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Evaluation of the Efficacy of Saliva as a Specimen in the Estimation of Biochemical Analytes in Diabetics.

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Abstract

Diabetes requires invasive blood sampling, making diagnosis and routine monitoring challenging. Saliva as a biological fluid is gaining popularity in monitoring health and diagnosis procedures. This study examined the suitability of saliva as a specimen for the analysis of specific analytes in diabetics.

A control group of 25 healthy individuals and 45 diabetics subjects were enlisted. Blood and unstimulated saliva samples were taken from each group. The enzymatic method was used to estimate salivary and plasma glucose levels, while the chemical method was used to determine albumin and total protein levels. A p-value of >0.05 was deemed statistically significant after the collected data were analyzed using the statistical package for social sciences (SPSS) 26. The fasting salivary glucose level in the test group was significantly higher than in the control group (p=0.01). The albumin and total protein levels did not differ significantly (p=0.38, p=0.93). Total protein, albumin, and postprandial salivary glucose levels were considerably higher in the test group than in the control group (p = 0.00). Saliva and plasma albumin levels in the test group showed a strong positive correlation (r=0.250, p=0.05). The receiver operating curve (ROC) provided good specificity and sensitivity in postprandial glucose and fasting saliva. This study shows new dimensions in using saliva as a specimen for diabetics and underscores its value in estimating biochemical analytes. Saliva proves important because its potential can be harnessed in point-of-care testing.

Keywords: Analytes, blood, diabetes, saliva, specimen.

Introduction:

Diabetes is a chronic condition that requires regular monitoring. This makes it challenging for both patients and healthcare professionals (Mrag *et al.,* 2020). Monitoring of biochemical analyte levels is essential in both health and disease. Blood is the most used fluid for laboratory diagnosis, and it is an exact medium that reflects the quality and quantity of various bodily analytes. Even in antiquity, blood collection remains an invasive procedure that poses potential risks to subjects, including transient discomfort, bruising, and infection at the venipuncture site, in addition to the possibility of anaemia depending on the amount of blood required (Williamson *et al.,* 2012).

Glucose is a ubiquitous and indispensable analyte mostly estimated in the blood to diagnose and monitor numerous disease conditions including diabetes. Maintaining adequate blood glucose levels is vital for survival (Giridharan, 2018). Drawing blood to monitor blood glucose levels is the traditional practice and abnormal glucose levels are a primary symptom of diabetes, a major public health problem. Insulin and glucagon are two hormones that interplay strictly to regulate and maintain glucose levels in the blood (Matschinsky and Wilson, 2019).

A plethora of diseases are typically evaluated, diagnosed, or monitored with serum total protein and albumin estimation. Protein plays a crucial

part in human biochemistry and is a building block in the body. Consequently, protein deficiency or excess leads to disease or even death (LaPelusa and Kaushik, 2021).

Routine monitoring of patients at frequent intervals with blood collection over time has increased trauma to patients (Puttaswamy *et al.,* 2017). Given the projections of a rapid rise in diseases, the continuous use of an invasive method may delay effective diagnosis. The advent of an easy, less expertise, non-invasive diagnostic tool with reduced interferences may proffer better solutions.

There is a need for a non-invasive procedure that can improve the evaluation of biological analytes, and saliva as a diagnostic tool could be a promising approach. In the past few decades, research has focused on alternative methodologies that involve using other body fluids as substitutes for blood for diagnostic purposes. Saliva is one of the most important of these which has been studied widely as a probable diagnostic tool (Lasisi and Lawal, 2019). Using saliva in diagnosing systemic diseases such as diabetes provides several advantages over blood since it is noninvasive, cost-effective, and easy to collect and store (Kumar *et al.,* 2024). There is dearth of data on saliva in samples in this region. This study aimed to assess the suitability of saliva as a potential biological sample for biochemical analyte estimation among diabetics.

Methods:

Study design: This was a comparative cross-sectional study. The sociodemographic and clinical information were obtained through a structured questionnaire. The study was in a tertiary healthcare in Sokoto, Nigeria. Ethical approval for the study was obtained from the Usmanu Danfodiyo University Teaching Hospital Health Research Ethics Committee (UDUTH/HREC/2021/V5). All the subjects gave written informed consent. The study was carried out between October 2021 and January 2022.

Sample size: The study sample size was determined using the standard formula for minimum sample size calculations (Charan and Biswas, 2013). The sample size was 45, hence 45 known diabetics and 25 healthy students were recruited as test and control respectively for the study.

Study subject selection: Diabetes patients aged 18 years and above attending Usmanu Danfodiyo University Teaching Hospital, Sokoto were included in the testing subjects while patients with salivary gland surgeries or those who refused to give a written informed consent were excluded.

Sample collection: Fasting and post-prandial saliva and blood samples were collected. Before sample collection, subjects were asked to fast overnight for 8 to 10 hours. Fasting unstimulated whole saliva and blood samples were collected through drooling or spitting and venipuncture, respectively.

Subjects were instructed to wash their mouth thoroughly with distilled water 2 to 3 times to prevent contamination and dilution by oral cavity and saliva. The subject sat in an upright position and a labeled wide-mouth sterile container was given. The subjects were instructed to keep their mouth open to pool the saliva on the floor of their mouth and to spit into the clean sterilized vial container gradually at the end of every 60 seconds for five minutes.

Blood samples were collected into lithium heparin and fluoride oxalate tubes. Saliva and blood containers were labeled properly, and patients' confidentiality was ensured. Before analysis, samples were refrigerated at 2^{o}C to 8^{o}C.

Sample processing: Collected samples were transferred into a centrifuge tube and centrifuged at 3000 revolutions per minute (rpm) for 10 minutes to obtain clear supernatant. The supernatant was subjected to glucose, protein, and albumin estimation using glucose oxidase-peroxidase, biuret, and bromocresol green (BCG) methods to estimate glucose, total protein, and albumin, respectively.

Data analysis: Data was entered in an Excel worksheet and analyzed using the Statistical Package for Social Sciences version 26.0 (SPSS). Data was expressed as means ± Standard

Deviation (SD), and Receiver operating characteristics (ROC) analysis was used to determine the sensitivity and specificity of saliva. Paired t-test and Pearson's correlation were used.

Results:
Table I shows the socio-demographic distribution among the study subjects. The highest frequency was among the age groups 51-60 and 21-30 for the test and control, respectively.

Table 1: Sociodemographic distribution of the study subjects.

Variables	Test n=45 (%)	Control n=25 (%)
Age Groups (Years)		
11-20	0(0.0)	2 (8)
21-30	5 (11.1)	23 (92)
31-40	10 (22.2)	0 (0.0)
41-50	9 (20)	0 (0.0)
51-60	11 (24.4)	0 (0.0)
61-70	9 (20)	0 (0.0)
71-80	1 (2.2)	0 (0.0)
Gender		
Male	18 (40)	20 (80)
Female	27 (60)	5 (20)
Ethnicity		
Hausa	33 (73.3)	8 (32)
Yoruba	4 (8.9)	3 (12)
Igbo	3 (6.7)	5 (20)
Others	5 (11.1)	9 (36)
Occupation		
Civil servant	9 (20)	1 (4)
Public servant	9 (20)	0 (0.0)
Business	5 (11.1)	0 (0.0)
Housewife	12 (26.7)	0 (0.0)
Student	1 (2.2)	24 (96)
Others	9 (20)	0 (0.0)
Educational Background		
Tertiary	21 (46.7)	23 (92)
Secondary	18 (40)	2 (8)
Primary	6 (13.3)	0 (0.0)
Marital Status		
Single	1 (2.2)	25 (100)
Married	43 (95.6)	0 (0.0)
Widowed	1 (2.2)	0 (0.0)

Key: n number of subjects

Table 1: Sociodemographic distribution of the study subjects.

Table 2 shows fasting plasma and salivary glucose, total protein, and albumin levels among test and control subjects. The mean ± SD of fasting plasma glucose in the test was significantly different compared to the control. The mean ± SD of fasting plasma total protein in the test was significantly different compared to the control.

Table 2: **Fasting plasma and salivary glucose, protein, and albumin levels among test and control subjects**

Parameters	Test (n=45)	Control (n=25)	p-value
Plasma			
Glucose(mg/dl)	106.28±41.86	41.68±42.03	0.00*
Protein(g/dl)	6.04±1.70	6.07±0.86	0.00*
Albumin(g/dl)	3.57±0.62	3.92±0.62	0.08
Saliva			
Glucose(mg/dl)	19.88±16.92	12.25±10.38	0.01*
Protein(g/dl)	1.04±3.98	0.53±0.57	0.38
Albumin(g/dl)	0.13±0.11	0.13±0.11	0.93

Key: Values are expressed as mean±S.D. *p-value $\leq$ 0.05 was considered statistically significant. n= number of subjects.

The mean ± SD of postprandial plasma glucose, total protein, and albumin in the test was significantly different from the control (Table 3), and the mean ± SD of postprandial salivary glucose, total protein, and albumin in the test was also significantly different from the control.

Table 3: Postprandial Plasma and Salivary glucose, protein, and albumin levels among test and control subjects

Parameters	Test (n=45)	Control (n=25)	p-value
Plasma			
Glucose(mg/dl)	143.03±49.49	93.60±13.04	0.00*
Protein(g/dl)	6.61±1.76	7.38±0.57	0.00*
Albumin(g/dl)	4.50±0.57	4.36±0.45	0.00*
Saliva			
Glucose(mg/dl)	23.5±18.42	18.6±3.27	0.00*
Protein(g/dl)	1.09±0.73	0.75±0.60	0.00*
Albumin(g/dl)	0.21±0.12	0.21±0.09	0.00*

Key: Values are expressed as mean±S.D. *p-value $\leq$0.05 was considered statistically significant. n= number of subjects.

The estimation of plasma glucose, total protein, and albumin with the corresponding saliva analytes was done with linear regression, as shown in the equations indicated in Figures 1, 2, and 3, respectively.

Table 4 shows ROC analysis in determining the sensitivity and specificity of saliva using glucose levels. The graph (Figure 4 and 5) shows the sensitivity and specificity of fasting and postprandial glucose levels in saliva, respectively. The fasting value in the area under the ROC curve is 0.623 with a p-value $\leq$ 0.05 and the postprandial value in the area under the ROC curve is 1.000 with a p-value $\leq$ 0.05. The cut-off value of fasting glucose is 2.7 and postprandial glucose is 45.8. The ROC curve shows 100% sensitivity and specificity in glucose detection.

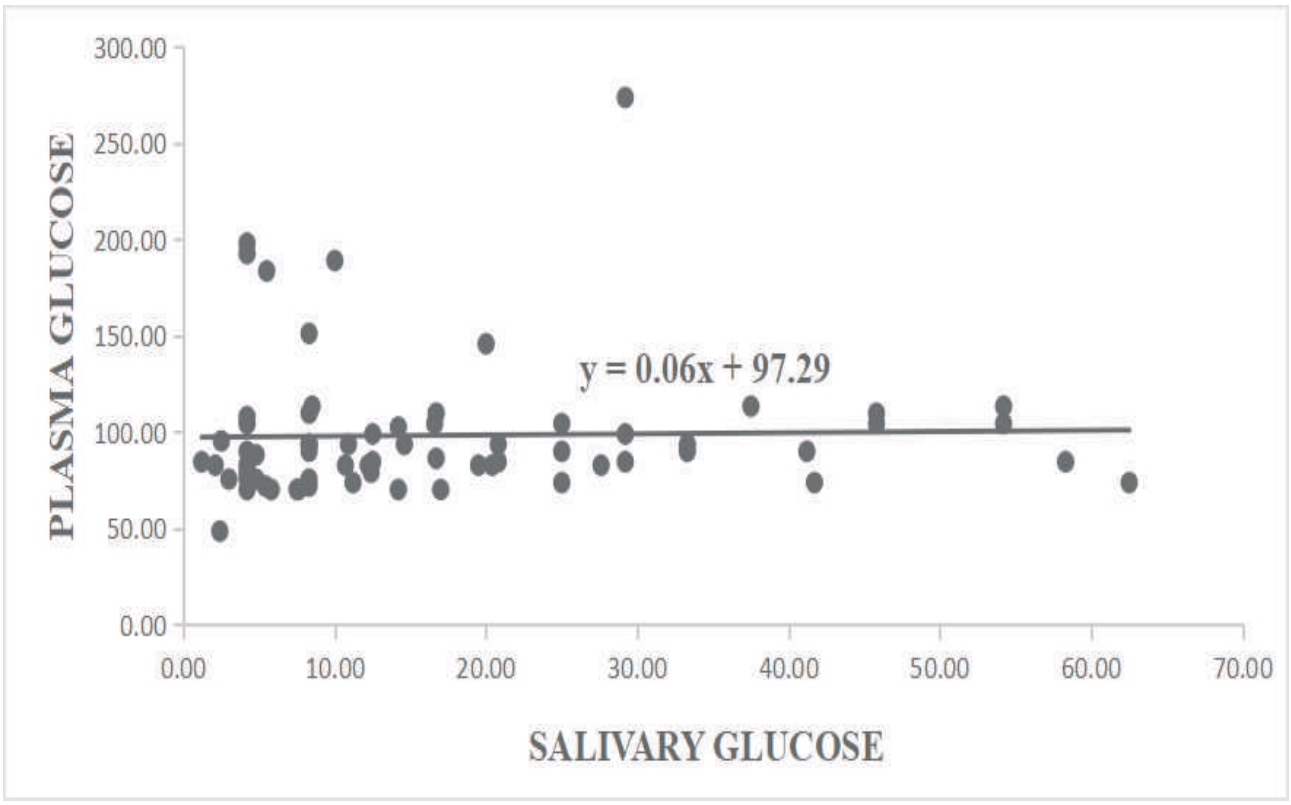

Figure 1: Correlation between salivary and plasma glucose of test and control subjects

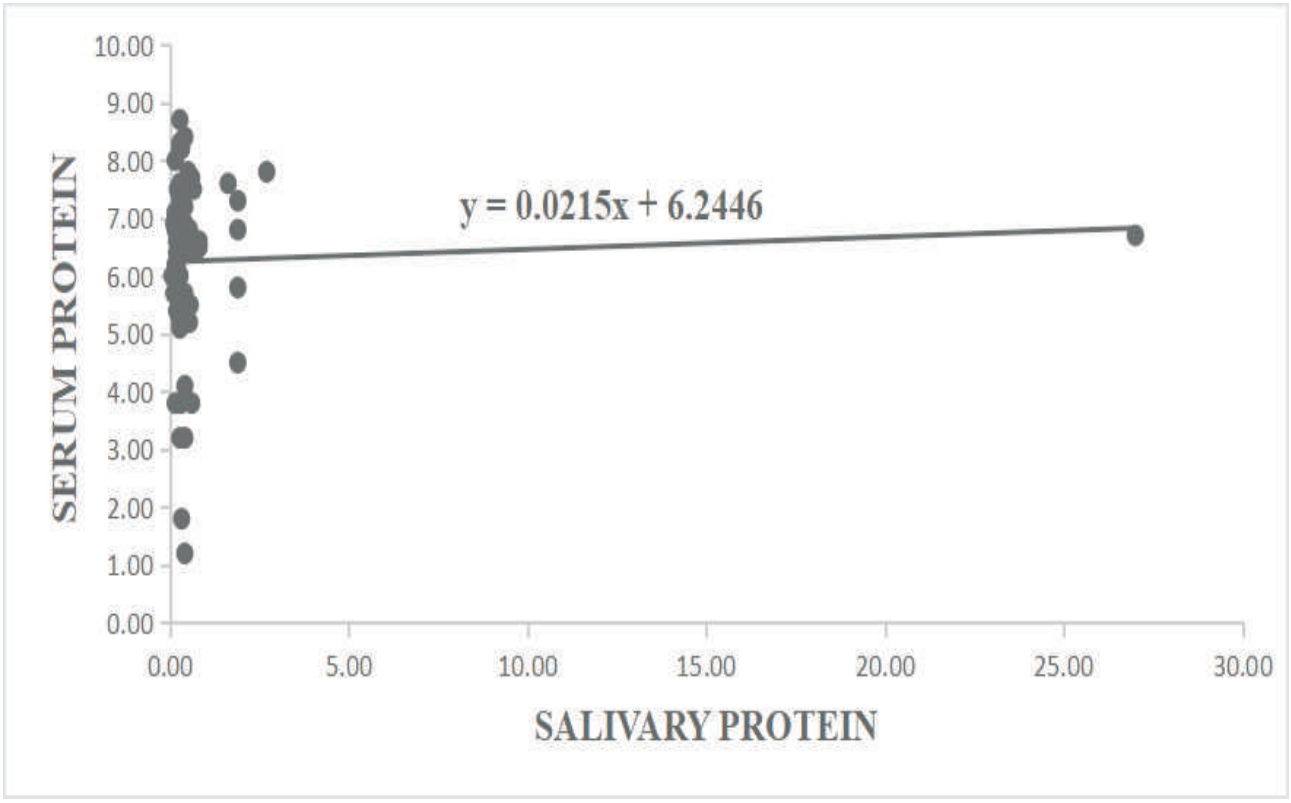

Figure 2: Correlation between salivary and serum total protein of test and control subjects

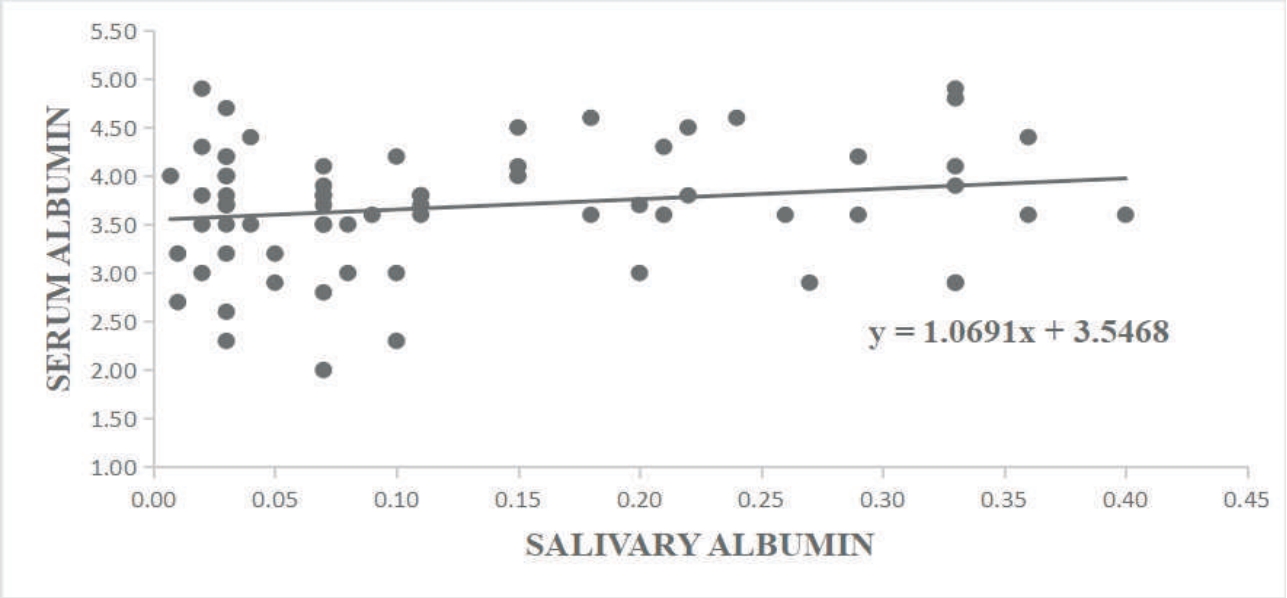

Figure 3: Correlation between salivary and serum albumin of test and control subjects

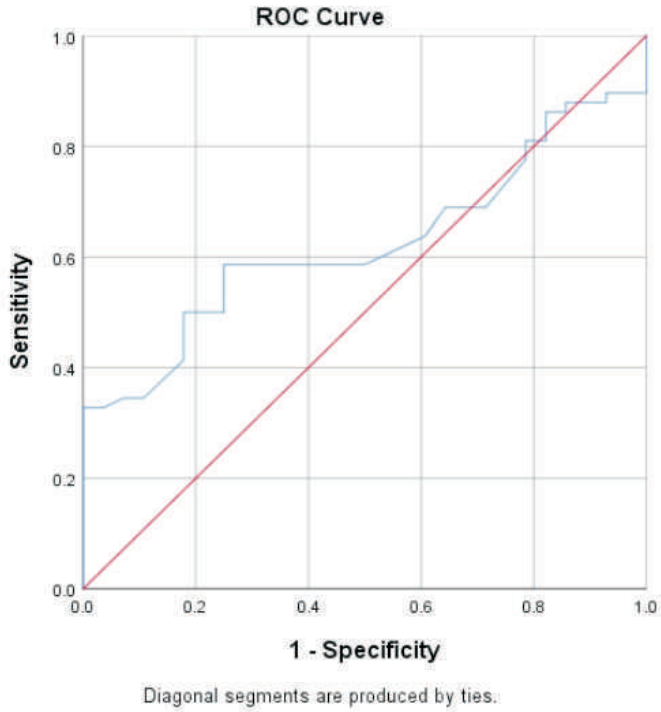

Figure 4: Receivers operating curve (ROC)Analysis

Table 4: Receivers Operating Curve (ROC) Analysis (Fasting saliva)

The area under the curve	95% Cl		p-value
	LB	UB	
0.623	0.507	0.739	0.000

Key: UB= upper boundary, LB= lower boundary

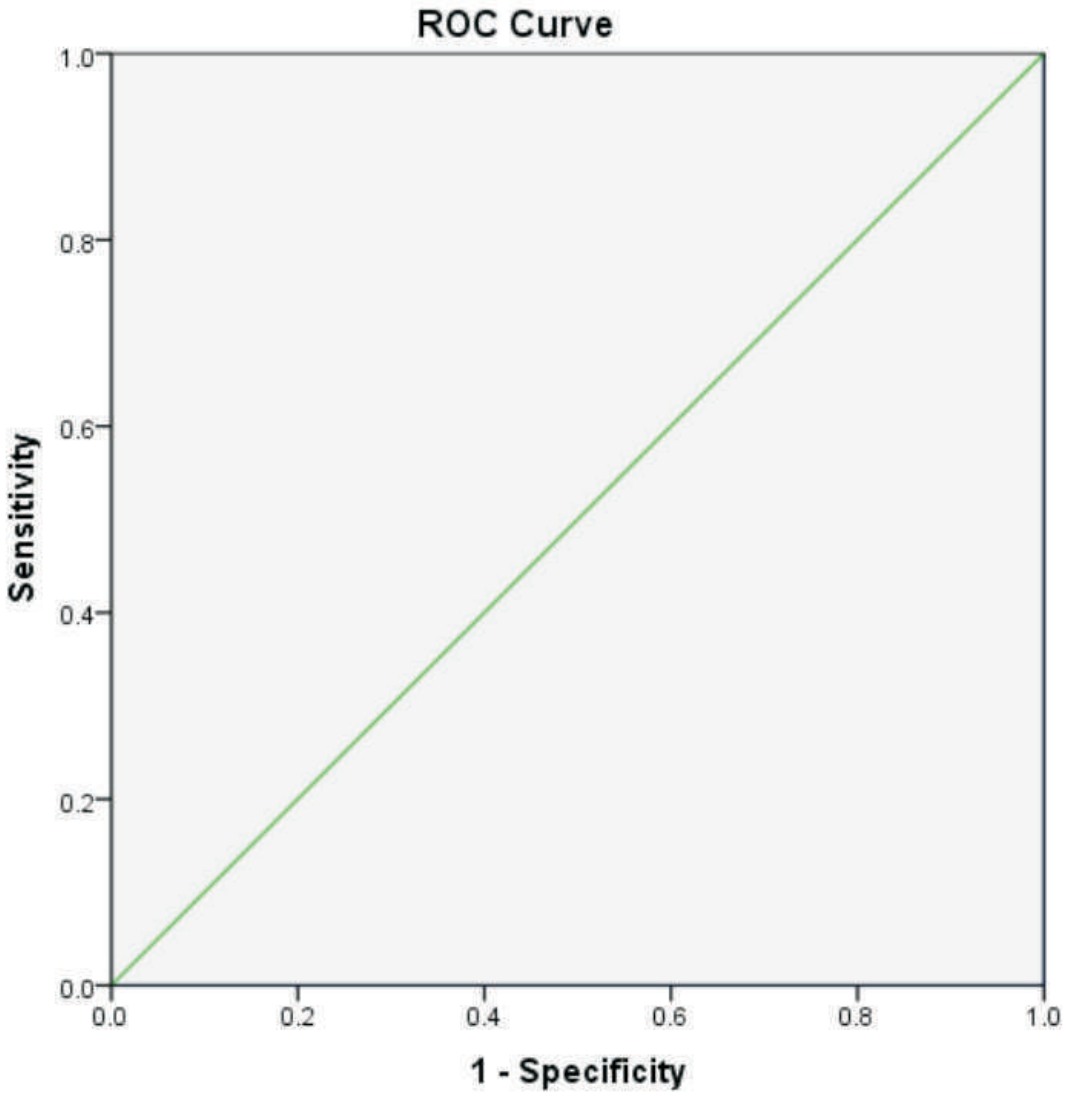

Figure 5: Receivers Operating Curve (ROC) Analysis

Table 5: Receivers Operating Curve (ROC) Analysis (Postprandial saliva)

Area under the curve	95% Cl		p-value
	LB	UB	
1.000	1.000	1.000	0.005

Key: UB= upper boundary, LB= lower boundary

Discussion:

Blood has long been the predominant body fluid used in the medical laboratory for diagnosing, monitoring, and follow-up diseases. Although saliva and urine are relevant, no proper document has shown an established cut-off for each analyte especially for the inexpensive, readily available, and non-invasive sample-like saliva.

With its invasive nature, blood drawing has been shown to cause discomfort, anxiety, and sharp pain in patients. Recently, there has been an increasing interest in salivary biomarkers in monitoring the onset, progression, and treatment outcomes, promoting health and well-being (Pérez-Ros *et al.,* 2021).

This study showed that fasting salivary glucose was significantly higher in test subjects when compared to the control which agrees with the work of Mrag and Friends (2020). This increase is possibly due to increased permeability of the basement membrane and leakage of the microvasculature which allows easy access of glucose into saliva (Kadashetti *et al.,* 2015). However, the study found no significant difference in fasting salivary total protein between diabetics and healthy individuals which contrasts the work of Naseri and colleagues (2018). Preanalytical factors like the medium and time of storage of the saliva sample could have been responsible for the discrepancy. This study also revealed that fasting salivary albumin was not significantly different between test and control subjects. This finding is consistent with a previous report (Hasan *et al.,* 2017).. This decrease may be due to a fall in serum albumin leading to the concomitant low level in salivary concentration, buttressing the hypothesis that some analytes have nearly similar concentrations in both serum and saliva.

The outcome of the study demonstrated a considerable rise increase in both saliva and blood levels of glucose, total protein, and albumin in both the test and control groups, which is consistent with the research conducted Abd-Elraheem and Mansour (2017) on postprandial blood and salivary glucose. However, the incremental levels of both salivary and blood glucose, total protein, and albumin did not display a uniform pattern two hours after consuming the meal, suggesting that the meal contained different constituents.

The established cut-off values of the salivary fasting and postprandial glucose analyte could be exploited in diagnosing and monitoring diabetes mellitus, a complex metabolic disease. In this study, values above 2.7 and 45.8 mg/dL for fasting and postprandial saliva samples, respectfully, buttress the assertion that saliva is a suitable sample for measuring glucose. Hence, saliva can be claimed to be a diagnostic fluid with a hedge over blood because of ease in its collection and availability.

Additionally, the ROC curve reiterates the sensitivity and specificity of saliva as a potential biological sample for diagnosis, yielding a sensitivity and specificity of 100%. To our knowledge, no cut-off value for fasting and postprandial salivary glucose has been reported in Nigeria. The expression of glucose, total protein, and albumin in saliva plays an important role in describing a patient's status in a physiologic and pathologic state, as demonstrated by the findings of this study. Hence, saliva could give a reliable and reproducible glucose value that mirrors the blood constituents. However, the study also had limitations, including a lack of individual cooperation and an unwillingness to participate due to misunderstandings regarding salivary analysis. The study findings showed that new dimensions in using saliva as a biological sample in diagnosis prove an important factor because of its potential to display biochemical analytes in human serum and its suitability in point-of-care testing.

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Conflicts of Interest: None

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