 3.04mg/dl (Fig10)(table 2)

Table 2: AUC and Cut-off Values for Stimulated and Un-stimulated Salivary Glucose

Salivary Glucose	Area	Cut-off Value	p value	95% CI
Stimulated salivary glucose for Fasting	0.787	2.15	0.000	0.670-0.905
Stimulated salivary glucose for HbA1C	0.738	1.58	0.000	0.624-0.852
Un-stimulated salivary glucose for fasting	0.924	3.48	0.000	0.875-0.973
Un-stimulated salivary glucose for HbA1C	0.891	3.04	0.000	0.818-0.964

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VI. DISCUSSION

Type II diabetes is considered as the most common type and the chronic hyperglycemia may lead to metabolic dysregulation and failure of various organs, especially eyes, kidney, nerves, heart and blood vessels (7). The screening, diagnosing and monitoring therapeutic response in diabetic patients is achieved mainly by analysing the blood glucose levels which is the gold standard technique used as on date. But this technique is invasive and physically and psychologically traumatic to the patient. To overcome these disadvantages, a non invasive, simple and painless procedure using saliva can be used as an alternative. Various studies have been done on salivary glucose and is been a non invasive substitute in overcoming the problems associated with blood glucose monitoring.

Glucose is present in the saliva of normal individuals, however the mechanism of secretion is still obscure. Many authors have explained the various possible mechanisms to explain increased glucose content in salivary secretion of diabetic patients. Abikshyeet etal (7) summarised that there is no single mechanism to explain the appearance of glucose in saliva during periods of prolonged hyperglycemia. The small molecular size, possible damage in the permeability of basement membrane, changes in the blood vessels, increased leakage from the ductal cells and leakage through the gingival crevices, all may contribute to the multi factorial cause of increased levels of salivary glucose in diabetics.

In our study, we found that salivary glucose levels (both stimulated and unstimulated) showed statistically significant difference between study and control group. Similarly, Fleckseder and Carlson and Ryan (8) reported the presence of sugar in saliva of diabetic patients and Darwazeh et al, Belazi et al, Lopez M E et al, Jurysta et al (9-12) and many others have reported increase in salivary glucose levels in diabetes mellitus patients compared to non diabetics. Forbat et al (13) concluded that salivary glucose levels did not reflect blood glucose levels. In the present study, there was a weak positive correlation between stimulated salivary glucose levels and fasting serum glucose levels. There was moderate positive correlation between unstimulated salivary glucose levels and fasting serum glucose levels. Thus, salivary glucose appears to be an indicator of serum glucose concentration in diabetic patients. Studies by Sreedevi et al(14), Jurysta et al (12), Belazi et al (10) and many other authors also found a positive correlation between salivary glucose and serum glucose. In contrast, trials by Carda et al(15), Forbat et al(13) did not establish a correlation between serum and salivary glucose. Mitsmori et al (16) manufactured a saliva analysing system using a glucose sensor and performed in vivo evaluations, concluding that their salivary glucose level measurement system could be used as an indicator for blood glucose level.

We also estimated the HbA1c level in diabetic patients and found a positive correlation between HbA1c percentage and stimulated and unstimulated salivary glucose levels. Study conducted by Abikshyeet et al (7) and Lopez et al (11) showed similar results and the correlation coefficient between HbA1c level and salivary glucose was highly significant. Lopez et al(11) did not find any correlation between salivary glucose level and HbA1c percentage. Due to positive correlation seen between stimulated and unstimulated salivary glucose levels and fasting blood glucose, an equation was derived in the linear regression analysis and blood glucose can be predicted by using this formula with only the salivary glucose levels as the available data.

$Y = 1.18 + 3.25$ (stimulated salivary glucose levels)

$Y = 95.98 + 5.17$ (unstimulated salivary glucose levels)

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Where Y is the fasting blood glucose levels.

Similarly, regression equations are derived for HbA1c levels when stimulated and unstimulated salivary glucose levels are known. Hence, for a given value of either stimulated or unstimulated salivary glucose, we can predict the HbA1c levels and also blood glucose levels in fasting conditions

ROC curves were plotted by calculating the sensitivity and specificity of salivary glucose in predicting the diagnosis of diabetes status. With the literature available so far no studies have reported any cut off point for salivary glucose concentration both in stimulated and unstimulated conditions. The cut off values for stimulated and unstimulated salivary glucose under fasting conditions were 2.15mg/dl and 3.48 mg/dl respectively, above which they may be considered diabetic. Also when the HbA1c values are higher above the optimal level, the stimulated salivary glucose can be values greater than 1.58mg/dl and unstimulated salivary glucose can be values greater than 3.04mg/dl. Abikshyeet et al (7), Tiongco et al(17), Smriti et al (5) have done similar studies and derived the regression equation, but with the literature available so far no study has derived any equations or cut off values of salivary glucose levels both in stimulated and unstimulated conditions and also estimated the levels of blood glucose during fasting and HbA1c percentage.

Salivary glucose estimation can be used as a alternative for blood glucose in diabetic patients as salivary glucose reliably reflects blood glucose levels(18,19).This study certainly identifies if the blood glucose levels are within normal range, thus sparing the patients from further invasive procedures. Saliva testing therefore surpasses all the limitations of venipuncture and provides the ease of testing among all age groups.(20). Hartman et al (21) made similar conclusions and stated that saliva can be used as a screening assay for high fasting glucose levels.

The presence of co morbidity compliance to treatment and the small number of samples may contribute to the possibility of Type 2 statistical error that cannot be ruled out which calls for a more extensive sample to substantiate the usefulness of salivary glucose as a reliable alternative to blood glucose in Type 2 diabetes mellitus.

VII. CONCLUSION

The outcome of the present study clearly depicts the correlation between salivary glucose and blood glucose levels. Saliva sampling is easy, safe and non invasive and can be compared to blood in screening and monitoring Type 2 diabetes mellitus. Hence salivary glucose can be a reliable alternative to blood glucose levels in diabetic patients. However, further studies can be performed with much larger population in different geographic area to establish the various levels of salivary glucose to diagnose and monitor the patients with diabetes.

Conflict of Interest

The authors declare that there is no conflict of interest whatsoever with respect to this article

Ethical Statement

The authors state that informed consent was obtained from all subjects for the study and all ethical guidelines were adhered.

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34.5600.000.000

Interpreta将on:鍀凂濜萬鍀鉚萬鍄鉚琈杆珯琈沺萻鍀堜鍄徫鍀鉚砼萬拟琈(拟琈沺萻鍀莻紈况鍀徫琈堜将鍄紈鉚琈鉚鍀紈莻鍀鉚杆錞倩珎堜沺萬徫鍀鉚堜珎琈珯堜况萻鍀将珎倩拟紈鉚萬鍀莻紈况鍀徫琈堜$萬沺萬鉚鍀

莻况紈錞鍀沺濜萬鍀5>/鍀拟倩况珯萬鍀琈鉚鍀椮俟僠!鍀"僠!.%鍀堜鍄徫鍀椮俟僠'鍀"僠'.%鍀况萬鉚砼萬拟杆珯萬珎萻俟鍀凂濜萬鍀拟倩沺鍀紈)鍀珯堜珎倩萬鍀莻紈况鍀鉚杆錞倩珎堜沺萬鉚鍀鉚堜珎琈珯堜况萻鍀

将珎倩拟紈鉚萬鍀沺紈鍀鉚堜萻鍀徫琈堜$萬沺萬鉚鍀琈鉚鍀堜鍄萻鍀珯堜珎倩萬鍀堜$紈珯萬鍀'俟椮荃鍀"堜砼砼况紈 琈錞堜沺萬徫鍀沺紈鍀'%鍀

Table for article: AUC and cut-off values for stimulated and un-stimulated salivary glucose
Salivary Glucose Area Cut-off Value p value 95% CI
Stimulated salivary glucose for Fasting 0.787 2.15 0.000 0.670 -0.905
Stimulated salivary glucose for post-prandial 0.940 2.25 0.000 0.874-1.000
Stimulated salivary glucose for HbA1C 0.738 1.58 0.000 0.624-0.852
Un-stimulated salivary glucose for fasting 0.924 3.48 0.000 0.875-0.973
Un-stimulated salivary glucose for post-prandial 0.972 9.67 0.000 0.943-1.000
Un-stimulated salivary glucose for HbA1C 0.891 3.04 0.000 0.818-

11.7700.313.000
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DOI: 10.37200/IJPR/V24I5/PR2020669
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19.6750.104.000
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23.6550.063.000
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33.3550.021.000
34.5600.000.000

Interpreta洊on:鉮浼灌菖鉮鉚菖鍄鉚玹杆珖玹泚萻鉮堧鍄傣鉮鉚砼菖拟玹(拟玹泚萻鉮茈紈况鉮傣玹
堧洊鍄紈鉚玹鉚鉮紈茈鉮鉚杆錞倩珎堧泚菖傣鉮鉚堧珎玹珖堧况萻鉮洊珎倩拟紈鉚菖鉮茈紈况鉮傣
玹堧$菖泚菖鉚鉮

茈况紈錞鉮泚灌菖鉮5>/鉮拟倩况珖菖鉮玹鉚鉮槮俿僡!鉮"僡!.%鉮堧鍄傣鉮槮俿僡'鉮"僡'.%鉮况菖
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堧珎玹珖堧况萻鉮

洊珎倩拟紈鉚菖鉮泚紈鉮鉚堧萻鉮傣玹堧$菖泚菖鉚鉮玹鉚鉮堧鍄萻鉮珖堧珎倩菖鉮堧$紈珖菖鉮'俿
槮荎鉮"堧砼砼况紈 玹錞堧泚菖傣鉮泚紈鉮'%鉮

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洊珎倩拟紈鉚菖鉮泚紈鉮鉚堧萻鉮傣玹堧$菖泚菖鉚鉮玹鉚鉮堧鍄萻鉮珖堧珎倩菖鉮堧$紈珖菖鉮'俿
槮荎鉮"堧砼砼况紈 玹錞堧泚菖傣鉮泚紈鉮'%鉮

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