Evaluation of Serum zinc levels and its correlation with obesity in type (2) Diabetes Patients

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□ ABSTRACT □

This study was conducted on 129 adult diabetics patients at Tishreen university hospital (TUH) and the Diabetes center of Lattakia, Syria with 51 matched healthy subjects. The prevalence of obesity was higher in the diabetics group compared with healthy subjects. Serum zinc levels were evaluated in all subjects and the results showed that 31.78% (n=41) of diabetes and 9.80% (n=5) of healthy controls were found to be deficient in serum zinc levels. In addition, a significant negative correlation was identified between serum zinc levels and HbA1C (r = -0.39, $p<0.01$), BMI (r = -0.618, $p<0.05$), body fat percentage (r = -0.67, $p<0.001$) and waist circumference (r = -0.264, $p<0.05$). therefore, this study demonstrated an association between decreased serum zinc levels and poor glycemic controls observed in obese diabetes patients.

Keywords: Serum Zinc levels , Type 2 Diabetes Mellitus, Obesity, Body Mass Index, waist circumference, body fat percentage

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تقييم المستويات المصلية للزنك وعلاقته مع البدانة لدى مرضى الداء السكري من النمط الثاني

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□ ملخّص □

شملتِ هذه الدراسةُ 129 مريضاً من مرضى الداء السكري من النمط الثاني من المراجعين لمستشفى تشرين الجامعي ومركز السكري في محافظة اللاذقية/ سوريا مع 51 مشاركاً من الفئة العمرية ذاتها كمجموعة الشاهد الأصحاء. كانت نسبة انتشار البدانة لدى مجموعة المرضى أعلى مما هي عليه لدى مجموعة الشاهد الأصحاء. تم تقييم المستويات المصلية للزنك لدى جميع المشاركين في الدراسة، وأظهرتِ النتائجُ أنّ 31.78% من مرضى السكري (n=41) و9.80% من مجموعة الشاهد (n=5) كان لديهم عوز في زنك المصل . إضافة إلى ما سبق، تبيّن وجودَ ارتباط سلبي هام احصائيا بين زنك المصل وكل من الخضاب الغلوكوزي ($r = -0.39, p<0.01$) ، مؤشر كتلة الجسم ($r = -0.618, p<0.05$) ، النسبة المئوية لشحوم الجسم ($r = -0.67, p<0.001$) ومحيط الخصر ($r = -0.264, p<0.05$). بالتالي، تُظهِرُ هذه الدراسةُ وجودَ علاقةٍ بين مستويات الزنك المنخفضة والضبط السيء لسكر الدم المشاهد لدى مرضى السكري المصابين بالبدانة.

الكلمات المفتاحية: زنك المصل، الداء السكري من النمط الثاني، البدانة، مشعر كتلة الجسم، محيط الخصر، النسبة المئوية لشحوم الجسم

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Introduction:

Type 2 Diabetes Mellitus (T2DM) is a chronic metabolic disorder characterized by persistent hyperglycemia which is caused by peripheral insulin resistance (IR) and pancreatic beta-cell dysfunction(1). Several factors are recognized to be a risk factor for IR such as overweight and obesity, aging, genetic defects, high sodium intake(2), stress states and some medications (3). Obesity is associated with a decreased number of insulin receptors with post-receptor failure to activate tyrosine kinase (4, 5). The association between obesity and IR has been studied, adipose tissue in obese individual is a major source for pro-inflammatory cytokines such as TNF-α and IL-6 that activate inflammatory pathways, Jun N-terminal kinase-1(JNK1) and inhibitor of kß kinase (IKKß) which leads to serine phosphorylation of insulin receptor substrate-1 (IRS-1) and cause IR (6, 7). State of obesity often hides other deficiencies, such as mineral deficiencies like zinc. Zinc is a micronutrient that plays an essential role in DNA synthesis, RNA transcription, cell division(8, 9). It also contributes in insulin biosynthesis, storage and secretion(10). In addition, it is important for zinc-α2-glycoprotein (ZAG) synthesis, an adipokine which secreted by various organs such as liver and adipose tissue(11). The most known biological role of ZAG has been a lipid mobilizing factor by reducing fatty acid synthesis and inducing lipolysis by stimulating expression of lipolytic enzymes, such as hormone-sensitive lipase (HSL) and reducing the expression of lipogenic enzymes such as Fatty acid synthase (FAS)(12). We aimed in this research to study the relation between serum zinc levels with obesity and glycemic control in T2DM patients. Body mass index (BMI), waist circumference and Body fat percentage (FAT%) were used to evaluate obesity in subjects while Glycated hemoglobin A1C (HbA1C) was used to evaluate glycemic control.

Method and Materials:

Study population

This study included 129 T2DM patients attended the diabetes center of Lattakia with 51 healthy participants as control group over 11 months from November 2020 to December 2021 in Lattakia City, Syria. A questionnaire with questions about age and medical history was completed by participants with a written informed consent.

Data measurement :

Body weight and height were measured to calculate BMI using the formula: weight/height^2 with weight in kilograms (kg) and height in meters. Based on BMI score, subjects were classified into 4 categories as follow (13):

Normal weight: BMI < 25 Kg/m^2, overweight: BMI 25-29.9 Kg/m^2 , obesity grade1: BMI 30-39.9 Kg/m^2 , obesity grade 2: BMI ≥ 40 Kg/m^2.

A person's body fat percentage was estimated indirectly by using the Deurenberg equation as follows:

Body fat percentage = 1.2 (BMI) + 0.23 (age) -10.8 (sex) -5.4

Age was expressed in years and sex was matched 1 for males and 0 for females.

The normal body fat percentage (FAT%) is 15-20% and 25-30% for men and women, respectively. Subjects FAT% more than 25% for men and 33% for women are considered as obese subjects. In addition, men with waist circumference more than 94 cm and women with waist circumference more than 88 cm were considered as obese subjects.

Exclusion criteria:

Patients taking zinc supplementation or suffering from chronic diseases that impair zinc absorption, such as Crohn's disease were excluded in this study.

Serum sample collection:

Venous blood samples were collected from all participants after an overnight fast. Two types of tubes were used for blood collection, EDTA anticoagulant tubes (for HbA1C test) and plain tubes (for biochemical tests).

plain tubes were centrifuged at 3000 rpm for 10 minutes and the serum obtained was used for fasting blood glucose (FBG) test while the remaining amount (1.5 ml) of serum was frozen using Eppendorf tubes at -40°C until zinc analysis at Tishreen University Hospital.

Analytical Methods and Instrumentation:

HbA1C was determined using the technique of High-performance liquid chromatography (HPLC) by Tosoh Automated Glycohemoglobin Analyzer (HLC®-723GX)/ India. Fasting blood glucose (FBG) and serum zinc were tested by HumaLyzer Primus; Semi-Automatic Microprocessor Controlled Photometer/ Germany. Biochemical assays for glucose were performed with commercially available kits from Biosystem® (Spain) while serum zinc concentrations were analyzed using a kit from Medichem Middle East® (Syria).

Statistical Analysis:

The data were analyzed using the Statistical Package for Social Sciences (SPSS) version 20 for Windows. Data are expressed as mean ± standard deviation (SD). Student's t test was used to compare means of different variables between two independent samples. Analysis of variance (ANOVA) of one factor was used to identify differences in means between three groups and more. Pearson's coefficient was performed to show the linear correlation between different variables. The results were expressed as a correlation coefficient (r) and a *P*-value less than or equal to 0.05 was considered statistically significant.

Results:

The distribution of participants by gender was homogeneous in both groups: 48% were men and 52% were women in the diabetics group, while in the control group, 45% were men and 52% were women.

The mean age was close between participants (46.63 ± 11.35 years for diabetics and 45.19 ± 10.60 years for the control group) as shown in (table 1).

Table1: the demographic characteristics of the study participants

Demographic characteristics	Diabetics group (Mean ± SD)	Healthy group (Mean ± SD)
Number of patients	n=129	n=51
Male	62	23
Female	67	28
Age (years)	46.63 ± 11.35	45.19 ± 10.60

(Table 2) shows a comparison between diabetics and controls according to the parameters examined. FBG, HbA1C were higher in the diabetic group and the differences in means were significant ($p<0.001$).

BMI, body fat percentage and waist circumference were higher in diabetes patients and the differences in means were significant ($p<0.05$, $p<0.001$, and $p<0.05$ respectively). Serum zinc levels were lower in the diabetics group than in controls (86.20 ± 34.99 µg/dL for diabetics and 132.86 ± 24.89 µg/dL for controls) and the difference in mean values was significant ($p<0.001$) as shown in (table 2).

Table 2: Comparison Of Serum Biochemical Parameters Between Diabetics And Controls Groups

parameter	Diabetics group	Healthy group	p-value*
FBG (mg/dL)	146.37 ± 56.34	79.47 ± 11.08	0.000
HbA1C %	8.25 ± 1.68	5.22 ± 0.71	0.000
Zinc (µg/dL)	86.20 ± 34.99	132.86 ± 24.89	0.000
BMI (kg/m^2)	29.05 ± 4.42	24.73 ± 1.07	0.001
Waist circumference (cm)	108.13 ± 17.59	94.62 ± 11.38	0.021
Body fat percentage %	28.10 ± 3.62	24.67 ± 4.81	0.000

*T-test to independent sample, **FBG**: Fasting Blood Glucose, **HbA1C**: Glycated hemoglobin A1C **, BMI:** Body Mass Index

According to the kit used , the accepted reference range for serum zinc in adults is 46-150 µg/dL, and the value of 46 µg/dL has been used as a cut-off as an indicator for zinc deficiency. We found that 31.78% of diabetics and 9.80% of healthy controls had zinc deficiency as shown in (Fig.1).

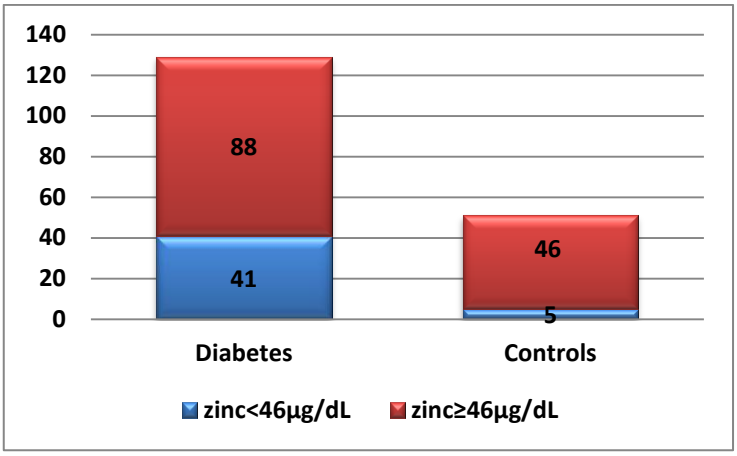

Figure1: Evaluation of zinc deficiency in subjects

(Table 3) shows the association of serum zinc levels with obesity and glycemic control in the diabetic group.

Table 3: Association of serum zinc levels with various parameters in diabetes.

parameter	value		n	Serum zinc (µg/dL) Mean ± SD	P-value	Correlation coefficient	p-value
HbA1C %	< 6.5		12	112.66±31.96	0.000	- 0.39	0.000
	6.5-7		22	103.25± 21.23			
	7.1-8		27	99.73±25.86			
	> 8		68	73.70±35.23			
FBG (mg/dL)	<110		13	113.25±30.25	0.021	- 0.301	0.001
	110-123		26	95.36±29.97			
	123-150		35	86.52±30.81			
	> 150		55	76.94±38.48			
BMI (Kg/m^2)	< 25		25	109.80±27.96	0.001	- 0.618	0.000
	25-29.9		61	100.81±26.45			
	30-39.9		39	83.38±20.70			
	≥40		4	71.54±13.25			
Waist circumference (cm)	men	< 94	4	86.76±24.35	0.034	- 0.246	0.002
		≥ 94	58	84.92±19.68			
	women	< 88	7	90.26±24.63	0.024		
		≥ 88	60	87.14±29.68			
Body fat percentage %	men	< 25%	2	89.32±12.23	0.012	- 0.67	0.000
		≥ 25%	60	85.68±28.61			
	women	< 33%	9	96.50±27.65	0.000		
		≥ 33%	58	87.57±9.01			

Diabetes were classified into 4 categories based on HbA1C levels and the results showed that diabetes with uncontrolled blood sugar (HbA1C > 8%) had lower serum zinc levels compared with patients with controlled blood sugar (HbA1C < 6.5 %) and the difference in

means between categories was significant ($p< 0.001$). In addition, diabetes with higher FBG concentrations had lower serum zinc levels and the difference was significant ($p<0.05$).

Based on BMI score, We observed a decrease in serum zinc levels in overweight and obese individuals compared with normal-weight patients ($p< 0.001$).

According to waist circumference, Subject with waist circumference more than 94cm for men and 88cm for women had lower serum zinc levels compared to subject with normal waist circumference and the differences in means between groups were significant ($p<0.05$).

For body fat percentage, subjects with FAT% more than 25% for men and 33% for women had lower serum zinc levels compared to subjects with normal FAT% and the differences in means were significant (for men: $p<0.05$, for women: $p<0.001$).

As a result of Pearson's test to investigate the correlation between serum zinc concentrations and the parameters studied, a significant negative correlation was identified between zinc levels and all of FBG (r = - 0.301, $p<0.05$), HbA1C (r = -0.39, $p<0.001$), BMI (r = -0.618, $p<0.001$), waist circumference (r = -0.264, $p<0.05$) and FAT% (r = -0.67, $p<0.001$).

Discussion:

T2DM is a metabolic disease associated with increasing the incidence of cardiovascular disease in patients(14, 15). Obesity plays a negative role in IR development and shown to be an additional risk factor for cardiovascular complications observed in diabetes patients(16). Previous studies have showed a decrease in specific serum minerals levels in T2DM patients such as magnesium(17) and this decrease accompanied by an increase in HbA1C levels (18). Zinc is a micronutrient that plays a fundamental roles in glucose metabolism(19). Several studies have reported an association between obesity and changes in serum zinc levels(20). In this research, we aimed to compare serum zinc levels between diabetes patients and healthy controls and analyzed the correlation between serum zinc concentrations and studied parameters. Serum zinc levels were estimated in all subjects , Diabetes patients appeared to have lower serum zinc levels compared to controls which was consistent with the result of Dasarathan et al. (21). In addition, the incidence of zinc deficiency was higher in diabetes group compared with controls. This finding is consistent with the study of Sakurai et al (22), they reported that 43.8% of diabetes patients have hypozincemia. Hyperzincuria is a major cause for hypozincemia(23) which could be explained by using some medication to treat hypertension(24). Furthermore, increasing adipose tissue is associated with changes in serum zinc levels(25).The importance of micronutrients in glycemic control have been studied before. In the case of zinc, our study showed that patients with lower zinc levels have poor glycemic control compared with those with normal zinc levels and a significant negative correlation was estimated between zinc and both HbA1C and FBG. Our results are consistent with the findings of previous studies(26, 27).

The importance of Zinc in insulin signaling pathways by inhibiting the tyrosine phosphatase activity of protein tyrosine phosphatase 1B (PTP1B)(28) is proposed to explain the association between decreased zinc levels and elevated HbA1C concentrations. Obese diabetics shown to have lower serum zinc levels compared with normal weight subjects and a significant negative correlation was identified between zinc and obesity index (BMI score, FAT% and waist circumference). This finding is consistent with the report of Rios-Lugo et al. (29) and Zohal et al. (30) . Inflammation state associated with

increasing adipose tissue appears to induce gene expression of Metallothionein and zinc binding protein Zip-14, which both works on entering zinc into different tissue (31).

Conclusion:

This study suggests that zinc has a negative correlation with all of BMI, waist circumference, body fat percentage and HbA1C levels. Low serum zinc levels detected in obese and overweight diabetes patients could give us an additional cause to investigate a possible association between obesity and poor glycemic control. Today, a large scale studies are needed to study the role of zinc supplementations in reducing obesity index and improving glycemic control in T2DM patients.

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