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The Suitability of Non-Invasive Sample in the Assay of Glucose in Diabetes Mellitus Diagnosis and Sex Difference

Agoro ES^{1*},
Nelson-Ebimie E²,
Soroh AE³ and Odegbemi JO⁴

Abstract

Plasma is the most patronized sample for the assay of glucose utilized as a focal biomedical marker for the diagnosis of diabetes mellitus. The procedure is painful and the cost implication is huge. Hence, the need for an alternative non-invasive sample void of painful sensation and of cheap cost implication is pertinent. In this study a total of 100 subjects were utilized and divided into two groups of fifty each. The first group constituted apparently healthy individuals, whereas the second was made up of subjects diagnosed with type 2 diabetes mellitus. Glucose was estimated quantitatively in the blood and the saliva using glucose oxidase method. Pearson correlation analysis was used to established relationship between plasma and salivary glucose, whereas *t*-student test was utilized to evaluated significant level based on sex difference. The result indicated that correlation was not observed between fasting blood glucose and fasting salivary glucose of diabetics as well as control subjects. However correlation was observed between fasting blood glucose and salivary glucose amongst diabetic subjects with glucose level above threshold value. The correlation coefficient value was +0.72 proving a significant correlation statistically. Also, statistical difference was not observed based on gender on studied plasma and salivary glucose. Therefore, the findings showed explicitly that saliva could serve as an alternative sample for glucose estimation and management especially for diabetics with evidently high glucose levels. Also, gender difference for customized diagnostics is of no diagnostic importance.

Keywords: Glucose; Saliva; Plasma; Diabetics; Threshold

- 1 Department of Chemical Pathology, Medical Laboratory Services, Federal Medical Center, Bayelsa State, Nigeria
- 2 Bayelsa State College of Health Sciences and Technology, Bayelsa State, Nigeria
- 3 Jasper Boro College of Education, Bayelsa State, Nigeria
- 4 Comprehensive Health Centre, Osun State

***Corresponding author:** Eni-yimini Agoro

✉ siragoro@yahoo.com

Department of Chemical Pathology, Medical Laboratory Services, Federal Medical Center, Bayelsa State, Nigeria.

Tel: 00213770321576

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Introduction

Medical Laboratory Scientists utilize various body fluids and tissues for the examination of specific marker absence or presence for the sole purpose of disease diagnosis and management. The efficiency of the Medical Laboratory is dependent on the use appropriate sample. Wrong or inappropriate sample will definitely yield wrong result irrespective of the cutting edge technology used. Wrong result is not just detrimental to the patients and the health team as a whole but creates an avenue for financial drain and man-hour wastage. In the hospital, most samples used for diagnosis are sourced through invasive methods which inflict pain on the patients.

Invasive method of sample collection involves the active puncture of the body cells or tissues, which in turn inflict pain

[1]. Examples of invasive samples include blood, vagina swabs, CSF etc. Contrarily, non-invasive method involves passive form of sample collection that does not involve any form puncture and pain [1]. Examples are urine, stool, saliva etc. Phlebotomy is the gold standard procedure most widely used for disease diagnosis and management. Despite the wide acceptance, the procedure is still plagued with some disadvantages, such as the painful sensation, vulnerability to injury or infection and huge monetary implication. Unlike blood, saliva collection is simple, timely and much cost effective [1]. Other advantages of saliva are the non-invasive nature, availability and the avoidance of pain or injury [1].

Blood is the fluid that the heart circulates through the body's arteries, capillaries, and veins [2], servicing the entire needs of cells and tissues in terms of nutrition, excretion and protection. It is mainly composed of plasma, a fluid in which red blood cells

(RBC), white blood cells (WBC) and platelets are suspended [3]. The plasma is a pale yellow liquid, occupying 55% of the total volume of blood, contains 90% water, the remaining 10% being proteins, carbohydrates, lipids, hormones, enzymes, vitamin and salts [3]. Saliva is a clear, alkaline, viscous fluid secreted from the parotid, submaxillary, sublingual, and smaller mucous glands of the mouth [2]. Approximately 0.8 to 1.2 L of saliva is produced daily. Saliva is a reservoir of varieties of biomolecules and substances such as viruses, bacteria, proteins, hormones and glucose [1].

Glucose is a six carbon chain monomer of carbohydrate utilized for the diagnosis and management of hyperglycaemia or hypoglycaemia. Hyperglycaemia is the increase in blood glucose levels above the normal range, whereas hypoglycaemia is below the normal reference range. In the nutshell, glucose is the diagnostic tool for diabetes mellitus. The World Health Organization (WHO) defined Diabetes Mellitus on the basis of laboratory findings, as a fasting venous plasma glucose concentration greater than 7.8 mmol/L (140 mg/dl) or greater than 11.1 mmol/L (200 mg/dl) two hours after a carbohydrate meal or two hours after the oral ingestion, even if the fasting concentration is normal [4]. The definition stands if only the laboratory investigation carried out more than twice still give diabetic range values [4]. The presence of glucose in urine is an indication of an overflow from the blood when plasma glucose is above the threshold value. Threshold value of plasma glucose is put at 11 mmol/l [4]. Whenever plasma glucose exceeds 11mmol/l, there is a spillover effect to the urine and possibly other fluids.

The report as portrayed by the International Diabetes Federation showed that in 2011, 366 million people worldwide were affected by diabetes and the number will hit 522 million by 2030 if appropriate system is not put in place. Currently, India is in the second position in the chart, after the People's Republic of China according to the International Diabetes Federation. Nigeria is currently estimated to have 1.56 million of diabetics as stated by International Diabetes Federation Africa (IDFA) in 2015. This value is estimated to double in 15 years if proper measures are not adopted.

Curbing the increase in persons with diabetes mellitus is multifaceted. However early diagnosis stands out as an anti-dote in prevention of early diabetes mellitus manifestation and its consequent complications. Early diagnosis aids the prevention of complications of diabetes mellitus and prolonged the time for its full manifestation. This is hampered by the phobia for phlebotomy. This singular procedure had discouraged a lot of individuals from the investigation. Hence the need for sample that its collection is painless and easy is apt. The findings on glucose in saliva could be traced to the early 19th century [2]. A lot of scientists had reported the presence of glucose in the saliva and further shown its usefulness in clinical decision making [5-16].

The study was aimed at bringing about alternative non-invasive sample in the analysis of glucose so as aid easy diagnosis of diabetes mellitus and customized diagnostics.

Materials and Methods

Study area

The study locations utilized for the research include Federal Medical Centre, Yenagoa, FMC Yenagoa Otuoke Outreach and the General Hospital Sagbama. The facilities are the major tertiary health facilities in Bayelsa State of Nigeria. Bayelsa State was created in 1996 and located within Latitude 40,151 North and Latitude 50 and 231 South. It is also within longitude 50,221 West and 60,451 East. It is bounded by Delta State on the North, Rivers State on the East and the Atlantic Ocean on the Western and Southern parts [17].

Study population

In this study a total of 100 subjects were utilized and divided into two groups of fifty (50) each. Group 1 was made of apparently health subjects and group 2 diabetic subjects. The age bracket was hinged between 18 and 60 years. Fasting blood and saliva were collected from the study subjects, exception of postprandial blood and saliva that was restricted to only group 2 subjects.

Ethical approval

The experimental protocol was approved by the ethics committee of all the study centers. Consents were also granted by individuals willingly before sample collection. The essence of the study was explicitly explained to the subjects without any iota of coercion.

Selection criteria for subjects

Subjects' recruitment and enrollment were based on the estimation of glucose in the blood. Subjects with normal glucose levels were categorized as group 1, whereas subjects with glucose level above reference range as stipulated by The Expert Committee on Diagnosis and classification of Diabetes mellitus, 1998 [16] as group 2. Subjects with life threatening disease and complication were excluded for the research.

Collection of samples

Blood: Blood samples were collected based on universally accepted procedure as depicted by Ochei and Kolhatar. 3 ml blood samples were collected from the subjects and subsequently emptied into fluoride oxalate containers. The samples were centrifuged at 300 revolutions per minute (rpm) for five minutes using the Vanguard V 6000 Centrifuge followed by the analysis.

Saliva: Collection of saliva was carried between 8.00-10.00 a.m. Spitting method was employed for the sample collection. Subjects seated upright and spitted into fluoride oxalate containers, the initial saliva were discarded. Subjects enrolled did no smoke or brush or eat or drink two hours prior to the time of saliva collection. The unstimulated whole saliva in the fluoride oxalate test tubes were then spun followed by the analysis.

Determination of salivary and plasma glucose

Glucose oxidase method was employed for the quantitative estimation Salivary and plasma glucose as specified by Randox Laboratories (United Kingdom). The SOP on the Randox Kit

Manuel was strictly followed, except that 0.2 ml was used for the assay of salivary glucose. The 0.2 ml saliva used is due to the low concentration of glucose in saliva as compared to plasma.

Statistical analysis

Pearson correlation and student *t*-test were used for the entire data analysis employing the instrumentality of SPSS program (SPSS Inc., Chicago, IL, USA; Version 15-21). Pearson correlational analysis was utilized to establish the extent of relationship between plasma and salivary glucose. However, Student *t*-test was used for comparison between plasma and salivary glucose, and plasma and salivary glucose in sexes. $P < 0.05$ was considered significant.

Results

Table 1 showed a significant difference between salivary and plasma glucose of controls and diabetics respectively. **Table 2** exhibited significant difference between plasma and salivary glucose between controls and diabetics. **Table 3** showed no significant difference between studied plasma and salivary glucose based on gender. **Table 4** showed that only above threshold salivary glucose correlated with that of plasma.

Discussion

The increasing incidence and complications of diabetes mellitus in Nigeria and the world at large is gradually becoming number one health challenge. This is mostly due to late diagnosis and unawareness. Early diagnosis is pivotal to effective control of diabetes mellitus and its complication. Diagnosis of diabetes mellitus is solely based on glucose analysis in the blood which requires invasive procedure. A lot of persons abscond screening due to the pain inflicted as a result of blood collection. The pain resulting from venipuncture or finger pricking is a major setback to the effective drive of diabetes reduction in Nigeria. Aside from the phobia of pain, the colossal funds allocated for sample collection is outrageous. This study was conducted basically to determine the suitability and desirability of saliva as a diagnostic tool for diabetes mellitus.

The study showed an explicit significant increase ($P < 0.05$) in fasting and postprandial salivary glucose when diabetes mellitus subjects were compared to the controls (**Figure 1**). The same was replicated for fasting and postprandial plasma glucose (**Figure 1**). However, weak correlation was observed using Pearson correlation between plasma and salivary glucose ($r = 0.22$) (**Table 3**). Contrarily, a strong correlation was observed ($r = 0.72$) between plasma and salivary glucose of the sub-group with glucose level above threshold value (**Table 4**). Hence, the finding has indicated that saliva is a suitable sample for the assay of glucose in patients whose glucose levels are above threshold value.

The findings of the study agrees with arrays of researches that reported increases in salivary glucose levels in diabetes mellitus patients in comparison to non-diabetics [8-29]. In terms of significant correlation between blood and salivary glucose, the work partly agrees with [29], though the correlation was observed in patients with blood glucose levels above threshold

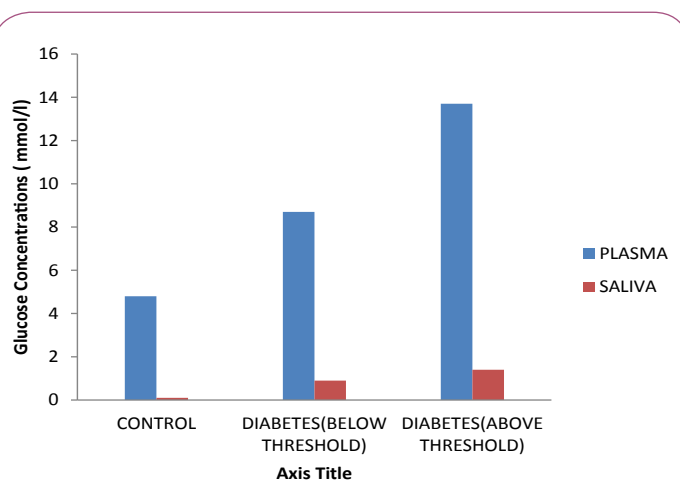


Figure 1 Graphical plot of plasma and salivary glucose in control, below glucose threshold and above glucose threshold subjects. It is showing a graphical relationship between plasma and salivary glucose concentrations in the controls, below glucose threshold and above glucose threshold subjects. The graphical plot showed that salivary glucose was lower than plasma glucose across the three study groups. The above threshold glucose subjects exhibited highest salivary and plasma glucose as compared to other study groups.

Table 1 Comparison of the mean concentrations of plasma and salivary glucose of controls and diabetes mellitus subjects. **Table 1** showed a significant difference between salivary and plasma glucose of controls and diabetics respectively.

Glucose Tests	Plasma	Salivary
Control FG (mmol/l)	4.8 ± 0.7	0.2 ± 0.1
DFG (mmol/l)	11.5 ± 4.2	0.4 ± 0.3
D2HPPG (mmol/l)	14.1 ± 3.1	0.6 ± 0.4

DFG- Diabetics Fasting Glucose
D2HPP- Diabetics 2 hour postprandial
All the data were represented in Mean ± SD. The data comparisons exhibited a significant difference ($P < 0.05$).

Table 2 Comparison of mean concentrations of the types of plasma glucose tests between controls and diabetes mellitus patients. It exhibited significant difference between plasma and salivary glucose between controls and diabetics.

Glucose Tests	Controls	Diabetics
FBG (mmol/l)	4.8 ± 0.7	11.5 ± 4.2
2HPPG (mmol/l)	5.8 ± 2.1	14.1 ± 3.1
FSG (mmol/l)	0.2 ± 0.1	0.4 ± 0.3
2HPPSG (mmol/l)	0.3 ± 0.2	0.6 ± 0.4

FSG- Fasting Salivary Glucose
2HPPSG- 2 Hour Postprandial Salivary Glucose
All the data were represented in Mean ± SD. The data comparisons exhibited a significant difference ($P < 0.05$).

value. Contrarily, the study disagree with [23,30,31] that could not establish a correlation between salivary and serum glucose.

The presence of glucose in saliva is not disputable, but the mechanisms involved in its secretion are the bone of contention in the scientific arena. There are handfults of schools of thought on this issue. According to Qureshi et al. [32] persistent

Table 3 Comparison of gender mean \pm SD concentrations of plasma biochemical. It showed no significant difference between studied plasma and salivary glucose based on gender.

Glucose Tests	Female	Male
FBG (mmol/l)	4.4 \pm 1.2	4.7 \pm 1.8
2HPPG (mmol/l)	6.0 \pm 1.4	6.5 \pm 1.1
FSG (mmol/l)	0.1 \pm 0.1	0.1 \pm 0.1
2HPPSG (mmol/l)	0.1 \pm 0.1	0.2 \pm 0.1

Parameters studied in Control Subjects.

FSG- Fasting Salivary Glucose

2HPPSG- 2 Hour Postprandial Salivary Glucose

All the data were represented in Mean \pm SD. The data comparisons exhibited a non-significant difference ($P>0.05$)

Table 4 Pearson's correlation analysis of the categories of subjects salivary and plasma glucose tests. It showed that only above threshold salivary glucose correlated with that of plasma.

Parameters	n	r
CFG	50	-0.19
DFG	50	0.22
ATFG	19	0.72*

CFG: Control Fasting Glucose, DFG: Diabetics Fasting Glucose, Above Threshold Fasting Glucose, *Significant

hyperglycemia leads to microvascular changes in the blood vessels, as well as basement membrane alteration in the salivary glands. This leads to increased leakage of glucose from the ductal cells of the salivary gland, thereby increasing the glucose content in saliva. Quoting the works of Harrison, commented that glucose is a small molecule that easily diffuses through semipermeable membranes. Thus, large amounts of glucose become available to saliva when blood glucose levels are elevated, as in diabetes. Alterations in the permeability, occurring as a result of basement membrane changes in diabetes, may be an additional explanation for the increased concentration of glucose in saliva. The elevated salivary glucose level in diabetes also confirms the effects of diabetic membranopathy, which leads to raised percolation of glucose from blood to saliva [33-37], thus altering the salivary composition in diabetes mellitus.

The authors of this study believe that the increase in salivary glucose was due to threshold mechanism and basement membrane leakage. The elevation of glucose in the blood above threshold value has the preponderance of causing a spillover effects on other fluids in the body. Most fluids in the body derive its nutrition and excretion from blood. This is suggestive to "leakage" across the basement membrane of the glands, particularly the parotid gland, when blood glucose levels increase beyond threshold value. Therefore, it can be inferred that in non-diabetics, because of the absence of the disease process, there is no change in the basement membrane permeability leading to less amounts of glucose in saliva as seen in control group.

The fact that diabetes mellitus affect both males and females is

not disputable [1]. However, the need for a biochemical profile with respect to gender is apt as gender based or customized medical regime is on the increase [1]. The findings of the study showed that was no significant difference ($P>0.05$) between the various glucose tests between males and females (**Table 3**). The finding has indicated that there is no need for a separate reference range for the management of diabetes mellitus. It further discourages customized diagnostics in the diagnosis and prognosis of diabetes mellitus.

However, it was observed that both serum and salivary glucose were higher in men than women based on the mean value. The finding was similar with some authors who revealed no significant differences in salivary glucose between sexes, although higher salivary glucose levels reported in males when compared with females [38,39].

The higher mean concentration of plasma and salivary glucose observed in males as compared to females is a proof of the vulnerability of male folks to diabetes mellitus. Factors attributed to the vulnerability include sex differences in fat distribution, insulin resistance, sex hormones, and blood glucose levels further support this notion. Males have more abdominal fats than females which is known contributor of diabetes mellitus [38]. On the contrarily, women have more peripheral fat-also denoted as "apple" versus "pear" shape. Thus, the phenomenon that men develop diabetes at a lower body mass index than women [39] can be explained by the fact that men have more visceral fat for a given body mass index than women and thereby a higher relative risk for developing type 2 diabetes. Also, insulin resistance favours men, hence the higher proportion of visceral and hepatic fat compartments associated with insulin resistance [38]. Another factor is the beneficial roles of estrogen to women; after menopause, insulin sensitivity declines, indicating that oestrogen may exert beneficial effect on insulin sensitivity in women. Oestrogen has also a beneficial effect on adipose tissue distribution. The preferential deposition of adipose tissue in the subcutaneous fat compartments in women compared with the visceral compartments in men seem to be related to the higher oestrogen levels in women compared with men [38]. In contrast, testosterone levels are significantly associated with central fat accumulation in both men and women [40].

Conclusion

The aim of the present study was to investigate if non-invasive sample such as saliva could be used in clinical practice as a sample of choice in the estimation of glucose. Consequently, the research revealed that saliva is suitable for diagnosis and management of diabetes mellitus among diabetics whose glucose level is above threshold value. Also, it supported the notion that gender should not be regarded in the management of diabetes mellitus and in customized diagnostics, but showed that diabetes mellitus is more prevalent among the male folks.

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