The relation of salivary glucose with dental caries and Mutans Streptococci among type1 diabetic mellitus patients aged 18-22 years

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ABSTRACT

Background: Diabetic is a chronic systemic disorder of glucose metabolism. That could be diagnosed using fasting and/or random plasma glucose and Glycated Haemoglobin (HbA_1c). Several biochemical and microbial alterations of saliva could affect dental caries occurrence and severity among diabetic patients. The aim of the present study was to assess the relation of salivary glucose with severity of dental caries and Mutans Streptococci, among uncontrolled and controlled diabetic groups in comparison with non-diabetic control group.

Materials and Methods: The total sample composed of adults aged (18-22) years. Divided into 25 uncontrolled diabetic patients ($HbA_1c > 7$), 25 controlled diabetic patients ($HbA_1c \leq 7$), in addition to 25 non-diabetic healthy looking individuals. Fasting blood sugar was determined for the diabetic patients. The diagnosis and recording of dental caries was according to severity of dental caries lesion through the application of $D_{1\_4}MFS$ (Manji *et al*, 1989) and stimulated salivary samples were collected under standardized condition (Tenovuo and Lagerlöf, 1994). Salivary glucose was estimated using spectrophotometric analysis. Viable count of mutans streptococci (on Mitis- Salivarius Bacitracin Agar) was determined.

Results: salivary glucose among uncontrolled diabetic group and controlled diabetic group were highly significant higher than control group ($p<0.01$). Analysis among uncontrolled diabetic patients and non-diabetic control group revealed that the salivary glucose correlate positively highly significant with caries experience represented DMFS ($p<0.01$), while among controlled diabetic group the correlation was not significant in positive direction concerning DMFS ($p>0.05$). The correlation between salivary glucose and Mutans Streptococci among three groups was highly significant in positive direction ($p<0.01$).

Conclusion: There are significant correlations between salivary glucose, severity of dental caries and mutans streptococci in uncontrolled diabetic group.

Keywords: Diabetic mellitus, Salivary Glucose, dental caries, Mutans Streptococci. (J Bagh Coll Dentistry 2015; 27(3):146-151).

INTRODUCTION

The World Health Organization described Diabetic mellitus as metabolic disorder of multiple etiology characterized by chronic hyperglycaemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action or both [(1)]. The two main types of diabetes mellitus are type 1 or insulin-dependent diabetes mellitus (IDDM) and type 2 or non-insulin-dependent diabetes mellitus (NIDDM) [(2)]. Type 1 diabetes as the name indicates, the patients are totally dependent on exogenous insulin therapy, because all the insulin-producing β cells in the Langerhans islets of the pancreas are ultimately destroyed [(3)]. It's only about 30 percent of the risk for type 1 diabetes is genetically determined, while the rest may be related to environmental factors [(4)].

Individuals with type 1 diabetes mellitus and poor glycemic control (FBS180mg/dl; $HbA_1c>8$) have elevated salivary glucose concentration as a result of hyperglycemia, reduction of the salivary glucose clearance, disturbance of the neuroregulatory mechanism of the salivary glands and increased permeability of the basal membrane of the parotid glands [(5,6)].

Amer *et al* [(7)] conducted a study on diabetic patients and found a significant association between the glucose levels in blood and saliva. The findings thereby indicated that salivary glucose evaluation may be a potential tool to monitor diabetics.

According to Tenovuo *et al* [(8)], the saliva of diabetics contains larger quantities of glucose owing to the leakage of glucose from the blood to the oral cavity, so the increased oral acidity as a result of the excessive amount of salivary glucose causes alteration in dental biofilm, predisposing to colonization by *Streptococcus mutans* and *Lactobacillus* and, thus, dental caries development [(5)]. Dental caries is the localized destruction of susceptible dental hard tissues by acids produced by bacterial fermentation of dietary carbohydrates [(9)]. Today, mutans Streptococci are considered to be the main aetiological microorganisms in caries disease, with lactobacilli and other microorganisms participating in the disease progression [(10)].

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Mutans streptococci have all the requirements for being a caries-inducing group of bacteria, individuals heavily colonized by mutans streptococci were thought to automatically be at high risk for caries [11,12]. Thus the greater amount of glucose in the saliva of diabetic patients stimulates bacterial growth, increases the production of lactic acid, reducing the pH and decreasing the salivary buffer capacities, which are risk factors for caries [5]; and therefore, correlation has been found with caries experience among diabetic patients[13]. However the correlation between diabetes mellitus and caries is controversy. High caries-experience among diabetics was reported by [14-16]. On the other hand, other studies showed low caries experience among diabetics [17,18]. While Lin *et al* [19] reported no difference in caries experience among diabetics and healthy group.

The present studies was conducted among patients with type1 diabetic mellitus aged 18-22 years in comparison to control group and to assess the relation of salivary glucose with severity of dental caries and Mutans Streptococci, among uncontrolled and controlled diabetic groups in comparison with non-diabetic control group.

MATERIALS AND METHODS

In the present investigation, the study group included 50 diabetic adults, with an age range of 18-22 years of both gender. They were examined at the Diabetic and Endocrinology Center, Al-Kindy Teaching Hospital in Baghdad City during the period from the first of November 2011 till the end of April 2012.

They were all with confirmed diagnosis of type IDDM with minimum duration of diabetes of at least 5 years (5-10years). The samples were divided into two groups based on the HbA_1c[2]: 25 uncontrolled type 1 diabetes mellitus ($HbA_1c > 7$) patients, 25controlled type 1 diabetes mellitus ($HbA_1c \leq 7$) patients and non-diabetic subjects as a control group were included 25 healthy students of both gender from college of dentistry/ university of Baghdad who did not suffer from any systemic diseases with an age range of 18-22 years and monitored their capillary blood glucose closely prior to the study, and matching with the study group. Caries experience was recorded according to the criteria Manjie *et al*. [20] this allows recording decayed lesion by severity.

Saliva was collected for diabetic patients at the same day of blood sample aspiration for HBA_1c assessment by measuring the absorbance of the glycohemoglobin and of the total hemoglobin fraction at 415 nm in comparison with a standard glycohemoglobin preparation carried through the test procedure (Human-Biochemical, 2011, Germany). The collection of stimulated salivary samples was performed under standard condition according to Tenovuo and Lagerlöf [21].

The salivary samples were taken to the laboratory for microbiological analysis. Saliva was homogenized by vortex mixer for two minutes. Ten fold serial dilutions were prepared using normal saline, two dilutions were selected and inoculated on the Mitis- Salivarius Bacitracin Agar (MSB agar) (The selective media for Mutans Streptococci) [22]. Identification of mutans streptococci includes: Colony Morphology; Gram's stain according to Koneman *et al* [23]; Motility; Catalase production [23]. CTA- mannitol media had been used to test the ability of Mutans Streptococci to ferment the mannitol [24]. Then Biochemical analyses of salivary samples were done by using spectrophotometric analysis. Salivary glucose level was measured by enzymatic method glucose-oxidase method, according to Srinivasan *et al* [25]. Glucose level concentrations of saliva were expressed in mg/dl.

$$\text{Glucose conc.(mg/dl)} = \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times 100(\text{Standard conc.})$$

Intra and inter calibration were performed to overcome any problem that could be faced during the research, and to ensure proper application of diagnostic criteria used in recording dental status through inter calibration. Statistical Analysis and processing of the data were carried out using SPSS version 18. The statistical tests included ANOVA and LSD tests, paired and Student's t-test and Pearson's correlation coefficients. The level of significance was accepted at $P< 0.05$, and highly significance when $P< 0.01$.

RESULTS

The percentage of dental caries occurrence in the present study was 100% in diabetes mellitus patients, while in control group (non-diabetic subjects) was 88%. Table **1** illustrates the severity of dental caries represented by grades of decayed fraction among study and control groups. The higher mean value was D_3 among uncontrolled diabetic group and D_2 among controlled diabetic group while among control group the higher mean value was D_1 than other grades of DS. However the mean values of all grades were found to be higher among uncontrolled diabetic group than other groups except for grade D_1 as the higher mean value was among control group than other two groups. Concerning these difference data analyses showed highly significant difference concerning grade D_1, D_2, D_3 and D_4 (F-value= 5.14, 15.26, 37.41 and 6.11 respectively $p<0.01$) among study and control groups. However L.S.D.

test revealed that the mean value was highly significant higher among uncontrolled diabetic group than control groups concerning D_2 (m.d. -4.56, $p<0.01$), D_3 (m.d.-16.96, $p<0.01$) and D_4 (m.d. -4.96, $p<0.01$). While opposite finding was found concerning grade D_1 where as the mean value was highly significant higher among control group than uncontrolled diabetic group (m.d. 2.00, $p<0.01$). The L.S.D. test also revealed that for controlled diabetic group the mean value of D_2 was highly significant higher than control groups (m.d. -4.20, $p<0.01$) and mean value of D_3significantly higher than control group (m.d.-4.52 $p<0.05$), and highly significant lower than uncontrolled group (m.d. -12.44, $p<0.01$), and the same picture was found concerning D_4 as the mean value for controlled diabetic was highly significant lower than uncontrolled group (m.d. -4.84, $p<0.01$).

Table **2** show salivary glucose level (mg/dl) (mean and standard deviation) among study and control groups. The highest salivary glucose value was represented in the saliva of the uncontrolled diabetic group, followed by the controlled diabetic group then the control group. Result revealed that salivary glucose was highly significant differ (F-value 51.892 $p<0.01$) among three groups. Further investigation using L.S.D. test showed that the level of salivary glucose among uncontrolled diabetic group and controlled diabetic group were highly significant higher than control group (m.d.-14.48, -11.56, respectively, $p<0.01$), while the level of salivary glucose between uncontrolled diabetic and controlled diabetic groups was not significant ($p>0.05$).

The correlation coefficient between salivary glucose and dental caries experience representing by DMFS and Ds in addition to its grades D_1, D_2, D_3, D_4 are shown in table **3**, analysis among uncontrolled diabetic patients revealed that the salivary glucose correlate positively with caries experience including DMFS, DS and its grades D_1, D_2, D_3, D_4 and these relations were highly significant only for DS and DMFS (r= 0.576 and 0.697 respectively $p<0.01$), and significant concerning D_3($r = 0.488$ $p< 0.05$), also the relation was not significant concerning D_1, D_2 grades. While among controlled diabetic group the correlations were not significant in positive direction concerning, D_2, D_3, D_4, DS and DMFS ($p>0.05$) opposite result was found for D_1 grade as the correlation was not significant in negative direction($p>0.05$). The same result was found among control group concerning Ds in addition to its grades D_1, D_2, D_3, D_4 as the relations were not significant in positive direction($p>0.05$) except for DMFS as the relation was highly significant in positive direction ($r = 0.536$ $p<0.01$).

Table **4** illustrates that the correlation of salivary glucose with Mutans streptococci was highly significant in positive direction among uncontrolled and controlled diabetic group and control group(r=0.508, r=0.621 and r=0.579 respectively $p<0.01$).

Table 1: Severity of dental caries represented by grades of D1-D4 (mean and standard deviation) among study and control groups

Severity of dental caries	Uncontrolled diabetic		Controlled diabetic		Control		Statistical analysis	
	Mean	±SD	Mean	±SD	Mean	±SD	F-value	p-value
D1	1.04	1.64	1.92	2.36	3.04	2.52	5.14*	0.00
D2	6.36	3.74	6.00	3.60	1.80	2.14	15.26*	0.00
D3	17.88	10.50	5.44	5.79	0.92	3.25	37.41*	0.00
D4	5.56	9.54	0.72	1.48	0.60	2.19	6.11*	0.00

***(p<0.01) Highly Significant, df=2**

Table 2: Salivary glucose level (mg/dl) (mean and standard deviation) among study and control groups

Salivary Variables	Uncontrolled diabetic		Controlled diabetic		Control		Statistical analysis	
	Mean	±SD	Mean	±SD	Mean	±SD	F-value	p-value
Salivary glucose	28.92	5.69	26.00	5.80	14.4	4.31	51.892*	0.00

*** (p<0.01) Highly Significant, df=2**

Table 3: Correlation coefficients between salivary glucose and caries experience among study and control groups

Severity of dental caries	Uncontrolled diabetic		Controlled diabetic		Control	
	Mean	±SD	Mean	±SD	Mean	±SD
D1	0.161	0.44	-0.061	0.77	0.370	0.06
D2	0.066	0.75	0.095	0.65	0.198	0.34
D3	0.488*	0.013	0.319	0.12	0.332	0.10
D4	0.229	0.27	0.116	0.58	0.191	0.36
DS	0.576**	0.00	0.293	0.15	0.298	0.14
DMFS	0.697**	0.00	0.399	0.051	0.536**	0.00

***(P<0.05) Significant, ** (P<0.01) Highly Significant**

Table 4: Correlations coefficients between salivary glucose and Mutans Streptococci among study and control groups

Salivary flora	Uncontrolled diabetic		Controlled diabetic		Control group	
	Salivary glucose		Salivary glucose		Salivary glucose	
	R	P	R	P	R	P
Mutans Streptococci	0.508**	0.001	0.621**	0.001	0.579**	0.002

*** (p<0.01) Highly Significant**

DISCUSSION

Diabetes and oral diseases often appear as the two sides of a coin, many patients with established oral diseases suffer from diabetes. Researchers in the dental field have suggested that oral diseases (periodontal disease and dental caries) should be included among the complications of diabetes [16, 26]. Most evidently, not all diabetic patients are at equal risk for oral diseases, and more attention has recently been paid to possible diabetes-related risk factors to identify subjects who are more prone to dental caries. The study groups selected aged 18-22 years, as at these ages the type 1 diabetes mellitus are predominate. However, in the present study it was difficult to have relatives patients as a control group, so, most of individuals among control group were from the students of college of dentistry, this could partly explained the differences in the severity of caries among study groups and control group. Since those students relatively differs from study subjects in their socioeconomic and behavior which play a role in the oral hygiene. Data of the present study showed that caries experience represented by DMFS and DS components among uncontrolled diabetes group was higher than that with both control diabetes and non-diabetes control group. This result in agree with the results reported by many researches [27-29].

However, Al-Dahan [30] reported an equal result of caries free subjects between control and diabetic groups. Further data analysis concerning grades of DS showed that the caries lesion severity represented by D_2, D_3 and D_4 were highly significant higher among uncontrolled diabetes group than both controlled diabetes and non-diabetes control group. In contrast, D_1 was highly significant higher among non-diabetes control group than uncontrolled diabetes group .The increased caries experience among uncontrolled diabetes group could be connected with complexity of the etiopathogesis of carious process and attributed to many diabetes-related changes in the salivary flow rate or glucose levels may include the effect of absolute or relative insulin deficiency, which impairs the function of salivary gland cells [31]. Individuals with type 1 diabetes mellitus and poor glycemic control have elevated salivary glucose concentration as a result of hyperglycemia, reduction of the salivary glucose clearance, disturbance of the neuroregulatory mechanism of the salivary glands and increased permeability of the basal membrane of the parotid glands [5, 6]. Hyperglycemia-related increased in the salivary glucose level have shown by the result of present study that shown a highly significant higher salivary glucose concentration among diabetic groups than control group. These results are in agreement with reports of other researchers [8,29]. Also this agrees with previously studies that showed the presence of glucose in saliva of diabetic patients probably reflect the high serum glucose concentration [33]. However, other suggested in their report that the measurement of salivary glucose concentration may also represent a simple, quick, and inexpensive method for screening of diabetic autonomic neuropathy [34]. On the other hand, Tenovuo *et al* [32] were found high blood glucose did not result in any notable elevation of salivary glucose in some subjects. Moreover, high levels of salivary glucose coming from both the serum (via gingival crevicular fluid) and the saliva [35].

The greater amount of glucose in the saliva stimulates bacterial growth (alteration in dental biofilm) predisposing colonization by *Streptococcus Mutans* and *Lactobacillus* [31]. This also shown in present study by highly significant positive relation between CFU of mutans Streptococci and salivary glucose among diabetic groups, as well as in previous studies [5,36]. One can suggest from the data of present study that elevation of glucose levels in the oral cavity will lead to increase acid production by cariogenic bacteria and reducing pH, thus decrease salivary buffer capacities [36] and this will enhance the cariogenic challenge and contribute to the development of the carious lesions as shown by analysis the data of present study the significant positive relation between salivary glucose and caries experience (DS and D_3).

The *Streptococcus Mutans* is the main microorganism responsible for the occurrence of dental caries in humans due to its ability to adhere to tooth surface [37]. High levels of these bacteria in saliva can be considered a reasonable indicator of a cariogenic environment in the mouths of uncontrolled diabetes subjects, this is also shown by the data of present study that showed the severity of caries lesion was highly significant (or significant) correlated in positive direction with salivary Mutans streptococci in all groups. These results are in agreement with reports of other Iraqi researchers [11,12,38]**.** In contrast, data of the present study showed that caries experience represented by D_1 among uncontrolled diabetes group was inverse not significant correlation with salivary mutans streptococci, this may be due to the frequency of D_1 and D_2 grades were low among uncontrolled diabetes group, and the frequency of D_4 grade was low among control group.

In conclusion, dental professionals need to have comprehensive knowledge of their patients' diabetes: knowledge that the patient has diabetes is not sufficient to assess the effects of diabetes with respect to oral diseases and dental treatment. This need is emphasized by the high and ever increasing number of patients with diabetes in Iraq. On the other hand, the members of the team responsible for diabetes treatment should pay attention to dental care and guidance to dental treatment. Finally, co-operation and consultation between all the members of the team responsible for the treatment of patients with diabetes is highly recommended.

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الخلاصة

المقدمة: داء السكر هو مرض مزمن ناتج من الاختلال في عملية ايض الكلوكوز, ويمكن الاستدلال عليه بقياس كمية السكر بالدم في حالة الصيام أو باستخدام اختبار الكلايكيتد هيموغلوبين. هنالك العديد من التغيرات البايوكيمائية والميكروبية في اللعاب, التي لها تأثير واضح في حدوث وانتشار تسوس الأسنان عند مرضى السكر.

أن الغرض من الدراسة هو تقيم علاقة كمية الكلوكوز باللعاب مع شدة تسوس سطوح الأسنان و بكتريا المكورات المسبحية الميوتنس لمرضى السكر ومقارنتهم بالأشخاص الأصحاء غير المصابين بداء السكر.

المواد وطرائق العمل: أشتملت الدراسة 75 شخص بعمر 18-22 سنة, 25 مريض بداء السكر غير المنضبط (HbA_{1C} <7) , 25 مريض بداء السكر المنضبط (HbA_{1C} ≥7) و 25 شخصا سليما غير مصابين بداء السكر كمجموعة ضابطة.تم قياس نسبة السكرفي الدم في حالة الصيام لجميع المرضى. كان التشخيص وحساب شدة التسوس من خلال تطبيق مؤشرDMFS-D_{1-4} (Manji *et a1*,1989).جمعت العينة اللعابية تحت ظروف موحدة وتم جمع عينات اللعاب المحفز اعتمادا على طريقة Tenovuo and Lagerlöf (1994). تم تحليل عينات اللعاب كيميائيا لغرض تحديد مستوى الكلوكوز باللعاب بأستخدام المطياف الضوئي اضافة الى تحليل اللعاب مايكروبايولوجيا لتحديد مستوى تواجد بكتريا المكورات المسبحية الميوتنس.

النتائج: اظهرت نتائج التحليل البايوكيميائي لعينات اللعاب ان مستوى تركيز الكلوكوز باللعاب عند مرضى السكرغير المنضبط ومرضى السكر المنضبط كانت مرتفعة وبفرق احصائي معنوي عالٍ مقارنة بمستواه في لعاب المجموعة الضابطة.ووجد ان مستوى تركيز الكلوكوز باللعاب ارتبط بعلاقة احصائية عالية المعنوية مع دالة سطوح الاسنان DMFS عند مرضى السكر غير المنضبط والمجموعة الضابطة.كما بينت النتائج ان مستوى تواجد بكتريا المكورات المسبحية الميوتنس ارتبط بعلاقة موجبة عالية المعنوية مع كمية سكرالكلوكوزفي اللعاب عند كل المجاميع الخاضعة للدراسة.

الاستنتاج: هنالك علاقة معنوية بين كمية سكر الكلوكوز, شدة تسوس الأسنان و تواجد بكتريا المكورات المسبحية عند لعاب مرضى السكر غير المنضبط.