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Biochemical Evaluation of Saliva in Insulin Dependent Diabetes Mellitus Children in Hilla City-Iraq

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Abstract

Objectives: Failure of pancreas to produce enough insulin due to beta cell loss is typically referred to as type1 diabetes mellitus (T1DM)

Methods: Oral examination and non -stimulated saliva samples were collected from TIDM children and healthy children (non –diabetic) as control group, with age ranging from 10 to 13 years old. Different materials have been used for evaluation purposes.

Aims of this study: Salivary parameters, like total protein, phosphate, alpha amylase, glucose and calcium concentration, and dental caries were evaluated and compared between control group and type1 diabetes mellitus (T1DM) children.

Results: Our results revealed that phosphate, glucose, and α-amylase shown significant variation, whereas salivary total protein count and calcium did not show any significant variation between the groups. Caries experience status was evaluated using the DMFT and dmft. The recent results found significant differences of DMFT between the TIDM children and control group. There was statistically significant positive correlation between glucose and DMFT value.

Conclusion: Early detection of TIDM children is important to prevent oral problems. Salivary parameters are helpful tools to recognize T1DM children at higher risk for developing caries. Good oral hygiene and dietary regime should be obtained.

Keywords: Type I diabetes, salivary parameters, dental caries

INTRODUCTION

Hilla city in Babylon province is located 100 kilometer south to Baghdad, which is the capital of Iraq. In this city, there is a center that is specialized for diabetic patients from which the children were selected.

Type 1 diabetes can affect children, commonly referred to as juvenile diabetes because most of these cases of diabetes were in children" [1]. However, T1DM can be accompanied by high and irregular blood glucose, often with ketones, and sometimes at low blood glucose levels. Other complications include the emergence of a negative regulatory response to hypoglycemia, infection, and gastric paralysis, which leads to irregular absorption of dietary carbohydrates [2].

Hyperglycemia in addition to damage to different systems in the body may weaken the functions of the salivary glands, resulting in a decrease in the flow of saliva and the alterations in the components of saliva. consequently, many dental and mucosal changes can occur which include the spread of different pathogenic bacteria , taste saliva is the body's natural protective mechanism against dental caries. saliva acts as a critical regulator and internal defense system against this disease through its physical and chemical properties as flow rate and buffer capacity and through its inorganic and organic constituents such as calcium bicarbonate, phosphorus and enzymes [3,4]. Dental decay caused by a breakdown made from acid bacteria [5]. It include signs of pain and difficulty in eating [6, 7]. Complications can include tissue inflammation surrounding the teeth, composition of abscess, infection or tooth loss [6,8]. The reason of decay is an acid microbes which dissolves solid tissues of the teeth, acid is produced from microbes when diet, sugar residues are broken on surface of the tooth [9].modifications in saliva components can affect development, signs and the severity of oral changes in diabetics to detect salivary components in DM may be useful in the understanding and management of oral manifestations [10].This study has been carried out to assess salivary changes in both groups, and notice any possible association between glycemic control and dental caries with T1DM children compared to non-diabetic children.

MATERIALS AND METHOD

Different materials had been used in this study as shown in table 1-1

Table (1-1) Chemical Kits used in this study

Name of the Kit	Company Origin
Calcium	Biolabo France
Phosphorus	Biolabo France
Alpha amylase	Biolabo France
Glucose	Linear chemical
protein	Biolabo France

METHODS:

TIDM children and control group

The study protocol was approved by research Ethics committees in Hilla city. Parents of the participants signed a consent form. Forty TIDM children attending Murjan Medical Center for Diabetic were selected. Exclusion criteria were the use of any medication that could affect or could cause failure to cooperate with the oral examination, and forty healthy (non -diabetic) children were selected from children attending Pediatric Department at the

College of Dentistry, University of Babylon considered as control group matching the study group in most of the variable .The study was conducted during the period from September 2017 to May 2018. Both genders were participants in this study with age ranging from 10 to13 years old.

Collection of Saliva Samples

Non stimulated whole saliva were collected from the participants the 5 ml of saliva samples were collected around 9:00 o'clock in the morning, while they were in a seated position and their head inclined forwards and ask them to spit it into a measuring sterilized cup over a period of 5 minutes, and immediately was centrifuged at 3000 rpm for 20 minutes to get clear supernatants. Lastly, they were stored at -20ºC for later evaluation of the all parameters decided to evaluate in this study [32].

Procedure for evaluation salivary parameters.

The pipette in the test tubes has been well identified.	Blank	Normal	Assay
Work reagent	1 mL	1 mL	1 mL
Demineralized water	20 µL		
Normal		20 µL	
Sample			20 µL
Mix well. Let stand for at least 10 minutes at room temperature. Read the absorbance at 600 nm (578-612) against the blank reagent.			

Calculation

Calculate the results as follows:

Result = $\frac{Abs\ (Assay)}{Abs\ (Standar)}$ x Standard concentration

Oral examination

The oral examination was performed for both groups by specialized dentist, children were seated in a reclining chair during the examination which were conducted under artificial lighting using dental mirror and explorer. Caries were recorded following WHO (1987). Dental caries was reported by the DMFT/dmft.

Statistical Analysis'

Statistical analysis was performed with statistical package for social science; SPSS. SPSS version 17 for windows. T-test was used for analysis of (dmft/DMFT) index. Biochemical parameters were used to compare between TIDM children and control groups.The ($P \leq 0.05$), ($P \leq 0.001$) were considered significant, highly significant respectively. Person's correlation was used to examine the relation between biochemical parameters and dmft/DMFT in both groups.

RESULTS

Evaluation of the Biochemical Changes in Saliva

Biochemical characteristics of saliva in TIDM children were calculated and obtained. The results were compared with the saliva parameters of control group. The results found that phosphate, glucose, and α-amylase shown significant variation, whereas salivary total protein count and calcium did not show any significant variation between the groups, as shown in table (1-2).

Caries Experience Examination

Caries experience was evaluated using the DMFT and dmft. The results revealed that there were significant differences in caries experience in permanent dentition (DMFT index) value between the TIDM children and control groups. On the other hand, there were non-significant differences of caries experience in primary teeth (dmft) between the diabetic and control groups, as shown in table (1-3).

Figure '(1-1) shows the correlation coefficient between DMFT values with glucose. In both TIDM and control groups, there was a significant positive correlation between DMFT with glucose ($r = 0.66$), ($p<0.05$), while there was no significant correlation between DMFT with calcium, phosphate, protein ($p>0.05$).

Table (1-2) shows the mean value for salivary calcium, total protein, phosphorus, glucose, and alpha-amylase enzyme among TIDM and control group in children.

Salivary parameter	Group	N	Mean	Std. Error Mean	Mean Difference	T Value	P Value
Calcium (mg/dl)	Control	40	7.58	± 0.30	0.81	1.45	0.14**
	TIDM	40	6.77	± 0.46			
Phosphorus (mg/dl)	Control	40	87.27	± 2.94	17.17	2.74	0.008*
	DM	40	104.44	± 5.52			
Total protein (mg/dl)	Control	40	232.85	± 7.51	5.65	0.39	0.69**
	TIDM	40	227.20	± 12.25			
Glucose (mg/dl)	Control	40	15.52	± 0.97	8.57	2.68	0.009*
	TIDM	40	24.10	± 3.04			
Alpha Amylase (U/L)	Control	40	2824.20	± 24.56	182.750	5.67	0.00*
	TIDM	40	3006.95	± 20.84			

*Significant : $p<0.05$, **No significant: $p>0.05$.

Table (1-3) reflects caries experience by DMFT/dmft for both dentition in control and TIDM children.

Caries Experience	Group	N	Mean	Std.Error Mean	Mean Difference	T value	P value
DMFT	Control	40	1.22	±0.22	1.42	3.36	0.001*
	TIDM	40	2.65	± 0.35			
Dmft	Control	40	0.60	± 0.10	0.27	1.13	0.26**
	TIDM	40	0.87	± 0.22			

Highly significant:*p <0.001, No significant: **p>0.05

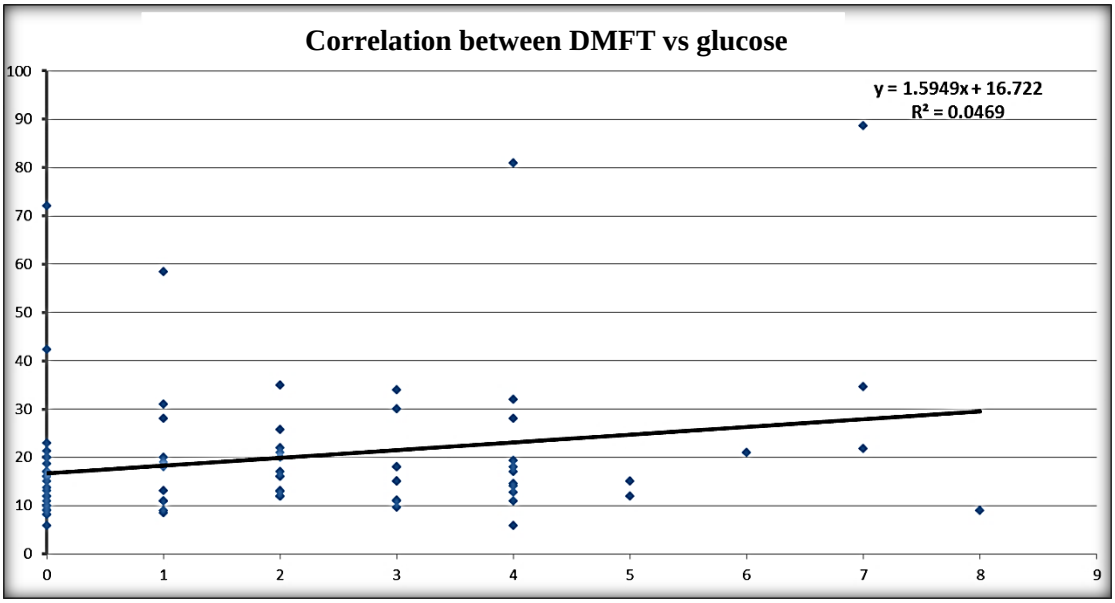

Figure (1-1) Correlations coefficient among DMFT and glucose

DISCUSSION

Evaluation of the Biochemical Changes in Saliva

Saliva is resemble to bloodstream to the mouth because it play a role in building and maintenance of oral health .Our results showed there were no statistically significant differences in salivary calcium levels between the two groups. The obtained results were agreed with other study [11]. Calcium level decreases because of the low insulin, which leads to a stimulating activity on the proliferation of osteoblast and calcium homeostasis impairment, increasing glucose increases the secretion of calcium that is proportional to the degree of glycosuria [12].

This study showed a significantly higher level of salivary phosphate in TIDM children as compared to the control group This finding was in agreement with other study [13]. This may be due to a decreased flow rate of saliva and phosphorus secretion in the saliva of degraded periodontal proteins in DM patients [12].

Our finding revealed that there was no significant difference in the salivary total protein level in TIDM children comparing with the control group, which is in consistent with the findings of other study [14] .Mata *et al*. reported significantly higher level in TIDM children [15] in contrary to the study that recorded higher level in control group [17], these differences in recording may be due to daily variation, speed and time of centrifugation of the samples. [18].

Continuous hyperglycemia leads to changes in microvascular vessels in the blood vessels, as well as basement membrane changing in the salivary glands this leads to increased glucose leakage of the salivary gland cells in the channels, which increases glucose in the saliva. [20] .Although decrease salivary glucose were recorded in diabetics patients [19], the present work reported significantly higher salivary glucose level in TIDM children vs control group and this may explain the significant positive relation between DMFT values with glucose in both groups. This result agreed with other study [31]. Increase salivary glucose levels with increased DMFT index can play a significant role in the occurrence of caries and preventive program should be conducted.

Salivary alpha amylase plays an important role in binding and adhesion of certain type of bacteria. Different factors may affect the production of this enzyme like stress, hormonal and metabolic changes in diabetes mellitus patients, insulin deficiency in TIDM leads to more disorders of amylase in the blood type II diabetes [22].Our work resemble other study [13] by having higher levels of alpha amylase in TIDM children vs control, in contrary to other that reported lower level [21].

Caries Experience Examination

Concerning dental caries experience in primary dentition there was not any significant differences between both groups. This result agreed with other studies [23] because of the modification in the diet with reduced amounts of intake of refined carbohydrates by diabetics. Thus reducing the formation of an acidic environment, especially sucrose,

it is recommended that there be an increase in the amount of protein that enhances the ability of buffering saliva [24]. In this study, DMFT value was significantly higher in TIDM children when compared with control group counterparts), This result was in agreement with other studies [11,25] due to low flow rate saliva and dry mouth may lead to dental problems and are consistent with high DMFT rate [26]. Increased risk of tooth decay may be associated with some factors such as poor oral hygiene, few dental visits, deficiency of controlled metabolism of diabetes, or lack of control of blood glucose levels [27] Others reported low prevalence of dental caries among diabetics [28] and some authors did not find any significant difference in the DMFT index between TIDM children and control group [29], this may be due to good metabolic regulator that prevents the most dangerous alterations in saliva such as high glucose, low pH and a good diet for diabetics, rich in fiber and low carbohydrates, can slow the production of plaque and proliferation of microbial bacteria [30].Awareness about the oral health status of children with T1DM is important to understand the many risk factors involved for establishing appropriate preventive and therapeutic approaches against oral complications.

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