Molecular Study of SOD1 gene in Type 2 Diabetics patients in Iraq

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Abstract

Diabetes is a group of metabolic diseases characterized by high blood sugar caused by defects in the secretion of insulin or the work of insulin or both. Superoxide dismutase (SOD1) is the first and most important line of antioxidant defense systems against ROS (Reactive oxygen Species). The Zn/Cu-SOD (SOD1) gene is located in chromosome 21 (q22.11) in humans. The study aimed to assess the possible association of the SOD1 gene in the pathological forms of diabetes 2 for Iraqi people. The study included patients with type 2 diabetes mellitus (n=50) middle-aged people (age group 35-75 years) and healthy control (n=30). The samples have taken from patients attended Kadhimiya Hospital in Baghdad Governorate. DNA was extracted from the blood samples. Then, the purity and concentration was measured. Next, the gene was determined by PCR using Thermal cycler system. The study showed that the size of the SOD1 gene was 278 base pairs. Standard, especially for people with type II diabetes. A system was added using the BLAST (Basic Local Alignment Search Tool) and National Center Biotechnology Information (NCBI) software available on NCBI and www.Ncbi.nih.gov.bioEdit, indicating that the Cu / Zn SOD gene polymorphism was present. Genetic diversity was found on the form of substitution mutations for purine bases with purine bases and pyrimidins in all samples. The result appeared Genetic variations (transition) C> T, G> A and transversion A> T were completely absent in healthy but they were present in patients with type 2 diabetes mellitus, no genetic variants (C> G) were shown in healthy and patient specimens. It was absent. This may be due to the fact that the insistence between G and C is triple insisting. were present suggesting that the Cu / Zn SOD polymorphism may be associated with exposure to type 2 diabetes among Iraqis Mutations in patients with type 2 diabetes have emerged among the Iraqi community.
1-Introduction

Diabetes mellitus (DM) is a metabolic condition affecting populations globally. It is associated with an imbalance between pro-oxidant mechanisms and the antioxidant defenses, contributing to oxidative-stress, and this is associated with an increased susceptibility to endothelial dysfunction, atherosclerosis, insulin-resistance and impaired pancreatic β-cell function. (Mirhafez, 2016).

Diabetes causes significant damage in the functions of different body tissues (WHO, 2015). The effects of diabetes included long-term damage and dysfunction of various organs. Diabetes may appear with distinctive symptoms such as thirst and increasing of urine (ADA, 2012), weight loss and in its most severe forms may develop into acidosis or hyperglycemia which cause coma; the absence of effective treatment may cause death and increases of complications risk including kidney disease, heart disease, cardiovascular disease and stroke. This sugar complication can cause coma and non-ketoacidosis Hyperosmolar Nonketonic (Hussam and Shouip, 2014). Unhealthy diet and a sedentary lifestyle are important drivers of the current global epidemic. (Zheng, 2018).

Previous Studies have been shown that antioxidant enzymes including Superoxide dismutase (SOD) has a role in the prevention of these diseases and eliminate the produced oxygen. Superoxide Oxide dismutase (SOD1) Cuzn-SOD is one of the antioxidant enzymes that protect cells from the harmful effects of Reactive Oxygen species (ROS) and break them down into $\text{H}_2\text{O}_2$ and $\text{O}_2$ (Halliwell et al, 1999; Gareth, 2019).

It has been shown that oxidative stress in diabetes patients can be accelerated not only due to increasing of reactive oxygen species (ROS) production caused by hyperglycemia, but also due to decreasing of antioxidant defense system by SOD. There are three types of SOD form: 1- SOD1 (CuZn–SOD) is one SOD kinds and estimated with 50-80% of total. 2-SOD (Mn-SOD), manganese (SOD2), and third one - the extracellular form of (EC-SOD) or (SOD3). SOD is the product of distinct genes and can stimulate the same reaction (Faraci et al 2018).

The main objective of this study is to explain if the genes of antioxidants (SOD1) effect in type II diabetes in Iraqi patients, and also to detect the genetic variations of $SOD1$ gene and its effect on type II diabetes in Iraqi patients.
SOD1 gene encodes superoxide dismutase (EC 1.15.1.1). SOD1 gene is located on the long arm of chromosome (21q22.11) (Sherman et al, 1983). Familial amyotrophic lateral sclerosis (ALS) is caused by the mutations in the copper (Cu) / zinc (Zn) superoxide dismutase 1 (SOD1) gene (Kaur, 2016). SOD1 gene is associated with Amyotrophic Lateral sclerosis (ALS) (Conwit, 2000; Redler, 2012). Down syndrome occurs due to triglyceride chromosome 21. The oxidative stress appears to be due to the doubling of the CU-ZN SOD1 quality found in chromosome 21. (Pallardoe et al, 2006)

2-Materials and Methods

2-1. Samples collection

The peripheral blood specimens were collected from (80) people, 50 patients with sort 2 diabetes; 30 cases as healthy people (control). All the samples were collected from Al-Imamain Alkademain hospital in Baghdad-Iraq, for the period from the 28th of November 2018 until the end of January 2019. Blood was collected in special 3 ml tubes containing Ethylene Diamin Tetra acetie acid (EDTA) as an anticoagulant for DNA extract on. The tube was shaken well and the patient was diagnosed by diabetes specialists in the hospital where a special form was organized for each patient Clinical data including information on presence of any complication, history of other disorders, age, gender, blood sugar level and lipid profile

Table 1: Clinical data of the study subjects

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Sort 2 diabetic patients</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n = (50)</td>
<td>n = (30)</td>
</tr>
<tr>
<td>Age</td>
<td>35-75</td>
<td>40-70</td>
</tr>
<tr>
<td>Gender(M/F)</td>
<td>25-25</td>
<td>14-16</td>
</tr>
<tr>
<td>Hypertension</td>
<td>38</td>
<td>3</td>
</tr>
<tr>
<td>Family history</td>
<td>42</td>
<td>2</td>
</tr>
<tr>
<td>Hypercholesteolema</td>
<td>30</td>
<td>2</td>
</tr>
</tbody>
</table>

2-2 DNA Extraction Genomic

DNA has extracted from blood samples that collected from people with type 2 diabetes and healthy people using Geneaidg Syn TM DNA Extraction Kit according to manufactured protocol. 200 μL of the blood was used. The DNA isolated and stored at ~20°C. The quality of the DNA was checked in 0.7 percent agarose gel electrophoresis and quantified using UV spectrophotometer. DNA purity was measured using nanodrop device and the DNA bands were observed. The purity ranged between 1.8 and 2.0. This purity and concentration are suitable and recommended and reliable for further genetic analysis by using PCR technique.
2-3 PCR Analysis of SOD1gene

In this study, thermal cycler system was utilized to amplified gene SOD1 DNA was incubated in a total reaction mixture of 20 μL containing both the forward primer 5’ CTATCCAGAAACACGGTGGGCC 3’ and the reverse primer 5’ TCTATATTTCAATCAAATGCTACAAAACC 3’ (~10 picomoles).

2-4 Primers solutions

A stock buffer solution was prepared for both forward and reverse primers by diluting each of them in distilled water that was free of the DNA-l, and then diluted by adding 10 μl of stock solution of each primer to 90 μl of enzyme-free distilled water to obtain a final concentration of 10 picomol/μl for each primer.

The following steps are followed:

1- PCR PreMix: it was dissolved at room temperature and mixed for homogenization prior to use. It is contain of, MgCl2, 200 μM deoxynucleotide triphosphate and, 5 units of Taq DNA polymerase, 10x PCR buffer pH-8.3.

DNA was denatured at 94°C for 5 min initially prior to amplification. The PCR amplification conditions were as follows: 30 cycles consisting of 30 sec denaturation at 94°C, 30 sec annealing at 59°C, and 1 min extension at 72°C. The final extension included 5 mins at 72°C. The PCR product (278 bp) was confirmed by 2% agarose, gel electrophoresis.

2- Primer solution of target gene was dissolved at room temperature, and mixed for homogenization before use.

PCR product was separated on 1.5% agarose electrophoresis and visualized by exposure to ultra violate light 300nm after ethidium bromide staining

3-Results and Discussion

PCR thermal cycler performed the amplification of SOD1 gene using specific primer. The results showed a product with 278 base pair using Molecular ladder (ladder) with 100-2000 base pair for 75 minute with 75 volt as shown in figure 1. We found 100 percent homology between the SOD1 gene and the submitted DNA sequence. The encoded region of the SOD1 gene contains five exons and four introns, and this gene encodes the enzyme found in the cytoplasm and in the lysosomes of the lobule cells (Crapo et al, 1992).
Figure 1: SOD1 gene electrophoresis in diabetes patients with type 2 on agarose gel (1.5%) using ladder (100-2000 bp) with 75 volt For 1.5 hour

3-2-The sequencing of amplified product of SOD1 gene for Study Groups

The sequencing of amplified product of sod1 gene of control and patients from type 2 Diabetes Mellitus The samples were sent for direct sequencing to Macrogen Company in Korea. Our sequences were compared with the reference sequence of in national center biotechnology information (NCBI) Gene Bank.

3-3 Scanning of SOD1 gene.

After alignment of product amplification of SOD1 gene for first group patients (Forward) showed eight transversion adenine to cytosine A>C, guanine to thymine G>T, T>A, A>C, and G>T, two Transition T>C and A>G from the Gene Bank, found that part of SOD1 gene having 94% compatibility with standard in Gene Bank.

Homo sapiens superoxide dismutase 1 (SOD1), RefSeqGene (LRG_652) on chromosome 21

Sequence ID: NG_008689.1.

<table>
<thead>
<tr>
<th>Score</th>
<th>Expect</th>
<th>Identities</th>
<th>Gaps</th>
<th>Strand</th>
</tr>
</thead>
<tbody>
<tr>
<td>277 bits(306)</td>
<td>4e-72</td>
<td>168/178(94%)</td>
<td>0/178(0%)</td>
<td>Plus/Plus</td>
</tr>
</tbody>
</table>

1F, Query
1ACTACTTGTAAATATGTGCTAAGATCCTCCCTTGTAAACCCCACTTTGCTTTTGAACCTT
60

http://doi.org/10.36295/ASRO.2020.232432
Figure (2): Alignment analysis of SOD1 gene of patient group with Gene Bank of NCBI. Query represents from the sample; Subject represents a database of (NCBI).

After alignment of product amplification of SOD1 gene for first group (Reverse) having four Transversion A>C, and A>T, one Transition G>A from the Gene Bank, found that part of SOD1 gene having 97% compatibility with standard in Gene Bank SOD1 gene of patients group as shown in figure (3) under sequence ID: NG_008689.1
Figure (3): Alignment analysis of SOD1 gene of patients group with Gene Bank of NCBI. Query represents from the sample; Subject represents a database of National Center Biotechnology Information (NCBI).

After alignment of product amplification of SOD1 gene for second patients group (Forward) having eight Transversion A>C, G>T, T>A, C>A, and A>C, three Transition T>C and A>G from the Gene Bank, found that part of SOD1 gene having 94% compatibility with standard in Gene Bank l (1%).

**Query**

ACTACTTGTAAAAATATGTGCTAAGATCATCTTGTAAACCACCTTTTGCTTCAGAAGC
60

**Subject**

ACTACTTGTAAAAATATGTGCTAAGATCATCTTGTAAACCACCTTTTGCTTCAGAACTT
11957

Query 61

GCTGACTCATCTAAACCCCTGCTCCCAAATGCTGGAATGCTTTTACTTCCTGGGCC
120

**Subject**

GCTGACTCATCTAAACCCCTGCTCCCAAATGCTGGAATGCTTTTACTTCCTGGGCTTAAA
120
Figure (4): Alignment analysis of SOD1 gene of patient second group with Gene Bank of NCBI. Query represents from the sample; Subject represents a database of (NCBI).

After alignment of product amplification of SOD1 gene for second group (Reverse) having six transversion A>T, T>A, T>G, and G>T, three transition T>C and A>G from the Gene Bank, found that part of SOD1 gene having 96% compatibility with standard in Gene Bank SOD1 gene of patients group as shown in figure (5) under sequence ID: NG_008689.1.
After alignment of product amplification of SOD1 gene for third patient group (Forward) having two transversion T>G, and T>A, one Transition T>C from the Gene Bank, found that part of SOD1 gene having 96% compatibility with standard in Gene Bank SOD1 gene of patients group as shown in figure (6) under sequence ID:NG_008689.1.
Sbjct 12077
GGAATTGACAAATGGGGACACTTAAAACGATTTGGTTTTGTAGCATTTATTGAATATAG
12135

Figure(6): Alignment analysis of SOD1 gene of patient group with Gene bank of query
represents from the sample; Subject represents a database of NCBI

Third group (Reverse) having five transversion A>T, and T>G, two Transition G>A and T>C
from the Gene Bank, found that part of SOD1 gene having 97% compatibility with standard in Gene
Bank SOD1 gene of patients group under sequence ID: NG_008689.1.

The results showed that C> G, G> A, and C> T did not appear in the control samples. Neither the
polymorphism C> G was shown in the samples of the patients or healthy subjects. This may be
attributed to the type of the bound between G, C where it is triple. The polymorphism ratio (T> A) in
the patient samples was highest and appeared in all samples. There were 2 samples where genetic
diversity was altered (A> G). There were 4 samples (A> T) transversion was shown and was not shown
in healthy samples us the table 2.
Both genetic and biochemical description of SOD1 proves that SOD1 gains importance in the development of diseases such as cancer, heart failure, Down's syndrome (De la Torre etes, 1996), diabetes (Flekac, 2008) and amyotrophic lateral sclerosis. (Rosen, 1993). It has been reported that the SOD's involvement in the pathogenesis of diseases is due to changed SOD activity and ROS absorption. It was also showed that the SOD1 gene polymorphism effects SOD activity. Reduced activity of the antioxidant enzymes and weakening of total antioxidant capacity may increase the susceptibility of diabetic patients to oxidative injury.

Some studies revealed that hyperglycemia produced marked oxidation impact as evidenced by a significant increase in lipid profile, lipid per oxidation products, as well as a significant decrease in the total SOD activity. studies conducted by Sayed et al. and Fujita et al. have reported that the enzymatic activity of SOD was significantly decreased in diabetic patients with retinopathy [Sayed, 2013] and

Table(2): Genetic percentage of infected samples studied in sequence is p6, p5, p4, . p3, p2 ,p1, standard samples = C8, and C7.

<table>
<thead>
<tr>
<th>Control samples</th>
<th>samples infected</th>
<th>Genetic variations</th>
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<tbody>
<tr>
<td>Percentage%</td>
<td>Repeat</td>
<td>Percentage%</td>
</tr>
<tr>
<td>%17.64</td>
<td>3</td>
<td>% 11.59</td>
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<tr>
<td>-</td>
<td>-</td>
<td>7.2</td>
</tr>
<tr>
<td>%5.88</td>
<td>1</td>
<td>% 2.8</td>
</tr>
<tr>
<td>-</td>
<td>-</td>
<td>2.8</td>
</tr>
<tr>
<td>%23.52</td>
<td>4</td>
<td>% 1.59</td>
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<tr>
<td>-</td>
<td>-</td>
<td>15.9</td>
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<td>%5.88</td>
<td>1</td>
<td>% 7.2.</td>
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<tr>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>%11.76</td>
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<td>%14.4</td>
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<tr>
<td>%5.88</td>
<td>1</td>
<td>% 2.8</td>
</tr>
<tr>
<td>%11.76</td>
<td>2</td>
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<td>3</td>
<td>%17.39</td>
</tr>
<tr>
<td>%100</td>
<td>17</td>
<td>%100</td>
</tr>
</tbody>
</table>

A = Adenine, T = Thymine, G = Guanines, C = cytosine

المجموع

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nephropathy [Fujita, 2009]. Clinical studies conducted in different populations have shown a decrease in SOD activity in type 2 diabetic patients when compared to controls.

The results of some study demonstrate that oxidative stress in D.M can be accelerated not only due to increased production of ROS caused by hyperglycaemia but also by reduced ability of antioxidant defense system caused at least partly by SNPs of some scavenger enzymes (Flekac, 2008). In this study the expectation value is defined to give an estimate of the number of times expected to get the same similarity coincidental and the lower the value of E. This indicates that the degree of similarity was high between sequences which give greater confidence. The value of a very close to zero means that these sequences are identical and the bit Score: statistical measure of the moral similarity and the higher value indicates that the high degree of similarity and if dropped from the class of 50 point, the sense that there is no similarity mention. The study is needed to investigate the other SNPs (Mn-SOD and EC-SOD) in SOD gene to elicit the potential role of antioxidant defense and susceptibility in type 2 diabetes mellitus.

REFERENCES.


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