Clinical Oral Findings and Salivary Analysis of Patients with and Without Diabetes Mellitus

Introduction

The use of saliva as a diagnostic tool has grown to become very popular in the management of systemic diseases. Changes in saliva composition often reflect the general state of health especially in some disease conditions such as diabetes mellitus [1], HIV/AIDS [2], Renal diseases and psychological stress [3,4]. In addition to underlying systemic diseases, salivary production is also affected by age and sex of the patients.

Salivary secretion occurs in two stages; first is the primary secretion by acini cells. At this first stage, the acini cells of salivary gland secrete the primary saliva from the substrate made available through the plasma. This first formed saliva is essentially an ultrafiltrate of the plasma and is called primary saliva [5]. The second step is the modification of the primary saliva as it flows down the duct; this is done by the ductal epithelial cells. This result is the formation of secondary saliva which will eventually be secreted into the mouth [6]. Second is the modification process which comprises secretion and selective reabsorption of substances into and from the plasma respectively. Due to the presence of similar anchor protein called claudin 16 at the epithelia tight junctions of salivary glands, renal tubules and breast; the mode of action of salivary ductal epithelia cells has been likened to that of respective cells in urine (renal tubules) and breast milk production [7]. The modification process on the saliva at this secondary stage is largely dependent upon the present plasma concentrations of the respective metabolites. The implication is that the higher the concentration of markers of systemic problems in the blood, the higher will be the concentration in the saliva and this is a good rationale for using saliva as a non-invasive replacement for plasma in diagnosis. A good example is the blood sugar, when the blood sugar is high as it happens in DM patients; it implies a corresponding increase in the salivary glucose. The same will also be applicable to some other substances in the blood such as urea, creatinine, antibody concentration, hormones and drugs.

The average unstimulated salivary flow rate of healthy adult ranges from 0.1-0.5 ml/min while the stimulated flow rate was found to be higher, 1-2 ml/min. Studies had shown that patients with diabetes mellitus have lower flow rates [8-10]. Glucose is not a constituent of saliva in a healthy individual.

The pH of saliva of patients with oral lesions has been shown to be at variants with apparently healthy patients [11]. Diabetic patients had acidic saliva compared to non-diabetic patients. Salivary urea and creatinine also differs in diabetic and non-diabetic patients [8,12].

The prevalence of DM is on the increase daily probably as a result of changing to westernized diet as well as sedentary lifestyle. Diabetes is also associated with complications such as CVS, renal failures, amputation, infection and oral problems [13]. It therefore leads to reduce quality of life and the burden on the community at large is quite enormous. Early diagnosis and management of this condition is therefore important and all hands must be on desk to achieve
this all important goal. The routine/conventional test is the use of plasma sugar which may be painful and require more skills when compared with saliva collection. Saliva is a potential alternative fluid for screening, diagnosis and monitoring the patients. Several studies have been conducted to look into salivary parameters in diseased condition but many did not relate the salivary findings to the severity of the oral symptoms and saliva parameters. The present study is designed to determine salivary physical and chemical constituents in diabetes and non-diabetes individuals, relating the findings with the severity of oral signs and symptoms.

Materials and Methods

This study was designed as a comparative cross-sectional study comparing the clinical features and salivary analysis of diabetic and non-diabetic patients who presented at Obafemi Awolowo University, Ile Ife from January, 2019 to June, 2019. Two groups of subjects were selected: Group a (Diabetic patients) were selected from the pool of patients who presented at Endocrinology clinic for treatment. Group B were apparently healthy and non-diabetic volunteers among staff and students of Obafemi Awolowo University, Ile Ife. Group B subjects were subjected to urinalysis screening test using Combic 9 urinalysis kit, only those with negative test were recruited. The subjects were selected using simple random method.

Ethical approval

Ethical approval was sought and obtained from the Research and Ethics Committee of the institute of Public Health Obafemi Awolowo University. Each participant was well informed and gave their consent before undertaking the study on voluntary basis. Participants were also free to decline from participating in the study at any time during the study period. Information obtained from the participants was treated with utmost confidentiality.

Sample size calculation

Saliva flow rate was used to calculate the sample size since variation in saliva flow is a common finding in many cases of systemic illness. Sample size was calculated using the formula as applicable to studies comparing two means as reported by Eng (2003) [14].

\[
N = \frac{4 \delta^2 (Z_{\alpha/2} + Z_{\beta})^2}{D^2}
\]

Where, \(N\) is the total number of samples requires for the two groups \(Z\) is a constant value of 1.96 at a clinical significance of 0.05. \(Z\) is also a constant which equals 1.645 at a statistical power of 0.95. The symbol \(\delta\) is the assumed SD of each group. Oyetola et al. had reported the mean saliva flow rate as 0.398 SD 0.25 mL/min [15], therefore \(\delta\) will be taken as 0.25. D is the total width of an expected Confidence Interval (CI) and was set at 1.3. With the power of 90% and the significance level of 0.05, a sample size of 180 subjects was obtained and rounded up to 200 to allow for attrition

\[
N = \frac{4 \times 0.25^2 \times (1.96 + 1.645)^2}{1.3^2}
\]

\(N= 180\)

To allow for attrition, \(N=200\). Each group will therefore require 100 participants.

Data collection

The tool used for the data collection was questionnaire. Section A collected information on the participants’ biodata such as age, sex, tribe, occupation and so on. Section B has information on oral and general symptoms of diabetes. Section C contains the salivary and blood findings.

Saliva collection

Spitting method, as reported by Srivastava et al. 2018 was used to collect saliva for this study [16]. Samples were collected between 10hrs and 12 hrs to minimize the effect of diurnal variation on the quantity and quality of saliva. Participants were asked to avoid food intake one hour before the procedure. Subjects were given saliva jar and were asked to spit into the jar for five minutes. Saliva collection time was determined using a stop watch. Salivary flow rate was calculated by dividing the total volume collected by a factor of 5 to get the flow rate in ml/min. Thereafter, the saliva was transported to the laboratory immediately for analysis.

Laboratory procedures

Estimation of salivary urea: RandoxKits BT29 4QY, United Kingdom was used to estimate salivary urea. The kits followed Urease- Berthelot method of urea estimation. The ammonia is the measured photometrically by Berthelot’s reaction. The procedure for the measurement is essential as contained in the manufacturer instructions and the salivary concentration of urea was calculated using the formula:

\[
\text{Absorbance of the sample (saliva) } \times \text{ Standard Concentration} \quad \text{Saliva}\text{=}\text{Absorbance of standard}
\]

Standard concentration is a constant =169 µmol/L

Estimation of salivary creatinine

This was done using RandoxKits BT29 4QY , United Kingdom. The kits operate on the principle that creatinine in alkaline solution reacts with picric acid to form a colored complex. The procedure for the measurement is essential as contained in the manufacturer instructions.

The salivary concentration of creatinine was calculated using the formula:

\[
\text{Absorbance of the sample (saliva) } \times \text{ Standard Concentration} \quad \text{Saliva}\text{=}\text{Absorbance of standard}
\]

Standard concentration is a constant =13.10 mmol/L

Estimation of salivary glucose

This was done using RandoxKits BT29 4QY, United Kingdom. The kits followed Urease- Berthelot method of urea estimation. The procedure for the measurement is essential as contained in the manufacturer instructions.

The salivary concentration of glucose was calculated using the formula:

\[
\text{Absorbance of the sample (saliva) } \times \text{ Standard Concentration} \quad \text{Saliva}\text{=}\text{Absorbance of standard}
\]

Standard concentration is a constant =6.25 mmol/L

ISSN: 2377-987X

Citation: Oyetola EO, Abimbola TA, Adesina OM, Egungjobi SM, Adebayo OF, et al. Clinical Oral Findings and Salivary Analysis of Patients with and Without Diabetes Mellitus. J Oral Biol. 2019; 6(2):6.
Estimation of salivary pH

This was done using pH meter. The instrument was first calibrated by using standard buffer solution of 4.18, 8 and 11.5 respectively and thereafter 0.0 L/ml of the sample (saliva) was carried with a pipette to come in contact with the sensitive part of the probe of the pH meter. The pH of the saliva shows immediately on the screen.

Statistical analysis

Data analysis was done using STATA 13 statistical software (StataCorp, College Station, Texas). Descriptive statistics were used to characterize socio-demographic variables (such as age and sex) and the presence of symptoms. Analyzing for descriptive factors for salivary constituents and age pf participants with continuous variables include checking for mean, median, mode, and range as appropriate. The continuous variable was checked for normality test using Shapiro-Wilks test. Comparison of the mean values of salivary constituents at the dichotomized age groups and sex were done using students-test and Wilcoxon rank-sum test as appropriate. Statistical significance was set at p<0.05.

Results

Sociodemographic of the participants

A total of 200 subjects participated in the study, 92 (46%) males and 108 (54%) females. Their mean age + SD was 54+12.23. Majority of the participants were trader with at least primary education. Yoruba ethnicity (95.5%) was the most common tribe. More than three-quarter of the participants were above 40 years old (Table 1).

Table 1: Sociodemographic of the Participants.

| Characteristics | Subjects with Diabetes | Subjects without Diabetes | All Subjects | P value |
|-----------------|------------------------|----------------------------|--------------|---------|
| Sex             |                        |                            |              |         |
| Male            | 32(34.8)               | 60(65.5)                   | 92(100)      |         |
| Female          | 68(63.0)               | 40(37.0)                   | 108(100)     |         |
| Total (%)       | 100                    | 100                        | 200 (100)    |         |
| Mean age (SD)   | 54.13±12.05            | 55.48±12.4                 | 54.81±12.23  | 0.2184  |
| Age Category (years) |                   |                            |              |         |
| <20% (%)        | 1 (1)                  | 0 (0)                      | 1 (0.5)      |         |
| 21-30 (%)       | 3 (3)                  | 3 (3)                      | 6 (3)        |         |
| 31-40 (%)       | 9 (9)                  | 10 (10)                    | 19 (9.5)     | 0.409   |
| 41-50 (%)       | 18 (18)                | 29 (29)                    | 47 (23.5)    |         |
| 51-60 (%)       | 31 (31)                | 29 (29)                    | 60 (30)      |         |
| >60 (%)         | 38 (38)                | 29 (29)                    | 67 (33.5)    |         |
| Total (%)       | 100 (100)              | 100 (100)                  | 200 (100)    |         |
| Ethnicity       |                        |                            |              |         |
| Yoruba          | 94 (94)                | 97 (97)                    | 191 (95.5)   |         |
| Ibo             | 5 (5)                  | 3 (3)                      | 8 (4)        | 0.496   |
| Hausa           | 1 (1)                  | 0 (0)                      | 1 (0.5)      |         |
| Total (%)       | 100 (100)              | 100 (100)                  | 200 (100)    |         |
| Occupation      |                        |                            |              |         |
| Unemployment    | 6 (6)                  | 5 (5)                      | 11 (5.5)     | 0.999   |
| Civil servants  | 27 (27)                | 26 (26)                    | 53 (26.5)    |         |
| Trading         | 44 (44)                | 45 (45)                    | 89 (44.5)    |         |
| Schooling       | 2 (2)                  | 2 (2)                      | 4 (9)        |         |
| Retiree         | 2 (2)                  | 2 (2)                      |              |         |
| Total (%)       | 100 (100)              | 100 (100)                  | 100 (100)    |         |
| Education Status|                        |                            |              |         |
| Illiterate      | 6 (6)                  | 14 (14)                    | 20 (10)      | 0.107   |
| Primary         | 18 (18)                | 20 (20)                    | 38 (19)      |         |
| Secondary       | 27 (27)                | 30 (30)                    | 57 (28.5)    |         |
| Tertiary        | 46 (46)                | 36 (36)                    | 82 (41)      |         |
| Post graduate   | 3 (3)                  | 0 (0)                      | 3 (1.5)      |         |
| Total (%)       | 100 (100)              | 100 (100)                  | 200 (100)    |         |

Wilcoxon rank-sum test * Statistical significant

oral symptoms and health practices

More than three quarter of the participants had never visited a dentist before, especially those without diabetes (p=0.18). History of tooth ache, spontaneous toothy loss, gum bleeding, taste impairment and mouth odor were significantly higher among diabetes p=0.0001, 0.006, 0.0001, 0.0001 and 0.001 respectively. Most of them brush once daily using tooth brush and toothpaste only (Table 2).

Sex variations in physical and biochemical analysis of salivary constituents of subjects

The mean salivary flow rate is higher in males than females and was significantly lower in diabetes patients, p=0.0077. The saliva of Diabetes subjects was found to be significantly more acidic compare to those without diabetes (p=0.0081). Salivary glucose was higher in female diabetic subjects, the difference was statistically significant, p=0.004. Salivary creatinine and urea was significantly higher in diabetes, p=0.00001 and 0.0001 respectively (Table 3).

Age variations in physical and biochemical analysis of some salivary constituents

Mean salivary flow rate is slightly higher among subjects above 50 years, but the difference was not statistically significant, p=0.226. Older subjects also had more acidic saliva the difference also not
Discussion

The physical and chemical properties of saliva are strongly associated with the changes in internal or external environment of the individuals. Patients with diabetes, therefore, show some changes in the saliva which can be a pointer to diagnosis and disease monitoring. In addition to the chemical and physical properties of diabetes patients, the present study revealed the association between salivary parameters, sex and age.

In this study, diabetes mellitus was found to be more frequent among men when compared women with male to female ration of 3:2. This finding is consistent with the reports of a United Kingdom based review by Siddiqui et al. which showed a higher prevalence of DM among males [17]. The sex variation of diabetes mellitus is attributed to lifestyle changes, predisposition to predisposing factors, genetics and environments [18,19]. This also showed that diabetes is more frequent among population of older age group which is consistent with the findings of Alva et al. 2017 who reported that the risk equation of diabetes is successful in middle age adult than young population [20].

Patients with diabetes mellitus often present with oral problems as a result of the disease process, associated complications and...
Salivary glucose concentration is a reflection of plasma glucose concentration. Consistent with other studies, salivary glucose of diabetic participants was significantly higher than that of the non-diabetes [12,23,25]. Primary saliva, from where the secondary saliva in the mouth is formed, is essentially an ultra-filtrate of the plasma and it’s a direct reflection of the level of plasma glucose. Salivary urea is also significantly higher in males consistent with other studies. Higher blood sugar among males is due to male predilection of DM.

Like salivary glucose, the concentration of urea and creatinine in saliva reflect the plasma values and are significantly higher in diabetic patients [26]. Increase plasma urea is a basic sign of renal problems. Consistent with scientific literature, salivary urea is significantly higher in diabetic patients [8,25]. Renal impairment (nephropathy) is not uncommon in diabetic mellitus; this may be responsible for the higher values. Higher urea level may also be found in higher protein diet and exercise. This study showed a higher urea concentration in saliva of females consistent with other studies.

Qualitative changes in saliva with respect to age are commonly reported in the literature [12,24,26]. In this study we report higher PH in subjects older participants above 50 years this may be connected to renal impairment which tends to happen at older age group. Likewise, salivary glucose, urea and creatinine concentration was found to be significantly higher older individuals. Increasing age predisposing to renal problems may also be responsible to the findings.

Salivary analysis has showed significant variations among diabetic and non-diabetic patients, as revealed in this study which was done among African population. Also, the relationship between the salivary and plasma concentration of the substances measured further reaffirm the potential use of saliva in the diagnosis and monitoring of diabetic patients. This become especially in resource limited countries.

Clinical Significance

This study had shown recent African data on salivary parameters and oral findings among diabetes and non diabetes. Major findings
were reduced salivary flow rate, increase salivary glucose, increased urea and creatinine concentrations among diabetes. The significant association between prevalence of oral lesions among diabetes as well as significant qualitative changes in saliva of diabetes is a potential non invasive tool of monitoring treatment outcomes of diabetes and also could enhance the multidisciplinary management of this distressing metabolic disease in our resource limited environment.

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