Evaluation of Some Levels of Biochemical Parameter in Saliva and Serum in Patients with Type 2 Diabetes Mellitus

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Abstract

Background: It has been observed that increased concentration of glucose and total sailic acid in serum of patients with diabetes mellitus type2. The current study salivary and serum sialic acid and glucose were checked in a sample of type 2 diabetic subjects.

Aims of the study: This study was conducted to find out any correlation between serum and salivary levels of SA and glucose in type 2 diabetic patients.

Subjects & Methods: A total of (80) subjects were enrolled in this study, they were (40) healthy individuals (control group) and (40) patients with diabetes mellitus type 2 (patients group). Blood and saliva were collected at the same visit from each subject only when they were 12 hr fasting at 8-10 am. Parameters studied in this work included: Serum glucose (G), serum total sialic acid (TSA), saliva glucose and saliva total sialic acid (TSA).

Results: There was significant elevation in saliva glucose level in patients group as compared to control group. There was a significant elevation in saliva TSA level in patients group as compared to control group. There was a significant positive correlation between serum and saliva parameters in diabetes patients.

Conclusion: Serum and salivary glucose and TSA increase in diabetic patients with significant positive correlation.

Key words: Saliva glucose, Saliva TSA, Diabetes mellitus. Serum glucose, Serum TSA, Diabetes mellitus.

Introduction:

In recent years, the use of saliva sampling as a non invasive qualitative and quantitative technique has become increasingly important ,in particular among those who suggested that saliva might be the best substitution for plasma in clinical areas [1,2]and in the areas of pharmacy-kinetic studies and drug monitoring [3]. Previous studies have made it clear that for many drugs the monitoring of saliva is a real alternative for determining plasma Levels [4].

However, a clear interpretation of the quantitative significance of their secretion into saliva has not yet been achieved [5]. Blood or plasma provides an estimate of the current circulating concentration of biochemical parameters of interest while saliva does not. This is because the concentration of any substance in saliva depends on the rate of saliva flow which itself is governed by many stimulating factors.

Composition of saliva:

Whole saliva is a complex mixture of major and minor salivary gland secretions, serum product, gingival fluid, blood debris, epithelial cells and cell products [6].

Saliva contains organic and inorganic constituents such as (Ca^{+2}, Mg^{+2}, Na^{+1}, K^{+1}, urea, amino acid, glucose, protein, glycoprotein's and amylase) [7].

Diabetes mellitus is a common disorder and type 2 D.M: (previously referred to as non- insulin dependent diabetes or adult onset diabetes) is a term used for individuals who have insulin resistance and usually have relative (rather than absolute) insulin deficiency [8]. The patients are usually adults over age 40 with some degree of obesity, and obesity itself causes some degree of insulin resistance. Diabetes has been considered as a risk factor for impaired oral health. It is known that diabetic patients have higher rates of tooth loss, periodontal disease and soft tissue diseases than non diabetic patients [9].

Glucose hypothesis:

It has been proposed that hyperglycemia, which is **the** distinguishing feature of diabetes mellitus, causes vasculopathy via different mechanisms [10]. Exposure of endothelial cells to high glucose leads to augmented production of superoxide anion which may quench nitric oxide and participate in endothelial dysfunction in DM [11].

Sialic acid (SA):

Is a family of acylated derivatives of neuraminic acid usually found on the terminal end of the carbohydrate portion of glycoprotein or glycolipids in cell membranes. The carbohydrate portion may influence cell –to cell interaction affecting cohesion ,adherence and antigenicity[12]. Increased SA concentration have been reported during inflammatory processes ,probably resulting from increased levels of richly sialyated acute phase glycoproteins[13] .The level of serum SA was significantly elevated in patients with rheumatoid arthritis, enclosing sodalities and systemic lupus erythematosus [14].

SA in saliva could be used possibly as non invasive marker for alcohol abuse [15], SA in saliva is mostly bound to mucin and not free in solution

Aims of the study:

There are different underlying causes for progression of cell damage in diabetes. For this reason a protocol has been suggested for studying these markers.

1-The study of certain inflammatory markers (serum sialic acid ,saliva sialic acid)and to find any correlation between them in D.M type 2.

2-To determine the levels of serum and saliva glucose and to find any correlation between them in D.M type 2.

Patients & Methods:

Patients:

Eighty subjects were enrolled in this study; they were divided into 2 groups:

1-Control group. This includes 40 apparently healthy subjects with out any signs or symptoms of diabetes mellitus or any other systemic diseases. They were 27 females and 13 males .The age range was from 20 to 32 years with a mean age of 34 ± 10.54 years.

2-Type 2 diabetic patients: They were 28 females and 12 males. The age range was from 32 to 70 with a mean age of 51.7 ±9.9 years. These patients were on oral hypoglycemic tablets. Subjects visiting the clinics of the national diabetes center AL- Yarmouk teaching hospital.

Blood and saliva were collected at the same visit from each subject only when they were 12hr fasting at 8-10 a.m..

Saliva:

Five to six ml of un-stimulated (resting) whole saliva

Was collected-after an individual was asked to rinse his mouth thoroughly with distilled water to insure the removal of any possible debris or contaminating materials. The first mouth full of saliva was discarded to spit all the saliva during 20 minutes into plastic polyethylene tubes provided for sample collection .The collected saliva was centrifuged at 3000 r.p.m. for 15 minutes , then the clear supernatant of saliva was taken and stored in deep freeze (-20 Cº) until the day of assay.

Methods:

1-*Estimation of serum and saliva glucose*: It was estimated by using enzymatic colorimetric test using kit supplied by Syribo of Eurobio laboratories, Parise-France[16].

Reagents:-

Phosphate buffer ph7.0	100 mmol / L
GOD	18000 U /L
POD	1000 U / L
4 -amino antipyrine	0.4 mmol / L
Phenol	0.4 mmol / L
Standard R_3	100 mg /dL

Procedure:

Ten µ l of sample added to 1 ml of reagent, mixed gently , incubated at 25 Cº for 30 minutes or at 37 Cº for 15 minutes ,then read the optical density at 505 nm wave lengths in spectrophotometry.

Reagent	**Test**	**Standard**	**Blank**
standard	-	10 µl	_
Sample	10 µ l	_	_
Working reagent	1ml	1 ml	1 ml

Calculation :-

$$\frac{\text{Optical density (O.D) sample}}{\text{Optical density (O.D) standard}} \times \text{standard concentration}$$

2-Estimation of TSA:

The principle of this adopted method depends on the formation of chromogen on addition of resorcinol reagent into the test tube[17].

1. **Resorcinol stock (2% w/v**).
2. **$CuSO_4$ (0.1 M):** prepared by dissolving 4 gm of the salt in 250 ml distilled water.
3. **(Resorcinol – HCL)** reagent: 10 ml of the stock + 9.75 distilled water + 0.25 ml 0.1 M $CuSO_4$, the volume was completed to 100 ml with concentrated HCL (prepared daily).
4. **Butyl acetate /Methanol (85:15) reagent:**
5. **Standard Sialic Acid:** The standard Sialic acid different concentrations (0 – 38) µg / ml were prepared by serial dilution with distilled water.

Procedure:

1- 20 µl of sample or standared Sialic acid solution and 980 µl of distilled water placed in test tubs, vortexes and placed on ice.

2-To each assay tube, 1 ml of resorcinol reagent was added, and placed in a 100 C^o. Boiling water bath for exactly 15 minutes flowed by 10 minutes on ice bath.

3-2 ml of butyl acetate / methanol (85:15 v/v) was added to each tube, then vortexes and centrifuged for ten minutes at 3000 rpm .The extracted chromophore was read at 580 nm.

4-The standard curve was prepared and used for determination of Sialic acid [18] fig 1.

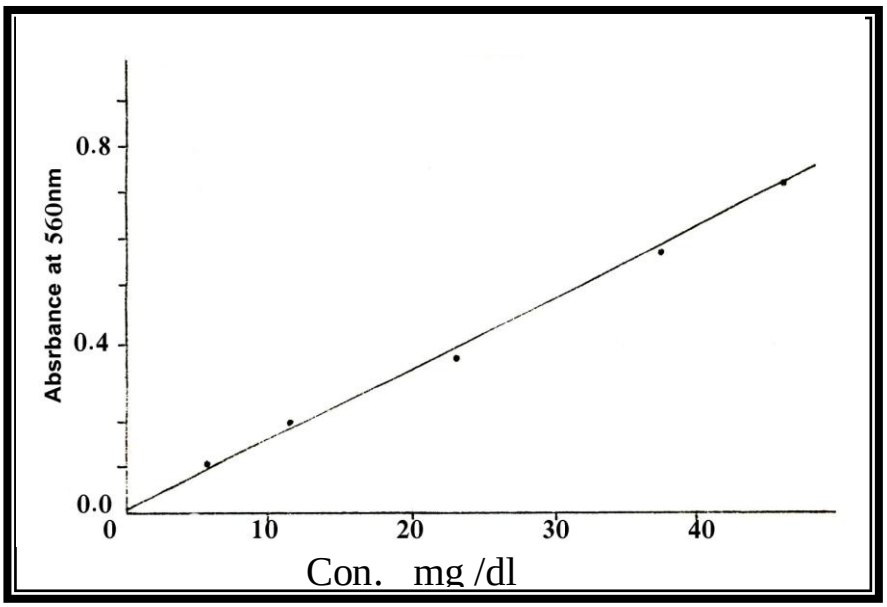

Figure 1: Standard curve for determination of Sialic acid concentration in human sera

Results:

Table 1, Figure 2 show that serum glucose levels in patients with D.M. type 2 (Mean 147.8 gm/dl, SD 38.59) as compared to healthy controls (Mean79.614gm/dl , SD9.522). The difference was significant $p<0.0005$.

Table 1, Figure 3 show that saliva glucose levels in patients with D.M. type 2 (Mean 24.45 gm/dl, SD 7.8) as compared to healthy controls (Mean 15.08 gm/dl, SD5.99). The difference was significant $p<0.0005$) .

Table1: Serum and salivary glucose and TSA levels in diabetic subjects with the control group

	D.M. Type 2 **n= 40**	**Control** **n =40**
***Serum glucose mg/dl(Mean ±SD)** ***Saliva glucose mg/dl (Mean ±SD**	**147.8 ± 38** **24.4 ± 7.8 15**	**79.61 ± 9.52** **15.08 ± 5.99**
***Serum TSA mg/dl (Mean ±SD)** ***Saliva TSA mg/dl (Mean ±SD)**	**61.41± 12.44** **15.62 ± 5.7**	**56.06± 6.56** **4.995± 3.008**

* Significant difference between control and patients ($p<0.005$)

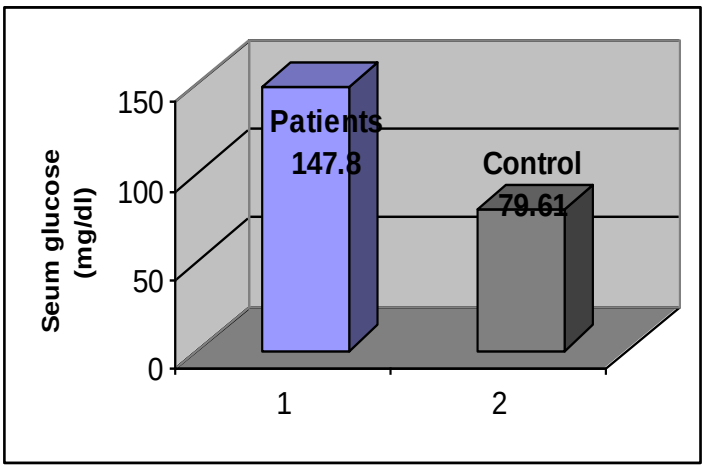

Figure 2: The mean levels of serum glucose (mg/ dl) in patients with type 2 D.M. as compared to control

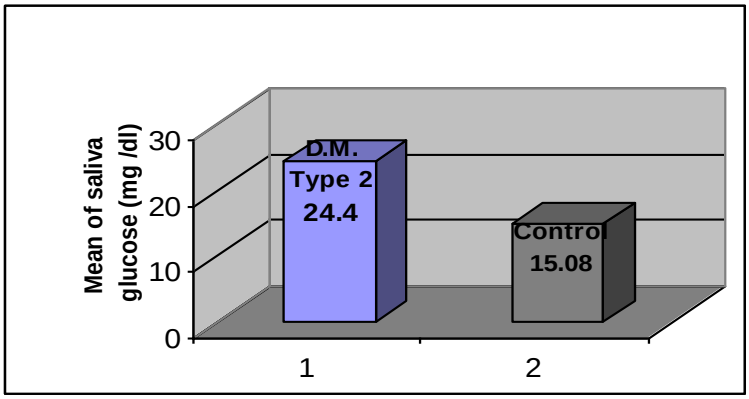

Figure 3: The mean levels of saliva glucose (mg/ dl) in patients with type 2 D.M. as compared to control.

Table 1, Figure 4 show that serum total sialic acid TSA levels in patients with type 2 D.M. (Mean 61.46 mg/dl, SD 12.44) as compared to healthy controls (Mean 56.06.6 mg/dl , SD 6.56). The difference was significant p<0.005.

Table 1, Figure 5 shows that saliva TSA levels in patients with D.M. type 2 (Mean 15.622 mg/dl, SD 5.7) as compared to healthy controls (Mean 4.99 mg/dl, SD 3.008). The difference was significant p<0.0005.

Figure 6 showed positive correlation between serum and salivary levels of glucose (r = 0.5).

Figure 7 showed a positive correlation between serum and saliva total sialic acid TSA (mg/dl) in patients with diabetes mellitus type 2 and revealed that r for these groups was r = 0.4705.

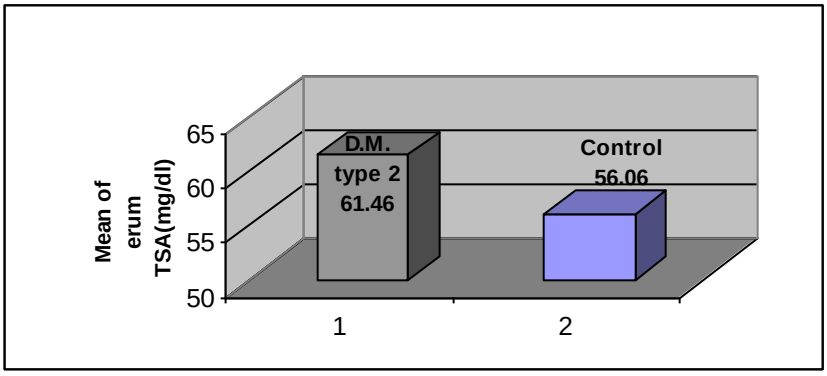

Figure 4: The mean levels of serum TSA(mg/ dl) in patients with D.M. type 2 as compared to control

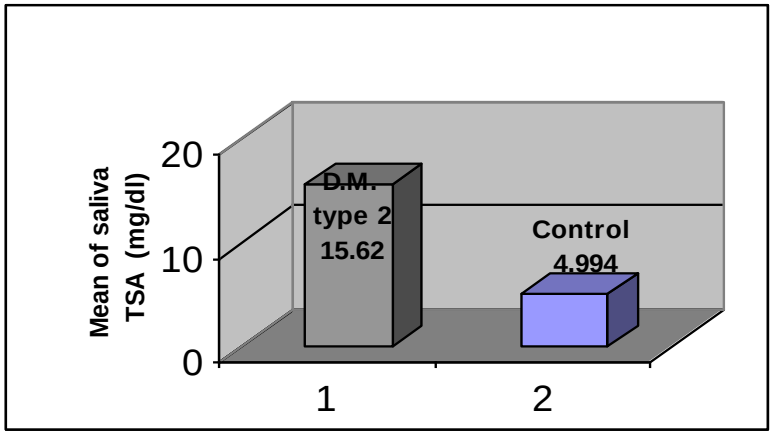

Figure 5: The mean levels of saliva TSA(mg/ dl) in patients with D.M. type 2 as compared to control.

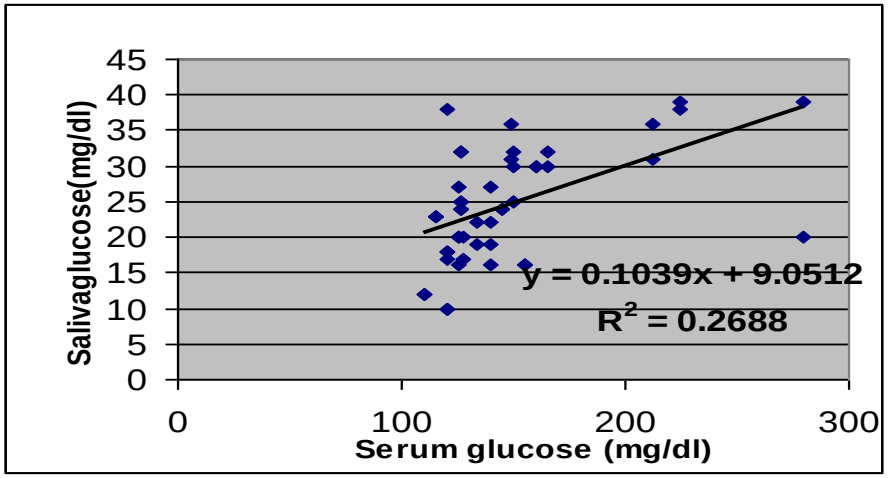

Figure 6: The correlation between serum glucose(mg/dl)and saliva glucose (mg/dl) in type 2 diabetic patients .

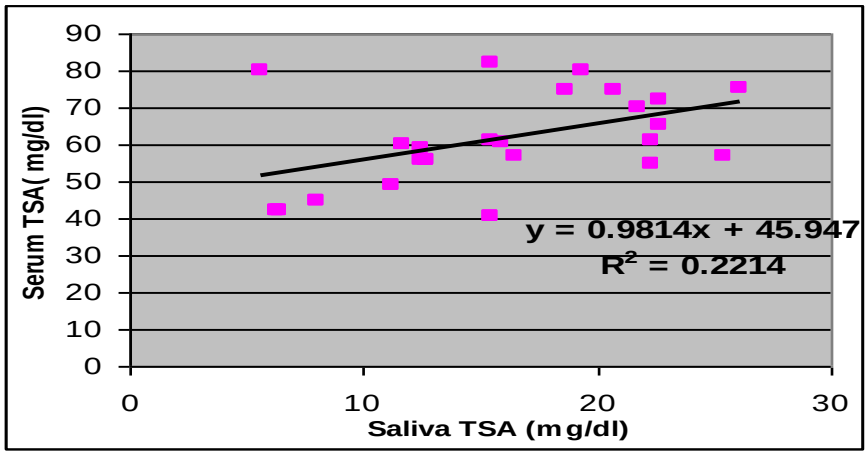

Figure 7: The correlation of serum TSA(mg/dl) with saliva TSA (mg/dl) in patients with D.M. type2

Discussion:

The prevalence of DM was found to be higher in women than in men . This is in agreement with the work of Ishihara ,M.1984, Levitt,N.S.1993 and Abdul –Ridha , F.,M,1998 [19,20,21] reported lower prevalence of DM in men than women and this discrepancy increase with age.

Salivary glucose was significantly higher ($p< 0.0005$) in diabetic patients than in control group, and it was significantly positive correlated with serum glucose. Tenovuo, 1986, ;Mahmood,1997 and Abdul-Ridha, 1998 found that saliva and serum glucose were significantly higher in diabetic patients than control [21,22,23].

The increase in saliva glucose in diabetic patients may be agreement by the fact that diabetic patients had gingival inflammation causing increased capillary fragility in reticular tissue. This condition will allow more glucose to pass from the hyperglycemic blood. Also in diabetic patients there is an increase in membrane permeability. The concentration of glucose in parotid saliva elevated at least 2h after glucose / food intake in individuals both IGT and manifest DM with a significant correlation between serum and saliva glucose[12,24].

Serum and saliva sialic acid were significantly higher in diabetic patients ($p< 0.005$, $p<0.0005$) .This result was similar to the result of Crook, 1993 and Chen, 1995 [25,26] who found that serum SA was elevated in diabetic patients.

The reason for the elevation of serum TSA in NIDDM is that much of circulating SA is covalently attached to glycoproteins , particularly of the acute-phase group. NIDDM itself may be an acute phase disease since in NIDDM, serum level of the acute phase proteins are increased[25]. The increase in SA concentration has been associated with many diseases. In diabetes, probably resulting from increased levels of richly sialyated acute –phase glycoproteins[27].

The results revealed that serum and saliva parameters were significantly positively correlated in diabetic patients, but not in controls. This could attributed to the fact that the severity of disease which could affect in some way the oral mucosal membrane ,like permeability of the basal membrane increased , which make a close a proximity between serum and saliva parameters.

In conclusion: There was :

1-A significant increase in serum and saliva glucose of patients with diabetes mellitus.

2-Serum and saliva total sialic acid (inflammatory marker) levels were significantly increased in diabetic patients, which may be a good indicator of elevated acute phase proteins.

3-There was a significant positive correlation between serum and saliva parameters in diabetic patients.

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