Investigation of SOD2 Gene Polymorphism in the Patients with Type Two Diabetes Disease in Babylon Province

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Abstract

Diabetes is a defect with body of human that causes blood glucose (sugar) levels to increase higher than normal. This is also named hyperglycemia. The free radical theory of many illness that reactive oxygen species (ROS) participate a key function in accumulation of cellular damage, The results of PCR-SSCP for SOD2 gene illustrated that two different haplotypes according to the numbers of bands in the SOD2 gene including 5 and 6 bands. While, these haplotype was detected between two groups; in diabetes patient groups and control, the results indicate that was association between 3 and 4 bands in patients as compared with a control group. the patients groups, had greater levels of SOD in contrast with control groups result a t significant differences (P≤ 0.05). Conclusion PCR-SSCP Is a good screening method to detection SOD2 snps gene in patient with diabetes type 2 disease

Keywords: Antioxidant, SOD2, Diabetes, Oxidative, SSCP, PCR.

Introduction

Diabetes Mellitus (DM) might be a perpetual issue that influences very different people of any age, race and sex. There are numerous intense and ceaseless inconveniences including this disorder [1]. Cardiovascular diseases, diabetic nephropathy, pathology and retinopathy are major and regular intricacies of diabetes. These inconveniences are influencing the framework, kidney, retina and peripheral nerves in diabetic patients and can additionally bring high value for each people and society. Late evidence presents oxidative worry as a key member being developed and movement of diabetes and it’s little and large scale vascular intricacies [2, 3].

Oxidative stress is induced by aerobic metabolism and to deal with this oxidative stress animal and human cells have developed a present inhibitor defense system, that consists of SOD (SOD), enzyme (CAT), antioxidant (GPx) and glutathione enzyme together with variety of low molecular-weight antioxidants such as ascorbate, a-tocopherol and [4] glutathione. a rise in ROS-elicited oxidative injury to DNA and other bio molecules could impair traditional functions of tissue cells and result in human aging and [5] disease.

Aging may be a complex method involving morphological and biochemical changes in single cell and within the whole organism. Oxidative stress is characterized as partner debilitated imbalance between ROS creation and inhibitor resistances, prompting the amassing of oxidative item. It’s engaged with a few sicknesses, for example, inflammatory or cardiovascular maladies [6].

The expulsion of free radicals by exogenous inhibitor mixes may so be a successful preventive measure against varied maladies. Antioxidant enzymes, for example, (SOD) assume a key part in diminishing oxidative stress. Numerous basic sorts of SOD are known: they're oligomeric proteins co factored with copper and Zn, or Mn, or iron. Grass subunits show a two-area structure: one space contains helices and in this way the second comprises of each helices and "-sheets.

The metal restricting site happens between these 2 areas and in this manner the ligands of the metal particles are made out of basic amino corrosive and aspartate side-chains [7]. In mammals, there are three isoforms of SOD, created by distinct genes: copper/zinc...
SOD (Cu/Zn-SOD), a homodimer of thirty two kDa, localized within the cytoplasm or mitochondrial inter-membrane space; Mn SOD (Mn-SOD), a homotetramer of eighty eight kDa, localized within the mitochondria (matrix and inner membrane), and Cu/Zn type (EC-SOD), associate extracellular tetrameric glycoprotein of a hundred thirty five kDa [8]. Some microorganism contains an iron SOD (Fe- SOD), others Mn SOD, and a few contain both [7].

The primary part of SODs by and large oxygen consuming living beings is to kill the O$_2^-$ made inside the cytoplasm, mitochondria and endoplasmic reticulum of cells. In any case, the SOD will likewise have an oxidant impact because of the separation of the O$_2^-$ produces H$_2$O$_2$ that is lethal to cells. it's to evacuate this unsafe H$_2$O$_2$ that the nearness of others inhibitor frameworks, for example, CAT and GPx catalysts, progresses toward becoming necessary[9].

SOD2 is encoded by the atomic SOD2 factor situated on the human chromosome 6q25 and in this manner the Ala16Val polymorphism has been known in deoxyribonucleic corrosive a couple of the human SOD2 quality. Ala16Val (rs4880) is that the most regularly considered SOD2 single ester polymorphism (SNP) in SOD2 factor.

This SNP changes the aminoalkanoic corrosive at position sixteen from fundamental amino corrosive (Val) to amino corrosive (Ala), that has been appeared to cause a conformational change inside the target arrangement of Mn SOD (MnSOD) and conjointly influences MnSOD action in mitochondria. Ala16Val SNP and changes inside the movement of the SOD2 cell reinforcement protein are identified with adjusted movement and danger of different illnesses [10].

Material and Method

Sampling

25 blood samples were collected from patient with primary diabetes whom visits marjan hospital /Babylon /Iraq and twenty five samples as control.

DNA Extraction

Genomic DNA from whole blood cells was extracted and purified using Extraction and purification Kit from Favergen Company (Taiwan).

PCR Amplification

The targeted sites of DNA were amplified using specific primers: One primer was used for identify SOD2 (rs4880), obtained from Bioneer, IDTDNA (USA). Primer: Forward sequence was 5-GCTGTGCTTTCTCGTCTTCAG -3, and the reverse sequence was 5-TGGTACTTCTCTCCGTGTAGC -3. Final product of 20μl reaction volumes containing 1.5 μl of forward and reverse primer,12.5 μl of Green Master Mix ,3μl of Genomic DNA and the volume of reaction was completed up to 20 ul by adding one.5 ul of nuclease free water.

Amplification was carried out during a thermo-cycler (Eppendorf) programmed for 5 minutes at 94°C; for 35cycles one minute at 94°C, one minute at 60°C and one minutes at 72°C; and a final extension of 5 minutes at 72°C. Amplification products were electrophoreses in agarose gels and so visualized by staining with ethidium bromide. Normal molecular markers were also included in every electrophoresis run. Ultraviolet trans-illuminated gels were photographed.

PCR-SSCP

The PCR-SSCP method includes subsequent steps viz; PCR amplification of the gene fragments, resolution in no denaturing PAGE and illustration image using ethidium bromide. The PCR amplification procedure for all the SSCP primers used is same excluding the annealing temperature, that varies between primers.

- The Single Strand Conformation Polymorphism analysis of amplified gene fragments is applied using Bio Rad protein II xi Cell vertical gel Electrophoresis unit (Bio Rad laboratories).
- The gel sandwich is assembled on a clean surface parturition down the long rectangular plate initial, then two spacers of equal thickness on the long edges of plate and therefore the short plate is placed on the oblong plate. the two glass plates with spacers between them are fitted well with correct alignment by adjustment the bulldog clamps.
The bottom side of the gel sandwich is sealed using 10 milliliter of 12-tone system gel mix. The gel sandwich is kept in slanting position and the solution mixed with 50 μl APS and 20 μl TEMED is injected between the two glass plates using syringe fitted with 10 μl tip and allowed to polymerize for 10 minutes.

After polymerization the assembled gel sandwich is placed in alignment slot of casting stand. The 12% native PAGE gel mix (25 ml) is prepared by adding APS (100 ul) and TEMED (40 μl) at a time and mixed well. This gel combine is filled from top of gel sandwich using syringe swimmingly with none bubble and clamps are immediately applied over the comb to make sure sharp wells. The gel is kept undisturbed a minimum of 45 minutes for chemical process.

After polymerisation the comb is removed and wells are flushed with zero.5X buffer. The gel sandwich is placed in electrophoresis tank with notched plate facing towards the buffer reservoir. The reservoir of the electrophoresis tank is full of zero.5X TBE and therefore the gel is given prerunat 200 volts at constant temperature for minimum 45 minutes. Ice cooled water circulation with electric pump is applied to central cooling core of assembly to keep up constant temperature.

About 4 μl PCR products and 12 μl of a form amide dye is prepared in PCR tube and denatured at 95°C for 10 minutes in the Biometra PCR machine. When denaturation the samples are immediately kept in ice chilled box and kept in 20°C deep freeze for ten minutes.

After completion of prerun the wells are flushed again using buffer. The samples are loaded on a non-denaturing 12% acrylamide: bisacrylamide (49:1) gel with gel loading tip and immediately electrophoresis is performed in 0.5 X TBE (pH 8.3) volts/cm for 100and 3 hours at temperature depending on the optimized conditions for every primer.

After completion of the electrophoresis for needed time the glass plates are removed from the assembly. There once gel is subjected to ethidium bromide staining to visualize SSCP band patterns.

**Determination of Superoxide Dismutase (SOD) Activity**

50 ul of serum was blended with 0.2 ml of 0.1 M EDTA [containing 0.0015% NaCN], 0.1 ml of 1.5 mM NBT and phosphate buffer (67 mM, pH 7.8) in an aggregate volume of 2.6 ml. In the wake of including 0.05 ml of riboflavin, the absorbance of the arrangement was estimated against refined water at 420 nm [16].

**Results**

**Genotyping Study**

The Figure (1) showed SOD2 gene amplification product. The Figure (2) showed polyacrylamide gel electrophoresis of SSCP-PCR of SOD2 gene (208) bp amplified product.

![Figure 1: Agarose gel electrophoresis of amplified products of SOD2 (rs4880)](image)

M; refers to DNA size marker lane 1 –8 lane refers to the patterns of amplified products of SOD2 (208 bp).
Selected lanes 1-9 refer to the PCR-SSCP pattern of SOD2. “C” refer to the control while “P” refer to the groups of patients. All the obtained SSCP gels were aligned with each other to show how many haplotypes where, two types of SSCP band patterns were observed in SSCP gels. The single stranded (ssDNA) DNA bands, which occupy the upper portion of the gel and the double stranded (dsDNA), which occupy the lower portion of the gel were observed. The variation of ssDNA in SSCP gels is relied to identify the genetic pattern of each amplified, and the condition for SSCP –PCR.

Table 1: The DNA polymorphism distribution of SOD2 gene by the number of bands and their association with Diabetes patients and control groups

<table>
<thead>
<tr>
<th>DNA Polymorphism of SOD2 gene</th>
<th>Patients Group NO. (%)</th>
<th>Control group NO. (%)</th>
<th>P-value</th>
<th>OR</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>5bands*</td>
<td>2 (8%)</td>
<td>9 (36%)</td>
<td>0.037</td>
<td>6.46</td>
<td>1.23–34.01</td>
</tr>
<tr>
<td>6bands</td>
<td>23 (92%)</td>
<td>16 (64%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total number</td>
<td>25</td>
<td>25</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*P ≤ 0.05.
S.E: Standard error.

Result of SOD2 Activity

The data demonstrated that the patients groups had greater significant differences (P≤ 0.05) levels of SOD in contrast with control groups result as show in Tables (2).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control (Mean ± S.E)</th>
<th>patients (Mean ± S.E)</th>
<th>P-value of groups</th>
</tr>
</thead>
<tbody>
<tr>
<td>SOD2</td>
<td>219.19±36.07</td>
<td>506.14±62.00</td>
<td>0.0001*</td>
</tr>
</tbody>
</table>

T-test. *P ≤ 0.05.
S.E: Standard error.
Discussion

The consequences of SOD2 gene illustrated that two different haplotypes according to the numbers of the SOD2 gene including 5 and 6 bands. While, conversely, these haplotype was detected between two groups; in diabetes patient groups, the results indicate that was association between 5 and 6 bands in patients as compared with a control group. Detrimental effects caused by ROS happen as a result of lopsidedness between the arrangement and inactivation of those species. Inactivation and expulsion of ROS depend on responses including the antioxidative resistance framework.

The action of SOD and catalyst guarantee a proficient searching activity against receptive oxygen intermediates, hence keeping them from expediently diffusing into oocyte layers all through the measure of continued meiotic activity [11]. Mutations or variations in antioxidant (Ala16Val polymorphism) will decrease its activity, which successively will increase oxidative stress. Defensive aftereffect of MnSOD against diabetes has been additionally appeared by changed investigations, securing part of “CC” (Ala/Ala) genotype was first announced in T1DM cases and control subjects with A16V (Ala/Ala) genotype was first announced in [12]. Changes or varieties in antioxidant (Ala16Val polymorphism) will diminish its action that progressively will increment oxidative pressure. This technique is a fundamental path physiological component inside the advancement and movement of diabetes and its vascular complexity. As Ala16Val polymorphism will diminish MnSOD action, it makes Val bearers less protection from oxidative pressure due to limited inhibitor potential and thus distressing conditions like hyperglycemia itself and performance of various pressure touchy sign pathways by Reactive Metabolites (RMs) prompt any harms rather than Ala/Ala and Ala/Val transporters. Through such theory that given in regard to A16V SNP, a few examinations researched regardless of whether this polymorphism is said to etiology of kind a couple of diabetes or diabetes difficulty in an extremely test of populace [13].

The Ala16Val polymorphism of SOD2 could be a hazard issue for diabetes among Japanese Americans. Consequences of this investigation propose that an inadequacy of ROS searching identified with the deficiency of Ala allele could bring about glucose intolerance [14]. This substitution of C to T (GCT to GTT), that is alanine to basic amino corrosive, brings about basic modifications in the mitochondrial focusing on area from β-sheet to α-helix, that actuates a 30-40% expansion in MnSOD action in mitochondria [15].

References


