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Influence of diabetes disease on concentration of total protein, albumin and globulins in saliva and serum: A comparative study

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Abstract

Background: Diabetes mellitus (DM) is a heterogeneous metabolic disease which is characterized by hyperglycemia and long term complication. The aim of current research to study the influence of both types of diabetic diseases on concentration of total protein, albumin and globulins. And to look out the possibility of using saliva rather than serum, as a diagnostic fluid to follow the changes in these parameters.

Materials and Methods: A total of 109 patients with Diabetes disease, 72 with type I (T1DM) and 37 with type II (T2DM) 68 age and gender matched healthy individuals were the samples of the present study. Concentration of total proteins, albumin and globulins were measured in both saliva and sera samples of the patients and the healthy individuals (control group).

Results: Total protein measurement results revealed presence of highly significant decrease in serum and highly significant increase in saliva samples of patients with both types of the disease. Albumin concentration was found to decrease significantly in sera and saliva samples of patients with both types of diabetic and highly significant increase was observed in globulins concentration in sera and saliva samples of the patients groups in comparison to the control groups.

Conclusion: the result of the present study suggests the possibility of using measurement of total protein concentration and globulins concentration in saliva of type 1 of diabetes as a reflection of the changes that occur in these parameters in serum of type 1 diabetes mellitus (T1DM).

Keywords: Diabetes mellitus; total proteins; albumin; globulins.

تأثير مرض السكري على تركيز البروتين الكلي والألبومين و الكلوبيولين في اللعاب والدم: دراسة مقارنة

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الخلاصة:

الخلفية: داء السكري (DM) هو مرض استقلابي غير متجانس يتميز بارتفاع السكر في الدم وضهور مضاعفات على المدى الطويل. يهدف البحث الحالي إلى دراسة تأثير كلا النوعين من مرض السكري على تركيز البروتين الكلي والألبومين و الكلوبيولين وتنظر الى إمكانية استخدام اللعاب بدلا من الدم، والسائل التشخيص لمتابعة التغييرات في هذه المعايير.

المواد وطرق: تضمنت الدراسة ما مجموعه ١٠٩ مريضا يعانون من مرض السكري، ٧٢ من النوع الاول و ٣٧ مع 68 IIمن النوع الثاني. تم قياس تركيز البروتين الكلي والألبومين و الكلوبيولين في كل من اللعاب والأمصال عينات من المرضى والأصحاء (المجموعة الضابطة).

النتائج: نتائج قياس البروتين الكلي كشفت وجود انخفاض كبير جدا في الدم وزيادة كبيرة للغاية في عينات لعاب المرضى الذين يعانون من كلا النوعين من المرض. وقد وجد أن تركيز الألبومين انخفض بشكل ملحوظ في مصل الدم واللعاب لدى المرضى الذين يعانون من كلا النوعين من السكري. بالعكس من ذلك وجدت زيادة معنوية في تركيز الكلوبيولين في عينات مصل الدم واللعاب لدى مجموعات المرضى بالمقارنة مع مجموعة السيطرة.

الاستنتاج: نتائج هذه الدراسة تشير إلى إمكانية استخدام قياس التركيز البروتين الكلي و تركيز الجلوبيولين في اللعاب من النوع ١ من داء السكري بوصفها انعكاسا للتغيرات التي تحدث في هذه العوامل في مصل الدم من النوع ١ من داء السكري.

الكلمات المفتاحية: داء السكري. مجموع البروتينات. الزلال. الجلوبيولين.

Introduction

Proteins play a central role in cell function and cell structure, serum and saliva contain mixture of protein differing in origin and function, the amount of protein in the vascular depends on the balance between the rate of synthesis and the rate of catabolism or loss[(1)] .It is a well-established and evidence based fact that plasma protein level may suffer changes during disease [(2)] .The use of plasma protein in the diagnosis of various diseases and provide pathophysiological information has increased markedly over the past decade, of the more than 100 plasma proteins which have been characterized from a basic biochemical stand point; relatively few have well documented clinical significance[(3)].

Diabetes mellitus (DM) is a heterogeneous metabolic disease which is characterized by hyperglycemia and long term complication [4]. Monitoring people with diabetes involves repeated estimation of plasma glucose by finger pricks or by intravenous blood sampling [5]. Hence, a noninvasive procedure for glucose measurement almost precious under circumstance [5].

Incidentally an array of oral manifestation has been reported in the diabetic population, such as dental caries, unexplained dentalgia, oral mucosal lesions, infection, burning, mouth syndrome and taste disorder [6]. Oral physicians tend to play a pivotal role in detecting and diagnosing this endocrine disease on the bases of the oral signs and symptoms [7]. Recently many researchers have been directed toward using saliva components as surrogate for systemic biomarker in blood[1].

Saliva has long been viewed as unique yet complex body fluids, like plasma and serum. Saliva is easy to collect by noninvasive method and preservation is inexpensive. The diagnostic value of saliva lies into components, flow and structure of gland [8].

Therefore the aim of current research is to study the influence of both types of diabetic diseases on concentration of total protein, albumin and globulins. Also this study aim to look out the possibility of using saliva rather than serum, as a diagnostic fluid to follow the changes in these parameters.

Materials and Methods

This study included two groups of diabetic patients, 72 cases type I diabetes mellitus (T1DM) and 37 cases type II diabetes mellitus (T2DM). These groups were matched with a two groups of age matched healthy individuals (68 control), All patients were admitted to; AL-Kindy Hospital, Center of Endocrinology and Diabetes Baghdad, Iraq. They were histologically proven under the supervision of specialists.

(1) Samples:

Serum and saliva collection:

Fasting whole blood was collected (5.0 ml) from the patients and healthy individual, kept in tube without any anticoagulant at room temperature for 1 hr. Then the tube was centrifuged (2000×g) for 10 min., the clear serum was pipetted into clear dry test tube and then stored at (-20)^{o}C for subsequent analysis .Unstimulated saliva was collected in the

fasting state, after thoroughly rinsing the mouth with saline solution[9]. It was centrifuged (2000×g) for 10 min. and the supernatant was stored at (-20) ^{o}C until being used for different investigations.

Exclusion criteria

Cushing's disease, acromegaly , chronic pancreatitis, pancreatactomy, pregnancy manifestation of nephropathy, chronic renal failure, malignancies and chronic or acute inflammatory dis-ease, patients who were taking aspirin, lipid lowering therapy, history of smoking or alcohol drinking were excluded .

(2) Total protein determination:

Total protein concentration in sera samples was determined using modified Biuret method [10]. While modified Lowry mothed by Hartree [11] was used to determine saliva protein. Bovine serum albumin (BSA) was used as a standard. Protein concentration of sera and saliva were expressed in g/dl.

(2) Albumin determination:

Serum and saliva albumin was determined by dye binding method[12] using kit manufactured by AGAPPE company. In this method the measurement of albumin is based on its quantitative binding at pH 4.2 with bromocresol green to form blue green complex.

(3) Globulins determination:

The concentration of globulin in the sera and saliva samples of healthy and diabetes patients was calculated ,using the following equation:

$$C_{globulins\ (g/dl)} = C_{total\ proteins(g/dl)} - C_{albumin(g/dl)}$$

Results

When the result of the above mentioned biochemical parameters in serum samples were compared between the patients (T1DM and T2DM) with corresponding control group (Table 1). A highly significant alteration that was characterized by highly significant decrease (p=0) in total proteins concentration and albumin concentration and highly significant increase (p=0) in globulins concentration were observed. But when these concentration were followed in saliva samples (Table 2), the obtained results reflect presence of highly significant increase (p=0) in total proteins concentration and globulins concentration, while the concentration of albumin appeared to decrease (p=0 in both patients groups.

The correlation between the level of these parameters in saliva with that of serum (Table 3) showed positive correlation in total protein concentration and globulins concentration in (T1DM 3**(A)**) (Pearson correlation of total protein concentration: 0.299 correlation is significant at the 0.05 level (2-tailed), Pearson correlation of globulins concentration: 0.331 Correlation is significant at the 0.01 level (2-tailed)).

Discussion

In the current study in serum was found markedly decrease in patients groups (Table 1). Generally it was reported that the variation in plasma protein concentration can be due to any of following three changes:- in the rate of their catabolism ,rate of their anabolism and in the volume of distribution [13]. On the other hand it is well known that each protein has characteristic half-life in the circulation for example the half-life of albumin in normal healthy adult is approximately 20 day and in certain diseases, the half-life of the protein may be markedly altered[14]. This can be added to the cause of the observed decreased protein concentration.

The observed increased in concentration of the total salivary proteins in the current studied patients groups(Table 2) supports the results obtained by previous work which reported high significantly total proteins in stimulated and unstimulated saliva of T2DM patients of different age group[15]. While It disagrees with other study reported presence of non-significant differences [16].This contradiction can be explained by the fact that these studies might have incorporated different methodology and metabolic control state of patients, different disease stages, different type of saliva, or different type of diabetes [17].

The observed elevation in the salivary total proteins concentration can be due to many reasons among them is that diabetic patients are characterized by having dry mouth (hyposalivation), which reported to cause an increase in total proteins concentration[18]. It is worth to mention that the present study patients were suffering from hyposalivation. Moreover this elevation could also be explained by the increase permeability of the basement membrane that reported to occurs in T1DM, such increase in permeability cause abnormal binding of serum protein to salivary gland basement membrane and enhanced leakage of these proteins through gingival crevice [18]. Another reason for such increase can be attributed to the active periodontal disease, which is commonly

found in diabetes they are derived from the gingival fluid and not the saliva[19]. Also the change in size that observed of parotid glands in DM patients because of xerostomia[20] this might be responsible for the observed biochemical disorders[21]. Moreover amylase and IgA level, were reported to be elevated in T1DM patients[15] such elevation may be the plausible reason for the observed increase in salivary total proteins.

Albumin concentration is the most abundant protein in plasma[13], a reduction in albumin concentration was observed to occur in sera of both types diabetic patients (Table 1). Such reduction was reported to occur in inflammatory process and chronic inflammatory diseases [22]. This result is in agreement with the studies that reported a decreased albumin concentration in different condition and diseases such as: hereditary defects, liver diseases, malnutrition, malabsorptive diseases[23]. The observed decrease in plasma albumin concentration that was accompanied with increased globulin concentration may be explained as follow: elevation of the glucose concentration occurs due to loss of the normal feedback inhibition of gluconeogenesis in the liver, this lead to increased breakdown of fats and proteins [24] and the conversion of glucogenic amino acid to glucose [25]. Liver is responsible of synthesis acute phase reactants which is a marker for inflammation state [26], among these proteins a group of several proteins called positive acute phase reactants [27], like α1-antitrypsin, α1- acid glycoprotein, C- reactive protein , globulins and ceruroplasmin. These proteins reported to increase significantly during acute inflammation, surgery, infection and tumors [28]. Moreover the inflammatory process including the so called acute phase response and chronic inflammatory are the most common cause of decrease in albumin concentration, and since diabetes mellitus has been reported to be an inflammatory state state[4], this may be one of the reasons that lead to the observed decreased in albumin concentration. Furthermore the reasons for such decrease may be due to:(1) Heamdiultion [29] , DM patients suffer from increased urinary [30] that result because of high glucose concentration, such increase lead to the cell pumping the water(hydration) to the blood stream which cause increased urinary and also dilution of glucose concentration and protein concentration .(2) Loss of extravascular space, as vascular permeability increase in the area of inflammation [31].(3) increase consumption by cell locally [29].(4) decreased synthesis as the result of direct inhibition by

cytokines[29]. Cytokines was reported as one of the many sequelae that lead to the generation of ROS as the root cause which underlies the development β cell dysfunction, impaired glucose tolerance and its complications[4]. The sources of oxidative stress is unclear, it may be form glucose autoxidation, protein glycation and glycoxidation, which leads to tissue damage[32]. Albumin serve as antioxidant[33], since it acts as sacrificial antioxidant, which is able to inhibit the generation of free radicals by a mechanism that involve the immediate attack of albumin itself by free radical, so they become no longer free in solution [34].

The positive acute phase proteins are globulins, so the observed increased in the globulin concentration in the present study (Table 1)results from the increase in the synthesis of cute phase proteins reported to occur in diabetic patients[4].

The results of the current study showed that both types of diabetes express a decrease in the salivary albumin concentration (Table 2). These results in contrast to previous studies which report high level or no change in albumin concentration in patients with T1DM compared to control[6, 35]. Albumin is regarded as serum ultra-filtrate to the mouth [36], it may diffuse into mucosal secretion[37]. Therefore the decrease in serum albumin concentration leads to decrease its concentration in saliva. As regards to salivary globulin concentration an increased was observed in both types of the disease (Table 2). This increase may explained on the bases that saliva in general contains array of proteins that have distinct biological functions, most of them have antibacterial, antimicrobial and antibodies[38], defend the oral environmental against any noxious agent, such proteins have been reported to increase in case of inflammation[39]. Another reason for such increase may be due to increase the leakage of the immunoglobulin family through damage of the oral mucosa.

Conclusions:

Diabetes mellitus disease affects the total protein, albumin and globulin concentration in serum and saliva at different degree. Saliva can be used as a diagnostic fluid to follow the changes in total protein and globulin concentration in type 1 diabetic patients only.

Table (1): Mean concentration of total proteins, albumin and globulins in sera samples of control, T1DM and T2DM patient groups.

Groups	Sample no.	Mean ± SD (g/dl)		
		Total proteins	Albumin	Globulins
Control group 1	32	7.680±0.557	4.871±0.177	2.809±0.547
T1DM	72	5.850±0.391**	2.241±0.189**	3.609±0.408**
Control group 2	36	7.752±0.527	4.865±0.176	2.886±0.526
T2DM	37	5.745±0.262**	2.253±0.245**	3.491±0.353**

Table (2): Mean concentration of total proteins, albumin and globulins in saliva samples of control, T1DM and T2DM patient groups.

Groups	Sample no.	Mean ± SD (g/dl)		
		Total proteins	Albumin	Globulins
Control group 1	32	0.189±0.013	0.115±0.012	0.073±0.014
T1DM	72	0.251±0.028**	0.087±0.015**	0.164±0.026**
Control group 2	36	0.191±0.020	0.113±0.018	0.077±0.010
T2DM	37	0.253±0.030**	0.096±0.025**	0.157±0.021**

Table (3): The correlation between saliva and serum protein profile in T1DM and T2DM patients groups, where
A: Male T1DM.
B: Female T2DM.

Saliva \ Serum	Total protein	Albumin	Globulins
Total protein	0.209*		
Albumin		0.065	
Globulins			0.331**

(A)

Saliva \ Serum	Total protein	Albumin	Globulins
Total protein	-0.007		
Albumin		0.064	
Globulins			-0.191

(B)

**Correlation is significant at the 0.01 level (2-tailed).
*Correlation is significant at the 0.05 level (2-tailed).

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