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Investigation of Blood Selenium and Zinc in Type2 Diabetes with TCF7L2 (rs7903146 C/T) Genotypes

Maryam Bakhtiari Boroujeni[1], Mohammad Fazilati[1], Hossein Salavati[1], Saeed Habibollahi[1], Abbasali Palizban[2]*

[1] Department of Biochemistry, Payame Noor University19395-4697, Tehran, Iran

[2] Department of Clinical Biochemistry, Faculty of Pharmacy and Pharmaceutical Sciences, Isfahan University of Medical Sciences, Isfahan, Iran

*Correspondence should be addressed to Abbasali Palizban, Department of Clinical Biochemistry, Faculty of Pharmacy and Pharmaceutical Sciences, Isfahan University of Medical Sciences, Isfahan, Iran; Tel: +983117922591; Fax: +983117922591; Email: palizban@pharm.mui.ac.ir.

ABSTRACT

TCF7L2 (rs7903146) as one of the most significant single nucleotide polymorphism, is one of the associated genes with type 2 diabetes. This case-control study was performed on the whole blood of 87 patients with type 2 diabetes and 45 healthy people. Selenium and zinc concentrations in whole blood were measured using flameless atomic-absorption spectrometry. The genotype of TCF7L2 was achieved by real-time PCR and high-resolution melt (HRM). In order to data analyzing, the t-test and one-way analysis of variance (ANOVA) were used. The difference of zinc levels and also selenium levels in whole blood for both healthy (Zinc: 164.75 µgr dL^{-1}, selenium: 140.71 µgr L^{-1}) and patients (Zinc: 136.77 µgr dL^{-1}, selenium: 111.66 µgr L^{-1}) groups was significant (P-value <0.05). The different levels of zinc and selenium in healthy genotype group (TT, CT, CC) and type 2 diabetic patient genotype (TT, CT, CC) group was also significant (P-value <0.05). The presence of the T allele as a risk factor led to reducing zinc levels in healthy subjects. Significant reduction in zinc in the diabetic CT versus healthy CC and in diabetic TT versus healthy CT can be considered as a result of the T allele risk. Amount of selenium in TT genotype in both control group and diabetic patients was reduced.

Keywords: Diabetes Type2, TCF7L2 (C/T) genotypes, Selenium, Zinc, rs7903146.

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1. INTRODUCTION

Type 2 Diabetes Mellitus (DM) is described as a component of metabolic syndrome in 1988 (1). Type 2 DM (non-insulin dependent DM) is the most common form of DM characterized by hyperglycemia, insulin resistance and relative insulin deficiency (2). Type 2 DM is found as the results of the interaction between genetic, environmental and behavioral risk factors (3). Some genes were discovered that are significantly associated with developing type 2 DM (like TCF7L2) (4). TCF7L2 (transcription factor 7-like 2) regulates proglucagon gene expression (through WNT / β-catenin) and then the production of glucagon-like peptide-1 (GLP-1) (5). The TCF7L2 gene has 836.215 nucleotide pairs, 14 exons and 13 intron that located on chromosome 10 in the 10q25.3 region (6). In the diabetes prevention plan, carriers of the T allele (rs79013146 or rs12255372) had lower values for insulin/glucose ratio and also insulin response compared to CC homozygotes (7). Micronutrients may be involved in the complex processes of the development in the complications of diabetes mellitus (8). Zinc is an essential micronutrient involved in the physiology of insulin and there is a close structural and functional relationship between them (9-12). The close relationship between zinc structure and insulin function was first identified by Scott (13). Zinc may participate as an integral component of antioxidant enzymes or as a cofactor in a variety of enzymatic processes of glucose and lipid metabolism (12, 14-17). Oxidative stress can reduce insulin secretion and increases insulin resistance in some experimental cases and can play a causal role in the pathogenesis of diabetes (18-22). Selenium is an essential trace element which is involved in the complex system of defense against oxidative stress (23, 24). The relationship between the level of blood Selenium and Zinc in Type 2 diabetes with TCF7L2 (rs7903146 C/T) genotypes has not been investigated yet and could be a noticeable issue for diabetic patients. The aim of this study was to investigate

the relationship between TCF7L2 variant and the amount of Zinc and selenium against diabetes.

2. MATERIALS AND METHODS

2.1. Materials

$Zn(NO_3)_2.6H_2O$, SeO_2 and other chemicals were purchased from Merck, Germany. DNA extraction kit was purchased from Genet Bio, South Korea. Real-time PCR kit was provided by QIAGen, Germany. Other used materials were obtained from valid sources.

2.2. Apparatus

Most important devices that used in this research were including Rotor Gene 6000 Real-Time PCR Machine (Corbett RotorGene, Germany), atomic absorption spectrometer (GBC GF3000, Australia) and spectrophotometer (NanoDrop® ND-1000, USA). Other devices were provided by standard companies.

2.3. Design of study

In this case-control study, the samples (132 subjects) were classified into two groups (healthy (45) and type 2 diabetic (87)). It is should be noted that 32 males and 55 females were found as type 2 diabetic patients while in the healthy group, males and females were 21 and 24 respectively.

2.4. Inclusion and exclusion criteria

Inclusion criteria were based on: patients who are defined by world health organization (WHO) as type 2 diabetes: FBS > 126 mgr dL^{-1} or 2-hpp glucose > 200 mgr dL^{-1}.

Exclusion criteria were based on: patients with type 1 diabetes, liver disorders, infectious, cancer, and autoimmune diseases, as well as patients who have supplements containing zinc and selenium.

2.5. Study procedures

The study group consisted of 55 type 2 diabetic patients for both genders and without any other diseases. The diagnosis experiments were performed by the specialized medical staff in the laboratory of Shahid Fayaz bakhsh hospital in Tehran, Iran. According to the diagnosis criteria of WHO, 45 healthy individuals were considered as a control group that did not any diabetic status in their first degree families and their fasting and 2-hpp blood were normal. A sample of whole blood (4 ml) was taken from brachial artery after 12 hours fasting. To prepare the samples, 300µl HNO_3 was added to 300µl of whole blood from each patient. Then samples were placed to a water bath at 60 °C for 60 minutes; in another step, 100 µl H_2O_2 were added to samples and the shaking process followed for 30 seconds. Then, samples were placed in water bath at 37 °C for 120 minutes. Afterwards, the samples were shaken for 30 seconds and were centrifuged at 12000 rpm for 15 minutes. A GBC GF model 3000 flameless atomic absorption spectrometer was used in order to the determination of zinc and selenium levels. A nitrate mixture of [0.1% Ni^{+2}] was added as a modifier of selenium to both standard solution and samples. According to the catalog of this atomic absorption, the determination of selenium was at wavelength of 196.0 nm and Zn was at 213.0 nm**.** A blank sample was used for setting of zero absorbance of the spectrophotometer. Calibration curves were prepared for zinc and selenium separately. The atomic absorption was performed using a series of zinc and selenium solutions with concentrations of 10, 20, 30, 40 and 50 µgr L^{-1}. Finally, whole blood elements were determined. Purification of DNA was done by Genet Bio kit based on offered protocols (25). Afterwards, Genotype was determined by Corbette Rotor-Gene model 6000 Real-time PCR and HRM. By performing PCR, the fragment containing the considered polymorphism of whole genome was isolated and amplified to desire numerous. Components such as 12.5 µl HRM PCR master mix ×2, 2 µl primer mix (10x), 8.5 µl RNase-free water and 2 µl template DNA were used in PCR. When PCR process was completed, the temperature was decreased to 65 °C and increasing gradually every 2 seconds for 1 °C until 95 °C. Determination of sequences was done by Bioneer, South Korea.

2.6. Analysis of data

All data analysis was performed by SPSS 22.0 statistical program. In this study, the data were analyzed by T-test, one way ANOVA and other statistical calculations such as correlation coefficient (R). If P-value was less than 0.05, it was considered statistically significant.

3. RESULTS AND DISCUSSION

3.1. Analysis of biochemical factors in both healthy and type 2 diabetes groups

Information on biochemical factors in two healthy groups (45) and type 2 diabetes (87) was summarized in Table 1. Statistical analyses of mean and significant levels of biochemical factors in both groups were calculated using t-test and P <0.05 was considered statistically significant.

Table 1. Information on the mean biochemical factors in both healthy and type 2 diabetic patients

Biochemical factors	*Control group (healthy)*	*Patient group (type 2 diabetes)*	*P-value*
FBS	89.27±2.09	183.84±7.03	0.001
TG	99.29±9.15	170.51±8.72	0.006
Total cholesterol	161.29±4.84	180.85±4.93	0.012
LDL	101.98±3.19	97.89±4.06	0.503
HDL	56.77±5.63	45.23±1.23	0.009
HbA1C	4.72±0.05	7.69±0.15	0.001

Statistical analysis of the biochemical factors between the two groups showed that the difference in fasting blood sugar (FBS), triglyceride (TG), total cholesterol, high-density lipoproteins (HDL) and hemoglobin A1c (HbA1C)

was significant between the two studied groups (P <0.05). However, the difference in low-density lipoprotein (LDL) cholesterol levels was not significant between the two groups. Significant differences in FBS and HbA1C between the two groups showed a good choice between the two groups because the difference between the two groups was only in the case of diabetes and the absence of diabetes.

3.2. Investigation standard atomic absorption for selenium and zinc

Figure 1 shows the linear relationship between absorbance and zinc concentration (R2 was 0.9928) and also Figure 2 shows the linear relationship between absorbance and selenium concentration (R^2 was 0.9942).

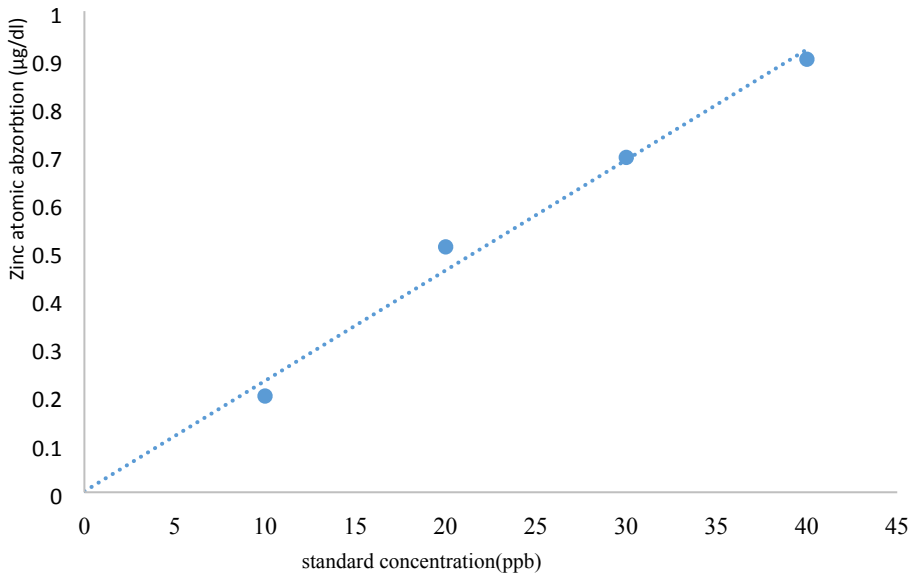

Figure 1. Calibration curve of zinc

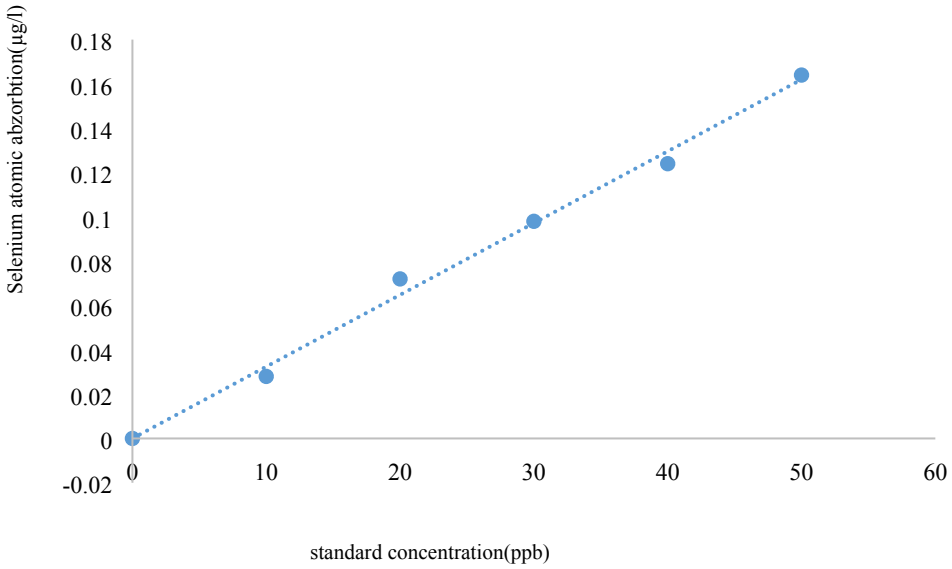

Figure 2. Calibration curve of selenium

3.3. Investigation of zinc and selenium changes in blood levels in healthy and type 2 diabetes groups

The t-test was used to evaluate the zinc and selenium level between healthy and type 2 diabetic patients. As shown in Table 2, Table 3, Figure 3 and Figure 4, in 45 healthy samples and 87 type 2 diabetic patients, the mean of zinc in whole blood were 164.75 µgr L^{-1} and 136.77 µgr L^{-1} respectively. Also, the mean of selenium in whole blood was 140.71 µgr L^{-1} and 111.26 µgr L^{-1} respectively.

Table 2. Comparison of Zinc level in healthy subjects and type 2 diabetic patients

	Group	Sample (n)	Mean µgr dL^{-1}	Standard Error (SE)	Standard deviation (SD)	P-value
Zinc	Control group (healthy)	45	164.75	3.53±	23.71	0.001
	Patient group (type 2 diabetes)	87	136.77	3.91±	36.50	

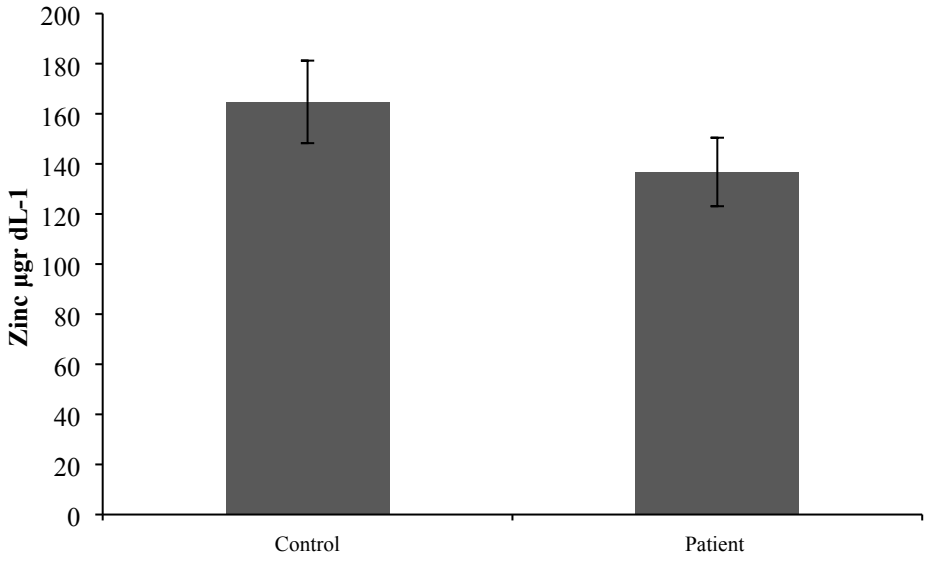

Figure 3. Column chart, Zinc level in healthy subjects and type 2 diabetic patients

Table 3. Comparison of Selenium level in healthy subjects and type 2 diabetic patients

	Group	Sample (n)	Mean µgr dL^{-1}	Standard Error (SE)	Standard deviation (SD)	P-value
Selenium	Control group (healthy)	45	140.71	4.91±	32.99	0.001
	Patient group (type 2 diabetes)	87	111.26	5.65±	52.74	

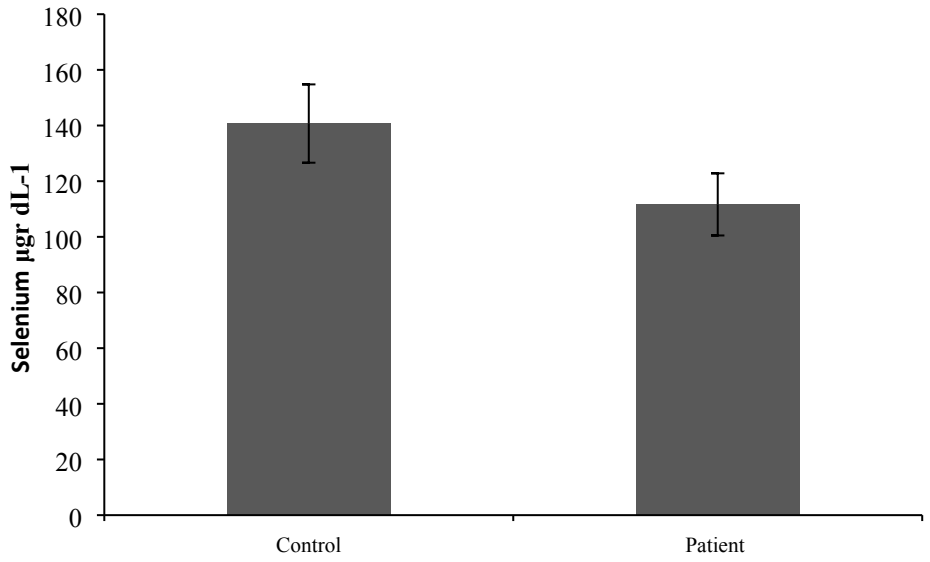

Figure 4. Column chart, selenium level in healthy subjects and type 2 diabetic patients

The results of this experiment showed that the difference of mean for zinc and selenium was significant in healthy subjects and type 2 diabetics patients and the mean level of zinc and selenium was significantly decreased in the

patient group compared to the healthy group (P <0.05). The effects of age and sex on zinc and selenium blood levels in both healthy and diabetic groups were also evaluated, but the results were not significant (P >0.05).

3.4. Determining the genotype of the gene TCF7L2, rs7903146

After investigation the gene of TCF7L2, rs7903146 and determining the sequence of the desired component, three types of healthy genotype (TT, CT and CC) were introduced. Also, three types of genotype (TT, CT and CC) were also introduced in type 2 diabetic group. The frequency of the genotypes was summarized in Table 4.

Table 4. Frequency distribution of TCF7L2 genotypes in both control and patient groups

Groups		Frequency	Percent
Control	CC	21	**46.67**
	CT	17	**37.78**
	TT	7	**15.55**
Total		45	**100**
Patient	CC	16	**18.4**
	CT	27	**13.03**
	TT	44	**50.57**
Total		87	**100**

3.5. Analysis of zinc variation among TCF7L2 genotypes

For analysis and determination of zinc and selenium differences in 6 groups of rs7903146 genotype one-way ANOVA (post hoc method) was used (P <0.05). The results were summarized in Table 5; as shown in this table, the difference of these elements in whole blood levels was significant in 6 groups.

Table 5. ANOVA test and determination of zinc and selenium in whole blood levels among 6 genotypes

Zinc	*F*	*dF*	*P-value*
	4.613	5	0.001
Selenium	*F*	*dF*	*P-value*
	2.27	4	0.04

Selenium levels in diabetics were reduced significantly compared to healthy ones. It was also found that the TT genotype in both healthy and diabetic groups reduced selenium, which this reduction was more in the healthy group (Figure 5). Zinc level decreased significantly in type 2 diabetic patients. In healthy people, the TT genotype reduced zinc levels. The presence of T allele (as a risk factor for diabetes) was a factor in reducing zinc in the healthy group (Figure 6). This showed that the T allele in the healthy TT genotype could play a role in reducing zinc levels. Considering the significant decrease in zinc level in the CT genotype of diabetes, compared with the healthy CC, the risk of T allele in this decrease and the incidence of diabetes can be noted. The presence of an additional T allele in the TT genotype compared to healthy CT significantly reduced the level of zinc, which could be the cause of diabetes.

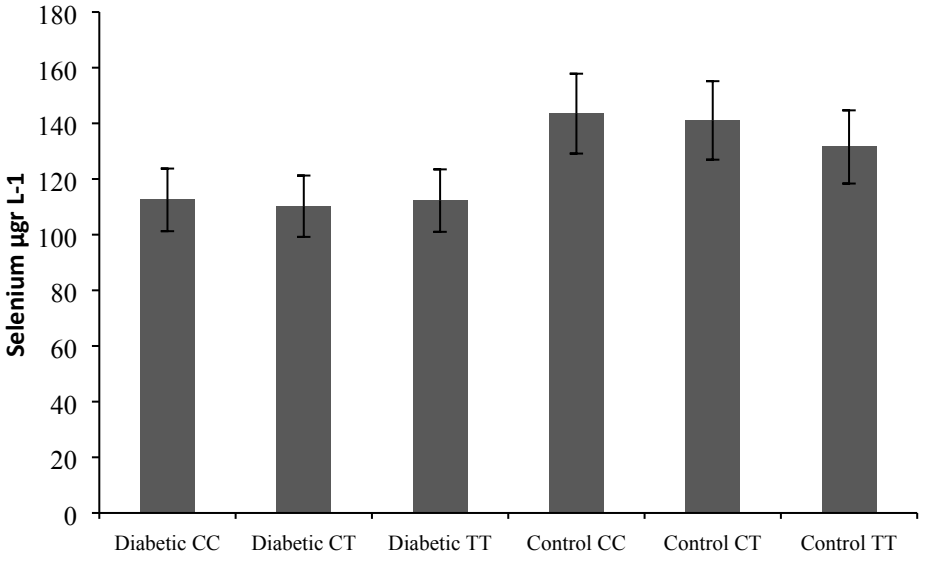

Figure 5. Column chart, selenium level in whole blood levels among 6 genotypes

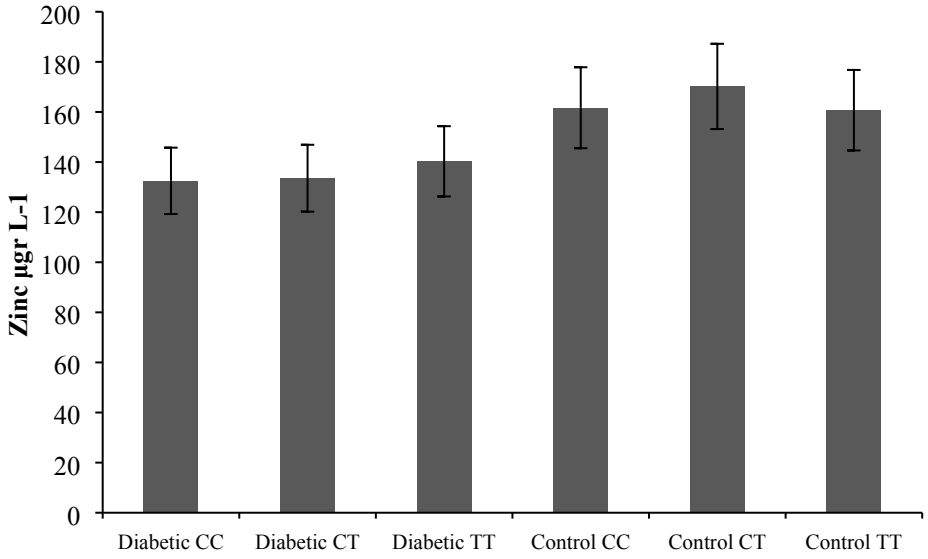

Figure 6. Column chart, zinc level in whole blood levels among 6 genotypes

Diabetes Mellitus is considered as a disease in which blood glucose concentrations increase due to defects in insulin secretion, insulin action or both of them (7). According to a report, 2 million people are diagnosed with type 2 diabetes and the disease prevalence in individuals 25-64 year-olds is about 7.7% (26). Genetic factors play an important role in the development of type 2 diabetes. Although, many genes leading to type 2 diabetes are still unknown, while it is known that this disease is polygenic and multifactorial. Various genetic locus are involved in the susceptibility to this disease. Also, environmental factors play a role in the phenotypic development of it (27). Therefore, identifying genes that make a person susceptible to this disease is important. One of the known genes is TCF7L2 as the strongest molecular pathology in diabetes. This gene is a transcription factor and one of the components of the WNT messenger pathways. The exact adjustment and control of this pathway are essential for normal growth of the pancreas and its islets in the fetus (28, 29). Observations suggest that the risk of TCF7L2 variants is associated with a decrease in the secretion of GLP-1 in the intestine and low insulin secretion (30). A group of researchers showed strong evidence about the association between the allele T polymorphism rs7903146 with type 2 diabetes (5). The results of a study indicated a significant association between rs7903146 polymorphism and the risk of type 2 diabetes. Therefore, the mutant allele rs7903146 (T allele) increased the risk of type 2 diabetes (1.57 times) (31). In a case-control study, the relationship between TCF7L2 rs7903146 genetic variation was investigated and a close association was found between the T allele and type 2 diabetes (32). Zinc as an antioxidant is involved in the production and secretion of insulin, the consumption of glucose by cells and many vital processes of the body including the activity of the immune system, and in the mechanism of wound healing (33, 34). Animal studies also indicated that zinc can stabilize insulin hexamers and improves the binding of insulin to liver cell receptors and prevents its degradation by plasma membranes (35, 36). Zinc is required as superoxide dismutase cofactor (37). Oxidative stress and tissue damage caused by the produced non-removal free radicals are due to the decreased activity of this enzyme (38). Complications of diabetes are also largely related to oxidative stress (39). Also, the effect of sex and age-related confounding factors on zinc and selenium levels in whole blood of both healthy and type 2 diabetic groups were investigated but there was no significant and had not any effect on the present study ($P < 0.05$). Previous studies have reported that the mean concentration of LDL in type 2 diabetic patients is not significantly different with non-diabetic subjects, but the qualitative changes in LDL may be found. Particularly, diabetic patients have smaller and denser LDL particles that are glycosylated easier and more susceptible to oxidation; this will be led to increase the risk of cardiovascular diseases (40, 41). In the present study, there was a significant decrease in the level of zinc in patients with type 2 diabetes compared to control group ($P < 0.05$). Other studies have shown that zinc plasma levels in diabetic patients were reduced in comparison with the control group, which is confirmed by the results of this study (42, 43). In previous studies, the lack of serum zinc levels in diabetic patients was reported due to an increase in serum glucose levels, impaired absorption of zinc intestines and increased urinary excretion of it (44, 45). Selenium is a major component of selenium enzymes, which acts as a redox agent. These enzymes catalyze reactions that disable reactive oxygen species, such as hydrogen peroxide and organic hydroperoxides; thus prevent of harmful oxidation in the cell (46). The risk of free radicals is associated with the development of complications in diabetes mellitus. The deficiency of selenium in humans produces a decrease in the activity of glutathione peroxidase and increases oxidation reactions and conversely, free radicals will be produced (47). Since selenium is essential for growing plants, its levels in plant foods are depended on the condition of the soil. In fact, studies showed that the selenium content of cereals is

variable considerably in different countries and even in different regions of a country (48). In addition, the presence of sulfur and copper also prevents selenium adsorption, and its absorption is a function of the concentration of these elements (49). In the present study, there was a significant decrease in selenium levels in the whole blood of patients with type 2 diabetes compared with the control group (P <0.05). Previous studies reported a significant reduction of serum selenium levels in diabetic patients (50-52). In this study, using real-time PCR and HRM, the rs7903146 TCF7L2 genotype was determined in two healthy and diabetic groups. It was correlated with the results of an atomic absorption spectrophotometer, which determined the concentration of zinc and selenium in the blood of both healthy and diabetic groups. Genotypes were studied in six groups (TT, CT, CC, healthy, TT, CT, CC type 2 diabetes) and ANOVA test was used to determine the relation between these six genotypes and the amount of selenium and zinc in the blood of individuals; the found differences were significant (P <0.05).

4. CONCLUSION

The change in the chemistry of trace elements is observed in patients with type 2 diabetes. Zinc as an essential trace element that has antioxidant action, plays an important role in the functioning of several enzymes. Selenium is a component of selenoproteins and enzymes which are important for the essential antioxidant actions. It was important to evaluate the relationship between rs7903146 polymorphism of the TCF7L2 and the amount of these elements in healthy subjects and type 2 diabetic patients**.** Previous studies and the present study on rs7903146 polymorphism of the TCF7L2 gene provide the presence of T allele as a risk factor for type 2 diabetes. Therefore, in the present study, reduction of zinc and selenium levels in diabetic and healthy individuals can be related to the presence of this allele.

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AUTHORS CONTRIBUTION

This work was carried out in collaboration among all authors.

CONFLICT OF INTEREST

The authors declared no potential conflicts of interests with respect to the authorship and/or publication of this paper.

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