DOI Number: 10.37506/v14/i1/2020/ijfmt/192966

# The Association between Mn-SOD Gene Polymorphism and Peripheral Neuropathy in Type2 Diabetic Patients of Babylon Province-Iraq

**Asma'a H. Mohamed[1], Haider K. Zaidan[2], Ali H. Al-Saadi[3]**

*[1]Department of Radiology Techniques- Al-Mustaqbal University College/Iraq, [2]Departement of Scholarships and Cultural Relations, Ministry of Higher Education and Scientific Research, Baghdad-Iraq, [3]Department of Biology-College of Science-University of Babylon/Iraq*

## Abstract

**Background:** Oxidative stress has been known to be implicated in the onset and development of impaired insulin secretion and insulin resistance and both are involved in diabetes. The mechanisms involved in oxidative stress-induced diabetic peripheral neuropathy include the generation of reactive oxygen species ROS, excesses reactive nitrogen species RNS, lipid peroxidation, DNA damage, and reduction in cellular antioxidants. Polymorphisms in genes responsible for encoding these antioxidant enzymes causes the development of diabetic peripheral neuropathy (DPN). **Aim:** this study was aimed to indicated the role of genes encoding manganese (Mn-SOD) superoxide dismutase in the pathogenesis of DPN in a type2 diabetic patients of Babylon province. Ala(-9)Val polymorphism of Mn-SOD gene polymorphism were studied in type2 diabetic patients with (n=30) and without DPN (n=30).

**Results:** Polymerase chain reaction (PCR) technique were used for detection Mn-SOD polymorphisms. This technique included the use of PCR primers (Forward and Reverse) to produce a restriction site in the amplified Mn-SOD gene product just with the polymorphic base. Then, the product of (PCR) was digested with Bsh TI restriction enzyme to detect Ala(-9) polymorphic position. The results of Ala(-9)Val polymorphism showed that the frequency of Ala/Ala, Ala/Val, and Val/Val were 63.3%, 20%, and 13.3% in healthy control subject and 36.6%, 33.3%, and 30% in diabetic without neuropathy countered by 23.3%, 20%, and 56.6% in diabetic with neuropathy. This proposed that the Ala(-9)Val polymorphism in the Mn-SOD gene is significantly associated with a risk for progression of diabetic peripheral neuropathy.

**Conclusions:** Homozygote pattern Ala/Ala were more frequent in control groups compared with homozygote pattern Val/Val were significantly more frequent in diabetic peripheral neuropathy patients.

***Keywords:*** *Oxidative stress, diabetic neuropathy, SOD, Mn-SOD polymorphism.*

## Introduction

The exposure to high levels of circulating glucose and fatty acids especially in non-insulin sensitive tissues like eye, kidney, and nervous system results in oxidative and nitrosative stress(1) leading to imbalance between oxidants and antioxidants in favour of the oxidants and consequently diabetic complications(2).

Oxidative stress has been known to be implicated in the onset and development of impaired insulin secretion and insulin resistance and both are involved in diabetes(3).The mechanisms involved in oxidative stress-induced diabetic peripheral neuropathy include the generation of reactive oxygen species ROS, excesses reactive nitrogen species RNS, lipid peroxidation(4), DNA damage, and reduction in cellular antioxidants triggering the disruption of lipids of the myelinated structure of nerves leading to loss of axons and damaging the microvasculature of peripheral nervous system(5) causes hyperexcitability in the afferent nociceptors and central neurons resulting in generation of spontaneous impulses within the axons and dorsal root ganglions of the nerves forming the pain that are associated with diabetic peripheral neuropathy(6) .

SOD catalyze the diversion of superoxide anion into hydrogen peroxide and oxygen:

$$O_2^- + O_2^- + 2H^+ \; H_2O_2 + O_2$$

SOD activity was primarily reported by McCord and Fridovich in 1969[7] they consequently demonstrated that SOD is the key antioxidant enzyme implicated in the detoxication of superoxide radicals and it plays an essential protective roles against cellular and histological disruption that are causes by ROS[8] and also has an important role in inhibiting inflammatory response which is closely correlated with minimizing hyperalgesia, several forms of SOD have been found in all mammalian tissues with different locations within the cell, these are metalloproteins each containing a distinct metal ion in its center: intracellular Cu-Zn-SOD or SOD 1, Mn-SOD or SOD 2, and extracellular EC-SOD or SOD 3, and each of which is produced by a special gene[9].

Mitochondrial SOD (Mn-SOD) is present in the mitochondrial matrix in two separating forms, dimeric Mn-SOD and tetrameric Mn-SOD and each has a subunit contains one Mn (III) ion[10]. Mn-SOD is generated in a constitutive manner, but can also be triggered by cytokines such as IL-1 and TNF, endotoxin, and by numerous oxygen metabolites in particular cell types having important role in occurrence of tissue damage in the case of oxidative stress, also has been believed that transcriptional regulation of Mn-SOD is mediated by the activation of nuclear transcription factor $_K$B (NF-$_K$B) propped by oxidants[11] .

Mn-SOD or SOD2 gene is the only known antioxidant enzyme present in matrix of mitochondria suggesting that is the first line of defense against free radicals production, and it located on chromosome 6q25.3 consisting of five exons interrupted by four introns and the promoter which control the gene expression, structural and/or functional SNP of the Mn-SOD encoding gene, and have an important role in maintenance of cellular ROS levels[12] .

In human there are at least 190 SNPs have been reported, the best known functional SNP that is associated with diabetic peripheral neuropathy is Ala-9Val SNP is a Restriction Fragment length Polymorphism (RFLP) with chromosomal position in the codon 56 (2 exon at position 9) of Mn-SOD gene, the subsituation of C to T (GCT to GTT) that is alanine to Valine results in structural changes of the mitochondrial targeting sequence of the enzyme leading to less efficient transport of Mn-SOD into the mitochondrial matrix and can compromise the capacity to neutralize superoxide radicals in the cell[13] .

## Materials and Method

### Study subjects

The study samples were collected in Marjan teaching hospital in AL-Hilla City/Babylon province-Iraq. Subjects in this study comprised from (30) patients suffer from type 2 diabetes with peripheral neuropathy, (30) patients without peripheral neuropathy as a positive control group with duration of disease (1-5, >5-10, >10 years) and with average age between (35-65 year). In contrast, the study included (30) apparently healthy people aged between (35-65) as control matched with disease group. The presence of type 2 diabetic peripheral neuropathy or not were diagnosed for all patients by a specialized doctor.

Venous blood samples were collected from fasting patients and control subjects after a period of fasting 8-10 hours by vein puncture using 5ml disposable syringes, 2 ml was placed into EDTA tubes mixed gently for 3 minutes and then being divided into two parts: the first part used in hematological tests and especially for $HbA_{1c}$ assay and the second part was stored in – 20 °C for using later for genetic analysis.

**Table (1): Clinical features of the study groups.**

| Group / Indicates | Healthy Control group (n = 30) | Type2 patients without DPN group (n = 30) | Type2 patients with DPN group (n = 30) | P value of group |
|---|---|---|---|---|
| | Mean ± SD | Mean ± SD | Mean ± SD | |
| Duration(Years) | ……… | 7.76 ±1.95 | 12.33 ± 7.64 | 0.01* |
| Age (Years) | 49.06 ± 10.06 | 50.76 ± 9.64 | 51.73 ± 8.04 | 0.53 |
| BMI (kg/m$^2$) | 27.58 ± 2.22 | 27.48 ± 0.69 | 28.44 ± 2.57 | 0.99 |
| $HbA_{1c}$ | 9.92±0.96 | 8.45±0.03 | 4.89±0.26 | 0.001* |

**(Mean ± SD): Mean ± Standard Deviation; n: number of samples; *Significant at P ≤ 0.01**

DNA extraction and genotyping of Mn-SOD

The manufacturer protocol (Geneaid/Korea) was followed for extraction the DNA from frozen blood samples by using some components of the extraction kit. The purity of extracted DNA were determined by using Nano-droop apparatus. Sequences of primers used for PCR amplification of Mn-SOD include the:

Forward strands **F**: '5-CCAGCAGGCAGCTGGCACCG-3' and

Revers strands **R**'5-TCCAGGGCGCCGTAGTCGTAGG-**3'** , the band size of about (91 bp).

Polymorphic sites were amplified by using polymerase chain reaction technique (PCR). The PCR reaction mixture considered of < 250ng/µl template DNA, 400µM of each dNTP, 12.5 µl buffer of 1U Go Taq DNA polymerase (Promega), 10 µM of each primer and 3 mM $MgCl_2$ in 25 µl of total reaction volume.

Amplification reactions were carried out by using GTC Series thermocycler (Cleaver Scientific/ UK) apparatus. The following program was set in the thermocycler after determination of the optimum annealing temperature to amplify Mn-SOD gene (55ºC for 30 seconds + 37 cycle). PCR-RFLP technique included the addition of restriction enzyme BshTI of about (0.5 µl to 10 µl PCR product), incubated at 37ºC overnight.

**Statistical analysis**

SPSS version 23 was used for analysis the clinical variables whereas, Chi-square test used for comparison genetic frequencies of Mn-SOD between patients and control group. Odds ratios (ORs) with confidence intervals (95% CL) and their associated P-Values were used to calculate the results. A P-Value of ≤ 0.05 considered statistically significance.

## Results

**Mn-SOD genotyping**

The results of Mn-SOD gene genotyping show that the PCR product had one band about (91bp) for both type2 diabetic patients and control group as shown in figure (1).

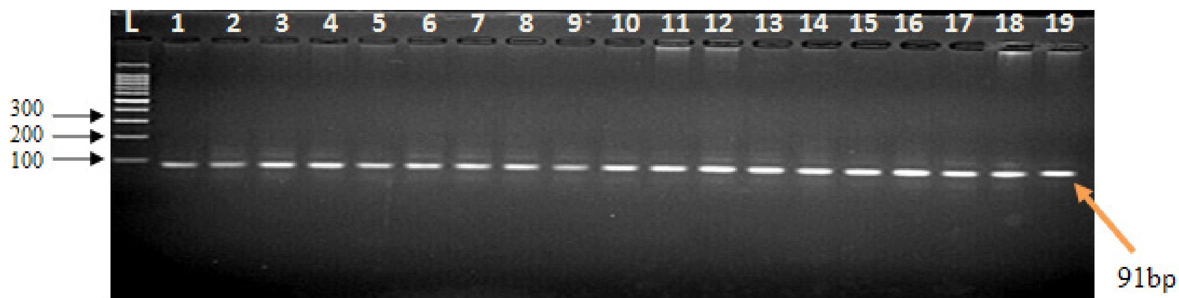


**Figure (1): The electrophoresis pattern of PCR product for Mn-SOD gene, 1% agarose, 75V, 20mA for 1h.**

**RFLP-PCR for Mn-SOD gene**

The results of PCR-RFLP of Mn-SOD for type2 patients with and without DPN and control group by using Bsh TI restriction enzyme show that the homozygous AA pattern has one band about (91bp), the homozygous VV pattern has two bands about (17 and 74 bp) and the heterozygous AV patterns has three bands (17, 74, and 91 bp) as shown in figure (2).

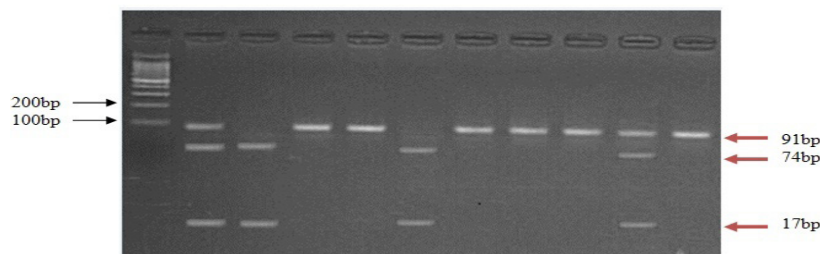


**Figure (2): Electrophoresis pattern of RFLP-PCR for PCR product (91bp) with restriction enzyme Bsh TI, 3% agarose, 75V, 20mA for 2h**

**The genotype and allele frequency of Mn-SOD gene polymorphism in type2 patients with and without DPN and control subjects**

The homozygote pattern (AA) were more frequent in control group (63.3%) than type2 patients with DPN (23.3%) with odd ratio (0.1762), while the homozygote (VV) were more frequent in type2 patients with DPN (56.6%) than control group (13.3%) with odd ratio (8.5000), as shown in table (2).

**Table (2): The genotype distribution of Mn-SOD gene polymorphism in type2 patients with and control subjects**

| Genotype | Genotype Frequency % | | ODD Ratio | CI 95% | P-Value |
|---|---|---|---|---|---|
| | Control | Type2 diabetic patients with DPN | | | |
| AA | 63.3 | 23.3 | 0.1762 | 0.0572-0.5431 | 0.002** |
| AV | 20 | 20 | 0.8214 | 0.2398-2.8140 | 0.75 |
| VV | 13.3 | 56.6 | 8.5000 | 2.3714-30.466 | 0.001** |
| Allele Frequency% | A(0.63) | A (0.23) | | | |
| | V(0.37) | V (0.77) | | | |

****P≤0.01, DPN: Diabetic Peripheral Neuropathy**

On the other hand, the homozygote pattern (AA) were more frequent in type2 patients without DPN (36.6%) than type2 patients with DPN (23.3%) with odd ratio (0.52), whereas the homozygote pattern (VV) were more frequent in type2 patients with DPN (56.6%) than type2 patients without DPN (30%) with odd ratio (3.05) as shown in table (3).

**Table (3): The genotype distribution of Mn-SOD gene polymorphism in type2 patients with and without DPN.**

| Genotype | Genotype Frequency % | | ODD Ratio | CI 95% | P-Value |
|---|---|---|---|---|---|
| | Type2 diabetic patients without DPN | Type2 diabetic patients with DPN | | | |
| AA | 36.6 | 23.3 | 0.52 | 0.1705-1.6204 | 0.26 |
| AV | 33.3 | 20 | 0.50 | 0.1547-1.6163 | 0.24 |
| VV | 30 | 56.6 | 3.05 | 1.0533-8.8390 | 0.03* |
| Allele Frequency% | A (0.36) | A (0.23) | | | |
| | V (0.64) | V (0.77) | | | |

***P≤0.05, DPN: Diabetic Peripheral Neuropathy**

## Discussion

Antioxidant enzymes represents one of the crucial cellular protective mechanisms against oxidative stress in the human body so that the polymorphism of the antioxidant genes can lead to change the enzyme activity[14]. Mn-SOD is a mitochondrial enzyme which is responsible for the formation of $H_2O_2$ from

superoxide radicals, the variant allele of Mn-SOD has been associated with increased oxidative stress which is induced by diabetes mellitus leading to the development of diabetic neuropathy[15]. Oxidative stress can trigger the damage of neurons by nerve lipid peroxidation, the impairment of mitochondrial DNA, the respiratory chain inhibition, and the cross-linking of the neurofilament protein[16]. The oxidative disorders also cause rapid changes in glia cells, suffering, severe pain, disability, cardiac death and silent myocardial ischemia, all these disorders are some of the most important consequences of diabetic peripheral neuropathy[17] .

Shimoda-Matsubayashi *et al*.[18] indicated that the (Ala) allele has an alpha-helical structure which represents a common conformation for mitochondrial leader signals, while the (Val) allele may alter its conformation from alpha-helix to beta-sheet starting from the position (16) because of amino acid substitution, this meaning that the (Val) allele is lesser transported into the mitochondria than the (Ala) allele of the enzyme. In the inner mitochondrial membrane, the poor recognition of signal sequence by membrane receptor may results in mistargeting and impairment splitting of a particular sequences leading to reduction of enzyme activity of an imported protein like Mn-SOD within the mitochondrial compartment. Processing studies have been suggested that the basal level of the Mn-SOD activity may be most increased for (AA), followed by (AV), and then (VV) [19]. The (AV) dimorphic site is located in Mn-SOD gene within exon 2[20], found that Knock-out mice lacking exon (1) and (2) display a progressive motor disorders because of neuronal degeneration.

In mitochondria, the (V) allele of the Mn-SOD may be present in a lower concentration so that the homozygous (VV) should have lower resistance to oxidative stress than patients with other Mn-SOD alleles which is a common feature of diabetes mellitus with different aging and neurological impairment, the ineffective targeting of Mn-SOD may drop out the mitochondria with low defended against superoxide radicals results in protein oxidation, mutations of mitochondrial DNA and damage which are common in the development and progression of diabetic neuropathy, and neurodegenerative disorders like Alzheimer and parkinson's disease[21] .

**Ethical Clearance:** The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

**Conflict of Interest:** The authors declare that they have no conflict of interest.

**Funding**: Self-funding

## References

**1-** Zaidan HK, Tahrear MN, Amira KM, Al-Saadi AH, Moshtak Abdul-Adheem Wtwt MAA, Zena HK. The Metabolic Syndrome and Disturbances in Sex Hormones Levels in both gender. Advances in Life Science and Technology. 2015; 34:110-116.

2- Maysaa AH, Zaidan HK, Al-Saadi AH. Histopathological Changes of Pancreatic Tissues in Hyperglycemic Male Rats Treated with Mixture of Plants Extracts. International Journal of ChemTech Research. 2016 ; 9(06):501-5013.

3- Israa HI, Zaidan HK, Al-Ameri QMA, Mufeed JE, Al-Saadi, AH. The prevalence of Microalbuminuria in type2 diabetes mellitus patients in Al-Hussain Hospital in Karbala Province-Iraq. Research in Biotechnology. 2012 ; 3(2).

4- Zaidan HK, Mohammed AH. Glucagon like peptide-1 and C peptide level and their relationship with some physiological and biochemical variables of non-insulin dependent diabetes mellitus type 2 patient. Australian Journal of Basic and Applied Sciences. 2014 ; 8:1-10.

5- Casellini CM, Vinik AI. ''Recent advances in the treatment of diabetic neuropathy,'' Current Opinion in Endocrinology and Diabetes., 2006 ; 13(2):147-153.

6- Mohammed AH, Zaidan, HK. Evaluation of Vascular Endothelial Growth Factor level of diabetic peripheral neuropathy patients in Babylon Province. Journal of pharmaceutical Sciences and Research. 2019 ; 11(1): 247-250.

7- McCord JM, Keele B, Fridovich I. An enzyme based theory of obligate anaerobiosis: the physiological function of SOD. Proc Natl Acad Sci. 1971 ; 68:1024-1027.

8- Davari A, Talaei SA, Alaei H, Salami M. "Probiotics treatment improves diabetes-induced Impairment of synaptic activity and cognitive function: behavioral and electrophysiological proofs for Microbiome-gut-brain axis," Neuroscience., 2013 ; 240:287–296.

9- Li H, Xia N, Förstermann U."Cardiovascular effects and molecular targets of resveratrol," Nitric

Oxide., 2012 ; 26(2):102-110.

10- Zelko IN, Mariani TJ, Folz RJ. “Superoxide dismutase multigene family: a comparison of the CuZn-SOD (SOD1), Mn-SOD (SOD2), and EC-SOD (SOD3) gene structures, evolution, and expression,” Free Radical Biology and Medicine. 2002 ; 33(3):337-349.

11- Mohsen IH, Zaidan HK, Al-Saadi AH. Association of miR-499 gene polymorphism with some hormones in type 2 diabetes mellitus patients. Diabetes Care. 2013 ; 36: 1413–1421.

12- Bresciani G, Cruz IB, de Paz JA, Cuevas MJ, Gonzalez-Gallego J. The Mn-SOD Ala16Val SNP: relevance to human diseases and interaction with environmental factors. Free Radic Res. 2013 ; 47(10):781-792.

13- Freedman BI, Bostrom M, Daeihagh P, Bwden DW. Genetic factors in diabetic nephropathy. Clin J Am Soc Nephrol. 2007 ; 2:1306-1316.

14- Zaidan HK, Israa HI, Al-Saadi AH, Mufeed, JE, Al-Ameri QMA. Total and differential leukocytes count in type2 diabetes mellitus patients in Iraq. Research in Biotechnology. 2012; 3(2).

15- Zaidan HK, Wtwt MAA, Amira KM, Al-Saadi AH, Tahrear MN. The Metabolic Syndrome and Disturbances in Sex Hormones Levels in both gender. Australian Journal of Basic and Applied Sciences. 2014 ; 8(13): 27-33.

16- EL-Masry TM, Abou Zahra MA, Mohammed ME, Rawhia AK. Manganese superoxide dismutase Alanine to Valine polymorphism and risk of neuropathy and nephropathy in Egyptian type 1 diabetic patients. Rev Diabet Stud. 2005 ; 2(2):70-74.

17- Juda TM, Issa AM, Zaidan HK. The role of advanced Glycated end products and Microalbuminuria on developing diabetic retinopathy in type 2 diabetes mellitus. Medical journal of Babylon. 2014 ; 11(1):152-161.

18- Shimoda-Matsubayashi S, Matsumine H, Kobayashi T, Nakagawa-Hattori Y, Shimizu Y Mizino Y. Structural dimorphism in the mitochondrial targeting sequence in the human manganese superoxide dismutase gene. Biochem Biophys Res Commu. 1996 ; 226:561-565.

19- Low PA, Nickander KK, Tritschler HJ. The roles of oxidative stress and antioxidant treatment in experimental diabetic neuropathy. Diabetes. 1997 ; 46:S38-S42.

20- Melov S, Schneider J, Day B, Hinerfield D, Coscun P, Mirra SS, Crapo ID, Wallace DC. A novel neurological phenotype in mice lacking mitochondrial superoxide dismutase. Nat Genet. 1998 ; 18:159-163.

21- Hiroi S, Harada H, Nishi H, Satoch M, Nagai R, Kimura A. Polymorphisms in the SOD2 and HLA-DRBI genes are associated with nonfamilial idiopathic dilated cardiomyopathy in Japanese. Biochem Biophys Res Commun. 1999 ; 261:332-339.