Predictability of Type II Diabetes Mellitus from Salivary Surrogate Markers

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ABSTRACT

Introduction: In medicine, one of the oldest known ailments of defiant carbohydrate regulation is Diabetes Mellitus (DM). In less sophisticated centers, DM Diagnostic measures have reportedly become tedious as it is invasive; often worsening other ailments in trypanophobics. Umpteen times, studies have continuously attempted to devise non-invasive, diagnostic alternatives in an attempt to addressing the issue, hence the current study. In this study, we investigated the possibility of predicting serum sugar levels in type II diabetics, using selected salivary surrogate markers; Na^{+}, K^{+}, Cl^{-}, Ca^{2+} and Mg^{2+}, C-Reactive Protein (CRP), Glucose, and α-amylase.

Materials and Methods: 213 candidates; 173 Diabetics (Experimental group) and 40 non-diabetics (Control group) were recruited for the study. For each subject, saliva and serum were obtained, assayed, and compared for aforementioned electrolytes, glucose and protein levels. Whilst comparing differences in means between obtained variables for experimental and control groups, using the student t-test. Statistical measures of association (Correlation) and predictions (Regression Analysis) were also performed to determine the relationship between saliva and serum bioanalytes. While establishing equations for predicting serum bioanalytes from known salivary values.

Results: The study found a statistically significant increase in salivary electrolyte levels in diabetics, as well as an increase in salivary and serum levels of α-Amylase, CRP, and fasting glucose in the same subjects.

Conclusion: These salivary surrogate markers in diabetics could serve as a non-invasive means of predicting serum levels of the bioanalytes, especially glucose levels which is the hallmark for making a diagnosis of DM.
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Key words:
C Diabetes Mellitus, Salivary Surrogates, C-reactive protein, Alpha amylase

INTRODUCTION

Diabetes mellitus (DM) is an endocrine disease that develops as a consequence of lack of insulin (type 1 DM) or peripheral resistance to insulin (type 2 DM). It is characterized by relative or absolute insufficiency in insulin secretion and/or concomitant resistance to the metabolic action of insulin on target tissues.[1] It is a systemic disease that can affect every system of the body,[2] with hyperglycaemia being its major hallmark.

Hyperglycaemia is known to cause advanced glycosylation end products (AGEs),[3] among which HbA1c (Haemoglobin glycosylation) is used for monitoring long term glycemic control.[4] Several studies have shown glucose to be a key component in the biosynthesis of saliva and body fluids, even though numerous electrolytes (Na^+, K^+, Cl^-, and HCO_3^-, Mg^{2+} and Ca^{2+}) have also been implicated. However, these electrolytes are accountable for the osmolarity and buffering of body fluids, causing them to maintain pH, with the goal of keeping the integrity of the body's epithelial lining.[5] For saliva, this compositional functions are also incomplete in the absence of salivary glucose and protein, which are constituents of glycolipids in mucous surfaces; making them flexible, fluidy and polarized. With normal saliva production though, Alamad(2019) found that absence of such glycolipids in oral epithelium may result in hypo-salivation.[7]

Studies have suggested that protracted hyperglycaemia may impair salivary gland functions, leading to reduced salivary flow and changes in saliva composition. Consequently resulting in dental and mucosal proliferation of various pathogenic microorganisms, taste alterations, burning mouth sensation, dental caries, coated tongue and halitosis.[8] With the growing interest in non-invasive diagnostic medicine, researchers desire in studying the similarities between salivary and serum biomarkers as a diagnostic tool for systemic diseases has increased.[9] Numerous reports on human saliva compositions have been closely linked to body fluid electrolytes and glucose constituents, and may provide helpful clues to the estimation of serum glucose levels by non-invasive means.[10,11,12] Several reports have also envisaged the non-invasive possibility of predicting human blood groups from "saliva prints".[13, 14, 15] This study was conducted to confirm these assertions and to establish a verifiable means of extrapolating serum markers of (type 2) diabetes mellitus (glucose, electrolyte and surrogate analytes) from the obtained saliva levels.

This study evaluated biomarkers (electrolytes, glucose, amylase, CRP) of type 2 diabetes in both serum and saliva with the hope of generating regression equations which can be used to predict serum markers when saliva values are known.

MATERIALS AND METHODS

Study Design

The study was a randomized experimental trial, carried out in General Hospital, Warri, Delta State, Nigeria. Diabetics who visit the general hospital regularly for checkup were the targeted population and from records available in the diabetic unit of the hospital, it's about 5,824 patients per annum. Adopting standards from the American Diabetes Association, 40 apparently healthy subjects (control) and 173 diabetics (Experimental group) between ages 25-65 years were recruited. For each subject, saliva fluids were assayed for constituent electrolytes, proteins, and sugar levels, and mapped against their corresponding serum levels for same variables.

Ethical approval was obtained from the office of the Chief Medical Director, General Hospital Warri, and a letter given (PROTOCOL NUMBER: CHW/ECC VOL 1/173; Registered on 9th of October, 2018). Verbal consent was obtained from each of the participants and study lasted for 24 weeks.

Sample and Sampling Technique

Using the random sampling technique, a total of 173 participants (diabetics) were drafted from aforementioned population, with 40 control(non-diabetics) subjects. The decision to sample subjects (sample size) was informed by the statistical relation employed by Singh and Masuku, (2014)

$$n = \frac{(Z\,\alpha/2)\,2\sigma 2}{d2}$$

Where Z α= standard normal deviate at 95 % confidence interval = 1.96

σ = standard deviation of the characteristic of interest in the target population

d = the margin of error = 0.02

Substituting the values in the above formula, the sample size (n) was calculated as

$$n = \frac{1.96\ x\ 1.96\ x\ 0.12\ x\ 0.12}{0{,}02\ x\ 0.02} = 138.29 = 139$$

To make up for attrition, 10 % of the sample size was added to make up for non-responses which was 13.83 =14 = 153.

However, a total of 173 diabetic subjects were enrolled for the study (To be able to adjust for attrition).

Inclusion Criteria

Subjects who volunteered to participate in the study were selected. This selection was based on the knowledge that selected subjects were certified type 2 diabetics by their physicians, and between the ages of 25-65 years, irrespective of gender. Non-diabetic (65-90 mg/dl) subjects in apparently good health were included as controls.

Exclusion Criteria

Patients suffering from type 1 diabetes mellitus, or confirmed pregnant, physically and/or mentally challenged were excluded from the study. Individuals with chronic renal failure, hyperthyroidism, pancreatitis, pancreatic cancer or hypercholesterolemia were also excluded from the research. Those under medications like steroids, tricyclic antidepressants, epinephrine, diuretics, oestrogen, lithium, and salicylates, were also excluded.

Saliva Collection

Two millilitres (2ml) of unstimulated saliva samples were taken between the hours 7.00am and 8.00am from the subjects after an overnight fast. The participants were asked to produce sputum (after rinsing their mouths with deionized water) into plastic vials[17]. Obtained saliva samples were then centrifuged at 6000 rpm for 10 minutes before use and the supernatant analysed immediately.

Serum Collection

Five milliliters (5ml) of subjects' intravenous blood was obtained from the median cubital vein of the cubital fossa in their forearm, using a 5ml disposable syringe. The blood sample was collected and transferred into a fluoride oxalate tube and a plain container which was centrifuged at 6000 rpm for 10minutes to obtain plasma and serum, respectively.

Determination of Salivary and SerumBio-analytes Concentration

Salivary and serum glucose [18,19], amylase[20], CRP[21], and electrolytes[22,23,24] were estimated using standard procedures and documented methods.

Statistical Approach

Statistical measures of central tendencies (means) and dispersion (standard deviations) were performed to compare and contrast between measured variables. Level of significance for all test calculations was set at p values < 0.05. One-way analysis of variance (ANOVA) was performed in order to check differences in mean values between the assayed groups. Correlation and regression analyses were conducted so as to correlate serum and saliva parametric values and be able to predict serum data from established saliva values by the fitted regression equations.

RESULTS

Table 1: Correlation of Saliva and Serum Bio-Analytes of Diabetics and Non-Diabetic

Bio-Analytes	Diabetics (Exp. Grp)			Non-Diabetics (Control Grp)		
	Saliva	Serum	r	Saliva	Serum	r
Electrolytes						
Na+ (mmol/L)	139.71±3.21	141.11±4.42	-0.239	81.22±1.64	86.22±2.52	-0.013
Cl- (mmol/L)	24.11±12.08	98.31±2.66	-0.091	21.00±9.68	91.92±1.91	-0.016
K+ (mmol/L)	14.67±3.95	6.94±1.03	0.011	18.40±7.74	3.95±0.67	-0.060
Ca^{2+}(mmol/L)	2.23±0.87	10.24±1.41	-0.047	1.04±0.99	7.10±1.01	0.251
Mg^{2+}(mmol/L)	0.90±0.11	8.33±4.30	0.138	0.01±0.02	4.22±1.61	-0.045
Amylase (U/L)	153.26±56.1	90.22±11.00	0.019	129.20±19.80	68.03±2.21	0.030
Glucose (mg/dl)	0.9±0.34	231.31±90.90	-0.039	0.4±0.02	86.38±6.63	0.133
CRP (mg/L)	9.42±2.83	10.61±1.93	-0.076	2.89±0.84	2.92±0.08	0.245

Above table compares saliva and serum bioanalytes for diabetics and non-diabetics. Here, saliva and serum values are expressed as mean± standard deviation. r = pearson product moment correlation coefficient (PPMCC). From PPMCC, negative r values indicate negative correlation, while positive values mean positive correlation. Again, r values > .05 indicate weak correlation, and r < .05 means strong correlation

Table II: Comparing the Means of Saliva and Serum Bio-Analytes in Diabetics

Bio-Analytes	Saliva	Serum	p-value (ANOVA) R		r (PPMCC)	Remark
Na+**(mmol/L)**	139.71±3.21	141.11±4.42	0.003	Sig	-0.239	NC
Cl-**(mmol/L)**	24.11±12.08	98.31±2.66	0.054nSig		-0.091	NC.
K+**(mmol/L)**	14.67±3.95	6.94±1.03	0.105nSig		0.011	PC.
Ca^{2+}**(mmol/L)**	2.23±0.87	10.24±1.41	0.004	Sig	-0.047	NC.
Mg^{2+}**(mmol/L)**	0.90±0.11	8.33±4.30	0.018	Sig	0.138	PC.
Amylase (U/L)	153.26±56.1	90.22±11.00	0.009	Sig	0.019	PC.
Glucose (mg/dl)	0.9±0.34	231.31±90.90	0.026	Sig	-0.039	NC.
CRP (mg/L)	9.42±2.83	10.61±1.93	0.040	Sig	-0.076	NC.

Serum and Saliva values are expressed as mean± Standard Deviation. r*values are expressed as coefficients of Pearson Product Moment correlation (p), where negative p values indicate negative correlation(NC), positive values mean positive correlation(PC). Again, r > .05 indicates weak correlation, and r < .05 means strong correlation. Sig. =statistically significant difference at p < .05 and Nsig not statistically significant as returned by ANOVA (When experimental group is compared with control group values).*

Table III: Regression Coefficients with Simple Linear Equations for Estimating the Relationship between Saliva and Serum Bio-Analytes in Diabetics and Non-Diabetics

Bio-Analytes	Diabetics		Non-Diabetics	
	Saliva	Serum	Saliva	Serum
	(Y = bx + a)		**(Y = bx + a)**	
Na+**(mmol/L)**	(-0.336) x + 185.32		(- 0.038) x + 142.30	
Cl-**(mmol/L)**	(-0.159) x + 42.51		(-0.043) x + 103.45	
K+**(mmol/L)**	(0.129) x + 15.37		(-0.909) x + 8.89	
Ca^{2+}**(mmol/L)**	(0.118) x + 1.81		(0.077) x + 0.15	
Mg^{2+}**(mmol/L)**	(0. 002) x + 0.004		(-0.001) x + 0.00	
Amylase (U/L)	(0.081) x + 157.89		(0.333) x + 105.67	
Glucose (mg/dl)	(0.009) x + 0.01		(0.001) x + (0.003)	
CRP (mg/L)	(-0.031) x + 9.88		(0.316) x + 2.01	

Y = dependent variable (Serum) to be estimated, b = Regression coefficient of Assayed Bio-Analyte (variable), a = Y -intercept of Correlated Bio-Analyte (with Serum) i.e. the expected mean value of Y when all x variables are equal to zero, and x = Independent variable (Saliva)

Correlating the bio-analytes in the saliva and serum of the diabetic and non-diabetic subjects, in the diabetics, a positive correlation was found for potassium, magnesium and alpha amylase, while in the non-diabetics, a positive correlation was found for calcium, alpha amylase, CRP and glucose.(Table 1). When the saliva and serum of the diabetic subjects were compared, sodium, calcium, magnesium, alpha amylase, CRP and glucose demonstrated a strong correlation (Table 11). Comparing the saliva and serum of the diabetics bio-analytes, one-way analysis of variance (ANOVA) at $p<0.05$ returned a statistical significance for sodium, calcium, magnesium, alpha amylase, CRP and glucose (Table 2).

DISCUSSION

Comparison of the average saliva electrolyte compositions of diabetics with those of non-diabetics shows an increased mean value of sodium in diabetics against non-diabetics. This increase in salivary sodium of diabetics may have resulted from glucose capacity to act as an osmotically active substance, pulling sodium in a symport fashion across concentration gradient. Moreso, glucose is known to increase osmolality of tissues to electrolytes, specifically Na^+, resulting in the movement of water out of cells.[25]

Salivary Cl^- mean value was higher in diabetics compared to non-diabetic subjects while the salivary K^+ level showed a lower mean value in diabetics when compared to non-diabetics subjects. Physiologically, reduced levels of potassium ion (K^+) concentration (seen in the saliva of diabetics) can be traced to the decrease in salivary fluid output as a result of potassium efflux. This is suggestive of the damaging effect of DM on secretory activity of the salivary gland in diabetic subjects as explained[26]

The study also observed salivary Ca^{2+} with an average value in diabetics as against non-diabetic individuals upon comparison. Calcium, which is the fifth most abundant element in the human body[27] is known to play a very crucial role in bone mineralization. Proline rich proteins are known to prevent enamel demineralization with special support from calcium. In the salivary glands, calcium is also a key functional electrolyte that drives and partakes in the biosynthesis of saliva. Therefore, higher calcium concentration could be a risk factor in the development of periodontal diseases. This possibly explains the reason for lower salivary Ca^{2+} concentration in non-diabetics than diabetics seen in this study. Salivary Mg^{2+} levels in this study also showed a higher mean value in diabetic individuals compared to the non-diabetics. Magnesium is closely linked to calcium and the same factor that affected calcium ions could apply to magnesium.

Salivary amylase level was observed to have a higher mean value in diabetics compared with non-diabetes individuals. This finding however disagrees with those of a previous study on salivary amylase activities in ailments like diabetes mellitus, especially those reported on salivary amylase concentrations.[28]These

differences may be due to variation in stress levels, hormonal and metabolic changes in DM patients compared with non-diabetic individuals. Also, in this study, salivary C-reactive protein (CRP) had a higher mean value in diabetics when compared to non-diabetic subjects. Evidences from a previous study suggest that inflammatory and metabolic factors associated with diabetes such as high glucose, adipokins, modified lipoproteins and free fatty acids may trigger CRP production by endothelial and smooth muscle cells, as well as monocytes/macrophages[29]. For salivary glucose levels, current study found a statistically significant increase with higher mean value in diabetic than non-diabetic participants. The possibility of this can be traced to consistent serum build-up of glucose resulting from hyperglycaemia; causing a high concentration gradient in return that drives more glucose into the saliva of diabetic sufferers. This finding is in consistency with previous findings[30,31,32]; and explicitly linked to the consequences of high glucose level from which saliva is formed. Result on glucose from this study agrees with a previous study[33]

Conclusively, this study finds a significant correlation in saliva and serum values of diabetics and non-diabetics for electrolyte levels, amylase activity, glucose concentration and CRP levels. The established regression equation is suggestive of saliva as a means by which serum levels of these bioanalytes may be predicted using saliva, especially glucose which still remains the hallmark for diagnosing DM.

Declaration

1. **Ethical approval and Consent to Participate:** Ethical approval was obtained from the office of the Chief Medical Director, General Hospital Warri, representing the management board and consent was obtained from each of the participants.
2. **Availability of data and materials:** The records referred to in this study are domiciled in the General Hospital Warri Diabetic Records Unit. The patients were assessed on their clinic days.
3. **Competing Interest:** The author(s) declare that they have no competing interest.
4. **Funding:** The research was self-funded.
5. **Author's Contributions:** Ojieh and Wilson designed the research and drafted the original frame work. Agbonifo-Chijiokwu and Ogagayere handled the analysis, while Nwogueze managed the statistical analysis and result interpretation. The final manuscript was drafted by Ojieh and Wilson.

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