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SALIVA AND BLOOD GLUCOSE PARAMETRIC ANALYSIS AMONG INDIVIDUALS ATTENDING KENYATTA UNIVERSITY TEACHING REFERRAL AND RESEARCH HOSPITAL IN NAIROBI, KENYA

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ABSTRACT

Background: **Diabetes mellitus is a glucose metabolic disorder which occurs when the body is unable to regulate blood glucose. High blood glucose level is toxic to body organs and leads to organ failure and death. Blood glucose levels are regulated through homeostatic process by glucagon and insulin hormones which act antagonistically.**

Objective: **To determine saliva and blood glucose levels of patients attending Kenyatta University Teaching Research and Referral Hospital in Nairobi, Kenya.**

Material and Methods: **A cross sectional correlational study was carried out with a sample size of 105 participants at the KUTRRH diabetic clinic. Sample size was tabulated from a table published by Karimollah Hajian-Tilaki and confidence interval set at 95% with a significance margin of error at 5%. Blood and saliva glucose levels were determined using Bs120 Mindray biochemistry auto analyzer. Raw data was entered in Microsoft Excel spread sheet and then exported to SPSS version 26.0 for statistical analysis.**

Result: **The mean random saliva glucose levels of diabetic individuals was 0.367±0.040 mmol/l whereas the mean random saliva glucose of non-diabetic individuals was 0.023±0.002 mmol/l ($p<0.001$). The Pearson's correlation coefficient between random saliva glucose and random blood glucose in diabetic and non-diabetics were $r=0.65$ and $r= 0.51$ respectively ($p<0.001$).**

Conclusion: **Our study concluded that saliva specimen have a measurable level of glucose. The levels are different between diabetic and non-diabetic and therefore can be used in assay for screening and diagnosis of diabetes mellitus.**

INTRODUCTION

Diabetes mellitus is a glucose metabolic disorder which occurs when the body is unable to regulate blood glucose. It is a non-communicable disease that causes high health and socioeconomic burden to the affected population (1). All cells in the body require glucose as an immediate principal source of energy. Glucose in the blood is acquired from dietary provision (2). Intestines which form part of digestive system, absorbs glucose and disperse it to all the body cells through bloodstream. Glucose in body cells then undergoes glycolysis for energy provision. Blood glucose levels are regulated through homeostatic process by glucagon and insulin hormones which act antagonistically. This is done through autonomic motor and sensory nerves that control metabolism in the liver, muscle and adipose tissue (3). These two endocrine secretions are released by the pancreas. Glucagon is a function of pancreatic alpha cells secretion whereas insulin constitutes secretions by beta cells (4). When glucose levels in blood are high, insulin hormone is released resulting in change of glucose molecules into glycogen. Alternatively, when the level of blood glucose is low, glucagon is released and stimulates breakdown of glycogen into glucose and increases the concentration of blood glucose (5). Therefore, secretion of these hormones is mainly dependent on blood glucose levels. Diabetes mellitus, generally referred to as diabetes, is a metabolic disorder associated with high glucose in blood circulation. People with diabetes disease do not produce adequate insulin or insulin activity is reduced, leading to high blood glucose. The primary test for diabetes mellitus is HBA1C (6). This blood test does not require the patient to undergo fasting before sample collection. It shows the average blood glucose level relating to the past three months. It is used to determine the percentage concentration of blood glucose found attached to hemoglobin in erythrocytes. Other glucose screen for diabetes diagnosis includes random blood glucose, fasting blood glucose and oral glucose tolerance test (OGTT). RBG and FBG are invasive tests commonly used for diabetes screening, diagnosis and management. (6). One in every nine live new born deliveries in Africa is affected by hyperglycemia during pregnancy.

Kenya has a diabetes prevalence rate of 2.4% (7) .The World Health Organization envisages a rise to 4.5% by 2025. Undiagnosed diabetes creates a public health concern with severe public health consequences especially in Africa and Kenya. It is therefore vital to examine the problem and risk factors for diabetes at national level to advice policy and national programs (7).

According to a previous study, 14% of the population has undiagnosed diabetes while another 18% are considered to be in pre-diabetic phase (8). This study explored saliva as a potential specimen for non-invasive diabetes screening and diagnosis in Kenya. A recent study reported that glucose is a biomarker in human saliva and can be used for diagnoses, management and prognosis of diabetes (9). The high numbers of undiagnosed individuals in the population indicates that there is a need for exploring and developing an easier and effective way for diabetes diagnosis. In addition, a non-invasive way of screening diabetes mellitus will lead to increased efficiency and ensure timely diagnosis. Saliva provides a less cost effective method to screening of large populations. In addition, it can widely be applied for cheap and fast diagnosis of diabetes mellitus (10).

This study finding has enriched the literature on studies conducted in parts of Asia, America and Europe. Previous studies present contradicting findings on the association between saliva glucose and blood glucose.

MATERIALS AND METHODS

Study area: This study was conducted at Kenyatta University Teaching, Referral & Research Hospital (KUTRRH), Kenya. The hospital is a Level 6 Tertiary Hospital offering specialized referral services with a bed capacity of 650 patients. The hospital is located along Northern bypass in Roysambu sub county, Nairobi County.

Study design: A cross sectional design was used in this study where participants were requested to give a sample of both saliva and blood both random and fasting after signing an informed consent form.

Study Population: The study population was defined by the number of patients attending KUTRRH diabetic clinic. KUTRRH offers a diabetic clinic on every Monday with an average of 20 -25 patients per clinic. The study population constituted a large representation of the general population in Kenya. This study recruited 105 participants during the period starting February 2022 to July 2022

Inclusion Criteria:

Willing participants who were diseased (diabetic mellitus patients) herein referred to as DM and their controls (non-diabetic participants) also referred to as NDM were enrolled to the study. The age of participants ranged between 18 years and 90 years with equal representation of both genders.

Exclusion criteria:

Individuals who presented with a history of gum disease, tonsillitis, or inflamed buccal mucosa were excluded from the study because any bleeding would contaminate the saliva sample and interfere with the saliva glucose level. All repeat patients attending the clinic were excluded to avoid selection bias. Volunteers below 18 years and above 90 years were also excluded. Participants unwilling to consent were also excluded.

Sample size determination

Sample size was tabulated from a published table by Hajian-Tilaki. Use of published tables reviewed in a study, can constitute a valid way of sample size determination (11). The table is based on sensitivity and specificity of the gold standard method and allowable margin of error in the research. Both the fasting plasma glucose level and post-prandial plasma glucose levels had sensitivities of (93.47%) and specificities (99.68%), respectively (12). A 95% confidence level was adopted in the study and the allowable margin of error was estimated at 0.05%. The sample size was then tabulated from the table. Tabulated sample size was 50 participants in every stratum of diseased and non-diseased totaling to 100 study subjects.

Table 1

Sample size determination for each study group of both non-diseased and diseased in estimation of diagnostic accuracy (AUC) and marginal errors with 95% confidence level. Published by Karimollah Hajian-Tilaki -2014

AUC	d = 0.03 n	Marginal d = 0.05 N	Errors d = 0.07 N	d = 0.10 n
0.60	665	240	122	60
0.65	648	234	119	59
0.70	620	232	114	58
0.73	596	215	110	54
0.75	576	208	106	52
0.78	540	194	99	49
0.80	510	184	94	46
0.83	458	165	85	42
0.85	418	151	77	38
0.88	347	125	64	32
0.90	293	106	57	27
0.93	202	73	38	–
0.95	137	50	–	–
0.98	42	–	–	–

Laboratory procedures

Participants' recruitment: The purpose of the study was clearly explained to the volunteers at the clinic. Those who accepted to participate were registered with a special research code after signing the consent form.

Blood specimen collection:

Participants were requested to sit comfortably at the phlebotomy chair. Brief description of expected level of pain and duration was explained. Up on composure, phlebotomy site was identified at the median cubital vein of the forearm and disinfected using 70% methylated spirit. Using 5mls syringe and needle gauge 23, five millilitres (5mls) of blood was drawn. Three millilitres (3mls) of the blood was dispensed to an EDTA vacutainer and two millilitres (2mls) dispensed to fluoride vacutainer. Labelling was done using the unique code identifier from the master register for research. Fluoride blood was then centrifuged at 2000 rpm for two minutes and then plasma was harvested for glucose analysis (FBS and RBS) using Mindray Bs 120 Chemistry auto analyser (13).

Saliva specimen collection.

Saliva sample was collected in a wide mouth sterile container. This was done by asking the participants to void approximately 2 mls of unstimulated saliva the container. To achieve consistency in saliva collection, the container was demarcated with an indelible mark for 2mls. For random saliva sample, participants were requested to rinse the mouth with water thrice, and then void approximately 2 mls of saliva sample in the container. Subsequently, coded details of the participants were recorded in the container for confidentiality.

Laboratory analysis

Blood and saliva samples were analyzed in the laboratory using a Bs 120 Mindray automated chemistry machine. Prior to testing, the machine was calibrated using multi sera calibrator and controlled using both level 1 and level 2 commercially acquired control cells from Mindray. The machine had a linearity of up to 36 mmol/l and a glucose sensitivity of 0.0167 mmol/l. In this study, 10 ul of sample were used for each run following the manufacturer's

protocol. The machine was incorporated with Glucose GOD-POD test algorithm. (13)

Sample Preparation

Fasting and random blood samples were centrifuged at 2,000 rpm for 2 minutes to obtain the plasma for analysis. Saliva samples were analyzed directly from collection after a 30-minute liquefaction time. When processing was not done immediately, samples were refrigerated at 2-8 °C for later analysis but not more than 48 hours of collection.

Blood and Saliva Analysis using Mindray Bs 120 Auto analyzer.

Saliva and blood samples were analyzed for glucose level using Mindray Bs 120 Auto analyzer. Glucose level was determined using the glucose oxidase peroxidase method. The output signal is directly proportional to the amount of glucose present in the specimen (13). The test principle is based on the capability of glucose oxidase enzyme to catalyze the oxidation of β- D-glucose to D-glucono- δ-lactone (Gluconic acid) with the release of hydrogen peroxide. An enzyme peroxidase then catalyzes the hydrogen peroxide to release oxygen that in turn oxidizes hydroxybenzoic acid (phenol) and 4-aminoantipyrine to form a quinoneimine which is a red dye. The dye is then quantitatively determined spectrophotometrically at 510 nm. This test is highly specific for β-D-glucose and does not act on α-D-glucose. (14). The reactions that take place are as follows:

$$\text{Glucose} + O_2 + H_2O \xrightarrow{\text{Glucose Oxidase}} \text{Gluconic acid} + H_2O_2$$

$$H_2O_2 + \text{Phenol} + \text{4-aminoantipyrine} \xrightarrow{\text{Peroxidase}} \text{Quinoneimine} + H_2O$$

Saliva glucose analysis

100 mls of saliva was dispensed in a sample cuvette and loaded in a sample tray of the Bs 120 Mindray machine. The sample code was keyed in, glucose test selected, and machine switched on to run the test. A read-out of glucose level was displayed after 15 minutes and recorded in the data collection tool (13).

Blood glucose analysis

100 mls of plasma was dispensed in a sample cuvette and loaded in a sample tray of the Bs 120 Mindray machine. The sample code was keyed in, glucose test selected, and the machine switched on to run the test. After duration of 15 minutes a read out of glucose level was displayed and this was recorded in the data collection tool. (13)

Blood HBA1C analysis

Four microliters (4 ul) of whole blood or EDTA sample was added in the reagent pack provided. The pack was then mixed by inversion five to eight times. The reagent pack was then inserted in the cartridge in the A1C Clover machine. After 5 minutes a read out was obtained. This was recorded in the data collection tool awaiting data analysis (15).

Ethical considerations

This study was approved by Kenyatta University Ethical Review Committee (KU-ERC). A research permit was also obtained from the National Commission for Science, Technology and Innovation (NACOSTI). In addition, a KUTRRH research authorization was issued by the hospital Research and Clinical trials Committee and a permit from the county government of Nairobi and Ministry of education. All the participants were taken through an introduction for purposes of clarity in what the study pertains and informed that the information was confidential and was only used for research purposes.

Data Analysis

Raw data were entered in Microsoft Excel spread sheet and then exported to Statistical

Package for Social Sciences (SPSS) version 26.0 for statistical analysis. Normality of continuous data was determined using skewness and Kurtosis (values between -2.99 to +2.99). Descriptive statistics were expressed as mean, standard error of the mean, frequency, percentages and ranges. Inferential statistics were computed using unpaired t-test for two independent samples, whereas Pearson's correlation analysis was conducted to explore the relationship between the two variables of diabetic and non-diabetic, saliva glucose and blood glucose and fasting sample and random sample. A probability (p) value of less or equal to 0.005 was considered significant. ($p \leq 0.005$). Data were presented in tables, pie charts and graphs. Statistical applications and interpretations were then reviewed by a qualified statistician.

RESULTS

Characteristic of study participants

In this study, a total of 105 individuals attending Kenyatta University Teaching Research and Referral Hospital (KUTRRH) diabetic clinic were enrolled, of whom 54 (51.43%) were female and 51 (48.57%) were male. The participants were 42.91 years old on average, with a standard error of the mean of 1.69 years (range of 21 to 86 years).

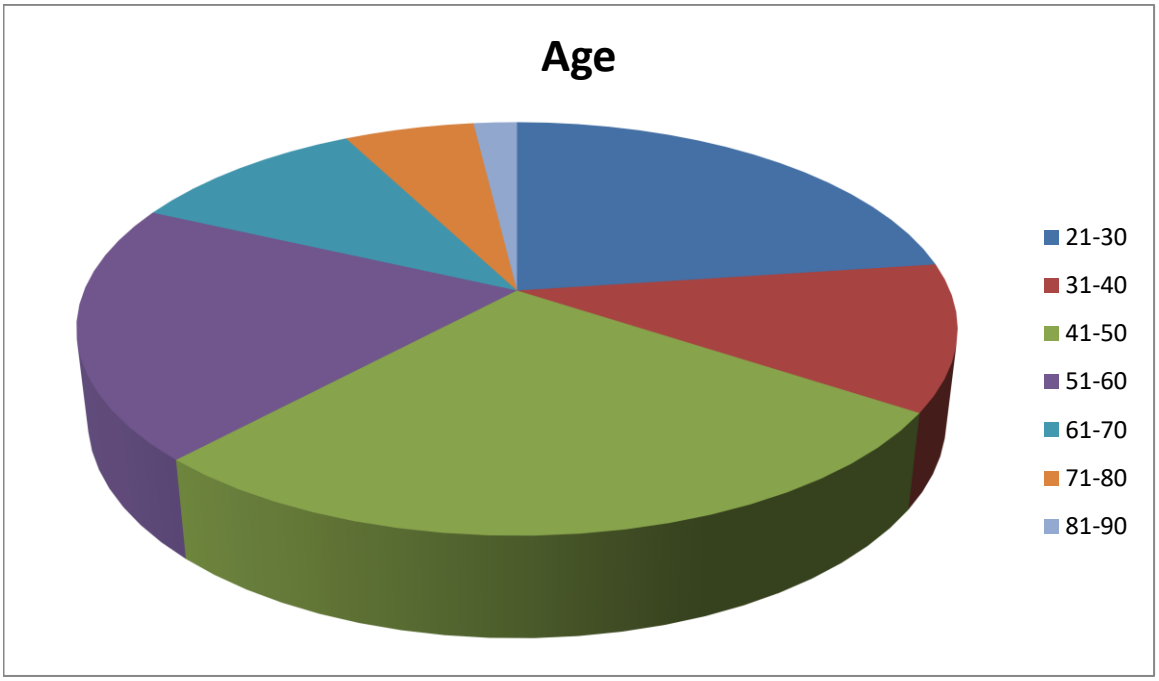

Figure 1 Distribution of age in years among participants.

Figure 1 shows distribution of age of participants arranged in classes from 21-30 years to 81-90years. The youngest participant was 21 years old while the oldest was 86years.

Table 2

Participant demographic characteristics

Variable	Statistics of participants
Gender	
Male	51 (48.57%)
Female	54 (51.43%)
Age (years)	
Mean age	42.91
Standard deviation	17.30
Minimum	21
Maximum	86
Standard error of mean	1.69
Diabetes status	
Diabetic	55 (52.38%)
Non-diabetic	50 (47.62%)
Sample size	105

Frequencies distribution of gender, age in years, and diabetic status. Age is further presented in mean, standard deviation and standard error of the mean.

The study comprised of 55 (52.4%) diabetic participants and 50(47.6%) non-diabetic participants (Table 2).

Table 3

Characteristic of diabetic and non-diabetic participants

Variable	Diabetics	Non-diabetics
Gender		
Male	29 (52.7%)	24 (43.6%)
Female	26 (47.3%)	31 (56.4%)
Age (years)		
Mean age	50.52	32.75
Standard deviation	15.12	15.03
Standard error of mean	2.04	2.03

Distribution in gender and age with respect to diabetic status. The frequencies are tabulated in percentage of total outcome.

As shown in table 3, diabetic male participants were 29 (52.73%), while 26 (47.27%) were female. The diabetic participants also had a mean age and a standard error of the mean of 50.53 and 2.04 years old, respectively. On the other hand, among the non-diabetic participants, 21 (42.00%) were male, while 29 (58.00%) were female. The non-diabetic participants had a mean age and standard error of mean of 32.75±2.03 years old respectively.

Saliva and blood glucose level among patients attending KUTRRH.

The blood glucose levels of random samples from diabetic and non-diabetic participants were 13.49±0.61 mmol/L and 5.57±0.10 mmol/L, respectively. Fasting blood glucose levels in diabetic and non-diabetic individuals were 8.17±0.25 mmol/L and 4.70±0.09 mmol/L, respectively. The fasting blood glucose levels in diabetics were significantly higher compared to those of non-diabetic individuals ($p<0.001$; Table 3).

The random saliva glucose levels in diabetic and non-diabetic participants were 0.367±0.040 mmol/L and 0.023±0.002

mmol/L, respectively. The random saliva glucose levels of diabetic patients were significantly greater than the levels in non-diabetic participants (p<0.001; Table 4). The fasting salivary glucose levels of diabetics and non-diabetics participants were 0.254±0.034 mmol/L and 0.014±0.001 mmol/L, respectively.

Table 4

Blood and saliva glucose levels and HbA1c levels among diabetics and non-diabetics participants

Variables	Diabetics	Non-diabetics	p value
Blood sugars			
Random blood glucose (mmol/L	13.49±0.61	5.57±0.10	<0.001
Fasting blood glucose (mmol/L)	8.17±0.25	4.70±0.09	<0.001
Saliva sugars			
Random saliva glucose (mmol/L)	0.367±0.040	0.023±0.002	<0.001
Fasting saliva glucose (mmol/L)	0.254±0.034	0.014±0.001	<0.001
HbA1C (%)	9.37±0.30	5.07±0.08	<0.001

Values are expressed as mean ± standard error of the mean. Comparison between diabetics and non-diabetics was performed using independent student t-test. p≤0.005 was considered statistically significant.

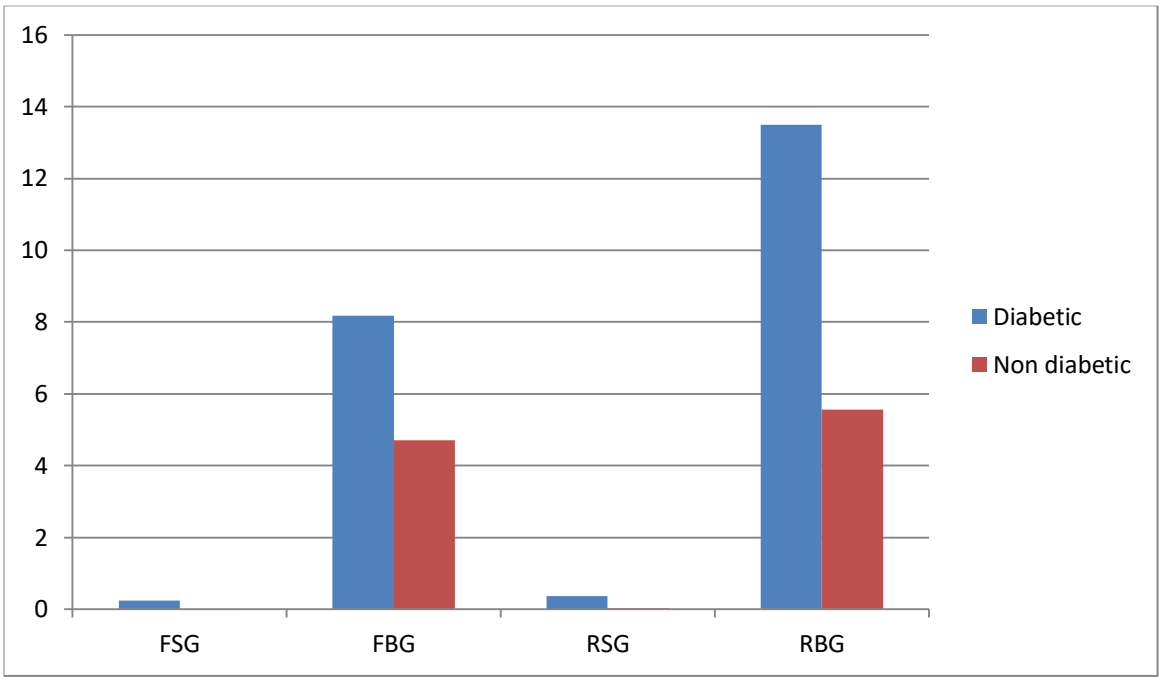

Figure 2 Correlation of mean saliva and blood glucose level in diabetic and Non diabetic

DISCUSSION

Saliva glucose level

Saliva glucose was assayed at fasting and random levels. Random saliva glucose levels in diabetic individuals had a mean of 0.367±0.040mmol/L while the random saliva glucose of non-diabetic individuals had a mean of 0.023±0.002mmol/L. The random saliva glucose levels of diabetic patients were significantly greater than those measured in non-diabetic participants (p<0.001). Fasting saliva glucose levels of diabetic individuals had a mean of 0.254±0.034mmol/L while the non-diabetic counterpart had a mean level of 0.014±0.001mmol/L. The fasting saliva glucose levels of diabetic patients were substantially higher than those measured in non-diabetic individuals (p<0.001). However, in both fasting and random saliva

glucose, the P values were less than 0.05 and therefore significant for the study. The presence of glucose in the saliva of non-diabetic individuals as well as diabetic patients has been attributed to both paracellular and intracellular pathways (16). The findings of this study also conform to a study done by Gupta which concludes by noting that saliva glucose may serve as a potentially non-invasive marker to monitor glycemic control in diabetic patients (17). In a more recent study, Shahin (18) noted that salivary glucose levels, showed a significant elevation in the saliva of diabetic cases as compared to controls (3.67 vs. 0.8 mg/dl respectively– $p < 0.001$). This study findings also concurred with another study which reported that salivary glucose had mean values of 2.1 and 0.813 mg/dl in cases and controls respectively, with highly significant difference between the two groups ($p < 0.001$) (19). However, the mean saliva glucose in this study was low (0.014mmol/L) as compared to the outcome of a study by Shahbaz et, al, which was (0.045mmol/L) (21). This was probably due to the fact that the two studies used different approach in sampling (20) and also partly due to use of different machines with different linearity range for analysis of glucose. Serdar in his study notes that the use of unstandardized sample size may yield different outcomes in research. (20).

CONCLUSION

The findings in this study have shown that saliva glucose is measurable among patients attending KUTRRH albeit too low. Saliva glucose can therefore be assayed for screening, diagnosis and monitoring of diabetes. However, due to low saliva glucose levels obtained especially in non-diabetic saliva fasting glucose, this study recommends further studies and research to ascertain the outcome.

Limitations

This study encountered a challenge when performing assays that include a repeat visit. This is due to the fact that trust is bestowed upon the participant to come for a repeat without fail while fasting. Again the levels of saliva glucose are too low and presented with difficulty when assaying due to machine linearity.

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