Association between Serum Sialic Acid and Some Trace Elements in Type 2 Diabetic patients

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Abstract:

Background: Diabetes Mellitus (DM) is a metabolic disorder of multiple etiologies characterized by chronic hyperglycemia with disturbances of carbohydrate, fat, and protein metabolism.

Objective: This study was designed to evaluate the status of serum total sialic acid (TSA), zinc (Zn), copper (Cu), iron (Fe), magnesium (Mg), and manganese (Mn) levels, and their correlation with each other in type 2 DM patients.

Materials & Methods: A total study subject of 80 were examined, including 52 patients with type 2 DM diagnosed and treated in the National Diabetes Center, Al-Mustansirya University, and 28 healthy individuals as a control group matched for age, sex, and body mass index (BMI). For the study, six groups were formed, categorized as follows; Group1 (G1): 28 male and female control subjects; Group2 (G2): 52 male and female type 2 DM patients; Group3 (G3): 13 male control subjects; Group4 (G4): 24 male type 2 DM patients; Group5 (G5): 15 female control subjects; Group6 (G6): 28 female type 2 DM patients. All subjects had not taken vitamin or mineral supplements for at least 2 weeks before sampling; Blood samples were drawn after an overnight fasting for the determination of serum glucose, HbA1c, TSA, Zn, Cu, Fe, Mg, Mn, and total cholesterol (TC).

Results: It was found that total diabetic patients had higher TSA, Fe, Cu/Zn, and Fe/Zn levels, and lower Zn, Cu, Mg, Mn, and Cu/Fe levels when comparing with control groups. Although, it was found that serum TSA, Fe, Cu/Zn, and Fe/Zn levels were higher and Zn, Cu, Mg, Mn, and Cu/Fe levels were lower in male and female diabetic patients than in the respective control subjects, there was only a positive correlation between Fe and Mn levels in male type 2 DM patients. There was a negative correlation between Fe-Cu, Cu-Fe/Zn, and Mn-Cu/Zn levels, and positive correlation between TSA-Mg in total diabetic patients. There was a positive correlation between TSA-Mg, and Zn-Cu levels while a negative correlation between TSA-Cu/Zn, Fe-Cu, Mn-Cu, and Zn-TC levels in female diabetic patients.

Conclusion: Our results suggest that TSA and selected trace elements Zn, Cu, Fe, Mg, and Mn levels have altered in type 2 DM; moreover, they have interactive connection with diabetes mellitus. There are also many sex-related positive or negative correlations between the altered parameters in DM patients. These parameters might have a diagnostic role in patients with diabetes mellitus.

Keywords: diabetes, Sialic acid, traces elements, zinc, copper, iron, magnesium, manganese.

Introduction:

Diabetes Mellitus (DM) is a metabolic disorder of multiple etiologies characterized by chronic hyperglycemia with disturbances of carbohydrate, fat, and protein metabolism, resulting from defects in insulin secretion, insulin action or both[1,2]. It is not a simple disorder of glucose homeostasis, but it is also accompanied by various degenerative manifestations such as accelerated aging, cardiovascular disease, and microvascular lesions leading to retinopathy and glomerulopathy [2-4]. The incidence of diabetes increases from year to year, especially type 2 of diabetes, which is the most common type. More than 90% of cases of diabetes are of type 2).[5]

In mammals, sialic acid occurs as a terminal sugar of glycoprotein and glycolipids [2,4], and is a marker of an acute phase response[2]. An elevated total serum sialic acid (TSA) concentrations is a risk factor for cardiovascular mortality in human [3,4,6]. Cardiovascular disease is the common cause of death in diabetic patients [6].

In type 2 diabetes, the serum sialic acid concentration is elevated in comparison with non diabetic subjects [7], especially in those with metabolic syndrome [8]. However, in type 1 diabetes serum sialic acid concentration is not elevated in comparison with non diabetic subjects [7].

There is accumulated evidences that the metabolism of several trace elements is altered in diabetes mellitus and that these factors might have specific roles in the pathogenesis and progress of this disease [9,10]. The alteration in the metabolism of several essential minerals including copper (Cu), zinc (Zn), manganese (Mn), and magnesium (Mg) have been associated with impaired insulin release, insulin resistance, and glucose intolerance in experimental animals and humans [11].

Therefore, we seek to answer the question of whether altered serum TSA, Zn, Cu, Fe, Mg, and Mn levels have an interactive connection with each other in DM.

Materials & Methods:

A total of 80 subjects were examined in this study including 52 (24 male, and 28 female) type 2

diabetes mellitus patients, who were refereed to the National Diabetes Center, Al-Mustansirya University, and 28 healthy individuals who served as a control group (13 male, and 15 female). For the study, six groups were formed, categorized as follows; Group1 (G1): 28 male and female control subjects; Group2 (G2): 52 male and female type 2 DM patients; Group3 (G3): 13 male control subjects; Group4 (G4): 24 male type 2 DM patients; Group5 (G5): 15 female control subjects; Group6 (G6): 28 female type 2 DM patients.

Subjects were matched in regard to age, sex, and body mass index (BMI). Weight and height were measured in indoor clothing without shoes, and the BMI was calculated as weight (kg)/(height in meters)^2. All the participants had not taken vitamin and mineral supplements for at least 2 weeks before sampling.

Blood samples were drawn by venipucture after an overnight fasting. Serum was used for biochemical assays (TSA, Zn, Cu, Fe, Mg, Mn, and total cholesterol TC, glucose), while 2ml of blood was put in EDTA tube, mixed gently and used for measurement HbA_{1c}. Hemoglobin A_{1c} program intended for the determination of Glycated hemoglobin (A1c) in human depended on high performance liquid chromatography and who supplied by Variant Company, USA. Glucose level was determined using kits supplied by Randox, UK. Total cholesterol TC, was determined using kits from bioMérieux, Marcy 1'toile, France). The method for TSA was essentially as described by Katopoidis and coworkers [12] with slight modification in the volume of sample used. An analysis of serum manganese sample was carried by using furnace atomic absorption spectrophotometery model (AA670) Shimadzu, Japan. Serum samples were diluted (1:2) with deionized water for Mn and measured directly at (279.5 nm). Analysis of serum zinc (Zn), copper (Cu), and magnesium (Mg), iron (Fe) were carried by using flame atomic absorption spectrophotometery model (AA670) Shimadzu, Japan. Serum samples were diluted (1:2, 1:2, 1:49, and 1:4) with deionized water for zinc, copper, magnesium, and iron respectively and measured directly at (213.9 nm, and 324.8 nm, and 285.2 nm, 248.3 nm) respectively.

Statistical analysis:

Data were analyzed using computer facility, the available statistical package of SPSS-11.5 (statistical packages for social sciences-version 11.5). Data were presented in simple measures of number, and mean ± SD. The significance of difference between quantitative variables was tested using student t-test for comparing between two means of independent groups. P value equal and less than 0.05 was used as the level of significance. Pearson correlation coefficient is significant at the 0.05 level (2-tailed)

Results:

Table (1) shows the clinical characteristics of type 2 DM patients and controls. They were similar of age, duration of disease, gender, and body mass index BMI. Age and BMI showed no significant differences between the controls and diabetics. Data shown in table (1) revealed that glucose and HbA_{1c} level of G2, G4, and G6 are significantly higher than in their respective control groups. Our results for glucose and HbA_{1c} are in agreement with findings of Chan *et. al.*, [13].

Table (1): Clinical characteristics of type 2 DM and controls.

Clinical characteristics*	Age (years)	Duration(years)	BMI (kg/m^2)	Glucose(mg/dl)	HbA_{1c} %
All					
G1; Control	38.2±0.6	-----	27.8±2.3	94.5±2.3	5.23±0.09
G2; Diabetic	39.3±0.5	10.0±0.5	28.3±0.2	198.1±13.7	9.73±0.34
P–value(G2vs.G1)	N.S		N.S	0.001	<0.0001
Male					
G3; Control	38.3±0.9	-----	27.3±0.1	94.61±2.96	5.26±0.14
G4; Diabetic	39.4±0.7	10.4±0.8	27.1±0.2	189.8±1.85	10.04±0.5
P–value(G4vs.G3)	N.S		N.S	0.001	0.001
Female					
G5; Control	38.1±0.8	-----	28.6±0.2	94.4±3.8	5.2±0.1
G6; Diabetic	39.3±0.7	9.7±0.6	29.2±0.3	205.5±1.62	9.46±1.7
P–value(G6vs.G5)	N.S		N.S	0.004	<0.000

*Values are expressed as means±SD, NS: not significant.

Table 2, 3, and **4** show the results of serum TSA, and selected trace elements levels of total, male, and female controls and type 2 DM patients, respectively. **In table2** diabetics had higher TSA, Fe, Cu/Zn and Fe/Zn levels (P< 0.001 for serum TSA, and Fe while P<0.01 for Cu/Zn and Fe/Zn levels). Patients had lower Zn, Cu, Mg, Mn, and Cu/Fe levels (P<0.001 for Zn, Mg, and Mn; P 0.01 for Cu/Fe levels; and P<0.003 for Cu level) when compared with control groups. **In table 3, and 4**, it was found that serum TSA, Fe, Cu/Zn, and Fe/Zn levels were higher (P< 0.001 for serum TSA, and

P<0.01 for Fe, Cu/Zn and Fe/Zn levels) while Zn, Cu, Mg, Mn, and Cu/Fe levels were lower in male and female diabetic patients than in the control subjects (P<0.01 for Zn, Mg, Mn, and Cu/Fe levels for male and female, and P 0.03 for Cu level for male, P<0.04 for Cu level for female). There was no significant difference in total cholesterol levels (TC) in total, male and female type 2 DM patients compared with their respective control groups.

There was a positive correlation between Fe-Mn levels in male diabetics. There was a negative correlation between Fe-Cu, Cu-Fe/Zn, and Mn-Cu/Zn levels, and a positive correlation between TSA-Mg in total type 2 diabetic patients. There was positive correlation between TSA-Mg, Zn-Cu levels and negative correlation between TSA-Cu/Zn, Fe-Cu, Mn-Cu, and Zn-TC levels in female diabetic patients **(Table 5)**.

Table (2): TSA, selected minerals, & total cholesterol (TC) levels of diabetic patients (G2), & controls (G1).

Parameters*	Controls (G1)	Diabetic patients (G2)	P value
TSA (mg/dl)	59.7±0.05	120.34±4.47	0.000
Zn (ppm)	1.193±0.015	0.588±0.012	<0.001
Cu (ppm)	1.265±0.014	1.1±0.022	0.003
Fe (ppm)	0.792±0.007	1.17±0.015	0.000
Mn (ppm)	0.04±0.003	0.018±0.004	0.000
Mg (ppm)	18.07±0.037	14.406±0.274	<0.001
Cu/Zn	1.062± 0.07	1.912±0.404	<0.01
Fe/Zn	0.983 ± 0.05	1.378 ± 0.236	<0.01
Cu/Fe	1.395 ± 0.238	1.081 ± 0.05	0.01
TC (mg/dl)	186.39±24.20	192.0 ± 47.59	N.S

*Values are expressed as mean±SD, NS: not significant.

Table (3): TSA, selected minerals, & total cholesterol (TC) levels of male diabetic patients (G4), & male controls (G3).

Parameters*	Male controls (G3)	Male Diabetic patients (G4)	P value
TSA (mg/dl)	58.8±12.89	116.08±24.21	<0.001
Zn (ppm)	1.216±0.07	0.58±0.1	<0.01
Cu (ppm)	1.266±0.06	1.075±0.16	0.03
Fe (ppm)	0.79±0.05	1.73±0.06	<0.01
Mn (ppm)	0.04±0.001	0.018±0.002	<0.01
Mg (ppm)	18.14±0.17	14.43±1.8	<0.01
Cu/Zn	1.04 ± 0.05	1.895 ± 0.383	<0.01
Fe/Zn	0.966 ± 0.06	1.411± 0.267	<0.01
Cu/Fe	1.359 ±0.260	1.081 ± 0.056	0.01
TC (mg/dl)	181.7 ± 41.02	183.8±25.37	N.S

*Values are expressed as mean±SD, NS: not significant.

Table (4): TSA, selected minerals, & total cholesterol (TC) levels of female diabetic patients (G2), & female controls (G1).

Parameters*	Female controls (G5)	Female diabetic patients (G6)	P value
TSA (mg/dl)	60.36±9.88	123.96±21.03	<0.001
Zn (ppm)	1.17±0.086	0.595±0.085	<0.01
Cu (ppm)	1.26±0.087	1.121±0.149	0.049
Fe (ppm)	0.78±0.05	1.17±0.09	<0.01
Mn (ppm)	0.04±0.002	0.018±0.003	<0.01
Mg (ppm)	18.01±0.2	14.37±2.13	<0.01
Cu/Zn	1.07 ±0.084	1.927 ± 0.424	<0.01
Fe/Zn	0.99 ± 0.057	1.35 ± 0.206	<0.01
Cu/Fe	1.426±0.217	1.08± 0.057	<0.01
TC (mg/dl)	188.6± 23.82	200.4 ± 37.49	N.S

*Values are expressed as mean±SD, NS: not significant.

Table (5): Correlation between parameters in total type 2 DM, males, and females.

Parameters*	r	P
All (G2)		
TSA-Mg	0.299	0.03
Fe-Cu	-0.344	0.01
Cu-Fe/Zn	-0.315	0.02
Mn-Cu/Zn	-0.286	0.04
Male patients (G4)		
Fe-Mn	0.630	0.02
Female patients (G6)		
TSA-Mg	0.421	0.02
TSA-Cu/Zn	-0.401	0.03
Zn-Cu	0.417	0.02
Mg-TC	-0.53	0.005
Zn-TC	-0.411	0.01
Fe-Cu	-0.411	0.03
Mn-Cu	-0.419	0.02

Correlation is significant if P is equal or <0.05 level (2-tailed)

Discussion:

The present study has shown that TSA and selected minerals have an association with DM. The TSA concentrations in total type 2 DM, female, and male are higher compared with normal control subjects. Our results consist with the results of Yilmaz *et al.*,[14] and Ekin, *et al.*[15]. The mechanism of increased serum sialic acid concentration in diabetic patients is unknown. However, the loss of sialic acid containing-glycoprotein from the vascular cells into the blood stream or cytokine-induced acute phase response (cytokines stimulate hepatic production of acute phase proteins with notable sialic acid content) might lead to the elevated serum sialic acid levels[16]. In addition, a declined renal function might also impair excretion of sialic acid-containing glycoconjugates, causing the elevated levels of serum sialic acid [١٦,17].

Many essential metals are shown to have interactive connection with DM [18], and alterations in the metabolism of several essential minerals, including Cu, Zn, Mn, Mg, and Fe have been associated with impaired insulin release, insulin resistance, and glucose intolerance. Abnormal metabolism of Zn, Cu, and Fe can lead to several chronic disorders such as diabetes or diabetic complications, and these pathologic conditions appear to be prevalent in Zn and Cu deficiency. As well as in Cu and Fe overload [5,19]. Jansen, *et al.* found that Zn supplementation of animals and humans has been shown to ameliorate glycemic control in type 1, and type2 diabetes [20]. In our study the Zn concentration was significantly lowered in type 2 DM than in controls, these results consist with the findings reported by Walter, *et al.*,[11]; Chen, *et al.*, [18]; and Anetor, *et al.* [21]. Unlike our findings, some studies did not find any evidence of an altered Zn status in diabetic patients compared with control subjects [22].

Decreased serum Zn concentration in diabetic subjects has been suggested to be mainly associated with diminished serum albumin and amino acid levels which are the circulating factors that transfer Zn [23]. In our study we did not investigated serum albumin and amino acid levels. However, decreased gastrointestinal absorption and tissue-specific absorption of Zn might also have a contributory effect [24].

In this study, serum Cu levels were decreased in type 2 DM patients so copper deficiency might be a contributor to glucose intolerance in diabetic patients[18], although, Chen *et al.*[25] found that Cu concentrations is decreased in both type of DM. Zargar *et al.* [26], showed that Cu level was lower in patients with poor glycemic control as compared with patients with good glycemic control and healthy control, in our study the mean HbA1c revealed that our patients have poor glycemic control.

Unlike our findings, some studies found increased serum Cu levels in diabetic patients compared with control subjects and this increased serum Cu concentrations is well correlated with macroaginopathy and microangiopathy, hypercholesterolemia, and hypertension that are associated with diabetes [11,27]. In our study, patients with complications were excluded.

In this study, serum iron levels were high in type 2 DM patients. The Fe status of diabetic patients has not been well studied, but Fe mobilization or uptake and utilization has been suggested to be delayed leading to higher serum Fe levels [28]. Liu *et al.* [29] Showed that iron overload

not only increased the risk of insulin resistance and diabetes but also associated with more cardiovascular disease in non-diabetic and diabetic subjects.

In this study, we also showed that serum Mg level was lower in diabetic subjects. This finding is compatible with other studies[21,30]. Hypomagnesaemia is was frequently described in diabetic patients[21,30]; However, there is no an exact elucidation of the mechanism of magnesium deficiency in diabetes mellitus. In presence of this illness, it is observed that inadequate metabolic control can affect the corporal concentrations of magnesium which might lead to development of hypomagnesaemia[31]. Moreover, some studies revealed that decreased serum levels of Mg in diabetic patients is the result of lowered tubular reabsorption and elevated urinary excretion[11].

In this study, an altered Mn metabolism has been associated with diabetes. Unlike our findings, some studies did not find any evidence of altered Mn status in diabetics compared with control subjects[11]. Deborah and coworkers found that a failure to ensure proper manganese levels may result in an abnormal glucose homeostasis as a consequence of depressed pancreatic function[32].

The most striking results of this study were the positive or negative correlation between parameters in. Although, only a positive correlation between Fe and Mn was observed in male type 2 DM patients, many positive and negative correlations were observed between the parameters in female type 2 DM patients. These results indicate that the correlations might be sex-related.

It is concluded that serum TSA, and selected minerals have associations with DM. There are also many sex–related positive or negative correlations between the altered parameters in diabetic patients. These parameters might have diagnostic usefulness or role in patients with DM.

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