Comparative Evaluation of Saliva and Serum Proteins in Diabetics and Normo-Glycemics

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ABSTRACT

One of the most probable markers of inflammatory response is serum protein. Recently, serum levels of these some proteins have been proven to be useful in clinical diagnosis. In this study, we comparatively investigated serum and saliva C - reactive protein (CRP), α -amylase and α -glucosidase activities in type II Diabetic and Normo-glycemic humans. Two hundred and thirteen (213) subjects of 173 Diabetics and 40 Non-diabetics (Control) were ethically recruited from the central hospital, warri, Delta State. For each participant, serum and saliva was collected and laboratorily analyzed for α -amylase, α -glucosidase and CRP levels, while comparing mean differences between groups with a student t-test and statistical measure of association (correlation). Result showed a statistically significant increase in CRP and α -amylase activity of diabetics than non-diabetic subjects, with a statistically significant increase in salivary and serum CRP, α -glucosidase and α -amylase levels across groups. This finding is suggestive that saliva and/or serum levels may be useful bioanalytes for non-invasive, alternative diagnosis of blood glucose levels. Similar studies that corroborate the efforts of this study is recommended. Further studies that assay other saliva and serum biomarkers may also be useful and thus recommended.

Keywords: serum, saliva, C - reactive protein and diabetes

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I. INTRODUCTION

As increasingly sophisticated techniques are available for the study of genes, proteins and bacteria, their application to saliva promises to extend the scope of oral diagnostics to the study of systemic disease as well as oral disease and metabolism [1]. Saliva is easily available for non-invasive sampling and analysis and with careful collection and handling presents possible opportunities for the identification of biomarkers for the two major oral diseases, periodontal disease and dental caries [2].

The impending use of saliva in diagnosis as well as in the regular monitoring of diseased subjects suffers from one possible constraint wherein in certain situations, including numerous autoimmune and/or inflammatory conditions like

Sjogren syndrome and primary biliary cirrhosis, graft-versus-host disease, IG-G4-related sclerosing disease, degenerative diseases like amyloidosis, granulomatous conditions including sarcoidosis, infections including HIV/AIDS, hepatitis C, malignant conditions like lymphomas and salivary gland agenesis or aplasia apart from drug-induced xerostomia caused due to drugs including anticholinergics, antihistaminics, antihypertensives, and neurotropic drugs, including sedatives and anxiolytics, antidepressants and antipsychotics, to name a few [3].

Of all known salivary health indicators, glucose, amylase, and total proteins appear to be most closely related to the oral environment in patient with Type II diabetes [4, 5 and 6]. Glucose is a small molecule that diffuses through the membranes of blood vessels, passing from the blood plasma

to the gingival fluid, through the gingival sulcus, and reaches the saliva [7]. The increase in blood glucose in Type II diabetes patients can cause higher levels of salivary glucose with the consequent loss of homeostasis and greater susceptibility to disease in the oral cavity [8]. Amylase plays an important role in the digestion of carbohydrates, which in turn acts as a potential factor in streptococcal adhesion to teeth and in plaque formation [9, 10]. Salivary total protein predominantly comprises of proline-rich proteins, mucin, amylase, immunoglobulins, statherins, and antibacterial factors which are responsible for most of the functions of saliva [5, 6].

In an India study performed on the salivary composition of diabetic individuals, very few results were reportedly contradictory in several aspects, suggesting the need for further investigative studies. Forbat et al., (2011) conducted a study to investigate the relationship between salivary and sera glucose levels in 31 diabetics in which parotid fluid samples were obtained by cannulation of the parotid duct. Glucose concentration was determined by glucose oxidase method using Beckmann glucose analyzer. The results revealed that salivary glucose concentration was independent of sera glucose levels [11].

Knowledge of the effects of hyperglycaemia on salivary composition and function remains equivocal, with oral cavity acting as the mirror of systemic disease and saliva. It is the key feature that involves various functions from digestive to immune system; making saliva a diagnostic marker for systemic diseases [12].

Evaluation of salivary parameters has been shown to be cost-effective and non-invasive for screening, diagnosis and monitoring of diabetes when compared to blood investigations which are painful and causes physical trauma and mental stress to patients, hence the need for this study. The non-invasive nature and simple collection of saliva allows for its ease of repetition, compliance and multiple collections; a potential aid in early diagnosis, monitoring of disease progression, or treatment responses with minimally trained personnel. This advantage of using saliva attract investigator who are looking for an alternative body fluid to simplify diagnostic procedures [13, 14]

II. AIM OF STUDY

This study evaluated the changes in salivary and serum electrolytes, glucose and amylase activity for Diabetic and non-Diabetic humans.

III. MATERIALS AND METHODS

A. Study Design

The study was targeted at human subjects, recruiting a cross section of type II diabetics from the general hospital, Warri, Delta State. Study fetched 40 healthy subjects (control) and 176 type II diabetics (Experimental) of between 25-65 years from the study area. For each subject, saliva and serum fluids were collected and assayed for C - reactive protein (CRP), α -amylase and glucose activities.

B. Population of Study

Study population was targeted at the total DM sufferers per annum that visit the central hospital, warri (DM Unit) for regular check-up.

C. Sample and Sampling Technique

A total of 213 participants (diabetics and non-diabetics) were randomly selected from population of DM sufferers within the study area, using the statistical relation of Singh and Masuku, 2014 [15];

$$n = \frac{(Z \alpha/2) 2\sigma^2}{d^2}$$

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

D. Saliva Collection

Two millilitres (2ml) of unstimulated saliva samples were taken between the hours 7.00am and 8.00am from selected subjects after an overnight fast [16]. Participants were asked to spit (after rinsing their mouths with deionized water) into plastic vials. Obtained saliva samples were then centrifuged at 6000 rpm for 10 minutes before being use. This was necessitated by the need to rid sample of any available contaminants; such as micro-organisms, food debris, etc. the supernatant were then analysed immediately.

E. Serum Collection

Five milliliters (5ml) of subjects' intravenous blood was obtained from the median cubital vein of the cubital fossa in their forearm, just by 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.

F. Inclusion Criteria

Subjects who volunteered to participate in the study were selected. This selection was based on the knowledge that selected subjects were certified diabetic by their physicians, must be between the ages of 40-75 years, irrespective of gender. Noteworthy is that inclusion criteria for control subjects was them being non-diabetic (65-90 mg/dl).

G. Exclusion Criteria

Patients suffering from type I 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. Subjects under steroid, antidepressant, estrogen, epinephrine and diuretic medications were also exempted from the study.

IV. DETERMINATION OF SERUM AND SALIVARY AMYLASES

A. Principle

Serum and salivary amylase activity was determined by the substrate method (kinetic enzyme assay). The ability of α -amylase to catalyze the hydrolysis of starch to maltose is the principle used to estimate amylase.

B. Procedure

The serum and saliva sample test tubes were labelled. They were then diluted to 1 in 100 (1 part of serum and 99 part of

saline and 1 part of saliva and 99 part of saline) and 25ul of serum and saliva sample were taken in test tube then 0.1ml of reagent solution was pipetted into each of 3 test tubes labelled 'blank', 'standard' and 'test' of serum and saliva. These were analyzed in an automatic analyzer. Finally, the test sample was aspirated and the readings were noted.

V. DETERMINATION OF SALIVA AND SERUM C - REACTIVE PROTEIN (CRP) LEVELS (ASSOCIATION FOR CLINICAL BIOCHEMISTRY, 2012)

A. Principle

In principle, c-reactive protein test is based latex agglutination reaction. By this, when latex particles complexed with anti-CRP are mixed with patient's serum or saliva containing CRP, and invisible agglutination reaction take place within 2mins

B. Procedure

First, all reagent including serum and saliva sample were brought to room temperature after gently mixing with latex reagent without diluting the selected samples. Next, one drop of test sample was added in glass slide for separate reaction circle thereafter one drop of CRP latex reagent was added to the circle and mixed with separate mixing stick while spreading the fluid over the entire area of the cell. This combination was then slowly tilt back and forth for 2 mins, while observing under for agglutination under artificial light.

C. Determination of Salivary and Serum Glucose Concentrations

Salivary and serum glucose estimation was performed using the glucose oxidase end-point method [17, 18, 19]. 20ul of standard was added to each of the test tubes against 20ul of sample. These were then mixed, and all the test tubes were kept in an incubator at 37°C for 20 min before aspiration. Reagent blank (.000). They were next, aspirated in the analyzer, followed by standardization, for which the reading was noted, and finally, the test sample was aspirated and the reading was noted. Results for saliva and serum were calculated and values were expressed as milligrams per deciliter (mg/dl).

D. Ethical Clearance

Ethical approval was sought and obtained from Central Hospital, Warri, and Bioethics Committee of the Delta State University, Abraka,, Delta State. Age and gender matched Non -Diabetics in apparent good health were included as control subjects.

E. Statistical Analysis

Obtained results were represented as mean standard deviation. Statistical analysis was done using the one-way analysis of variance (ANOVA) and post-hoc (tukey) test. Statistics was carried out with a graph pad prism software (version 8.0). A p-level less than 0.05 was considered as statistically significant.

VI. RESULTS

Fig. 1. Comparison of Saliva and Serum Amylase Activities in Diabetics and Non-Diabetics

Above Figure (figure 1) compares average saliva and serum α -Amylase levels in diabetics to Non-diabetic subjects. From the chart, student t-test returned a statistically significant increase in Diabetic α -Amylase levels as compared to Non-Diabetics (Control group I). a = statistically significant increase in saliva (at $p < .05$), b= statistically significant increase in serum of diabetics compared to non-diabetics

Fig. 2. Comparison of Saliva and Serum C-Reactive Protein levels in Diabetics and Non-Diabetics

Above Figure (figure 2) compares average saliva and serum CRP levels in diabetics to Non-diabetic subjects. From the chart, Student t-Test proved a statistically significant increase in Diabetic CRP concentration as compared to Non-Diabetics (Control group I). a= statistically significant increase in saliva (at $p < .05$), b= statistically significant increase in serum of diabetics compared to non-diabetics.

Fig. 3. Comparison of Saliva and Serum Glucose levels in Diabetics and Non-Diabetics

Above figure compares average saliva and serum glucose levels of diabetics to Non-diabetic subjects. From the chart, Student t-Test returned a statistically significant increase in Diabetic Saliva glucose concentration as compared to Non-Diabetics. a= statistically significant increase in saliva (at $p < .05$), b= statistically significant increase in serum glucose of diabetics compared to non-diabetics.

TABLE I: SUMMARY OF AVERAGE SERUM AMYLASE, CRP AND GLUCOSE LEVELS

Parameters	Diabetics (Exp.)	Non-Diabetics (Control)	Student t-Test (p-values)
α -Amylase (U/L)	90.22 $\pm$ 11.00*	68.03 $\pm$ 2.21	0.005
CRP (mg/L)	10.61 $\pm$ 1.93*	2.92 $\pm$ 0.88	0.035
α - Glucose (%)	81.11 $\pm$ 61.61*	76.25 $\pm$ 7.52	0.002

Table I (Above) shows serum α -amylase, CRP and α -glucosidase levels in diabetic and Non-diabetic subjects. From the table, t-test shows a statistically significant increase in serum levels of assayed parameters for Diabetics against Non-Diabetics.

VII. DISCUSSION

The goal of this study was to investigate the surrogate biomarkers in type II diabetic saliva, establishing, in essence, the possibility of estimating the concentrations of serum α -amylase and C-reactive protein (CRP) levels from salivary levels. Therefore, the controversy focused on the effects of type II diabetes mellitus (DM) on human serum and salivary alpha-amylase, and CRP levels. A total of two hundred and thirteen (213) individuals were ethically derived from the Central Hospital, Warri, Delta State, comprising one hundred and seventy three (173) diabetics (experimental group) and forty (40) non-diabetic (control group) individuals.

Diabetes mellitus (DM), which is an endocrine condition characterized by relative or absolute insufficiency in the secretion of insulin and / or concomitant resistance to insulin metabolism in the target tissue [20], is a systemic disease that affects all the body's systems [21].

DM diagnosis is usually conducted by calculating the amount of serum blood glucose by standardized methods that are intrusive, physically and psychotically traumatic to patients [22]. Therefore, the present approach discourages people from undergoing testing and results in a lack of adequate evidence for DM diagnosis [6]. Efforts have been

made in recent years to replace blood tests with other samples of biological material that could be collected through non-invasive procedures [23]. Because of its several benefits, Saliva has been developed as a potential diagnostic tool for disease surveillance, it clearly provides a cheap, simple and easy-to-use screening method.

Here, after contrast, salivary amylase levels were observed with a higher mean value in diabetic subjects (153.26) than in non-diabetic subjects. There was also a statistically significant improvement (at $p < 0.05$) for diabetics relative to non-diabetic participants in comparison with the student t-test. However, relative to non-diabetics (0.133 and 0.245), diabetics demonstrated a negative association between saliva and serum glucose and CRP levels (-0.03 and -0.076 respectively), which also had a clear positive correlation. Moreover, as compared with non-diabetics, amylase appeared to be positively associated, albeit poor in diabetic serum and saliva.

Multiple diabetic serum and saliva correlations, however, returned high negative correlation values for amylase and glucose levels with the exception of CRP. In situations such as diabetes mellitus, this finding conflicts with those of common literatures on salivary amylase activities, especially those recorded on salivary amylase levels [24], these differences may be due to differences in stress levels, hormonal and metabolic changes in DM patients compared to non-diabetic people. In comparison, in the current study, higher salivary amylase activity was observed in diabetic individuals.

Overtime, CRP surge in blood due to inflammatory response has been shown to have a preponderant increase in saliva as well. Evidences from previous studies 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 [25]. For salivary glucose levels, current study found a statistically significant increase with higher mean value (0.9) in diabetic than non-diabetic (0.4) 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 [24]; and explicitly linked to the consequences of high glucose level from which saliva is formed. The outcome was in line with previous studies by Panchbhai et al (2010). Changes in blood glucose levels were also identified in the current study for diabetics and non-diabetic participants. Diabetics are seen here in all analysed parameters with higher average values than non-diabetics. However, relative to non-diabetics, CRP was lower on average, while amylase was second, followed by serum glucose levels. A statistically significant increase ($p < .05$) was returned from the student t-test for amylase, CRP and serum glucose levels of diabetics relative to non-diabetics when compared between classes (Control group I).

Normal salivary CRP and amylase concentrations are apparently shown to be higher at all levels than serum diabetic glucose values, although marginally comparable for diabetics and controls. This apparent rise in the mean salivary amylase concentration was shown to be statistically important for diabetics (153.3 mmol/L) relative to non-diabetic participants at $p < .05$. For this cause, there was a sharp decrease in mean CRP levels between the classes, averaging

around 10.1 mmol/L and 10.61 mmol/L for diabetics and non-diabetics, respectively. For saliva and serum glucose levels, also shows a sharp upsurge in average values for diabetic saliva (0.4mmol/L) and serum (86.38 mmol/L) groups of sampled subjects. amylase is a digestive enzyme produced by salivary gland and pancreas that cleaves the glycosidic linkages in starch molecules to produce saccharides, altered expression of the amylase and cyclic AMP receptor in the parotid glands of diabetic patients leads to changes in secretory protein of human salivary gland thus contributing to altered amylase level and oral disease associated with diabetes [26]. According to vasconcelos et al., 2010, salivary and serum glucose elevation may be due to diabetic membranopathy that leads to leakage through the membrane and increased percolation from blood to saliva [27].

For non-diabetic subjects, the current study also compared saliva and serum glucose levels. The graph indicates that the level of non-diabetic salivary amylase increased similarly to the level of serum, even though the mean saliva levels were significantly higher (129.2 mm/L) than that of serum amylase (68.03 mm/L) for the same non-diabetic patients. The apparent explanation for this may not be far-fetched, and can be traced to physiologically elevated levels of serum amylase as opposed to salivary levels.

Remember that the bioavailability of raw materials in salivary biosynthesis, including salivary amylase, is a function of the serum levels of the same metabolites. The same is clarified for most other tissues and/or glands, not necessarily the salivary gland, which synthesize their products from available raw materials in the blood [28]. This finding indicates that other salivary constituents, other than salivary amylase, can rise high in the gland with elevated plasma levels, suggesting their activity as biomarkers in the diagnosis of saliva and/or serum increases in the metabolite level. Apparently, a low and extremely high level of saliva amylase is common in patients with hyperglycaemia and so on [24]. It is important to explore the biological mechanism underlying the observed association with a more advanced stage of moderate speech. While there was a rise in levels of diabetic C-reactive protein, there was a sharp decrease in non-diabetic CRP levels relative to diabetic CRP levels. Again, after a sudden drop in glucose levels, the level of salivary CRP was seen to increase at some point. Apparently, there appears to be a gradual decrease in salivary amylase, down to non-diabetic salivary glucose levels by CRP. Physiologically, this means that a decrease in salivary glucose levels can eventually affect non-diabetic saliva levels of CRP and alpha-amylase. This find, by implication.

VIII. CONCLUSION

The preservation of oral health and diagnosis of many illnesses as it applies to humans are involved in different salivary functions. It is known that salivary C-reactive protein (CRP) and/or glucose play important roles in maintaining good health through disease prognosis after proper laboratory procedures. In relation to particular health conditions, numerous studies have shown the relationship between these salivary proteins and glucose. Very few studies, however, have actually suggested the possibility of relating a single salivary CRP as alternative to diagnosing medical conditions in clinical practice. The findings of this term are more or less controversial, this research addressed the methodological issues in particular, as well as different methods of extracting saliva from diabetics and non-diabetics.

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