ISSN: 1812–1217

Alteration of Saliva in Insulin Dependent Diabetic Patients and its Relation to Their Periodontal Status

Rafah H Al-Maroof
BDS, MSC, PhD (Lect)

Department of Oral and Maxillofacial Surgery
College of Dentistry, University of Erbil

الخلاصه

الهدف: يهدف هذا البحث الى تحديد بعض التغيرات الفسلجيه و الكيمياء-حياتيه لمكونات اللعاب عند مرضى السكر من النوع الأول و المعتمد على الأنسولين بمقارنتهم بالأشخاص الطبيعين و ربط هذه التغيرات بحالة الفم الصحيه. **المواد وطرق البحث:**تم جمع عينات من اللعاب من 20 مريض بالسكر من النوع الأول ومن 15 شخص طبيعي بأستعمال طريقة البصق البسيطه. أستعملت النماذج لتحديد نسبة جريان اللعاب، كثافة الهيدروجين الأيونيه، وتراكيز البروتين الأجمالي، الكالسيوم، الصوديوم، و البوتاسيوم. تم فحص أسنان كل المشاركين بالدراسه لتحديد مستوى الصفيحه الجرثومية،التهاب اللثه، و الترسب الجيري. **النتائج :** أظهرت النتائج أنخفاض معنوي في نسبة جريان اللعاب، كثافة الهيدروجين الأيونيه، و تركيز الكالسيوم. بينما لوحظ أرتفاع معنوي في تركيز البروتين الأجمالي،والبوتاسيوم. أما فيما يخص مؤشرات حالة الفم الصحيه، فقد لوحظ أرتفاع معنوي في المؤشرات الثلاثه المستعمله. كما أشارت العمليات الأحصائيه الى وجود علاقه معنويه بين الصفيحه الجرثوميه مع جريان اللعاب ، كثافه الهيدروجين الأيونيه ، تركيز البروتين الأجمالي وتركيز الكالسيوم. **الاستنتاجات** : من هذه الدراسه نستنتج أن مرض السكر من النوع المعتمد على الأنسولين من الحالات المرضيه التي تسبب تغيرا في مكونات اللعاب وتغير حالة الفم الصحيه ترتبط مباشرة بالتغيرات التي تطرأ على اللعاب.

ABSTRACT

Aims: To determine some physical and biochemical characteristic of the saliva in insulin dependant diabetes mellitus (IDDM) patients compared to control group and to correlate the salivary variables to any alteration in the oral health status which is evaluated by recording the plaque index, gingival index, and calculus index. **Materials and Methods :** Salivary samples were collected from 20 IDDM patients and 15 healthy male with age range (20-28) using simple spitting method. Salivary flow rate, pH value, total proteins, calcium, sodium, and potassium concentration were determined for each sample. Clinical examination was performed for both, patients and control groups to determine the plaque index (PI), gingival index (GI), and calculus index (CI). **Results:** The salivary samples of IDDM patients showed significantly lower salivary flow rate, pH value, and calcium concentration when compared with control group, while total proteins and potassium concentration were significantly higher. The PI, GI, and CI were significantly higher in IDDM Patients. **Conclusions:**
Our results suggested that IDDM is one of the pathological conditions that alter the properties of salivary secretion. The oral health status in IDDM in addition to its relation to the systemic condition; it is also related to the salivary properties.

Keywords: Diabetes Mellitus, Saliva, Periodontal status

Al-Maroof RH. Alteration of Saliva in Insulin Dependent Diabetic Patients and its Relation to Their Periodontal Status. *Al–Rafidain Dent J*. 2010; 10(1):102-109.
Received: 22/9/2008 ***Sent to Referees***:22/9/2008 ***Accepted for Publication:*** 21/12/2008

INTRODUCTION

Salivary fluid is an exocrine secretion consisting of approximately 99% water containing a variety of electrolytes (calcium, sodium, potassium, chloride, magnesium, phosphate) and proteins represented by enzymes, immunoglobulin and other antimicrobial factors, mucosal glycoprotein, traces of albumin and some polypeptides and oligopeptides of importance to oral health[(1)].

At present, saliva represents an increasingly useful auxiliary means of diagnosis. Many researchers have made use of sailometry and sialochemistry to diagnose systemic illnesses and monitoring general health[(2)].

Salivary secretion rate and composition are altered in several systemic diseases such as, cystic fibrosis and hypertension. Further more, activity of the salivary glands is influenced by many drugs and hormones and consequently, interest in saliva as a diagnostic tool and monitoring serum levels of drugs and hormones is growing. The problem of salivary gland is

involvement in diabetics of special interest in a view of the clinical observation of frequent complaints of dry mouth, parotid swelling and prevalence of gingivitis in diabetic patient[3].

Diabetes mellitus is a complex and pernicious syndrome characterized by abnormalities in carbohydrate, lipid and protein metabolism that result either from profound or absolute deficiency of insulin related to destruction of the insulin-producing pancreatic beta cell (Type 1 or insulin dependent diabetes mellitus IDDM) or from target tissue resistance to its cellular metabolic effects (Type 2 or non insulin dependent diabetes NIDDM) [4].

Diabetes patients usually present altered salivary secretion that can cause disorder of hard and soft tissue of the mouth leading to cariostatic and gingival lesions[5].

The relationship between the diabetes and periodontal disease provide an example of systemic disease predisposing to oral infection. The periodontal disease has been reported as the sixth complication of diabetes[6].

Saliva is critical for preserving and maintaining the health of oral tissue and since this fluid undergoes many changes in diabetic patients,[3,5,7,8] and since many patients usually demonstrate different degree in gingivitis, periodontitis and alveolar bone loss[4,5,6,9]. The aim of this study was to describe the physiological and biochemical characteristic of whole saliva of IDDM patients to determine the most indicative parameters of this illness and their relation to the periodontal conditions of these patients.

MATERIALS AND METHODS

This study was conducted on 20 males with Type1 (IDDM) patients who were attending Diabetic Center in Al-yarmook teaching hospital in Baghdad. Their age range 20-28 years, and duration of disease not less than 8 years. They were treated with insulin only. Fifteen healthy volunteers with no reported systemic disease, no history of drugs within previous month and with the same age range were chosen as a control group.

Unstimulated whole saliva collected from each subject in the morning between 8:30-10:30 after rinsing the mouth with 30ml of distal water.

The salivary samples were collected using the simple sitting method over a period of 10 minutes. The samples were collected in sterile glass test tubes of 25ml. The samples were left to stand for 1 minute to clear the foam and thus allow measurements. The amounts obtained were divided by 10 to obtain the salivary flow rates in milliliters/minutes (ml/min.).

The pH of each sample was evaluated using the coring (CG-701) pH meter. Its electrode was cleaned in distal water following each measurement and adjusted following each 3 samples to ensure the precise measurement.

Salivary chemical determination

Salivary samples were transformed in ice boxes to laboratory and then each sample was centrifuged at 3500 rpm for 5 minutes. The supernatant was transformed to another test tubes and frozen at -4C° till time of chemical analysis.

For the assessment of total proteins, lowry[10] method was used. For calcium assessment colorimetric method was used and for the assessment of sodium and potassium the emission flame photometry [11], (Corning 410 &410 flame photometry) was used.

Clinical Examination

The level of oral hygiene was estimated by recording the plaque index (PI). gingival index (GI) and calculus index (CI). They were scored according to examination protocol that has been advocated by the World Health Organization (WHO)[12].

The clinical examination was carried out on dental chair in good condition of illumination using mirror and periodontal probe after collection of the salivary samples and the scores were recorded on a case sheet specific for each patient.

Statistical analysis

The statistical analysis was done using SSPS programme. Quantitative variable were expressed as the mean and standard deviation. Independent samples t-test was used for comparison between the diabetics and control group. Correlation between variable was examined using Pearson correlation test. Statistical significance for $p<0.05$ was accepted.

RESULTS

Physiological variables of saliva

The measurement of salivary flow rate (FR) demonstrated significant lower level in IDDM patients (0.21±0.03) ml/min. when compared with the control subjects (0.39±0.06)ml/min. The salivary pH which was measured directly after the collection process showed the same difference. Its measurement showed a significantly lower value in IDDM (6.8±0.16) than in control subjects (7.2±0.11) as shown in Table(1).

Table(1): The t-test between the mean and standard deviation (X^{-}±SD) of the physiological variables of saliva in IDDM patients and in control subjects

Sample / Salivary variables	IDDM (X^{-}±SD) N= 20	Control Subjects (X^{-}±SD) N=15	significance
FR ml/min	0.21±0.03	0.39±0.06	P <0.01
pH	6.8±0.16	7.2±0.11	P <0.03

Biochemical variables of saliva

Table(2) represented the measurements of biochemical salivary variables. It shows significantly higher level of total proteins concentration in IDDM patients (280±21.8) mg/dl when compared with the values obtained from the control group (232±24.3) mg/dl. On the other hand, calcium concentration in IDDM patients (2.7±0.25) mg/dl was significantly lower than its concentration in control group (3.5±0.6) mg/dl. On measuring the concentration of sodium in IDDM patients and in control subjects, no significant difference was noticed between these two groups, while the potassium level showed significantly higher level in IDDM patients (36.6± 6.03) mEq/L over the control group (30.7±5.03) mEq/L.

Table-2- The t-test between the mean and standard deviation ($\mathbf{X^{-}}$±SD) of the biochemical variables of saliva in IDDM patients and in control subjects

Samples / Salivary variables	IDDM (X^{-}±SD) N=20	Control Subject (X^{-}±SD) N=15	significance
Total proteins (mg/dl)	280±21.8	232±24.3	P<0.02
Calcium (mg/dl)	2.7±0.25	3.5±0.6	P< 0.001
Sodium (mEq/L)	3.04±0.25	3.01±0.37	NS
Potassium (mEq/L)	36.6±6.03	30.7±5.03	P< 0.01

Periodontal status

Following the Oral health surveys of the WHO, clinical examination was preformed for each subject of both IDDM and control group. Table(3) represented the mean and standard deviation of the plaque index (PI), gingival index (GI), and calculus index (CI), t-test demonstrate signifi-

cantly higher level of the PI (2.7±0.33), GI (2.64±0.44), and CI (2.7±0.34) in IDDM patients when compared to the records obtained from the control group PI (2.05±0.98), GI (1.5±0.97), and CI(1.9±0.92).

In an attempt to correlate the finding of the salivary variables to the oral health status parameters, Pearson correlation test was used and the results are represents in Table (4) which showed significant relationship between PI with salivary flow rate (r= -0.885), salivary pH (r= 0.642), salivary total proteins (r= 0.836), and salivary calcium concentration (r= 0.447). The GI demonstrate significant correlation with the salivary flow rate (r= -0.446) and salivary total proteins concentration (r= 0.470). While the CI demonstrate only significant correlation with total proteins concentration (r= 0.464) and sodium concentration (r= 0.561).

Table(3): The t-test between the mean and standard deviation ($\bar{X}$±SD) of the periodontal status (gingival index-GI-, plaque index-PI-, and calculus index-CI-) in IDDM patient and in control subjects

Samples / parameters	IDDM ($\bar{X}$±SD) N=20	Control subjects ($\bar{X}$±SD) N=15	Level of significance
PI	2.7±0.33	2.05±0.98	P<0.01
GI	2.64±0.44	1.5±0.97	P<0.01
CI	2.7±0.34	1.9±0.92	P<0.01

Table(4):Relationship between salivary variables and periodontal status parameters (PI, GI, and CI) in IDDM patients and control subjects

Indices / Salivary variables	PI	GI	CI
FR Ml/min	-0.885**	-0.446*	0.373
pH	0.642**	0.444	0.438
TP	0.836**	0.470*	0.464*
Ca	0.447*	0.05	0.034
Na	0.227	0.201	0.561*
K	0.162	0.208	0.048

** Correlation is significant at the 0. 01 level; * Correlation is significant at the 0. 05 level

DISCUSSION

Total or whole saliva refers to complex mixture of fluids from salivary glands, gingival fluid, non-adherent oral bacterial, food remainders, desquamated epithelium and blood cells [13].

The salivary flow rate index is a parameter allowing saliva flow to be classified as normal, low or very low [14]. In this study although the salivary flow rate of the diabetic patient significantly lower than in control, it was still above the lowest physiologic secretion rate (0.1ml/minute) [14]. This result comes in accordance with many previous studies, Ben-Aryeh *etal* [3] reported significant decrease in salivary flow rate in IDDM in children and adolescence, Lopez [7] reported the same result in diabetic children with age range 3-15 years old, Moore *etal* [15] reported decrease in both stimulated and unstimulated saliva of diabetic patient, and Mata *etal*[16] reported decrease in both stimulated and unstimulated saliva of both of diabetic patient (IDDM and NIDDM) and. On the other hand, Swanljung *etal* [17] and Edblad *etal*[18] reported non- significant change in salivary flow rate of

IDDM, in the age range 12-18 and 18-24 respectively, this disagreement with the result of this study may be due to the short duration of the disease.

The diminished flow rate in this study could be related to the irreversible changes in the salivary gland with the alteration of the basement membrane which play an important role in the diffusion of the fluid to and out of the salivary gland [19] or due to overall dehydration of the diabetic patient of different degree, the salivary gland will in turn cease secretion to conserve water [20].

The pH value of saliva in diabetic patients of this study was significantly lower than control subject, this result agreed with Lopez *etal* [7] whom reported acidic pH of IDDM children whole saliva, while Swanljung *etal* [17] and Edblad *etal*[18] reported non- significant change in the salivary pH value in patient with Type1 diabetes.The change in pH value is greatly related to the obvious reduction in the salivary flow rate, since the salivary pH is directly related to the salivary flow rate [1, 13].

Chemical determination of saliva

The total proteins concentration was significantly higher in IDDM patient, and this result supported the results obtained by previous literatures which reported significantly high concentration of total proteins in stimulated and unstimulated saliva of IDDM patients of different age group [3,7]. On the other hand, Ben-Aryeh *etal* [21] study recorded no difference in the level of salivary total proteins of stimulated whole saliva between IDDM and NIDDM and control subjects, while streckfus *etal* [22] study reported lower level in total proteins of NIDDM parotid saliva. The conflicts in the results could be explained by the fact that different studies examined patients in different disease stages, different type of saliva, or different type of diabetes.

In this study, the high concentration of the total proteins level in diabetic patient could be related to the increase in amylase and IgA level, since these parameters are reported to be elevated in IDDM[3,7], or may be due to the decrease in the flow rate since,total proteins has an inverse relationship with salivary flow rate [38].

Chemical determination also showed lower calcium concentration in IDDM patients and this result come in agreement with Lopez *etal* [7] while it disagree with result obtain by mata *etal* [16] whom reported elevated salivary calcium level in IDDM and NIDDM patients in both resting and stimulated saliva.

The low calcium level in this study may be due to the decrease in the salivary flow rate, since there is a direct relationship between the flow rate and calcium concentration[1], or it could be related to high protein level, since the calcium phosphate precipitation is bonded to specific salivary proteins[23, 24]. The lower calcium concentration may explain the increase in the incident of caries in diabetic patient[23, 25].

The significantly high salivary potassium level in this study supported the previous results which recorded an elevated potassium level in IDDM stimulated and unstimulated whole and parotid saliva[3, 26]. The primary fluid secretion by salivary acini is isotonic with plasma. Sodium then extruded and potassium is reabsorbed by an active mechanism involving Na^+k^+-ATPase, during passage of saliva through the duct, secondary process occurs and reabsorption of sodium and secretion of potassium takes place results in hypotonic secretion[27].The elevated concentration of potassium found in diabetic patients in this study might indicate an impaired activity of Na^+k^+-ATPase activity as a result of change in the basement membrane of salivary acini.

In this study, recording the plaque index, gingival index and calculus index demonstrated significant increase in the three indices in IDDM patients when compared with control subject. This result disagreed with Miralles *etal* [8] whom reported non-significant changes in plaque index and gingival index in IDDM patients with age range 18-50 years. The disagreement may be due to the wide range of patients' age and involvement of old age control subject with the possibility of having periodontal complications. On the other hand, the result of this study concerning the oral health status supported lots of published studies that reported significant increase in the three mentioned

indices in IDDM patients of different age group and duration of disease[29,30,31]. Rayan *etal* [32] stated that, the prevalence of gingivitis in children and adolescence is nearly twice that observed in normal subjects. Gingival bleeding is an indicator of inflammation and it has a positive correlation with the accumulation of plaque and calculus, these deposits are the most important pathogenic factor of periodontopathy [9,30]. Seemann *etal*[35] stated that the improvement of oral health in IDDM patients resulted in increase in the salivary flow rate and decrease in their PI.

The oral complications in the diabetic patient are most likely related to altered response to infection, micro-vascular change and possibly increased glucose concentrations in the saliva and gingival crevicular fluid which may be contribute to periodontal diseases[28].Increased in glucose level in gingival fluid may diminish the ability of the periodontal fibroblasts to contribute to periodontal healing. Increased salivary glucose results in additional bacterial substrate and plaque formation [29].

In this study it is possible that the vascular changes in diabetes mellitus results in an increase in gingival bleeding and in turn increase in plaque index and calculus index.

Although primarily related to the presence of dental plaque, periodontitis appear to be related to several pathological events associated with diabetes but the reason for the higher rate of periodontal destruction in people with diabetes is not completely understood. Many studies have been shown that microorganism in the periodontal flora were similar in people with diabetes and in those without diabetes. This suggests that differences in the host response to periodontal pathogens are related to the increased tissue destruction in diabetes [19,32,33].

In this study we attempt to correlate the changes in the GI, PI, and CI to the changes recorded in the salivary variables.

The significant relation between the decreased salivary flow rate and increased PI come in accordance with Farsi *etal*[34] and Seemann *etal*[35] whom reported significant relation between salivary flow rate and plaque index in healthy subjects of different age group. The increase in the plaque index will directly affect gingival index since the plaque status showed high association with gingivitis and calculus index[36] and this association may explain the significant relationship between salivary flow rate and gingival index.

The salivary pH is directly related to the flow rate and in turn it will be associated with the oral health indices. Farsi *etal*[34] reported same result, where they recorded significant relationship between salivary pH and GI and PI.

The significant relationship between total proteins and dental plaque accumulation come in accordance with Rudney *et-al*.[38]. Many investigators have looked at oral disease in relation to salivary proteins concentration, more plaque was seen in subjects with high level of antimicrobial proteins [39].

Although the level of salivary calcium was found to be lower than control subjects, the CI was significantly higher than control subjects. Yejin *etal*[40] and poff *etal*[41]reported no significant correlation existed between calcium phosphate supersaturation in saliva and the rate of calculus formation for both stimulated and unstimulated saliva. However, calculus formation is influenced by a variety of factors, such as salivary flow rate and inhibitor and promoters of calculus formation other than salivary supersaturation with calcium phosphate salts.

The direct relationship between total proteins and calcium index in this study may explain this result since, the matrix of supragingival calculus constitute 15.7% of the calculus dry weight and contains 54.9% proteins. Although salivary proteins are effective inhibitor of the mineralization reaction that take place in dental plaque, once adsorbed, their conformation may change to present surface that catalyze the nucleation of mineral phases [37].

The lowered flow rate, pH value and calcium level and the elevated potassium and total proteins concentration in diabetic patients indicate the involvement of the salivary glands in these patients. This could be due to altered microcirculation, periphral neuropathy or direct changes in the glands such as damaged basement membranes. As all the patients were on

insulin, it is difficult to differentiate between the effect of the disease and the effect of the treatment. These changes in salivary variables play an important role in the exacerbation of the oral health status, since many relationships were found to be significant. The determination of the possible alterations in the composition of whole saliva might also be helpful in understanding the increased severity of periodontal disease in diabetic patients. Further research is needed to clarify the influence of metabolic glycemic control on the composition of saliva and on oral health status.

CONCLUSIONS

Our results suggested that IDDM is one of the pathological conditions that alter the properties of salivary secretion. The oral health status in IDDM in addition to its relation to the systemic condition; it is also related to the salivary properties.

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