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Relationship between Serum Selenium and Copper Levels with Insulin Resistance in Patients with Diabetes type 2: A Case Control Study

Talal Al Jarah¹, Vahid Pouresmaeil^{2*}, Ali Es-Haghi¹¹Department of Biology, Mashhad Branch, Islamic Azad University, Mashhad, Iran.²Department of Biochemistry, Mashhad Branch, Islamic Azad University, Mashhad, Iran.

*Corresponding author: Vahid Pouresmaeil, Department of Biochemistry, Mashhad Branch, Islamic Azad University, Mashhad, Iran

Abstract

Background and Objectives:

Diabetes is one of the metabolic diseases in which various factors play a role. The aim of this study was to investigate the relationship between serum level of selenium and copper with insulin resistance (IR) in type 2 diabetes (T2D).

Methods:

This case control study was performed on 30 patients with T2D and 31 healthy individuals referred to Mashhad hospitals in 2020. The lipid profile, creatinine, uric acid, fasting blood sugar (FBS), HbA1c, blood pressure, smoking, exercise in all patients were extracted from medical document. Insulin level measured by ELISA, and serum levels of copper and selenium measured with Atomic Absorption. IR level was calculated from HOMA-IR formula.

Results:

30 T2D patients and 31 healthy individuals with a mean age of (52/3±10/4) and (50/4±10/2) years respectively, including 16 women in each group, were studied. BMI level (P=0/004), systolic pressure (P=0/031), FBS, HbA1c, insulin, selenium and copper levels and mean of IR in diabetic patients were significantly higher than the control group (P<0/001, each). Cholesterol, HDL, LDL, triglycerides, uric acid, creatinine, diastolic pressure, cigarettes and exercise were not significantly different between the two groups; But in the T2D group there is an inverse relationship between IR and cholesterol, HDL, LDL and systolic pressure (P<0.05). In this group, IR increases with increasing selenium (P<0.001), while copper and IR were not significantly associated.

Conclusion:

According to these results, Selenium as an essential trace mineral, is associated with diabetes and IR and its control can help to control diabetes.

Keywords: Type 2 Diabetes; Selenium; Copper, Insulin Resistance.

Introduction

Diabetes mellitus is a disease caused by the body's inability to produce insulin by pancreatic islet cells or the body's resistance to insulin [1]. The prevalence of diabetes in 2017 reached 451 million people worldwide [2]. The prevalence of this disease is growing with lifestyle changes, decreased activity and serious changes in eating habits, and over time the growth slope of this curve will become more intense, so that by 2045 the number of these people is expected to reach 693 million [3]. In Iran, however, the prevalence of this disease has a higher percentage, so that

the latest systematic study in 2019 states that about 36% of the population of Iran have diabetes [4]. Diabetes can increase the risk of cardiovascular disease, nephropathy, decreased renal function, ocular retinopathy, neuropathy, loss of sensation in four limbs, and wounds, especially in patients' feet [5]. There is currently no definitive treatment for diabetes, the most important proposed treatment is to adapt the body to the condition by using insulin injections or increasing the pressure on β cells to produce more insulin with drug therapies such as glibanglamide [6]. There are two types of the disease, the first of which usually begins

before the age of 21 and is caused by the destruction of the beta cells by the immune system, but the second type at any stage of life due to lack of response to insulin and decrease of glucose uptake [4]. Selenium is a metal that was initially known as a protective element against diabetes due to its inflammatory and antioxidant functions, but some studies have reported no link between serum levels of diabetes and selenium and even the negative effect of this element on the incidence of diabetes [7].

Some studies have suggested that selenium has an indirect effect on the function of insulin receptors at the level of myocardial cells by expressing the effect of selenium on adipokine, adiponectin, and other factors involved in cell membrane formation. This effect gradually reduces its effect on glucose uptake by reducing insulin function [8]. Some studies have shown that the selenium creates abnormal compounds with the substrate of insulin 1 and thronin protein kinase 2, causing a spatial deformation in these proteins and reducing their functional ability, which can cause insulin resistance in the body [8]. Various studies have also shown that copper is associated with oxidative stress and inflammatory cytokines, that cause oxidative stress, especially in diabetes, the level of copper is subject to changes [9,10]. The need for accurate knowledge of the processes involved in the development of diabetes due to the high prevalence and complications of the disease, for accurate control and prevention of its occurrence in the future is clearly felt. Therefore, due to the ambiguity about the relationship between copper and selenium elements with insulin resistance in type 2 diabetes, the present study was designed to investigate the relationship between selenium and copper with insulin resistance in type 2 diabetes.

Method

Participants

In this case-control study, 31 patients with type 2 diabetes were referred to university hospitals in Mashhad in 2020 whose disease was confirmed by an endocrinologist along with 31 healthy individuals were enrolled.

Inclusion criteria including age over 18 years, no heart disease and diabetic nephropathy, consent to participate in the study and the exclusion criteria including pregnancy, liver or kidney disease, hypothyroidism and hyperthyroidism, acute coronary syndrome, Stroke, cancer and patients taking vitamin and minerals supplements.

Procedure

Control group with two diabetic glycemic tests showed that they did not have diabetes and were matched with

the group of T2D patients in terms of age and sex. A written informed consent was received from all patients to participate in the study. Patient information including age, sex, duration of diabetes, demographic information, smoking, fasting blood sugar (FBS), HbA1c, blood pressure, lipid profile (TG, Chol., LDL, HDL), presence of other diseases, drug history and the patient's body mass index is recorded in the checklist. In this study, diabetes was defined by the following criteria: fasting blood sugar greater than or equal to 126 mg/dl (twice), oral glucose tolerance test greater than or equal to 200 mg/dl, glycosylated hemoglobin greater than or equal to 6.5%, and blood sugar greater than or equal to 200 mg/dl. Body mass index (BMI) is determined using the formula for weight in kilograms divided by height squared in meters.

In this study, first 5 ml of venous blood was taken from patients after 8 hours of fasting, the blood was centrifuged, the serum was separated from it and stored at -20 ° C until the day of the experiment. Selenium and copper have been measured with atomic absorption spectroscopy according to Zanao 2002 (20), Shimadzu Atomic Absorption Spectrophotometers AA-7000 Series. Serum insulin levels were measured by ELISA (Monobind Inc.) method and then the following formula, known as HOMA-IR formula,

$$\text{HOMA-IR} = \text{BS (mmol/lit)} \times \text{insulin(microunit/lit)} / 22.5$$

was used to determine insulin resistance. Finally, the raw data obtained from the serum levels of selenium, copper and insulin resistance of the two groups were calculated.

Statistical Analysis

The results of this study using SPSS 24 statistical software and using Chi-square methods, correlation coefficient test, logistic regression, t-test and Mann-Whitney test with a significance level of $P < 0.05$ Was analyzed.

Results

Among the patients participating in this study, 29 were male and 32 were female. The mean age of patients was 52.3 ± 10.4 and the mean age of the control group was 50.4 ± 10.2 , There was no significant difference between the mean age of the control group and the case group ($P = 0.338$). The mean height of the case group was 169.8 ± 8.8 cm and the mean height of the control group was 169 ± 10.1 cm which were not significantly different ($P = 0.734$). The mean weight of the case and control groups were 84.6 ± 10.9 and 75.4 ± 15.7 kg, respectively, which the mean weight of the case group was significantly higher than the mean weight of the control group ($P = 0.010$). The mean body mass index was 29.5 ± 4.8 in the case group and 26.2 ± 3.7 in the control

group, which the case group is significantly higher ($P=0.004$). The mean fasting plasma glucose in case and control groups was 128.3 ± 17.9 and 96.3 ± 16.3 mg / dl, respectively, which the case group was significantly higher than the control group ($P < 0.001$).

The mean HbA1C was $7.4 \pm 0.6\%$ in the case group and $0.5 \pm 0.6\%$ in the control group, which the case group was significantly higher than the control group ($P < 0.001$). The mean of total cholesterol in case and control groups was 180.4 ± 49.6 and 176.7 ± 49.6 mg / dl, respectively, which showed no significant difference between the mean total cholesterol of control and case groups ($P = 0.776$). The mean HDL in the case and control groups were 42.7 ± 13.4 and 43.1 ± 14.3 mg / dl, respectively, which showed no significant difference between the mean HDL of control and case groups ($P = 0.923$). The mean LDL in case and control groups were 87.8 ± 44.4 and 92.0 ± 45.1 mg / dl, respectively, which showed no significant difference between the mean LDL of control and case groups ($P = 0.719$). The mean triglycerides in the case and control groups were 160.4 ± 90.3 and 161.7 ± 65.8 mg/dl, respectively, which were not significantly different ($P = 0.949$). Mean creatinine in case and control men was 1.91 ± 0.95 and 1.24 ± 0.41 mg / dl, respectively. In men, the mean creatinine in patients was significantly higher than the control group ($P = 0.019$). The mean systolic blood pressure in the case and control groups was 138.8 ± 12.5 and 130.7 ± 16.0 mmHg, respectively, which the mean systolic blood pressure of the case group was significantly higher than the control group ($P = 0.031$). The mean of selenium in the case group was 113.0 ± 27.9 $\mu\text{g/l}$ and in the control group was 77.6 ± 18.8 $\mu\text{g/l}$ (**Figure 1**). The mean selenium in the patient group was significantly higher than the control group ($P < 0.001$). The mean of copper in the case group was 88.2 ± 56.4 $\mu\text{g/dl}$ and in the control, group was 48.9 ± 14.3 $\mu\text{g/dl}$ (**Figure 2**). The mean copper of the case group was significantly higher than the control group ($P < 0.001$). The mean insulin in the case group was 13.55 ± 16.63 $\mu\text{m} / \text{ml}$ and in the control, group was 3.55 ± 2.14 $\mu\text{g} / \text{ml}$ (**Figure 3**). The mean insulin of the patient group was significantly higher than the control group ($P < 0.001$).

The mean insulin resistance in the case group was 3.56 ± 3.86 and in the control group was 0.84 ± 0.57 $\mu\text{U} / \text{ml}$ (**Figure 4**). According to the student T test, the mean resistance of the case group was significantly higher than the control group ($P < 0.001$). According to logistic regression and the role of risk factors in causing the disease, these cases can be mentioned (**Table 1**). The risk of diabetes increases with age ($\text{OR} = 1.09$, $P = 0.002$) and with individuals aged 40 to 50 years is approximately 5.18 times higher than in

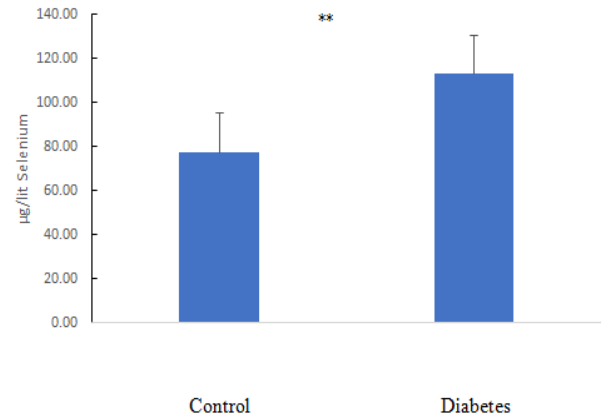


Figure 1: Selenium Distribution (ng/mL) in patient and control samples ($P < 0.001$).

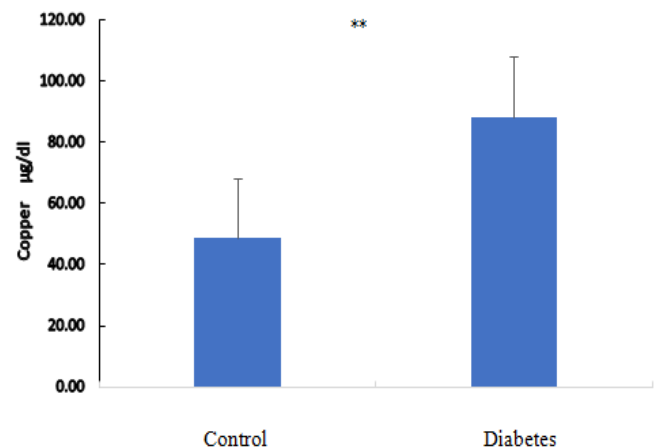


Figure 2: Copper Distribution (ng/mL) in patient and control samples ($P < 0.001$).

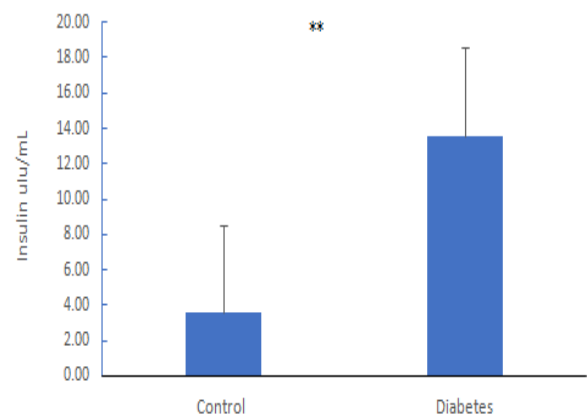


Figure 3: Insulin distribution (ng/mL) in patient and control samples ($P < 0.001$).

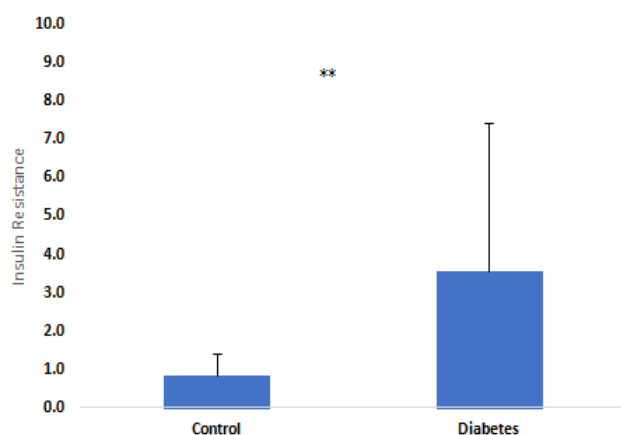


Figure 4: Insulin resistance distribution in patient and control samples ($P < 0.001$).

Table 1: Relationship between disease and risk factors.

Risk factor	Patient	Healthy	OR	CI 95%	P-Value	
Gender	Female	16	1	-	-	
	Male	14	0.93	55/2-34/0	893/0	
Age (year)	<40	3	1	-	-	
	40-50	10	5.18	14/24-11/1	0.036	
	>50	17	8	9.91	2.44-20.61	0.003
Weight (kg)	9/10 ± 6/84	7/15 ± 4/75	1.05	09/1-01/1	014/0	
Height (cm)	8/8 ± 8/169	1/10 ± 0/169	01/1	06/1-96/0	729/0	
BMI	<25	5	1	-	-	
	25-30	14	15	2.24	00/8-63/0	214/0
	>30	11	4	6.60	05/31-40/1	017/0
FBS (mg/dl)	9/17 ± 3/128	3/16 ± 3/96	12/1	18/1-05/1	001/0>	
HbA1C (%)	6/0 ± 4/7	6/0 ± 0/5	93/0	55/2-34/0	991/0	
Cholesterol (mg/dl)	6/49 ± 4/180	6/49 ± 7/176	00/1	01/1-99/0	772/0	
HDL (mg/dl)	4/13 ± 7/42	3/14 ± 7/143	00/1	04/1-96/0	921/0	
LDL (mg/dl)	4/44 ± 8/87	1/45 ± 0/92	00/1	01/1-99/0	713/0	
Triglycerides (mg/dl)	3/90 ± 4/160	8/65 ± 7/161	00/1	01/1-99/0	948/0	
Uric acid (mg/dl)	6/0 ± 9/5	0/1 ± 7/5	37/1	60/2-72/0	335/0	
Serum creatinine	8/0 ± 7/1	6/0 ± 4/1	80/1	90/3-83/0	136/0	
Systolic pressure	<129	5	1	-	-	
	>129	25	18	3.61	94/11-09/1	035/0
Diastolic pressure	4/13 ± 7/91	5/14 ± 7/88	02/1	05/1-98/0	406/0	
Cigarette (n)	6/1 ± 2/1	8/0 ± 7/0	48/1	27/2-11/1	019/0	
Daily exercises	7/0 ± 8/0	7/0 ± 0/1	95/0	98/0-93/0	032/0	

under 40 years ($OR = 5.18$, $P = 0.036$). This risk for over 50 years of age is approximately 9.91 times higher than under 40 years of age ($OR = 9.91$, $P = 0.003$). Weight gain increases the risk of diabetes ($OR = 1.05$, $P = 0.014$). The risk with a BMI over 30 is approximately 6.60 times higher than with a BMI under 25 ($OR = 6.60$, $P = 0.017$). The risk of developing

diabetes with a systolic blood pressure greater than 129, is approximately 3.61 times higher than healthy people without hypertension ($OR = 3.61$, $P = 0.035$). Smoking increases the risk of diabetes ($OR = 1.48$, $P = 0.019$) and increasing daily exercise reduces the risk of disease ($OR = 1.55$, $P = 0.032$).

According to Spearman test and correlation coefficient, the results showed that in the patient group, copper has no effect on insulin resistance ($R = 0.183$). There was a direct relationship between insulin and creatinine levels in the patient group ($P < 0.05$). The effect of insulin resistance on other variables can also be mentioned as follows: In the patient group, there was a direct relationship between insulin resistance and HbA1C ($P < 0.05$) and there is an inverse relationship between insulin resistance and total cholesterol, HDL cholesterol, LDL cholesterol and systolic blood pressure ($P < 0.05$).

Discussion

Diabetes is the most important and common autoimmune disease in the world, which is caused by a disorder in the production or effect of insulin in the body [11]. According to the World Health Organization, the outbreak of the disease will be around 366 million people by 2030 [11]. This disease with serious effects on the quality of life and function of various organs of the body can cause disability and even death secondary to disorders caused by disorders in patients; Therefore, prevention, identification of effective factors in the development and control of the disease and treatment of patients with diabetes has always been one of the main priorities of the medical community [12]. Various studies around the world have shown that in diabetes, the balance of inflammatory and non-inflammatory cytokines is disturbed and inflammation can be effective in the development and progression of diabetes, which is mainly associated with insulin resistance in type 2 diabetes [13]. Selenium play an important role in the human body in anti-inflammatory activities, and some studies have reported high levels of selenium in diabetic patients; Copper is also associated with oxidative stress and the level of inflammatory cytokines in the body [14]. Therefore, the present study was designed to investigate the relationship between serum levels of selenium and copper with insulin resistance in type 2 diabetes. In this study there was no significant difference between the two groups in terms of age and sex and in this regard both groups were matched. The mean level of body mass index was higher in T2D than the group of healthy individuals ($P < 0.05$). In a 2013 study by Supriya et al. [13] 100 diabetic patients were compared with 100 healthy individuals. the mean

age of patients with diabetes was 54.36 ± 11.25 years and the mean age of healthy individuals was 51.81 ± 10.25 years, which were similar to our study in terms of mean age. In a study conducted by Pfutzner et al. In 2006 in Germany [14] to investigate the association of CRP (C-reactive protein) with pancreatic beta cell dysfunction and insulin resistance in diabetic patients, 4270 patients with Type 2 diabetes was evaluated. The mean body mass index in the study of Pfutzner et al. [14] was 30.1 ± 5.5 kg / m².

The results obtained in this study were similar to the results obtained in our study. In a 2010 study by Akinloye et al. [15] that examined the association of cadmium, lead, arsenic, and selenium with type 2 diabetes, 50 people with type 2 diabetes were compared with 40 healthy individuals. In this study of Akinloye et al. in blood pressure was significantly higher in the diabetic group than the control group; The results of this study were in line with our study. The level of HbA1c in the study of Akinloye et al. [15] in the group of patients with diabetes was $9.95 \pm 3.12\%$, while in healthy individuals its level was $4.83 \pm 0.70\%$. Significance was higher in patients with diabetes ($P < 0.05$). The results of the study of Akinloye et al. [15] were completely in line with our study and showed that fasting blood sugar levels and HbA1c levels in patients with diabetes increased significantly compared to healthy individuals, given that diabetes Impaired glucose metabolism. This change is quite logical. In a 2014 study by Kim et al. In South Korea [9] that examined the relationship between chromium, selenium and copper levels in hair and insulin resistance in obese people compared with healthy people, HDL cholesterol levels in patients with Obesity which was significantly higher in the group of healthy individuals. Triglyceride levels in obese patients was statistically significant. Although the results of the study of Kim et al. [9] were in line with our results in terms HDL cholesterol and triglyceride levels, but our study, unlike the study of Kim et al. [9]. In a study by Siddiqui et al. [11], which aimed to investigate the relationship between selenium and glutathione peroxidase levels and nephropathy in patients with type 2 diabetes, 37 diabetics without albuminuria were compared with 38 healthy individuals. The mean uric acid level in the group of patients with diabetes was similar to our results. Creatinine level in the study of Siddiqui et al. [11] in the group of diabetic patients no significant difference was observed between the two groups; This result is in line with our study. In the study of Kim et al. [9], the mean systolic blood pressure in the obese patients was significantly higher in the obese group. Also, the level of diastolic blood pressure in obese patients was statistically significant. The results of the study of Kim et al. [9] were in line with our study. In the

study of Siddiqui et al. [16], the rate of smoking in the group of diabetic patients was significantly higher in the group of healthy people. In terms of daily exercise in the group of patients with diabetes there was a significant difference between the two groups ($P < 0.05$).

which seems to be a significant difference due to the strong recommendation to diabetic patients for exercise and weight loss. In the study of Akinloye et al. [15], the selenium level in the diabetic group was a significant difference between the two groups. In the study of Sedighi et al. [17], the level of selenium was a significant difference between the two groups ($P < 0.05$), And similar to the study of Akinloye et al. [15], selenium levels in the diabetic patient group were significantly lower than control group. Even in the study of Siddiqui et al., Who also examined diabetic patients with macroalbuminuria, selenium levels were significantly lower in the group of diabetic patients with macroalbuminuria than in diabetic patients without albuminuria. On the other hand, selenium, is associated with inflammatory cytokines, can cause secondary inflammation, which can increase its level in diabetics; These contradictions make it more difficult and complex to determine the relationship between diabetes and selenium levels in the body and indicate that this requires further study in a larger sample size to determine the exact mechanism of the effect of diabetes on serum selenium levels. In a study conducted by to evaluate copper, magnesium and zinc levels in patients with type 2 diabetes, 60 patients with type 2 diabetes were compared with 40 healthy individuals. In this study, the mean copper level in the group of diabetic patients was significantly higher than healthy individuals ($P < 0.05$); These results were in line with the results obtained in our study. In the study of Akinloye et al. [15], the serum copper level was difference between the two groups ($P < 0.05$). The results of the study by Akinloye et al. [15], like the study by, were in line with the results obtained in our study and showed that diabetes increases serum copper levels in patients with type 2 diabetes. In the study of Siddiqui et al. [16], patients with insulin resistance based on HOMA-IR formula in the group of patients with diabetes was significantly higher in the group of patients with diabetes ($P < 0.05$). In the study of Kim et al. (9), the level of insulin resistance significantly more in the group of obese patients than compared with healthy individuals. The results of the study of Kim et al. [9] and Sidighi et al. [17] were in line with our study. The study of the relationship between insulin resistance and serum levels of selenium and copper showed that with increasing selenium levels in diabetic patients the amount of insulin resistance increases. In a study by Lou et al. [6], which aimed to investigate the relationship between selenium levels and

the risk of diabetes and insulin resistance, patients were divided into three groups in terms of selenium levels, with increasing selenium levels in patients with diabetes which is in line with our results [18-20].

As a result of this study, selenium levels were associated with insulin resistance but there was no significant relationship between serum copper levels and insulin resistance. Therefore, increasing selenium levels in diabetic patients can affect the increase in insulin resistance and the progression of diabetes. The results of this study also showed that the incidence of diabetes is associated with increasing age, especially over 50 years, weight gain, increased body mass index, increased fasting blood sugar and increased systolic blood pressure.

Limitations

Since this study was conducted in only one center, the lack of sample size due to time constraints is one of the main limitations of this study; Also, in this study, due to lack of financial resources, it was not possible to follow up patients.

Conflict of Interest

The authors declare that they have no conflict of interest to the publication of this article.

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*Corresponding author: Vahid Pouresmaeil, Email: v.pouresmaeil@mshdiau.ac.ir; vahidpouresmail@yahoo.fr

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