Dental caries, Mutans Streptococci, Lactobacilli and salivary status of type1 diabetic mellitus patients aged 18-22 years in relation to Glycated Haemoglobin

Juman D. Al- Khayoun, B.D.S., H.D.D. [1]
Ban S. Diab, B.D.S., M.Sc., Ph.D. [2]

ABSTRACT

Background: diabetic mellitus is one of the serious systemic diseases that may cause general systemic changes, which may be reflected in the oral cavity. The aims of this study were to assess the severity of dental caries, Mutans Streptococci and Lactobacilli in addition to flow rate and pH among uncontrolled and controlled diabetic groups in comparison with non-diabetic control group.

Materials and Methods: Study groups consisted of 25 uncontrolled diabetic patients ($HbA_1c > 7$), 25 controlled diabetic patients ($HbA_1c \leq 7$), in addition to 25 non-diabetic healthy looking individuals. Their age was (18-22) years from both genders. 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. Salivary flow rate and pH were estimated. Viable count of mutans streptococci (on Mitis- Salivarius Bacitracin Agar) and lactobacilli (on Rogosa) was determined.

Results: The mean values of caries-severity were recorded to be highest among study groups compared to the control with statistically highly significant difference ($p<0.01$). Lowest values of salivary pH and flow rate were among study groups compared to the control with highly significant difference ($p<0.01$). Concerning Mutans Streptococci and Lactobacilli were found that the mean values of them for uncontrolled diabetic group were highly significant higher than both mean values of controlled diabetic group and control group.

Conclusion: Dental caries revealed higher percentage of occurrence and severity among uncontrolled diabetic group. Furthermore there was significant influence of the diabetic and the poor metabolic control on the salivary flow rat, pH, mutans streptococci and Lactobacilli that have an effect on caries occurrence and severity.

Keywords: diabetic mellitus, dental caries, Glycated Haemoglobin, Mutans Streptococci, Lactobacilli. (J Bagh Coll Dentistry 2013; 25(1):153-158).

INTRODUCTION

Diabetes is a chronic systemic disorder of glucose metabolism. 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) [1, 2]. Type 1 of diabetes account for 10 to 15 % of all cases of DM [3]. Glycated haemoglobin (glycosylate haemoglobin, HbA1c) is a form of haemoglobin used to identify the average plasma glucose concentration over prolonged periods of time. It is formed in a non-enzymatic pathway by haemoglobin's normal exposure to high plasmas levels of glucose, the measurement of glycosylated haemoglobin is one of the well established means of monitoring glycemic control in patients with diabetes mellitus [4].

Oral manifestations associated with diabetes are in most cases restricted to the uncontrolled or poorly controlled patient. There is evidence that diabetic patients have saliva secretion different from non-diabetic subjects [5].

However, the literatures contain contradictory results, Mata *et al.* [6], Siudikiene et al. [7] reported that both unstimulated (resting) and stimulated salivary flow rates are reduced in diabetic patients, whereas Lopez *et al.* [8] reported that only unstimulated salivary flow is reduced. In contrast, Swanljung *et al.* [9] and Edblad *et al.* [10] did not find any significant differences in salivary flow rates between diabetic and non-diabetic individuals. In general, the higher the flow rate, the faster the clearance and the higher the buffer capacity [11]. Sakeenabi and Hiremath [12] and Gawri *et al.* [13] concluded that decrease salivary flow rate, salivary pH and increase the levels of both salivary mutans streptococci and lactobacilli significantly correlated with caries experience. Studies on the concentration of Streptococcus mutans and Lactobacillus in the saliva of diabetics are inconclusive. Wallengren *et al.* [14] demonstrated that the inheritance of some types of HLA-DR4, most prevalent in type1 diabetics, was related to low salivary IgA activity against the Streptococcus mutans lead to increase the level of these bacteria. While other studies reporting decreased, increased or equivalent levels of the concentrations of these bacteria both in the saliva of diabetics and non-diabetics [7, 15]. The present studies was conducted among patients with type1 diabetic mellitus aged 18-22 years in comparison to control group and determine the occurrence and severity of following variable: dental caries and its relation to physicochemical characteristic of stimulated saliva (Salivary flow rate, pH). And

(1)M.Sc student Department of Pedodontic and Preventive Dentistry, College of Dentistry, University of Baghdad.
(2) Assistant Professor, Department of Pedodontic and Preventive Dentistry, College of Dentistry, University of Baghdad.

evaluate the quantitative distribution of Mutans Streptococci and lactobacilli in saliva and their relation to oral variable. In addition examine the relation of glycated haemoglobin(HbA1c) with dental caries, oral microorganisms and salivary variables (Salivary flow rate, pH).

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 HbA1c(1): 25 uncontrolled type 1 diabetes mellitus (HbA1c > 7) patients, 25 controlled type 1 diabetes mellitus (HbA1c ≤ 7) patients and non-diabetic subjects as a control group were included 25healthy 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.* [16] this allows recording decayed lesion by severity. Saliva was collected for diabetic patients at the same day of blood sample aspiration for HBA1c 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 [17]. Immediately after collection of saliva, through five minutes and disappearance of the salivary foam, the salivary flow rate was expressed as ml / mins and salivary pH was measured using a portable electronic pH meter. The salivary samples were then 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 for each microbial type and inoculated on the Mitis- Salivarius Bacitracin Agar (MSB agar) (The selective media for Mutans Streptococci) [18] and Rogosa Selective Lactobacilli agar (RSL) [19]. Identification of mutans streptococci and Lactobacilli includes: Colony Morphology; gram's stain according to Koneman *et al.* [20]; Motility; Catalase production [20]. CTA- mannitol media had been used to test the ability of Mutans Streptococci to ferment the mannitol. [21].

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. **Descriptive Statistics.** The statistical tests that were used in ANOVA test; L.S.D. test; Student's t-test; Pearson correlation coefficients and Paired sample t-test. 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%. Results revealed that caries experience represented by DS and DMFS were highly significant differ (p<0.01) among three groups (table **1**). Further investigation using L.S.D. test to DS revealed that both uncontrolled diabetic and controlled diabetic had highly significant higher DS than control group (m.d. -25.08 and -7.72 respectively p<0.01).Furthermore the uncontrolled diabetic had highly significant higher mean value of DS than controlled diabetic group (m.d. -17.36 p<0.01). On the other hand, using L.S.D. test concerning DMFS, showed that uncontrolled diabetic had highly significant higher mean than both controlled diabetic group and control group (m.d. -19.60 and -25.80 respectively p<0.01).While data of present study showed the mean difference between controlled diabetic and control group was not significant (p>0.05).

Salivary flow rate and pH among study and control groups are shown in Table **2**. Results revealed that salivary flow rate and pH were highly significant differ (F-value 160.03 and 183.94 respectively p<0.01) among three groups. Further investigation using L.S.D. test concerning salivary flow rate revealed that the mean value in uncontrolled diabetic group was highly significant lower than mean values in both controlled diabetic group and control group (m.d. 0.36, 1.77 respectively p<0.01), and the mean value of salivary flow rate among controlled diabetic group was highly significant lower than salivary flow rate in control group (m.d. 1.41 p<0.01). The same picture was found concerning salivary pH the mean value among uncontrolled diabetic group was highly significant lower than mean values in both controlled diabetic and control groups (m.d. 1.22, 2.14 respectively p<0.01), as

well as the mean value of salivary pH in controlled diabetic group was highly significant lower than mean value of salivary pH in control group (m.d. 0.91 p<0.01).

The viable count (CFU/ml) of salivary Mutans Streptococci and lactobacilli were highly significant differ (F-value 24.74 and 124.947 respectively p<0.01) among three groups. Table **3**. The highest CFU/ml of salivary Mutans Streptococci and lactobacilli values were represented in the saliva of the uncontrolled diabetic group followed by the controlled diabetic group then the control group.

Table **4** showed the correlation coefficients of salivary flow rate with caries experience for study and control groups. Analysis among uncontrolled diabetic group revealed that the relation between salivary flow rate and Ds was significant in negative direction (r= -0.423 p<0.05). Table **5** illustrates that the correlations were highly significant in negative direction between salivary pH and DMFS and DS (r=-0.655 and -0.663 respectively p<0.01). However among control group the relation between pH and DMFS was highly significant in negative direction (r = -0.820 p<0.01). Table **6** revealed that among uncontrolled diabetic group the salivary mutans streptococci correlate positively with caries experience including DMFS, DS and these relations were highly significant for DMFS and DS (r = 0.741 and 0.838, respectively p< 0.01). A significant positive relations were also found among controlled diabetic group concerning DS and DMFS(r = 0.429, and 0.432 respectively p< 0.05). The same finding found concerning control group but the highly significant relation was found concerning DS and DMFS(r = 0.566 and 0.652 respectively p< 0.01). Correlation coefficients of salivary Lactobacilli in relation to dental caries are seen in table **7.** This table revealed that among uncontrolled diabetic patient the salivary Lactobacilli correlate positively with caries experience including DMFS, DS and these relations were highly significant for DS and DMFS (r = 0.777 and 0.718 respectively p< 0.01). Among controlled diabetic group the salivary Lactobacilli correlate positively with DMFS, DS and these relations were highly significant for DS and DMFS (r = 0.534, 0.524 respectively p<0.01) The same finding was reported among control group as the salivary Lactobacilli correlate positively with caries experience and the correlation was significant concerning DS(r = 0.472 p<0.05).

Table **8** illustrates that among uncontrolled diabetic group the HbA_1c was positively correlated with caries experience represented by DMFS, DS and these relations were highly significant(r=0.586 and 0.574 respectively p<0.01).

DISCUSSION

Researchers in the dental field have suggested that oral diseases (periodontal disease and dental caries) should be included among the complications of diabetes [22, 23]. 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[24, 25, 26]. However, Al-Dahan, (1991) [27] reported an equal results of caries free subjects between control and diabetic groups.

Nonetheless, there is no consensus concerning the association between metabolic control and dental caries. In the current study positive highly significant correlation between HbA_1c and caries experience was found just among uncontrolled diabetes group. The elevation in the severity of dental caries among diabetic patients especially among uncontrolled diabetes group in the current study may be related to changes in the salivary physical properties involving the flow rate and salivary pH. A reduction in the flow rate of saliva and pH were reported among study groups. This may give an indication that the diabetes disease has an influence on salivary flow rate. These results are in agreement with reports of other researchers who found the same reduction in the salivary flow rate of diabetic patients [8, 6, 7, 28]. It seems likely that the thirst and dry mouth characteristic among uncontrolled diabetes group are related to the poor metabolic control of disease with increased diuresis and fluid loss, and that salivary flow rate is restored when the disease is well controlled among controlled diabetic

group. The possible explanation for the low salivation could be the neuoropathy of salivary gland[29]. Another explanation for the reduction of flow rate among the study groups especially uncontrolled diabetes group, is that the increase in glucose concentration in the blood may increase the osmolality of the glomerular filtrate and thus prevent the reabsorption of water as the filtrate passes down the renal tubular system. In this way the volume of urine is markedly increased in diabetes and polyuria and nocturia occur [30]. The decreased in the salivary flow rate among diabetic patients could give some explanation to the increased severity of dental caries by the significant inverse relation with DS that were reported by the data of present study as well as in previous Iraqi studies among diabetic [31, 32, 28]. Among uncontrolled diabetic group the salivary pH associated inversely with highly significant relation with DS and DMFS. This is consistent with other clinical studies that reported an inverse association between salivary pH and dental caries [33, 34]. But disagree with other studies that reported no association with caries [35, 36]. The *Streptococcus mutans* the main microorganism responsible for the occurrence of dental caries in humans. Due to its ability to adhere to tooth surface, the *Lactobacillus* is more related to a later stage of caries development [37]. Therefore, one can suggest that diminished salivary flow create an attractive environment for establishment of mutans streptococci and lactobacilli in the oral cavity of diabetic patients especially among uncontrolled diabetes group. 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 and lactobacilli in all groups. These results are in agreement with reports of other Iraqi researcher who found the same positive correlation between caries lesion severity and both mutans streptococci and lactobacilli [38, 39, 40].

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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Table 1: Caries experience DMFS and its component (DS, MS, FS) (mean and standard deviation) among study and control groups.

caries exper-ience	Uncontrolled diabetic		Controlled diabetic		Control		Statistical analysis	
	Mean	±SD	Mean	±SD	Mean	±SD	F-value	p-value
DS	31.44	16.95	14.08	7.59	6.36	6.73	31.69*	0.00
MS	2.00	4.33	1.20	2.61	0.60	2.19	1.21	0.30
FS	4.32	5.76	3.28	3.51	5.00	6.48	0.64	0.52
DMFS	37.76	16.83	18.16	9.57	11.96	8.61	30.27*	0.00

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

Table 2: Salivary flow rate (ml/min) and pH (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
Flow rate	0.84	0.19	1.20	0.31	2.61	0.52	160.03*	0.00
pH	6.08	0.34	7.31	0.36	8.22	0.46	183.94*	0.00

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

Table 3: Colony Forming Units (CFU/ml) of salivary Mutans Streptococci and Lactobacilli (mean and standard deviation $\times 10^5$) among study and control groups

Salivary Flora	Uncontrolled diabetic		Controlled diabetic		Control		Statistical analysis	
	Mean	±SD	Mean	±SD	Mean	±SD	F-value	p-value
Mutans Streptococci	397.04	133.66	232.64	101.87	185.00	96.23	24.74*	0.00
Lactobacilli	22.51	7.96	2.23	1.86	2.06	4.00	124.94*	0.00

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

Table 4: Correlation coefficients between salivary flow rate and caries experience among study and control groups

Caries experience	Uncontrolled diabetic		Controlled diabetic		Control group	
	Flow rate		Flow rate		Flow rate	
	R	P	R	P	R	P
DS	-0.423*	0.03	-0.251	0.22	-0.087	0.68
DMFS	-0.360	0.07	-0.216	0.30	-0.225	0.28

***(P<0.05) Significant**

Table 5: Correlations coefficients between salivary pH and caries experience among study and control groups

Caries experience	Uncontrolled diabetic		Controlled diabetic		Control group	
	pH		pH		pH	
	R	P	R	P	R	P
DS	-0.663*	0.00	-0.092	0.66	-0.283	0.17
DMFS	-0.655*	0.00	-0.081	0.69	-0.820*	0.00

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

Table 6: Correlations coefficients between salivary Mutans Streptococci and caries experience among study and control groups

Caries experience	Uncontrolled diabetic		Controlled diabetic		Control group	
	Mutans Streptococci		Mutans Streptococci		Mutans Streptococci	
	R	P	R	P	R	P
DS	0.838**	0.000	0.429*	0.03	0.566**	0.003
DMFS	0.741**	0.000	0.432*	0.03	0.652**	0.000

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

Table 7: Correlations coefficients between salivary Lactobacilli and caries experience among study and control groups.

Caries experience	Uncontrolled diabetic		Controlled diabetic		Control group	
	Lactobacilli		Lactobacilli		Lactobacilli	
	R	P	R	P	R	P
DS	0.777**	0.000	0.534**	0.006	0.472*	0.017
DMFS	0.718**	0.000	0.524**	0.007	0.318	0.12

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

Table 8: Correlation coefficients between HbA_1c and caries experience among uncontrolled and controlled diabetic groups

Caries experience	Uncontrolled diabetic		Controlled diabetic	
	HbA_1c		HbA_1c	
	R	P	R	P
DS	0.574**	0.003	0.214	0.30
DMFS	0.586**	0.002	0.195	0.34

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