Diagnostic perspective of saliva in insulin dependent diabetes mellitus children: An *in vivo* study

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Abstract

Background and Objectives: The absence, destruction, or loss of β-cells of pancreas results in type 1 diabetes (insulin-dependent diabetes mellitus [IDDM]). Presently, diagnosis and periodic monitoring of diabetes is achieved by evaluating blood glucose levels as it is relatively invasive and dreaded by children. In the light of this, present study was planned to compare salivary glucose values with blood glucose values and the biochemical characteristics of saliva in IDDM children were evaluated and obtained results were compared with the salivary parameters of normal children. **Materials and Methods**: Thirty IDDM children and 30 healthy children were selected for the study. Fasting blood sample and unstimulated salivary sample were collected from all the subjects and were subjected for analysis. **Results**: A weak positive correlation was noticed between fasting blood glucose and salivary glucose values in IDDM children. But a mean average of salivary glucose was high in IDDM children when compared with healthy children. The biochemical parameters like acid phosphatase, total protein count, and α-amylase were increased, whereas salivary urea did not show significant variation between the groups. **Conclusion:** With presently used diagnostic armamentarium, estimation of salivary glucose cannot replace the standard method of estimation of glucose in diabetic mellitus children. The established relationship was very weak with many variations.

Keywords: Biomarkers, glucose, insulin-dependent diabetes mellitus, saliva

Introduction

It would not be misleading to depict the noble prize in physiology awarded to Ivan Pavlov in 1994 in recognition of his research on saliva's composition and functions. Yet, it is apparently due to Pavlov's inadvertent influence that saliva has attained a sovereign status of a body fluid worthy of scientific exploration.[1] If the oral cavity is mirror of the body[2] the saliva provides life to the tissues, structures, and organs present in the oral cavity, as blood to the body. Saliva is a glandular secretion and a complex fluid composed of a variety of organic and inorganic components, proteins, enzymes, glucose, and hormones at a gradient comparable with the serum.[1,3,4] The role of saliva in oral health and general health has been a curious subject of continued research, and the study of salivary functions has been challenging because of the high physiological variability of this fluid when compared to other body fluids such as plasma. This complexity in its composition, origin and nature had hurdled the medical profession to select this fluid for the procedures of disease diagnosis. Hence in the past, the identity of saliva as a diagnostic medium had suffered, questioned, and contradicted. But with the advances in technology, recognition of disease specific biomarkers, the evolution of molecular diagnostics in the past decade and a constant quest toward noninvasive sample collection has empowered the saliva as means for disease diagnosis.[5-9] Saliva has also clearly substantiated its diagnostic protagonist in various clinical conditions those include human immunodeficiency virus antibodies, conjugated steroid hormones, certain drugs, etc., and in the others extents there is an enduring research.[3,9-15]

Hence, this study was planned with a clinical condition which requires regular monitoring, i.e., Insulin-dependent diabetes

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mellitus (IDDM), as this disease is the most common in pediatric age group. This particular age group children below 14 years were selected for the study purpose. IDDM is an endocrine, metabolic syndrome of childhood and adolescence and its incidence increasing worldwide, especially under 15-year-old age group. Currently, diagnosis of diabetes is achieved by evaluating blood glucose levels.[16,17] If at all the glucose levels in the blood maintain a positive correlation with salivary glucose levels it not only paves the pathway for easy and early disease diagnosis, but also act as a means for a continuous stress-free monitoring of disease prognosis. The similar studies in literature reported conflicting results, i.e., few studies reported positive and few reported a lack of correlation. Thus, this study attempted to correlate the salivary glucose with blood glucose level in insulin-dependent diabetic children and in the healthy control group and also to describe alterations in biochemical characteristics of saliva in IDDM children.

Materials and Methods

A sample of 60 children aged between 4 and 14 were selected, in which 30 children who were attending diabetic clinics diagnosed as IDDM, without any other systemic complications were selected as the experimental group (Group I). In the control group (Group II), 30 healthy children were selected after performing glucose tolerance test and basic medical examination by the physician. Institutional ethical committee clearance and an informed written parental consent were obtained before the start of the study. Children were advised to come in the morning with an empty stomach (8 h) for collecting the sample. The blood samples were collected by venipuncture with the help of 2 cc sterile disposable syringe. Approximately, 1 cc of blood was transferred to vaccutainer coated with clot activators and allowed for clotting; afterward blood sample was centrifuged for 1000 rpm for 10 min. Ten microliters of supernatant serum was mixed with a reagent for calculation of serum glucose using Trinder's method.

Unstimulated saliva collection was done based on guidelines given by the University Of Southern California School of Dentistry.[18] The subjects were asked to refrain from oral hygiene procedures like brushing with fluoridated toothpaste, at least 1 h prior to salivary sample collection. Drinking water was given to the subjects to rinse their mouth. Five minutes after the oral rinse, unstimulated saliva was collected in 50 ml sterile plastic containers by spitting method. 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 1 min. The patient spat the saliva every 60 s for a total of 5 min into the container. These samples were placed in an ice containers which were maintained at a temperature ranging from −20°C to −80°C and sent for laboratory investigations. Further the salivary samples were centrifuged at 5000 rpm for 10 min, fractionated, and cooled down for protein determination. The remaining saliva was frozen for further analyses of the biochemical determinations like salivary glucose, total proteins, alpha-amylase, urea, and acid phosphatase.

Salivary glucose estimation

Salivary glucose levels were estimated using the glucose oxidase method in a semiautomated analyzer. The sample (100 μl) was mixed with the reagent in the ratio of 1:3 and incubated at 37°C. The readings of salivary glucose were recorded after 10 times dilution of the standard.

Salivary alpha-amylase and total protein urea and acid phosphatase estimation

Salivary amylase levels were estimated using the direct substrate kinetic enzymatic method. Mean absorbance change per minute was calculated and expressed as units per liter. Total protein estimation carried out using pyrogallol red dye by the end point method, and values were expressed as mg/dl. Acid phosphatase was determined by the calorimetric method and urea by enzymatic method.

Statistical analysis

Data entry, database management, and all statistical analysis were performed using Statistical Package for the Social Sciences (SPSS 20.0 version, Delaware, Chicago, IL, USA) software package. Values were expressed as means ± standard deviation and a $P \leq 0.05$ was considered significant. The *t*-test was used to discern the interindividual variations and Karl Pearson's correlation test was utilized to describe the association between blood glucose and salivary glucose.

Results

A weak positive correlation of 0.161 was noticed between blood glucose and salivary glucose in diabetic children (*P* value 0.396), which was statistically nonsignificant. However, a slight negative correlation −0.148 observed in nondiabetic children, $P = 0.434$ [Table 1 and Figure 1a and b].

The biochemical characteristics of saliva in diabetic and nondiabetic children were analyzed utilizing independent sample *t*-test [Table 2 and Figure 3a and b].

Statistically significant increase in the mean values of salivary total protein (0.84 ± 0.64 g/dl and 0.43 ± 0.31 g/dl, $P = 0.004$), glucose (8.56 ± 4.39 mg/dL and 5.06 ± 1.73 mg/dL, $P = 0.000$), acid phosphatase (10.98 ± 5.79 and 6.57 ± 4.08 $P = 0.001$), and α-amylase (166,188.93 ± 365,717.3 and 10,439.3 ± 10,976.65, $P = 0.023$) were observed in diabetic children. The mean value of salivary urea did not show any statistically significant difference.

Discussion

Diabetes itself is a serious condition, it is proved to be a foremost risk factor for disorders like blindness, renal

failure, and micro- and macro-vascular diseases. Renowned studies on diabetes specify that higher the prevalence of complications, if the onset of the disease is at very young age and if the diagnosis and presentation of the condition are delayed and/or missed.[14,16,19,20] This has become a burning issue in underdeveloped, developing, and even in developed countries. Thus, current epidemic of the disease and presence of large diabetic population highly deserves a noninvasive method for its diagnosis and monitoring.

Saliva believed to contain all the medical information as blood (DNA, proteins, hormones, metabolites, and immune effectors) owing to its thin epithelial layer that separates the salivary ducts from the systemic circulation thus facilitating an easy exchange of substances between plasma and saliva.[6,11,21]

Considering saliva as an ultrafiltrate of blood, this study was designed to evaluate the correlation between salivary glucose and blood glucose levels in IDDM children and also to describe biochemical characteristics of saliva in these children, consequently it may pave the way to distinguish any specific salivary biomarkers to characterize specific disease states.

Table 1: Correlation between salivary glucose with FBS

Clinical parameters	Karl Pearson's correlation	Diabetic Saliva glucose	Nondiabetic Saliva glucose
FBS	Correlation	0.161	−0.148
	P	0.396 NS	0.434 NS

Statistical analysis: Karl Pearson's correlation. NS: Not significant; FBS: Fasting blood sugar

In this study, mean salivary glucose level in diabetic children was significantly ($P = 0.000$) higher in diabetic children when compared with nondiabetic counterparts. Similar results were reported by most of the previous studies.[16,22-27]

In this study, a weak positive correlation ($r = 0.161$) was noticed between fasting blood glucose and salivary glucose levels in diabetic patients [Table 1 and Figure 1]. Similar observations were also reported in few previous studies,[14,19,23,24] whereas a strong positive correlation has been reported recently by Mussavira *et al*.[28] This difference in the results can be attributed to the selection of the sample. This study has focused on IDDM children, whereas above said study focused on non-IDDM (NIDDM) adult patients. The other reasons would be in NIDDM subjects the duration of disease would be longer which would result in an alteration of the epithelial and neural component. Further in this study, the study sample was selected from the diabetic clinics thus there was most likely chance that the condition of the disease would be well under control and hence the complications. López *et al*. in 1987 reported a positive correlation between

Table 2: Salivary biochemical characteristics in diabetic and nondiabetic children

Biochemical parameters	Mean±SD			*t*	*P*
	Diabetic	Nondiabetic	Difference		
Total proteins (g/dL)	0.84±0.68	0.43±0.31	0.41±0.37	3.010	0.004 S
Glucose (g/dL)	8.56±4.39	5.06±1.73	3.5±2.66	4.066	0.000 S
Urea (g/dL)	28.3±9.78	25.01±7.32	3.29±2.46	1.475	0.146 NS
Acid phosphatase (U/L)	10.98±5.79	6.57±4.08	4.41±1.71	3.412	0.001 S
Alpha amylase (U/L)	166188.93±365717.3	10439.3±10976.65	155749.63±354741	2.332	0.023 S

Statistical analysis: Independent sample *t*-test. Statistically significant if $P<0.05$. SD: Standard deviation; S: Significant; NS: Not significant

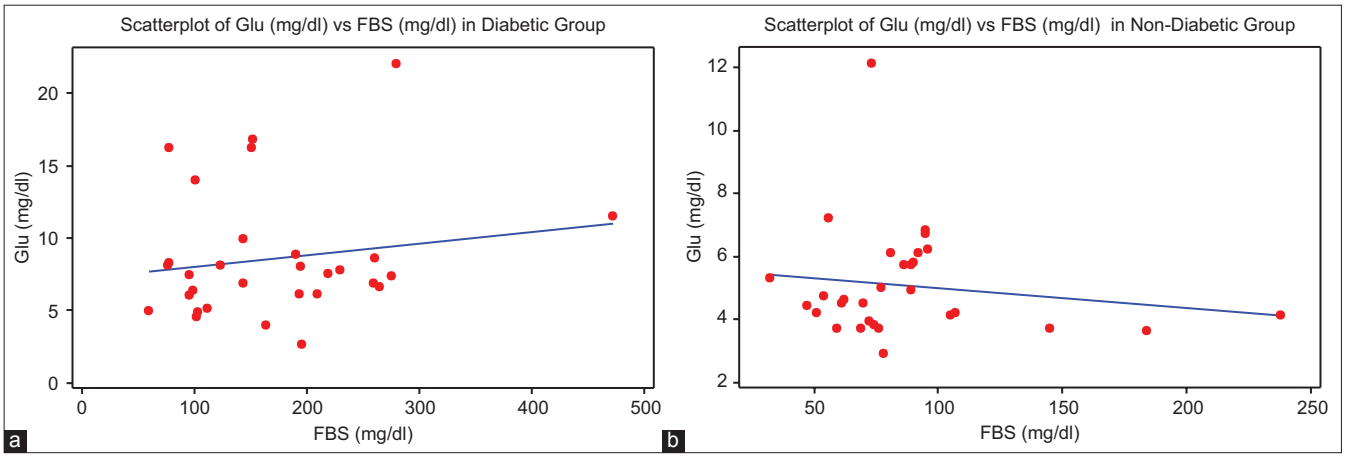

Figure 1: (a) Correlation between salivary glucose with fasting blood sugar in diabetes. (b) Correlation between salivary glucose with fasting blood sugar in nondiabetes

salivary and blood glucose levels only when blood glucose levels were approximately 15 mmol/L and suggested that similar to urine; there could be a threshold mechanism be existent for saliva at blood glucose concentrations of about 10–15 mmol/l.[23] In contrast, significant positive correlation was reported between fasting blood glucose and salivary glucose in NIDDM and uncontrolled IDDM.[12,15] The attained values in this study displayed high individual variations, i.e., in some subjects the correlation between blood and saliva glucose was high, whereas in others high blood glucose did not result in any notable elevation of salivary glucose. The reasons that could be attributed were individual variations in the permeability of glandular epithelium, multiple sources of glucose from which it will be secreted into saliva, varying degrees of microbial load in the oral cavity which may compete for utilization of secreted glucose for their metabolism and alter its availability for testing, occurrence, or absenteeism of gingival inflammation that may influence readings,[13] as selected sample of children are under the treatment for diabetes these drugs used may interfere with the secretion of glucose into the saliva and also there could be existence of threshold mechanism similar to renal threshold. Although there was no consistent increase in salivary glucose level along with blood glucose, the mean salivary glucose levels were higher in diabetics when compared with healthy nondiabetics.

Though the positive correlation established between blood glucose and salivary glucose was weak, it may perhaps a significant determination due to obtained negative correlation in healthy children [Table 1 and Figure 1b]. By standardizing the above-mentioned hindrances, a definitive positive correlation could be expected.

When biochemical characteristics of saliva were evaluated, increased levels of total protein content, acid phosphatase, and α-amylase were noted in IDDM children as compared to control children [Table 2 and Figure 2a and b]. In similar studies, akin, as well as contradicting findings have been reported in literature.[10,14,23,25,29-32] Among all the series of α-amylase levels were strikingly high [Figure 2b], but these levels will be increased in many disease conditions those include renal diseases, cardiovascular diseases, psychological conditions such as stress and pain, etc. Thus, studies correlating salivary α-amylase levels with blood glucose levels needs to be accomplished to consider α-amylase as a potential marker. It has been reported in literature that the diabetic patients who are under treatment with insulin having good metabolic control exhibit elevated levels of α-amylase. In this study, the drastic hike of α-amylase may be an indicator of above said statement[33] thus showing its prognostic value.

According to the results of recent research, the saliva can be described as clinically informative, biological (biofluid) fluid useful for novel approaches such as laboratory or clinical diagnosis and for monitoring the prognosis.

Considering salivary glycated protein instead of salivary glucose, searching for biomarkers that are not usually native of saliva but specifically appear during the absolute disease conditions, targeting at glandular saliva rather than the saliva collected from oral cavity in order to avoid confounding variables from oronasal mucosal secretions, gingival crevicular fluid, and from oral wounds and standardizing the local and systemic influencing factors may positively influence the dependability of saliva as diagnostic medium for diabetic conditions.

Conclusive Interpretations

In this study though the upsurge of salivary glucose was not always in correspondence with serum glucose, a significant increase in mean salivary glucose levels in IDDM children sustains the hope of salivary diagnostics for diabetic conditions.

Even though the salivary components like total protein, acid phosphatase, and α-amylase were increased in IDDM children when compared to healthy counterparts, the strikingly high values of α-amylase calls for an additional assessment of this association.

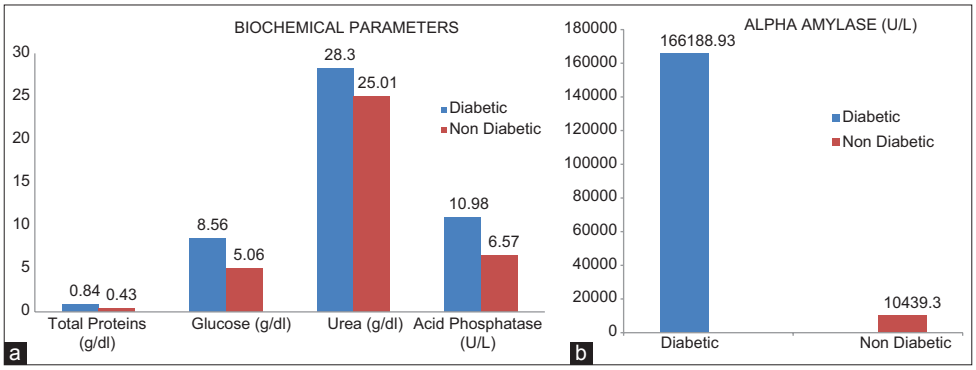

Figure 2: (a) Biochemical characteristics of saliva in diabetic and nondiabetic children. (b) ALPHA-amylase between diabetic and nondiabetic children. *Statistical analysis: Independent sample *t*-test. Statistically significant with *P* value 0.023

Though the saliva has already been established as a diagnostic medium for certain diseases due to the presence of locally produced and serum derived markers, use of saliva for diagnosing IDDM is still needs to be furthermore evaluated.

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Conflicts of interest
There are no conflicts of interest.

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