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A Case Control Study to Evaluate the Sensitivity of Salivary over Serum Glycated Protien Levels in Patients with Controlled Type 2 Diabetes Mellitus

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Abstract

Background: To evaluate the sensitivity of salivary over serum glycated protein levels in patients with type 2 diabetics mellitus.

Materials and methods: The samples for the present study comprised of two groups. Group A included 60 Age and sex matched healthy adult whereas group B consisted of 60 adults with controlled type2 diabetes mellitus. Blood samples (1.5 ml of venous blood) are collected with EDTA in the test tubes for the determination of glycosylated hemoglobin and Serum glucose level. Stimulated saliva was collected from both groups in a sterile container. Then the salivary samples were also subjected to glucose estimation and glycosylated hemoglobin by microcolumn method. Serum fructosamine and salivary fructoseamine was estimated by NBT reduction method (B.L. Somani et al 2010) using Schimadzu CL-750 Spectrophotometer and Colorimeter.

The results were statistically evaluated with one way ANOVA, Pearson's correlation test.

Results: The salivary fructoseamine levels were significantly increased in the case groups, when compared with controls. There was an increase in the salivary fractoseamine levels with an increase in HbA1c percentage. The correlation analysis revealed a significant positive correlation($P < 0.001$) between salivary and serum glucose levels and salivary glucose level with HbA1c.

Conclusion:Diabetes is known to influence salivary composition and function and therefore salivary fructoseamine can be used as a noninvasive diagnostic aid for diagnosis and monitoring of diabetes.

Keywords: Insulin, Hyperglycemia, Advanced Glycation End Product, Fructoseamine.

INTRODUCTION

Diabetes mellitus is the commonest endocrine metabolic disorder resulting in hyperglycemia either due to primary insulin deficiency or reduction in its biologic effectiveness or both. Asian

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Indians seem to be at a greater risk of developing this disorder. Currently we have 40.9 million people suffering from diabetes and the predicted estimate by the year 2025 is around 70 million throughout the world. The crude prevalence rate of diabetes in urban areas is about 9% and in rural areas, has increased to around 3% of the total population [1].

Diabetes mellitus (DM) is a metabolic disease characterized by dysregulation of carbohydrate, lipid and protein metabolism. The primary feature of this disorder is elevation in blood glucose levels (hyperglycemia). Untreated diabetes leads to significant complications of multiple organs including the eyes, nerves, kidneys and blood vessels. These complications are responsible for high degree of morbidity and mortality. When a disease with its resultant devastating effects become so widespread it becomes the duty of the diagnostician to combine their knowledge to search for the increasing number of unknown or uncontrolled diabetes so as to give them the benefit of adequate medical care and to provide adequate medical care to the known controlled diabetes. The oral tissues react and produce characteristic manifestations in DM. It includes advanced periodontal disease, high rate of dental caries, sialosis, xerostomia, abnormal taste, prolonged, recurrent fungal infections and burning mouth syndrome [2].

Saliva is a unique fluid, whose important role is to maintain the well-being of the oral cavity. Saliva acts as the mirror of the body and hence, is a perfect medium to be explored for disease, health and surveillance. The constituents of the saliva not only maintains the oral health but also plays a vital role in the diagnosis and assessment of various oral diseases like oral cancer, candidiasis and periodontal diseases.DM may lead to potential complications like the diabetes retinopathy, nephropathy, peripheral neuropathy, macroangiopathies. Hence, the investigative procedures must be routinely employed to diagnose and monitor the diabetes. The most routinely employed procedures are blood investigations. These procedures are invasive and offer much discomfort to the patient. So there is a pressing need for the development of non invasive procedure for diagnosing and monitoring diabetes. Saliva offers distinctive advantages, as saliva can be collected noninvasively with limited training, has fewer compliance problems and is cost effective [3]. This study was carried out to evaluate the role of saliva as a diagnostic tool by correlating serum glucose and salivary glucose in the patient and control groups.

There are other serum proteins beside hemoglobin that become glycated in the presence of hyperglycemia. Measurement of these glycated proteins can be used as an alternative to the HbA1c. The normal range for the fructosamine test is 2.0– 2.8 mmol/l.

Fructosamines are compounds that result from glycation reactions between a sugar (such as fructose or glucose) and a primary amine, followed by isomerization via the Amadori rearrangement. Biologically, fructosamines are recognized by fructosamine-3-kinase, which may trigger the degradation of advanced glycation end-products (though the true clinical significance of this pathway is unclear). Fructosamine can also refer to the specific compound 1-amino-1-deoxy-D-fructose (isoglucosamine), first synthesized by Nobel laureate Hermann Emil Fischer in 1886.

Similar to the hemoglobin A1c testing (which measures the glycation of hemoglobin), fructosamine testing calculates the fraction of total serum proteins that have undergone glycation (the glycated serum proteins). Since albumin is the most common protein in blood, fructosamine levels typically reflect albumin glycation. (Some fructosamine tests specifically quantify the

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glycation of albumin, or glycated serum albumin instead of all proteins.). Because albumin has a half-life of approximately 20 days, the plasma fructosamine concentration reflects relatively recent (1-2 week) changes in blood glucose.

In patients with diseases that reduce red blood cell lifespan, such as hemolytic anaemia or hemoglobinopathies such as sickle-cell disease, a hemoglobin-based A1c test can be misleadingly low. A1c results may also be falsely high or low in hemoglobinopathies because abnormal hemoglobin variants can interfere in the analysis. In these cases, fructosamine measurement can be used as a marker of blood sugar levels, as its measurements are based on albumin instead of hemoglobin.

In practice, fructosamine is rarely measured clinically (even in individuals with hemoglobinopathies or other red cell disorders) due to a number of pragmatic concerns. First, diabetes care is rarely changed in short (1-4 week) intervals, since diabetes medications can take months to reach a steady state. An exception to this is pregnancy, where medication needs can change more rapidly and fructosamine may help provide closer short-term monitoring. Second, fructosamine has higher variability than A1c tests. Third, the overwhelming majority of studies in diabetes care are based on A1c measurements, which can make fructosamine results difficult to interpret. Fourth, the A1c test is incredibly well standardized and trusted due to its nearly universal use. A variety of more advanced forms of the A1c test (e.g. some types of HPLC, immunoassay and capillary electrophoresis) can more accurately assay A1c levels during complex hemoglobinopathies and other conditions. However this does not overcome the effect on A1c results of reduced red cell lifespan.

MATERIALS AND METHODS

The case control study was planned & conducted during the period of September 2021 to August 2022 in the department of Oral Medicine & Radiology, Karpaga Vinayaga Institute of Dental Sciences to evaluate the sensitivity of salivary over serum glycated protein levels post-prandially in patients with type 2 diabetics mellitus.

. The patients for the study were selected among the outpatients who visited the department of oral medicine and maxillofacial radiology. The study groups comprises of 120 subjects in the age group of 25 – 60 years and were divided into two groups with each of 60 subjects. Group A – Age and sex matched 60 healthy adult were taken in the control group. Group B – 60 adults with controlled type2 diabetes mellitus.

A detailed case history of the patient with type 2 diabetes and a thorough clinical examination was done and recorded on a standard proforma. Screening of patient done based on diabetes status whether it is controlled or uncontrolled.

This is assessed based on serum glucose level. A formal ethical clearance to conduct the study was obtained from the ethical Committee of the college. Patients selected for the study were explained in detail about the condition affecting their oral cavity and the procedure they were subjected to. A formal informed written consent was taken from all of them.

Clinically proven cases of controlled type 2 diabetes mellitus were included for this study. Patients excluded from the study are those who with pregnant woman, chronic alcoholic, smoking habit,

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steroid treatment, diuretics, phenol barbitol, protease inhibitor. A detailed case history was recorded for all patients with special reference to diabetes mellitus status and duration of type 2 diabetes mellitus also recorded. Stimulated saliva is collected using 2% food-grade citric acid that is applied to the dorso lateral surface and the tip of the tongue every 30 seconds, and the patient is asked to spit the saliva as it pooled in the mouth, into a sterile container without swallowing, for 3 minutes. Once the saliva 2ml was collected, the plastic container will be placed in an ice carrier box and transferred to the laboratory for biochemical analysis. For Serum sample collection 1.5 ml of venous blood will be collected with EDTA/ heparin in the test tubes for determination of Glycosylated haemoglobin.And another 1.5 ml of venous blood will be collected with EDTA/ heparin in the test tubes for determination of Serum glucose level.

Glucose levels of stimulated saliva and serum glucose were estimated using GOD POD Method in Semiautomatic Analyser.

$$\frac{\text{Sample (A)}}{\text{Standard (A)}} \text{ x 100 (Standard conc.) = mg/dL glucose in the sample}$$

Conversion factor: mg/dL x 0.0555= mmol/L

Serum fructosamine was estimated by NBT reduction method (B.L. Somani et al 2010) using Schimadzu CL-750 Spectrophotometer and Colorimeter. Fresh 0.2 ml of serum was Taken in 1 ml of 9 gm/1 sodium chloride and Incubated at 37°C for 5 to 10 min. 1.0 ml of Pre-warmed NBT reagent was added and absorbance was measured at 530 nm at Interval of 5 min (Al) and 10 min (A2) using Schimadzu CL-750 Spectrophotometer. The ΔA (A2-A1) was calculated and the Results were expressed as ΔA/min.

CALCULATION: serum glucose concentration in the sample can be calculated using the following formula

$$\frac{\text{Sample (A)}}{\text{Standard (A)}} \times 100 \text{ (Standard conc.) = mg/dL glucose in}$$

Conversion factor: mg/dL x 0.0555= mmol/L

Salivary fructosamine was estimated by NBT reduction method (B.L. Somani et al 2010) using Schimadzu CL-750 Spectrophotometer and Colorimeter. Fresh 3 ml of serum was taken in 5 ml of 9 gm/1 sodium chloride and Incubated at 37°C for 5 to 10 min. 1.0 ml of Pre-warmed NBT reagent was added and absorbance was measured at 530 nm at Interval of 5 min (Al) and 10 min (A2) using Schimadzu CL-750 Spectrophotometer. The ΔA (A2-A1) was calculated and the Results were expressed as ΔA/min.

CALCULATION: salivary glucose concentration in the sample can be calculated using the following formula

$$\frac{\text{Sample (A)}}{\text{Standard (A)}} \times 100 \text{ (Standard conc.) = mg/dL glucose in the sample}$$

Conversion factor: mg/dL x 0.0555= mmol/L

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Glycated hemoglobin (HbA1c) of serum were estimated using micro-column method .Samples of blood (1.5 ml) collected into EDTA/ heparinised vacutainers were centrifuged (1500g, 10 min) and the plasma removed. After preparing the hemolysate, where the labile fraction is eliminated, hemoglobins are retained by a cationic exchange resin. Hemoglobin A1c is specifically eluted after washing away the HbA1a+b fraction, and is quantified by direct photometric reading at 415 nm.

STATISTICAL ANALYSIS

Statistical comparisons of salivary glucose and serum glucose levels in patients with type 2 diabetes mellitus done with SPSS version 13.0.One way ANOVA was used for the statistical evaluations whereas Pearson's correlation analysis was used to determine the correlation between salivary glucose and serum glucose.

RESULTS

INFERENCE STATISTICS

Table 1: Comparison of fasting Salivary Glucose between Control and Case Group

S.No	Group	N	Mean	SD	*f- value*	P –value
1	Case	30	164	10.26	58	**0.000*****
2	Control	30	83.6	5.35		

Unpaired t test, SD-standard deviation; ⦃***VHS= Very high significant P < 0.001⦄.
***Inference*:** On group comparison there was significant increase of p value (P < 0.001⦄ seen in case group.

Table 2: Comparison of fasting Serum Glucose between control and Case

S.No	Group	N	Mean	SD	*f- value*	P –value
1	Case	30	88.8	.560	58	**0.000*****
2	Control	30	59.1	.588		

Unpaired t test, SD-standard deviation; ⦃***VHS= Very high significant P < 0.001⦄
***Inference*:** On group comparison there was significant increase of p value (P < 0.001⦄ seen in case group.

Table 3: Comparison of postprandial Salivary Glucose between control and Case

S.No	Group	N	Mean	SD	*f- value*	P –value
1	Case	30	191.9	12.12	58	**0.000*****
2	Control	30	120.3	10.185		

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Unpaired t test, SD-standard deviation; ⋖***VHS= Very high significant P < 0.001⋗
***Inference*:** On group comparison there was significant increase of p value (P < 0.001⋗ seen in case group .

Table 4: Comparison of postprandial Serum Glucose between control and Case

S.NO	Group	N	Mean	SD	*f- value*	P –value
1	Case	30	114.1	.950	58	**0.000*****
2	Control	30	60.0	.566		

Unpaired t test, SD-standard deviation; ⋖***VHS= Very high significant P < 0.001⋗
***Inference*:** On group comparison there was significant increase of p value (P < 0.001⋗ seen in case group.

Table 5: Comparison of fasting salivary fructosamine between control and Case

S.NO	Group	N	Mean	SD	*f- value*	P –value
1	Case	30	.3900	.0649	58	**0.000*****
2	Control	30	.2603	.1575		

Unpaired t test, SD-standard deviation; ⋖***VHS= Very high significant P < 0.001⋗
***Inference*:** On group comparison there was significant increase of p value (P < 0.001⋗ seen in case group.

Table 6: Comparison of fasting serum fructosamine between control and Case

S.No	Group	N	Mean	SD	*f- value*	P –value
1	Case	30	1.7157	.17144	58	**0.000*****
2	Control	30	.9810	.17060		

Unpaired t test, SD-standard deviation; ⋖***VHS= Very high significant P < 0.001⋗
***Inference*:** On group comparison there was significant increase of p value (P < 0.001⋗ seen in case group.

Table 7: Comparison of postprandial salivary fructosamine between control and Case

S.No	Group	N	Mean	SD	*f- value*	P –value
1	Case	30	.71	.174	58	**0.000*****
2	Control	30	.48	.160		

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Unpaired t test, SD-standard deviation; ⋕***VHS= Very high significant P < 0.001⋕
***Inference*:** On group comparison there was significant increase of p value (P < 0.001⋕ seen in case group.

Table 9: Correlation between comparison of fasting salivary and serum fructosamine levels of control and case

	Fructose saliva	**Fructose blood**
Fructose saliva Pearson correlation Sig. N	1 60	.361 0.005 60
Fructose blood Pearson correlation Sig. N	.361 0.005 60	1 60

***Inference*:** Pearson correlation value is r = 0.361. It shows that positive correlation. The relation is very strong between them. As per our experiment, we obtain linear relationship among them. The r^2 value = 0.130321. Sig. value is .005 (which is less than .01), so we say the data supports our hypothesis.

Pearson's Correlation

Table 10: Correlation between comparison of postprandial salivary and serum fructosamine levels in control and case

	Fructose saliva	**Fructose blood**
Fructose saliva Pearson correlation Sig. N	1 60	.361 0.005 60
Fructose blood Pearson correlation Sig. N	.361 0.005 60	1 60

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Table 8: Comparison of postprandial serum fructosamine between control and Case

S.No	Group	N	Mean	SD	*f- value*	P –value
1	Case	30	1.91	0.194	58	**0.000*****
2	Control	30	.81	0.180		

Unpaired t test, SD-standard deviation; ⦉***VHS= Very high significant P < 0.001⦊
***Inference*:** On group comparison there was significant increase of p value (P < 0.001⦊ seen in case group.

CAUSAL ANALYSIS: Pearson correlation value is r = 0.361. It shows that positive correlation. The relation is very strong between them. As per our experiment, we obtain linear relationship among them. The r^2 value = 0.130321. Sig. value is .005 (which is less than .01), so we say the data supports our hypothesis.

DISCUSSION

Diabetes mellitus (DM) is a syndrome of abnormal carbohydrate, fat and protein metabolism that results in acute and chronic complications due to the absolute or relative lack of insulin [4].
Diabetes mellitus is reaching potentially epidemic proportions in India. The level of morbidity and mortality due to diabetes and its potential complications are enormous, and pose significant healthcare burdens on both families and society. Worryingly, diabetes is now being shown to be associated with a spectrum of complications and to be occurring at a relatively younger age within the country. In 2000, India (31.7 million) topped the world with the highest number of people with diabetes mellitus followed by China (20.8 million) with the United States (17.7 million) in second and third place respectively [5].
Oral manifestations include advanced periodontal disease, high rate of dental caries, sialosis, xerostomia, abnormal taste, prolonged or recurrent fungal infections and burning mouth syndrome. All these manifestations not only destroy the oral and dental structures but also give rise to clinical suspicion to oral diagnostician in routine and screening programs of the oral cavity [2].
Hence, the investigative procedures must be routinely employed to monitor the diabetes. The most routinely employed procedure is serum glucose investigation .This is achieved by repeated finger pricks or by intravenous blood sampling. In doing so, it causes unnecessary discomfort and mental trauma to the patients; therefore, a much simpler and non invasive technique is very much desirable [6].

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Saliva offers some distinctive advantage from other body fluids. It can be collected non invasively and by individuals with limited training. No special equipment is needed for collection of the fluid [7].

Most of the previous studies have demonstrated the raised salivary glucose level in diabetes. Numerous studies reported a significant correlation between salivary glucose level (SGL) and blood glucose level (BGL) in DM [8, 9]. As a matter of fact glucose is present in saliva of normal individuals; however, the mechanism of its secretion is still obscure.

In this study, a significant increase ($P < 0.001$) in the salivary glucose levels was seen in the case groups, when compared with control. These results were consistent with that proposed by Belazi MA et al [10] and Borg Andersson A et al [11].

Various hypothesis regarding increased the concentration of glucose in saliva have been put forward in the literature. The increased permeability of basement membrane in insulin dependent diabetes mellitus may lead to enhanced leakage of serum-derived components into whole saliva via gingival crevices. The small glucose molecule can easily diffuse via semi permeable basement membrane [12].

According to Qureshi et al., [13] and Sreedevi et al., [14] persistent hyperglycemia leads to microvascular changes in the blood vessels, as well as endothelial dysfunction in the salivary glands causing increased leakage of glucose from the ductal cells of the salivary gland, thereby increasing the glucose content in saliva.

Kumar S et al., [15] also concluded salivary glucose levels can be used as a noninvasive technique for the measurement of diabetic status of a patient in a dental set up.

On the contrary, Anjali Gupta et al., [16] concluded that salivary glucose levels did not reflect blood glucose levels. Hartman et al., [17] concluded that saliva glucose appears to have reasonable characteristics to serve as a screening diagnostic for high plasma glucose in children.

In this study, an significant positive correlation ($P < 0.001$) was seen between fasting salivary and serum fructosamine levels (Table 13). These results were consistent with the previous studies by (Amer S et al.,2001; Sreedevi et al., 2008; Jurysta C et al., 2009). On the other hand, Carda C et al., (2006) had conflicting results.

In this study, an significant positive correlation ($P < 0.001$) was seen between postprandial salivary and serum fructosamine levels (Table 14). These results were consistent with the previous studies by (Kapellas K. et al., 2001;). On the other hand, Carda C et al., (2006) had conflicting results.

CONCLUSION

In conclusion, our study emphasizes that the salivary fructosamine level can be used as a noninvasive diagnostic test. However, the promising results of this study should be further refined in the future with a larger sample size in larger population.

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