

Salivary characteristics of diabetic children

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Abstract

Aim: The objectives of this study were to evaluate the levels of glucose, cholesterol, protein and albumin in saliva, and to correlate the levels of glucose of the saliva to oral health and blood glucose of diabetic and non-diabetic children. **Methods:** 32 children with type 1 diabetes mellitus formed the study group (DC) and 32 non-diabetic children formed the control group (ND). The patients had their saliva collected and evaluated for glucose, cholesterol, total protein and albumin. Blood glucose analysis was also performed. The dental health status of the subjects was measured by DMFT index and def index. Independent Student's t-test was performed to compare metabolic status values in DC and ND groups. Correlation test was applied between blood glucose and salivary glucose (Spearman's correlation), and salivary glucose and DMFT/def (Spearman's test). **Results:** A statistically significant difference was observed between DC and ND considering salivary glucose ($p=0.000$). Elevated levels of cholesterol were evident in DC in correlation with ND. Total protein and albumin had increased values in DC (nonsignificant p value). The dental health status was not statistically different. **Conclusions:** Salivary parameters can act as adjuncts in assessing the overall metabolic status of the patient.

Keywords: saliva, diabetes, pediatric, glucose, cholesterol.

Introduction

Diabetes mellitus (DM) comprises a group of common metabolic disorders that share the salient feature of hyperglycemia. Several distinct types of DM exist and are caused by a complex interaction of genetics, environmental factors and lifestyle choices. The metabolic deregulation associated with DM causes secondary pathophysiological changes in multiple organ systems and imposes a tremendous burden on the individual with diabetes and the health care system.

Although all forms of DM are characterized by hyperglycemia, the pathogenic mechanisms by which hyperglycemia arises differ widely. The two broad categories of DM are designated as type 1 (IDDM) and type 2 (NIDDM). The incidence of childhood onset type 1 diabetes is increasing in many countries in the world, at least in the under 15-year-old age group. There are strong indications of geographic differences in trends but the overall annual increase is estimated to be around 3%. Two international collaborative projects, the Diabetes Mondiale study (DiaMond)¹ and the Europe and Diabetes study (EURODIAB)² have been instrumental in monitoring trends in incidence.

By 2010, it is estimated that annually some 76,000 children aged less than 15 years develop type 1 DM worldwide. Of the estimated 480,000 children with type 1 DM, 24% come from the South-East Asian Region, but the European Region, where the most reliable and up-to-date estimates of incidence are available, comes a close second (23%)³. The incidence rate of type 1 DM in India is 4.2/100,000 population per year.

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A wide spectrum of oral manifestations of DM has been reported ranging from xerostomia, taste impairment, sialosis, dental caries and periodontal disease to fungal infections, oral lichen planus and fissured tongue⁴. However, in children, there is no agreement in results that relate alterations of salivary chemical composition and oral health⁵.

An optimum metabolic control level correlates positively with the well being of the patient. Assessing the metabolic control of the patient has traditionally been done through testing of glycosylated hemoglobin, fructosamine, and glycoalbumin along with other criteria such as Body Mass Index, microalbuminuria and dyslipidemia⁶⁻⁷. Fructosamine reflects the glycemic control over the previous 1-2 weeks and has been correlated positively with serum albumin and serum total protein⁵. Saliva is a unique fluid and interest in it as a diagnostic medium has advanced exponentially in the last decade. Advances in technology have helped to move saliva beyond evaluating oral health characteristics to where it now may be used to measure essential features of overall health⁸.

Thus, the objectives of this study were to evaluate the levels of glucose, cholesterol, protein and albumin in saliva, and to correlate the levels of glucose of the saliva to oral health and blood glucose of diabetic and non-diabetic children thereby analyzing whether saliva-based glucose testing can be an effective alternative to blood-based glucose testing in children.

Material and methods

Study population

The study group comprised 32 diabetic children (DC) aged between 5 and 15 years with an already established diagnosis of type 1 DM. This group was selected from the patients attending the outpatient diabetes department, Institute of child health and research centre, Egmore, Chennai. The control group comprised 32 non-diabetic children (ND) without any other pre-existing systemic diseases. The study was conducted in collaboration with the Institute of Child Health and Research Centre and College of Dental Surgery, Saveetha University, Chennai with the ethical committee approval [(ihep no.) MDS 34/SU 63/06]. Parental informed consent was obtained for all patients before they were examined and samples collected.

Saliva collection and processing

Sample collection was performed during the morning hours when the subjects are in the fasting mode. Additionally, blood glucose values determined from venous blood samples drawn at the time of collection of saliva (fasting blood sugar) were also taken into account.

Standard UCLA procedure was used to collect the saliva. The subjects were asked to refrain from eating, drinking or other oral hygiene procedures, for at least one hour prior to collection. Drinking water was then given to the subjects, to rinse their mouth. Five minutes after the oral rinse, un-

stimulated saliva was collected in 50 mL falcon tubes by the method of spitting the saliva. The patient was asked to swallow the saliva present in the mouth and then to remain still without moving the tongue or swallowing the saliva for one minute. The patient spat the saliva every 60 s for a total of 5 min into a falcon tube to which sodium fluoride⁹ has been added. The tubes were then placed on ice. About 1.5 mL of saliva was collected from each subject. The samples were then centrifuged at 2,500 rpm for 5 min. The centrifugation resulted in a saliva sample which is free of large particulate debris and reduced viscosity, thereby allowing a more accurate and reproducible analysis.

Determination of glucose

Glucose was estimated by using a glucose kit (CREST BIOSYSTEMS, A division of Coral Clinical Systems, Goa, India) based on glucose oxidase-peroxidase method. It was standardized for saliva with 0.384mg/dL - as the lower value. The absorbance of the standard and the test material (0.01 mL/10 μ L) were measured against the blank without dilution within 60 min at a wavelength of 505 nm (Hg 546 nm)/ Green. Total glucose in milligram/deciliter (mg/dL) is then derived as Absorbance of test (Abs.T)/ Absorbance of standard (Abs.S) X 100.

Determination of Cholesterol

Cholesterol (CH) was estimated by using a commercially available cholesterol kit, which is based on cholesterol oxidase /PAP method. The absorbance of the standard and the test material (0.01 mL/10 μ L) without dilution were measured against the blank within 60 min at a wavelength of 505nm (Hg 546nm)/ Green. Cholesterol in mg/dL = Abs.T/ Abs. S X 200.

Determination of Total Protein

Total protein (TP) was estimated by using a commercially available test kit based on the biuret method. The absorbance of the standard and the test material (0.02 mL/20 μ L) are measured against the blank without dilution within 60 min at a wavelength of 550 nm (Hg 546 nm)/ yellow - green. Total proteins in gram (g)/dL = Abs.T/Abs.S x 8

Determination of Albumin

Estimation of albumin (ALB) was done by the colorimetric method based on the BCG method (commercially available test kits). The absorbance of the standard and the test material (0.01 mL/10 μ L) are measured without dilution against the blank within 60 min at a wavelength of 630 nm (Hg623nm)/ Red. Albumin in g/dL = Abs.T/Abs.S x 4.

Dental health status

The dental health status of the subjects was measured by the DMF-T index and the def index. The DMF index pertains to the permanent teeth (D - Decayed, M- Missing and F - Filled) and the def index (d - decayed, e - extracted, f - filled) to the deciduous teeth

$$\text{Average DMF} = \frac{\text{Total DMF}}{\text{Total number of the subjects examined}}$$

$$\text{Average def} = \frac{\text{Total def}}{\text{Total number: of the children examined}}$$

Statistical analysis

Independent Students't-test was performed to compare metabolic status values in DC and ND groups. Correlation test was applied between blood glucose and salivary glucose (Spearman's correlation), and salivary glucose and DMFT/def (Spearman's test).

Results

The mean value of salivary glucose (SG) of DC is 10.4 mg/dL and for ND is 17.6 mg/dL. The resulting $p = 0.000$ was 99.9% significant. A statistically significant difference ($p = 0.000$) was found between DC and ND with regard to cholesterol values. No statistically significant difference was found between DC and ND with regard to albumin ($p = 0.22$) and total protein ($p = 0.47$) (Table 1).

There was no significant correlation between salivary glucose (SG) and blood glucose (BG) within DC, but there was a significant correlation within ND ($p = 0.000$) (Table 2). The correlation between the DMFT status and the salivary glucose values of the subjects yielded nonsignificant results.

Discussion

Whole saliva is a mixture of the secretions produced by the three pairs of large glands and the smaller glands of oral mucosa (labial, lingual, buccal and palatal). It may also contain fluid from the gingival pocket (gingival, or crevicular fluid)⁹. The serum constituents in saliva are derived from the local vasculature of the salivary glands as well as from gingival fluid. The increased salivary glucose evident in the whole saliva can be due to several contributing factors or it could be a simple reflection of the blood glucose levels since saliva is an ultra filtrate of plasma. The salivary analytes are derived from plasma generally by three mechanisms (passive diffusion, active transport and ultra filtration) and are thus found in saliva as mentioned by Miller¹⁰.

A chronic disease like DM, with its emphasis on patient involvement in the control of the disease, and its attendant

Table 2 - Correlation between blood glucose and salivary glucose in diabetic children and non-diabetic children

| Group | Parameters | Correlation value | p value |
|-----------------------|------------------|-------------------|-----------|
| Diabetic children | blood glucose | 0.255 | 0.159(NS) |
| | salivary glucose | | |
| Non-diabetic children | blood glucose | 0.833 | 0.000** |
| | salivary glucose | | |

** - Correlation is significant at the 0.01 level (2-tailed)

problems, place a huge burden on the pediatric patients. Our efforts should be directed at making it an easier burden to bear. If saliva could be used for diagnosis and monitoring, diabetic children would not require daily invasive tests, they can just collect their saliva in a sterilized collection tube, which could then be used in biosensors for the real-time, sensitive and specific detection of salivary diagnostic analytes to give an overall view of the child's diabetic status. Very little information is available on the potential of saliva in diabetic children in India.

The salivary glucose values of the case subjects in our study were lower than the controls. This finding is similar to those of previous studies¹¹⁻¹³. Reuterving et al.¹¹ determined whether salivary flow rate and salivary glucose concentration in patients with diabetes mellitus influence of severity of diabetes. Salivary glucose concentration was lower during the period of better metabolic control. Marchetti et al.¹² found that the salivary glucose secretion rate was significantly lower (p less than 0.02) in diabetic patients with diabetic autonomic neuropathy than normal patients. Kanji et al.¹³ measured the plasma and salivary concentrations of glucose and cortisol during insulin induced hypoglycemic stress in healthy Nigerians. Salivary glucose levels (fasting and after intravenous insulin) were unaffected by hypoglycemia and did not correlate with plasma glucose at any time point.

We could not ascertain the reason for the low salivary glucose concentrations evident in DC in comparison to ND. One of the confounding variables may be that the cases in DC were under treatment as opposed to the cases in ND. More studies are needed on this facet.

The salivary concentration of Lipid soluble, unconjugated steroids such as cortisol, estriol, testosterone and progesterone, closely reflects the plasma concentration. The significantly high values of salivary cholesterol seen in the diabetic subjects could be an indication of the dyslipidemia, a

Table 1 - Metabolic status –blood glucose, salivary glucose, cholesterol, total protein and albumin - in the groups

| Parameters | Diabetic Children | | Non-diabetic children | | significance |
|------------------|-------------------|--------|-----------------------|------|--------------|
| | MEAN(mg/dL) | SD | MEAN(mg/dL) | SD | |
| Blood glucose | 326.34 | 104.33 | 84.5 | 5.94 | 0.000*** |
| Salivary glucose | 10.48 | 8.26 | 17.60 | 4.34 | 0.000*** |
| Cholesterol | 11.24 | 8.9 | 4.08 | 3.05 | 0.000*** |
| Total protein | 0.47 | 0.60 | 0.34 | 0.16 | 0.22(NS) |
| Albumin | 0.30 | 0.46 | 0.24 | 0.16 | 0.47(NS) |

*** - $p < 0.001$ (99.9% significant)

condition usually associated with diabetic subjects¹⁴⁻¹⁵.

Dyslipidemia is a common feature of DM and needs to be treated because of the potential complications especially atherosclerosis. Cholesterol being a lipid moiety passes into saliva by passive diffusion and has the potential to closely reflect blood concentrations.

The cholesterol of case subjects is statistically higher than that for the control subjects. Karjailanen et al.¹⁶ suggested that salivary cholesterol may be regarded as a transudate from serum, as suggested by Slomiany et al. The positive correlation of serum and saliva cholesterol values further supported the concept that at least part of the salivary cholesterol originates from serum. They also concluded that, in healthy adults, salivary cholesterol concentrations reflect serum concentration to some extent and can be used to select individuals with high serum cholesterol levels. Medical Laboratory Observer (2000) gives information about a saliva-based cholesterol test that has demonstrated the potential to correlate a quantitative result with patient blood cholesterol level.

Ben Aryeh et al.¹⁷ found no difference in salivary total protein, amylase, lactoferrin, or lysozyme among the three groups (IDDM, NIDDM, Controls) examined. Streckfus et al.¹⁸ recorded that all diabetic groups in their study demonstrated a significantly lower salivary total protein concentration when compared with controls, which is in contrast to our study values.

There is a lack of literature on total protein and albumin estimations from the saliva of diabetic subjects, and the available data do not show a consensus on the results. These varied results indicate, as suggested by Rantonen et al.¹⁹ that these proteins are subject to short-term variation. Improved ways of evaluation of these elements in saliva with sophisticated techniques or another way of assessing the metabolic control in saliva needs to be considered.

The diabetic group and the control group did not differ with regard to the dental status as opposed to the usual scenario where diabetic children are expected to have more carious lesions. This could be attributed to the reduction in the frequency of sticky food intake and food with high glycemic index and better oral hygiene practices by the diabetic children. Swanljung et al.²⁰ found that if the patients' IDDM is well controlled, their salivary and caries data does not differ from that of healthy controls. Blanco et al.²¹ found no differences in the number of decayed, missing and filled teeth based on metabolic control, evolution time and existence of late complications of diabetes.

The lack of correlation between the salivary glucose and blood glucose values in DC underscores the importance of more studies needed in this direction. Both saliva and blood samples were taken during morning hours when the case subjects were in fasting mode. One of the confounding variables may be the fact that the cases in DC were under treatment as opposed to the cases in ND.

The cholesterol in DC was significantly higher than that of ND. Further research is needed, especially on the correlation of salivary and blood values of cholesterol, to have a better

understanding of this relationship.

Total protein and albumin can be used to assess the metabolic status of diabetic subjects since both showed a slight increase as compared to controls. The dental caries experience correlation between the cases and controls was not significant and gives credence to the value of diet and oral hygiene measures in the subjects. Salivary parameters can act as adjuncts in assessing the overall metabolic status of the patient. Further studies should be performed to make these tests equivalent to a blood test.

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