Original Article

Comparative Assessment of Levels of Total Proteins in Saliva on Control and Diabetic Patients

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Abstract

Diabetic Patients frequently report for Dental problems; however assessment of their diabetic status though empirical is quiet challenging. Either patients do not know about their diabetic status or are not willing to get it done due to financial constraints or chances of cross infection. Some of them get it checked but do not report at proper time. Though serum analysis has always been a gold standard for diagnosis of various diseases, but serum assessment at dental clinic is not possible because of non-existence of such culture. In the recent past assessment of other body fluids such as Saliva as diagnostic tool has gained importance. Aim of this study was thus to assess level of Total Proteins in saliva of control and diabetics and correlate between Total Protein levels and incidence of caries and gingival health. Study was conducted on 90 subjects; 30 control and 60 diabetics. Sample was selected on the basis of history, HbA1c levels, serum sugar levels and salivary sugar levels. Un-stimulated whole saliva from diabetics and control group was then collected, stored and freezed at -20°C. Saliva samples were then defrosted centrifuged and its supernatant portion was analyzed by Dimension Clinical Chemistry System for Total Proteins. SPSS 17.0 was used for statistical analysis. Results showed that salivary Total protein levels were higher for the diseased group when compared with control group and were correlated statistically significantly with caries and poor gingival health.

Key Words: Serum Glucose Level, Salivary Total Protein Level, Diabetes Mellitus, Caries, Gingival Health.

Introduction

Considerable number of patients reporting for dental procedures has Diabetes Mellitus (DM).[1] Diabetes mellitus a common disease of 20th century has been linked with an increased risk of caries, gingivitis and periodontal disease.[2] Almost 6.2 million people of Pakistan are diabetics, ranking sixth largest diabetic population in the world.[3] In 1982 diabetes affected more than 3% of Pakistan's population and in 2008 it occurred in 10% of Pakistan population in both sexes 25 years or above.[4] This incidence definitely would have been increased in last 3 years. This reported incidence of DM in Pakistan is definitely much low than expected as most of the rural population didn't even know / report their diseases.[5,6] Diabetes mellitus is a chronic disease that requires long – term medical attention both to limit the development of its devastating

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complications and to manage them when they do occur. It is a disproportionately expensive disease; in the United States in 2002, the per-capita cost of health care was $13,243 for people with diabetes, while it was $2560 for those without diabetes. The emergency department utilization rate by people with diabetes is thus twice than that of the unaffected population.[7] Such data for Pakistani population is needed for understanding grievance of this disease.

Diabetes is a wide spread metabolic disease characterized by impaired insulin secretion or insulin resistance resulting in carbohydrate and fat abnormalities of metabolism and decrease sensitivity of tissues to insulin.[8] It is characterized by two types. Type 1 Diabetes mellitus and type 2 Diabetes mellitus. The type 1 has an abrupt onset and occurs usually in children and young adults. It presents with polydypsia, polyuria and polyphagia. There is negligible endogenous insulin due to viral or autoimmune destruction of beta cells of islets of langerhans.[9] Type 2 diabetes mellitus comprises an array of dysfunctions resulting from the combination of resistance to insulin action and inadequate insulin secretion. It`s disorders are characterized by hyperglycemia and associated with microvascular, macrovascular and neuropathic complications. Unlike patients with type 1 diabetes mellitus, patients with type 2 are not absolutely dependent upon insulin for life.[10]

Diabetes causes numerous oral changes. Several studies have mentioned different oral changes in diabetic children, which include decreased salivary flow, burning mouth and tongue, increased rate of dental caries and also influence on periodontium.[11] It is hypothesized that these oral changes may be attributed mainly to alterations in salivary glands, its biochemical constituents and decreased flow rate. Changed oral environment may cause increase in pathogenic bacteria and cause destruction of hard and soft tissues of mouth leading to cariogenic and gingival lesions. Salivary flow and its composition influence calculus formation, periodontal disease and caries.[11] Many of such patients need surgical interventions.

Diabetic Patients frequently report for Dental problems; however assessment of their diabetic status though empirical is quiet challenging. Though serum analysis has always been a gold standard for diagnosis of various diseases, but in the recent past assessment of other body fluids such as Saliva as diagnostic tool has gained importance as it is readily available; collection requires non-invasive approach, no chances of cross infection, can be performed at dental chair side and above all being the representative of various changes occurring in the body.[12-14]

Salivary constituents gets affected in diabetic patients; different studies have shown affected levels of salivary glucose, amylase and total proteins in diabetic patients. Role of total proteins though have been studied but extensive studies are needed to correlate its relation with dental & gingival problems.[15-17]

Aim of this study was thus to establish relationship between salivary total protein levels and diabetes and correlate total protein levels with the incidence of caries and gingival health.

Methodology

The study was conducted on 90 subjects; 30 control and 60 known diabetics as assessed from the medical history of the patients, HbA_1c Levels[18] reflecting levels of glycemia over the preceding 3 – 4 months (by using GLYCOSAL™ HbA_1c control kit, Product Code 901025J). Patients were then grouped into control group (HbA_1c level < 7.0%), Diabetic Group (HbA_1c level > 7.0%), serum sugar levels (Control Group 70 – 110 mg/dl and Diabetic Group > 110 mg/dl) and Salivary glucose levels (Control Group < 9 mg/dl and Diabetic Group > .9 mg/dl).[19]

Un-stimulated whole saliva from diabetics and control group was then collected, stored and freezed at -20°C and then brought to the laboratory in a ice chilled box. Saliva samples were then defrosted centrifuged and its supernatant portion was analyzed by Dimension Clinical Chemistry System for Total Protein levels. The total protein method is a modification of the biuret reaction.

Principles of Procedures

$\mathbf{Cu}^{++}$ + Protein $\xrightarrow{OH}$ Complex (absorbs at 540 nm).

Procedure

The TP Flex® reagent cartridge, Cat. No. DF73 is required to perform TP test.

Test Steps

Sample reagent delivery, mixing, processing and printing of results are automatically performed by the dimension system.

Test Conditions

- Sample size 15 microlitre
- Reagent 1 vol 85 microlitre
- Reagent 2 vol 85 microlitre
- Diluent vol. 315 microlitre
- Test temperature 37 degree C
- Wave length 54 and 700 nm
- Type of measurements biochromatic endpoint

Gingival status of the patients were assessed by CPITN Index and Caries was assessed by DMFT.

SPSS 17.0 was used for statistical evaluation. Descriptive Statistics were calculated for each variable for each subject. Total Protein levels were then compared between diabetic and control group.

Results

The study was conducted on 90 subjects; 30 control and 60 known diabetics as assessed from the medical history of the patients. Patients were then grouped into control group (HbA_1c level < 7.0%), Diabetic Group (HbA1c level > 7.0%), serum sugar levels (Control Group 70 – 110 mg/dl and Diabetic Group > 110 mg/dl) and salivary glucose levels (Control Group < 9 mg/dl and Diabetic Group > 9 mg/dl).

Total protein concentration (gm/dl) in saliva in patients with diabetes mellitus was compared with healthy population. The mean salivary total protein level in diabetic cases was 0.605 ± 0.131 ranging from 0.42 to 0.95 mg/dl and that of control was 0.25 ± 0.067 ranging from 0.10 to 0.40 mg/dl. From these data we can see that salivary total protein concentration in Saliva of diabetic patients was significantly higher as compared to healthy population ($t = 14.07$, $p = 0.001 < 0.05$) and may be a reason for increased caries and gingival incidence as shown in table 1.

Mean salivary total protein level among cases having good oral hygiene condition (healthy) was 0.57 ± 0.07, while mean salivary total protein level among cases having poor oral health (gingivitis / calculus) was 0.78 ± 0.16. Student's t-test was applied to show the difference between two means. It was shown that salivary total protein was significantly higher in cases having poor hygiene status than having good oral health ($p = 0.040 < 0.05$).

Mean salivary total protein level was calculated in all diabetic subjects having permanent/primary decayed teeth. The level of salivary total protein was correlated with the number of permanent teeth decayed. It was shown that mean total protein level increased with the increase in number of decayed teeth. Value of

Table 1: Comparison of Salivary Total Protein levels among study groups.

Study Groups	Salivary total Protein levels (gm/dl)			
	Minimum	Maximum	Mean	± SD
Controls (n = 30)	0.10	0.40	0.25	0.067
Diabetic Group (n = 60)	0.42	0.95	0.605	0.131

Figure 1: Comparison of Mean Salivary Total Protein levels among study groups

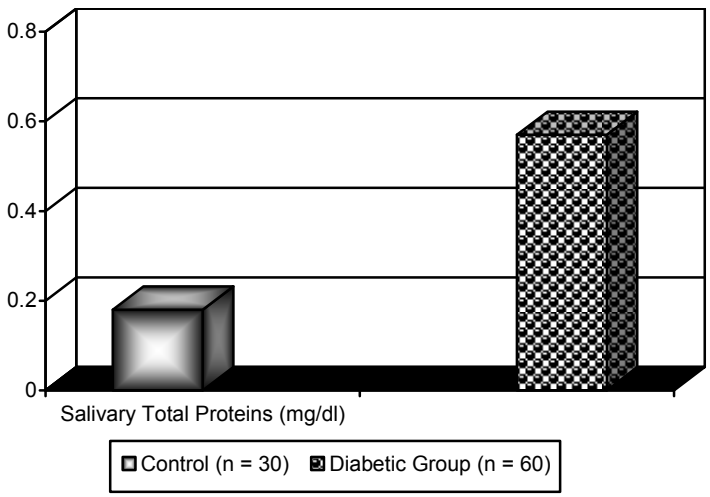

Table 2: Correlation of Total Salivary Proteins with gingivitis status among cases.

Salivary constituents	Gingivitis Status		p value
	Good (Healthy)	Poor Gingivitis / Calculus	
Salivary total protein (gm/dl)	0.57 ± 0.07	0.78 ± 0.16	0.040

Table 3: Levels of Salivary Protein among cases with reference to their decayed permanent / primary teeth.

No. of Permanent / Primary Teeth Decayed	Salivary Protein gm/dl	
	Cases n = 60	
	Mean	± SD
0.	0.59	0.10
1.	0.49	0.06
2.	0.59	0.14
3.	0.63	0.10
4.	0.69	0.11
5.	0.79	0.10
6.	0.62	0.05
7.	0.46	0.05
8.	0.69	0.19
9.	0.95	-

Pearson's correlation coefficient r = 0.320
P = 0.013 < 0.05, so correlation is significant

Pearson's correlation coefficient r was computed using SPSS software. For salivary total protein r = 0.320, which shows a positive correlation. Value of p = 0.013 < 0.05 showed that the correlation is significant.

Discussion

In present study total protein in the saliva samples of the diabetic children was high (p = 0.001 < 0.05) that is the mean values in diabetic and non-diabetic children was 0.6 and 0.25 mg/dl. These results matched with the other study showing that total protein concentration was raised in saliva of subjects with IDDM.[20,21] Lopez ME et al in their study concluded that salivary sugars, glucose, urea and total proteins were greater in diabetic patients than controls, while calcium values were decreased. Moreover Diabetic children had higher DMFT – dmft – deft and DMFS – dmfs – defs values compared to those of the control children despite their lower sugar intake.[17] Yavuzyilmaz E et al found n their study that the mean salivary total protein, amylase and secretory IgA levels in the DM group were 2.41 ± 1.0 mg/mL, 124.2 ± 79.7 U/mL and 6.86 ± 3.50 mg/L, all being significantly higher than the control group.[22]

In contrast to this study one study shows no significant differences in total protein in saliva between study groups and in this study mean of salivary protein in diabetic and control group was 1.4 and 1.26 g.[23] Panchbai et al[24,25] in their study evaluated saliva samples for levels of glucose, amylase and total protein, and assessed salivary flow rate in diabetics and healthy non-diabetics. A total of 120 age – and sex – matched participants were divided into 3 groups of 40 each; the uncontrolled diabetic group, the controlled diabetic group and the healthy non-diabetic group and concluded that Mean salivary glucose levels were significantly elevated in both uncontrolled and controlled diabetics, as compared to healthy non-diabetics. There were significant decreases in mean salivary amylase levels in controlled diabetics when compared to healthy non-diabetics. However salivary total protein levels were not found to be markedly affected in diabetes mellitus. Most of the previous studies established no significant differences between diabetics and non-diabetics.[16,26-29] Tenovuo J, et al in their study concluded that no differences between the study groups (Control group and diabetic group) existed in flow rate, protein content, amylase activity, or IgM.[26] While Streckfus et al estimated significant lower protein concentrations in diabetics and emphasized protein utilization by other biochemical metabolic pathways an overall systemic response to glucose intolerance.[30]

Statistically significant correlation existed in this study between the total protein levels which were raised for diabetic group and the incidence of caries. Nihon Eiseigaku Zasshi. In his study evaluated the relationship between salivary components (calcium: Ca, total protein: T-Pro) and dental caries, in 131 primary school children aged 11 years old and on inclusion of variables for the Ca / T-Pro ratio, numbers of

erupted permanent teeth, sex and salivary flow rate into the model as independent variables, concluded statistically significant association ($P < 0.05$) between the Ca / T-Pro ratio and the numbers of DMF teeth.[30] Javed F et al aimed their study towards establishing association between periodontal conditions, oral yeast colonisation and salivary proteins in subjects with type 2 diabetes (T2D) with reference to gender and concluded that Clinical and salivary parameters of periodontal inflammation (BOP and IgG (mug)/mg protein and total proteins) were higher in type 2 diabetic females with oral C. albicans colonisation compared to males in the same group.[31] Rao NS et al in their study concluded that whole salivary proteins were not associated with dental caries, except for the 17 kDa salivary protein, which might be risk marker for dental caries.[32]

Conclusion

It was concluded in this study that salivary sugar levels and Total protein levels were higher for the diseased group when compared with control group. Associated increased incidence of caries and poor gingival health was established.

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