Original Research Article

Investigate the SOD-1 gene variants rs4817415 and Their Relationship with the Physiological Antioxidant Index in Patients with T2DM

Zena Shakir Al-Tamemi[1], Suhad J. Hadi[1], Hawraa H. Naji[1], Mustafa G. Kadhim[1], Hamzah. H. K. Al-Shukri[1*]
[1]College of Veterinary Medicine, Al-Qasim Green University, 51013 Babylon, Iraq

*Corresponding Author: Hamzah. H. K. Al-Shukri
College of Veterinary Medicine, Al-Qasim Green University, 51013 Babylon, Iraq

Abstract: ***Background and Aim***: Type 2 diabetes mellitus (T2DM) is currently classified as a multi-gene hereditary metabolic disorder where the body fails to produce adequate insulin, resulting in irregular glucose regulation. Objectives: This research focuses on assessing the impact of SOD-1 gene variants rs4817415 and antioxidant index (AOI) in Iraqi individuals diagnosed with T2DM. ***Methods***: A total of 90 participants, consisting of 45 individuals with T2DM and 45 healthy controls, were included in this study. The antioxidant index (TAO-C/MDA) was evaluated using the spectrophotometer method, while SOD-1 gene variants rs4817415 were analyzed through RT-RFLP. ***Results***: The results of this study show that the T2DM and control groups had high statistically significant differences in TAO-C, MDA, and AOI levels (p-value < 0.05). The duration of T2DM in participants was strongly negatively correlated with TAO-C levels (r = -0.654), and it was significantly positively correlated with MDA levels (r = 0.412). According to the study's current findings, the AC hetrozygote genotype implied a statistically significant effect on the risk of type 2 diabetes (T2DM), with a P-value of 0.000 and an OR of 3.19 (1.56–3.09). Additionally, we discovered that the CC genotype and T2DM were significantly correlated, with OR= 1.45 (0.45-3.88). Additionally, the data indicated that the T2DM and CONT groups had statistically different distributions of SOD-1 gene variations, specifically (OR=3.11(1.12-4.09) rs4817415 and its alleles. ***Conclusion***: The findings suggest that the SOD-1 gene variants rs4817415 may serve as a risk factor for the onset of T2DM and contribute to further complications in affected individuals.
Keywords: T2DM, TAO-C, SOD-1, Gene Variants, rs4817415.

INTRODUCTION

Hyperglycemia caused by deficits in either the action or secretion of insulin, or both, is a hallmark of a class of metabolic illnesses known as diabetes [1]. Chronic hyperglycemia associated with diabetes is associated with long-term damage, dysfunction, and failure of many organs, especially the heart, blood vessels, kidneys, eyes, and nerves [2]. Numerous pathogenic processes contribute to the development of diabetes. These include autoimmune destruction of the pancreatic β-cells, which causes an insulin deficit, and abnormalities that result in resistance to the action of insulin [3]. Insufficient insulin action on target tissues is the fundamental cause of the abnormalities in protein, lipid, and glucose metabolism associated with diabetes. Reduced tissue responses to insulin or insufficient insulin production at one or more locations along the intricate hormone action pathways are the two main causes of insufficient insulin action [4]. Patients frequently have both impaired insulin action and impaired insulin synthesis, and it can sometimes be challenging to identify which abnormality—if either one alone—is the main cause of the hyperglycemia [5]. Obesity is primarily caused by excessive energy intake and subsequent storage, as well as inadequate energy expenditure that results in weight gain [6]. Over the past few decades, particularly in wealthy nations, it has grown to be a major healthcare concern. However, increased dietary consumption may have a hereditary cause, such as a lack of leptin, in some obese people [7]. A person is considered obese if their BMI is ≥30 kg/m2, and overweight if their BMI is between ≥25 and <30.16.

Since 1980, the prevalence of obesity and overweight has more than doubled, and the WHO estimates that there are currently over 2.1 billion people worldwide with a BMI of >25 [8].

The enzymes known as superoxide dismutases are responsible for catalyzing the conversion of superoxide into hydrogen peroxide and oxygen. In practically all oxygen-exposed cells, they are crucial to the antioxidant system [9]. Mammals have been found to have three different kinds of SOD, Extracellular superoxide dismutase (SOD3), mitochondrial SOD2, and cytoplasmic SOD1 are the three types of SOD that have been identified in mammals [10]. SOD1, or CuZnSOD, is a cellular defense mechanism against oxidative attacks and is found in human chromosome 21 (region 21q22) [11]. It may possibly be the most significant free radical scavenger enzyme in the lens, according to earlier research [12]. Numerous elements contribute to the pathophysiology of type 2 diabetes, such as genetic predispositions, favorable family history, ethnicity, and environmental and lifestyle factors [13]. The present study aims to assess the impact of SOD-1 gene variants rs4817415 and the antioxidant index as a risk factor for progression of T2DM in participants in the present research.

MATERIALS AND METHODS

In present study, there were 90 participants in this study, including 45 patients with Type 2 Diabetes Mellitus (T2DM) (24 men and 21 women, ages 33–62) and 45 age and gender-matched control subjects. The oxidative Stress and antioxidant markers were assessed by the colorimetric reaction between malondialdehyde (MDA) and its reagent called thiobarbituric acid (TBA) at (90-100°C) and pH at range of (2-3) for 15 minutes yields a pink product that can be detected with a spectrophotometric analysis at 532 nm. MDA will be measured from the individuals' serum samples using a recently improved technique as follows: As shown in Table 1, two sets of tubes will be made.

Table 1: Estimation of MDA protocol

No.	Reagent	Sample	Blank
1	Serum	150µl	-
2	TCA	1 ml	1 ml
3	TBA	1 ml	1 ml

Genotyping Analysis

A UV spectrophotometer was used to measure the amount of DNA that was extracted using the salting-out procedure. During electrophoresis, two microliters of genomic DNA samples were run on a 1% agarose gel to confirm the DNA's integrity. PCR Analysis: The target DNA was amplified using PCR. Online software (www.simgene.com/primer3) was used to design the forward and reverse primers. The SOD1 gene variations, in particular the rs4817415 genotype, as indicated in Table 2, were examined using RT-PCR.

Table 2: Set of primers of SOD1 gene variants rs4817415

Primer sequence (5'→3')	Amplicon length
SOD1 F: 5'-AATTCCTTACCCCTGTTCTA-3 SOD1 R: 5'-GGCAGATTTCAGTTCATTGT-3'	50 bp

Statistical Analysis

Frequency, percentage, mean, and SD were used for comparing the results in the study. The genotype analysis was done by the Hardy-Weinberg equation in all groups.

RESULTS

The medicinal properties of patient group is illustrated into Table 3:

Table 3: Patient group characteristic

PARAMETERS	T2DM N = 45	(%)	p-value
Age 33-49 50-63	27 18	60 40	0.109
Gender M F	24 21	53 47	0.098
BMI ≥30 <30	22 23	49 51	0.121

As shown in Table 4, this study showed a significantly significant difference in the antioxidant markers levels used in this research between the both groups, with a p-value of less than 0.05.

Table 4: Levels of TAO-C and MDA in study groups

| Study Groups	TAO-C (U/ml) mean± SD	P-value	MDA(U/l) mean± SD	P-value
T2DM n=45	23±3.8	0.0001	6.09±1.8	<0.001
CONTROL n=45	45±5.2		1.7±0.7	

On the other hand, the results show a strongly negative correlation between the T2DM group ages, according to TAO-C, while highly positive with MDA, and which accordingly gave r = - 0.654 and 0.412, correspondingly, as shown in Figure 1.
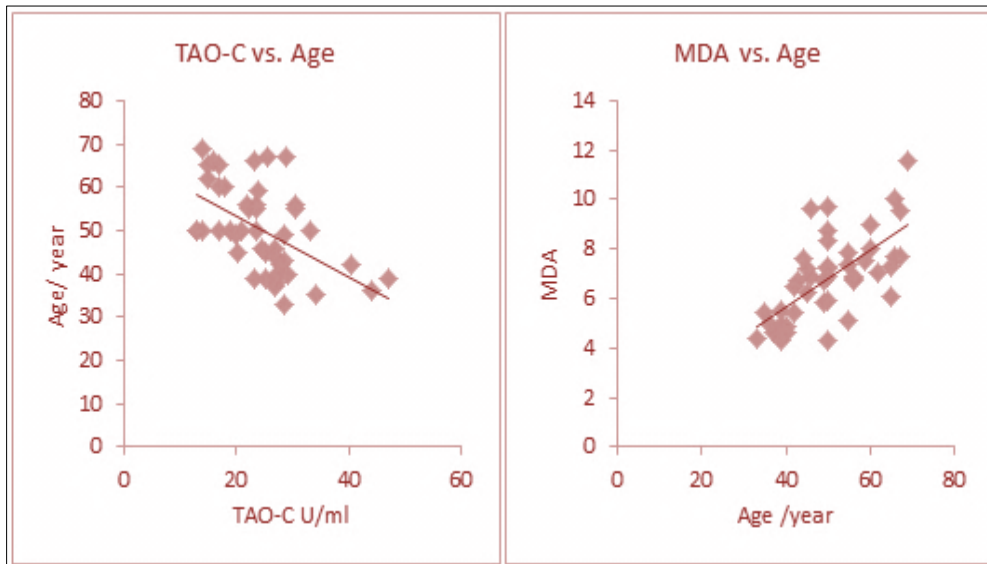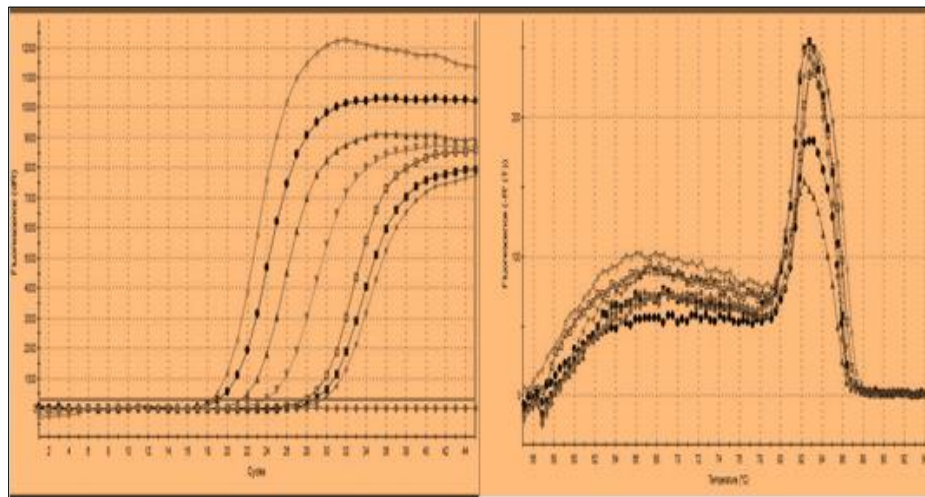

Figure 1: TAO-C and MDA (µmol/l) values in the T2DM group correlated with age (year)

Figure 3 illustrates our study's findings, which indicate a negative relationship between the T2DM group's AOI with BMI.
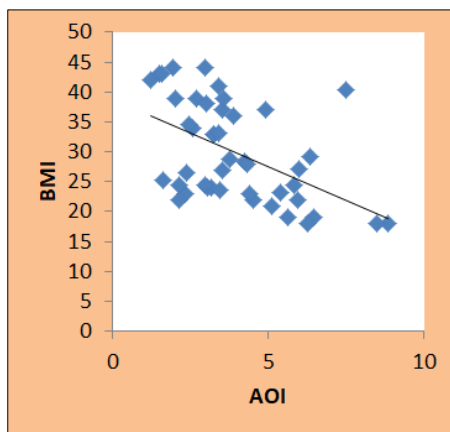
Figure 2: BMI and AOI levels in the T2DM group were correlated

The genotyping analysis of SOD1 gene variations rs4817415 was evaluated using RT-PCR, as seen in Figure 3:

Figure 3: SOD-1 gene variants rs4817415 amplification and melting curve by RT-PCR analysis

Table 4 displays the findings of the current investigation, which indicate the frequency of genotype polymorphism and allele frequency of SOD1 gene variations rs4817415. Differences in SOD1 gene variations rs4817415 are shown by the odd ratio statistical analysis test, as indicated in Table 5:

Table 5: Comparison of SOD1 gene variants rs4817415 incidence in T2DM and control groups

Genotypes	CONTROL n=45 (%)	T2DM n= 45(%)	Total	OR (CI 95%)	p-value
AA	8 (18)	10 (22)	18	1.0 (reference)	
AC	22 (49)	25 (56)	47	3.19 (1.56-3.09)	
CC	15 (33)	10 (22)	25	1.45 (0. 45-3.88)	<0.001
TOTAL	45	45	90		
A	66%	77%	3.11 (1.12-4.09)	<0.001	
C	44%	33%			

According to the study's current findings, the AC hetrozygote genotype implied a statistically significant effect on the risk of type 2 diabetes (T2DM), with a P-value of 0.000 and an OR of 3.19 (1.56–3.09). Additionally, we discovered that the CC genotype and T2DM were significantly correlated, with OR= 1.45 (0.45-3.88). Additionally, the data indicated that the T2DM and CONT groups had statistically different distributions of SOD-1 gene variations, specifically (OR=3.11(1.12-4.09) rs4817415 and its alleles.

DISCUSSION

A common metabolic condition called diabetes mellitus is typified by hyperglycemia brought on by either insulin resistance, decreased insulin production, or both. The autoimmune death of pancreatic β cells causes type 1 diabetes mellitus (T1DM), which is characterized by a near total lack of insulin production [14]. Patients with T1DM must therefore take exogenous insulin, either by subcutaneous injection or a continuous infusion pump, in order to maintain control of their plasma glucose levels. Nonetheless, type 2 diabetes (T2DM) is the most prevalent kind of the disease, which is distinguished by both insulin resistance and insufficient insulin production [15]. With 90% of cases worldwide and 4% of the adult population affected, type 2 diabetes is the most prevalent type of the disease [16]. In this study, we assessed the SOD-1 SNPs in individuals with T2DM and their relationship with antioxidant index (AOI). Age, gender, and BMI did not significantly differ between the T2DM and control groups, according to the results (p-value>0.05). MDA, a biological marker for lipid peroxidation brought on by oxidative stress, is the end product of the peroxidation process of polyunsaturated fatty acids and their esters [17]. According to the common results of multiple epidemiological research, diabetes mellitus and its consequences are responsible for at least one in twenty deaths; among individuals aged 35 to 64, this proportion increases to at least one in ten deaths. The findings of this study showed that the AA homozygote genotype significantly increased the incidence of type 2 diabetes (T2DM) (P-value=0.000) (OR=2.66(1.23-4.12)). Additionally, we observed a significant correlation between T2DM and the AC genotype (OR= 2.99 (1.22-4.98)). Additionally, the results show that the T2DM and CONT groups differ statistically (OR) in SOD1 gene variants rs4817415 and AC alleles. The results of this investigation were consistent with earlier studies [18, 19], which suggested that gene polymorphism has a role

in the development of a number of disorders [20]. In order to counteract the impact of many hereditary and natural modifiers, it is necessary to have large sample sizes for both the recognition of normal variations in the human genome with subtle effects on common disease risks like type 2 diabetes, whether they are legitimate or not, and the verification of typical human genetic variants that have subtle effects on common health risks like type 2 diabetes, whether they are genuine or not [21-23]. Based on the results, there was significant association between SOD-1 gene polymorphisms and in patients with progression of T2DM. Therefore, SOD-1 gene polymorphisms might be a potential marker for increased risk of T2DM.

CONCLUSION

The results of this research suggested that SOD-1 gene variants rs4817415 and Physiological Antioxidant Index promote complications in patients with T2DM.

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