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The association of manganese superoxide dismutase gene polymorphism (Rs4880) with diabetic macular edema in a cohort of type 2 diabetic Egyptian patients

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Abstract

Background: Diabetic retinopathy (DR) and diabetic macular edema (DME) are the leading causes of blindness in patients with diabetes. Increasing numbers of people with diabetes worldwide suggest that DR and DME will continue to be major contributors to vision loss and associated functional impairment for years to come. Oxidative stress is a key participant in the development and progression of diabetes mellitus (DM) and its complications. Antioxidant status can affect vulnerability to oxidative damage, onset and progression of diabetes, and complications of diabetes. Manganese superoxide dismutase (Mn-SOD) is a key mitochondrial enzyme in cell defense against reactive oxygen species (ROS). DR and progression to DME have been associated with polymorphism in the second exon of the *Mn-SOD* gene at the 16th amino acid (Ala16Val) in the mitochondrial targeting sequence (MTS) of the protein. The study aimed to investigate the association between Ala16Val *Mn-SOD* gene polymorphism and the susceptibility to DR and DME in type 2 DM (T2DM).

Results: In this study, 150 patients with type 2 DM were enrolled: 100 patients with DR with and without diabetic macular edema (DME) and 50 patients with type 2 diabetes with a duration of 10 years without DR. *Ala16Val* SNP of the *Mn-SOD gene* (*rs4880*) was detected by TaqMan real-time PCR. The results showed that the homozygous polymorphic variant VV between the DME group is significantly higher than the non-DME group (*P* 0.018) among the DR group.

Conclusion: *Mn SOD A16V* polymorphism itself may not be associated with DR; meanwhile, it may be implicated in the pathogenesis of DME.

Keywords: Diabetic retinopathy, Mitochondrial targeting sequence, Diabetic macular edema, *Mn-SOD* gene, *Ala16Val* (*rs 4880*)

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Background

Diabetic retinopathy (DR) is one of the most common microvascular complications of diabetes mellitus (DM). It is the main cause of blindness; it is subdivided into proliferative and non-proliferative subtypes. It may occur with or without diabetic macular edema (DME) [1]. DM results in the formation of advanced glycation end products and free oxygen radicals, leading to inflammation and the occurrence of DR and DME [2].

As the global prevalence of diabetes increases, the number of people with DR is growing, and it has been estimated to increase from 424.9 million in 2017 to 628 million, by 2045 [3]. DR may be classified as non-proliferative diabetic retinopathy (NPDR) and proliferative diabetic retinopathy (PDR) based on the presence of visible ophthalmological changes and the manifestation of retinal neovascularization [4].

DR is a multifactorial disease with an etiology that is complex. The exact mechanisms by which high blood glucose levels produce complications with diabetes is not entirely clear; however, it is known that hyperglycemia has metabolic effects that cause microvascular damage to the retina [5].

Different and heterogeneous factors such as hyperglycemia, growth factors, advanced glycation end products (AGEs), high levels of circulating or vitreous cytokines and chemokines, and reactive oxygen species (ROS) may trigger the inflammatory response of the retinal vasculature [6]. A large body of evidence, therefore, confirms that chronic inflammation is critical in the development of DR, particularly in the early stages [4].

Superoxide is a reactive oxygen species (ROS) molecule that should be maintained at nanomolar concentrations to prevent cell damage. Excessive quantities of ROS molecules cause damage to DNA and RNA proteins resulting in cell apoptosis, necrosis, and inflammation of endothelial vessels [7].

Manganese superoxide dismutase Mn-SOD is an antioxidant enzyme that causes mitochondrial superoxide radicals to be inactivated and dissipated into either ordinary molecular oxygen or hydrogen peroxide neutralized by catalase, resulting in protection against oxidative stress and cell damage [2].

Host genetic may contribute to the development of the diseases [8, 9]. The encoding gene of the Mn-SOD enzyme is located on the long arm of chromosome 6q25.2. A single nucleotide polymorphism (SNP) 47C/T in the promoter region of the *Mn-SOD gene rs4880* at the 16th amino acid position in the second exon results in changes in the activity of this enzyme produced by the gene and accumulation of ROS [1, 10, 11].

Mn-SOD activity is affected by *47C/T rs4880* polymorphism due to structural changes in the mitochondrial targeting domain, resulting in a 30 to 40% decrease in Mn-SOD activity [12]. This polymorphism encodes for either (A) alanine or (V) valine. The presence of valine (V allele) results in the production of unstable messenger RNA (mRNA) and a reduction in the transport of the enzyme to the mitochondrial matrix. This affects the severity of oxidative stress and reduces the efficacy of the enzyme, which means that the gene valley has a higher superoxide radical level than the A allele due to its lower ability to degrade ROS to H_2O_2 [10, 13].

Accumulated ROS due to *Mn-SOD* gene polymorphism affects the progression of retinal disease in which retinal capillary cells undergo accelerated apoptosis in diabetics, causing diabetic retinopathy-characteristic histopathological changes [14].

The objective of this study is to demonstrate the association of *Ala16Val Mn-SOD* gene variants with DR susceptibility and progression to DME in diabetes type 2 patients.

Methods

This is a case-control study from January 2018 to November 2019. The study included 150 patients with type 2 diabetes who were recruited from the Research Institute of Ophthalmology's outpatient clinic, Giza, Egypt. They were divided into two groups: group 1, 100 patients with DR with or without DME diagnosed based on the ophthalmology criteria [15]. Group 2 included 50 patients with type 2 diabetes without DR. The inclusion criteria for the patient group were type 2 DM for more than 5 years, age > 25 years, and the fundus examination showing typical DR blood vessel abnormalities, while the control group must be DR-free with a duration of more than 10 years. Patients with type 1 diabetes, diabetic patients with diabetic nephropathy or severe hypertension, and diabetes for less than 5 years were excluded from this study.

Both patients underwent a complete history of ophthalmological evaluation (visual acuity testing, Humphrey perimetry, optical coherence tomography, and fluorescein angiography), fundus examination, and laboratory investigations. Approval was obtained from the Ophthalmology department council of The Research Institute of Ophthalmology (reference no. I- 331016). All the procedures performed in the study were under the ethical standards of the Kasr Al-Ainy School of Medicine and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Specimen collection

Venous blood samples (5 ml) were withdrawn from all subjects and evacuated to 2 sterile ethylenediaminetetraacetate EDTA vacutainer tubes. The first one (3 ml) was used for DNA extraction while the second one (2 ml) was used for HbA1c measurement.

HbA1c assay

The HbA1c measurement was based on the turbidimetric inhibition immunoassay (TINIA) principle of the Dimension clinical chemistry auto analysis kits supplied by Siemens (Siemens Health Care Diagnostics Inc, 511 Benedict Ave/Tarrytown, NY10591) [16].

Genotyping of *Mn-SOD* (*rs4880*) variant

Analysis of *Mn-SOD* polymorphism (*rs 4880*) using TaqMan pre-designed probes and primers was performed on Applied Biosystems Step One Real-time PCR. Genomic DNA was extracted from peripheral blood leukocytes using QIA amp DNA Mini kit-QIAGEN (Hoffmann-La Roche AG, Germany). Genotyping was performed using TaqMan fluorescently labeled with FAM or HEX and following the protocol recommended by the supplier (Applied Biosystems, USA). Negative controls were performed using the same DNA-free reaction mixtures. The amplification protocol for the PCR was as follows: initial activation of AmpliTaq Gold DNA Polymerase at 95 °C for 10 min followed by 50 denaturation cycles at 92 °C for 15 s and annealing and extension at 60 °C for 1 min [2, 17].

After PCR amplification, an endpoint plate read was performed using Real-Time PCR System; the Sequence Detection System (SDS) software used the fluorescence measurements made during the plate read to plot fluorescence measurement values based on the signals from each well. Automatic allele calls have been made, and allele calls have been converted to genotypes [18].

Statistical analysis

Quantitative data were statistically described in terms of mean ± standard deviation (± SD) for parametric data, median for non-parametric data, and frequency and percentage for qualitative data. The odds ratio (OR) and the 95% confidence interval (CI) were calculated for the risk estimate. The comparison of numerical variables between study groups was performed using the Student *t*-test to compare 2 groups. To compare categorical data, the Chi-square (χ^2) test was performed. An exact test was used instead when the expected frequency is less than 5. *P* values of less than 0.05 were considered statistically significant. All statistical calculations were made using SPSS (Statistics Package for Social Sciences; SPSS Inc., Chicago, IL, USA) version 15 for Microsoft Windows.

Results

One hundred and fifty patients have been enrolled in this case-control study divided into two groups: group I with DR which is further divided into two subgroups with DME and non-DME, and group II as a control group. The demographic and laboratory data for both groups are shown in Table 1.

The results showed that the age of onset of diabetes was statistically significantly lower in group I than in group II ($P < 0.001$). As far as a treatment modality is concerned, group II showed a statistically significant increase in the use of insulin injections compared to group 2 ($P < 0.001$).

The results also demonstrated that there was a statistically significant increase in the polymorphic VV genotype among patients with DME compared to those without DME (*P* 0.018). The frequency distribution of different genotypes in both DME and non-DME groups is shown in Table 2 and illustrated in Fig. 1.

Table 1 Comparison of demographic and laboratory data of diabetic with DR group and diabetic without DR group

Index	Group 1 (diabetic with DR) (*n* = 100)	Group II (diabetic without DR) (*n* = 50)	*P* value
Sex			
Male, *n* (%)	6/100 (46%)	19/50 (38%)	0.351
Female, *n* (%)	54/100 (54%)	31/50 (62%)	
DM duration (years)*	18 ± 6.8	14 ± 5.3	0.000
Age of onset of diabetes (years)*	40 ± 10.5	46.7 ± 8	0.000
Blood pressure state			
Hypertensive	55/100 (55%)	35/50 (70%)	0.077
Normotensive	45/100(45%)	15/50 (30%)	
Treatment modality			
Insulin therapy	77/100 (77%)	20/50 (40%)	0.000
Oral hypoglycemic therapy	23/100 (23%)	20/50 (60%)	
HbA1c (%)*	8.7 ± 1.6	8.55 ± 2	0.561
FBS (mg/dl)*	196 ± 81	184 ± 87	0.394

Data are presented as number and percentage or * mean and SD, *P* value < 0.05 is significant
FBS fasting blood sugar

Table 2 Frequency distribution of different genotypes and alleles in DME and non-DME groups

Mn-SOD genotypes and alleles	Patients with DME, *N* 53	Patients without DME, *N* 47	*P* value
AA (wild)	8 (15.1)	12 (25.5%)	0.018
AV (heterozygous)	22 (41.5%)	25 (53.2%)	
VV (homo-mutant)	23 (43.4%)	10. (21.3%)	
Wild allele (A)	50 (51%)	37 (37.8%)	0.062
Mutant allele (V)	48 (49%)	61 (62.2%)	

Data are presented as number and percentage, *P* value < 0.05 is significant
DME diabetic macular edema

The frequency distribution of different genotypes and alleles in both DR and non-DR patients had no significant difference between both groups concerning to the different distribution of genotypes (*P* 0.3) or the distribution of alleles (*P* 0.120, OR 1.465, 95th CI 0.9–2.3) (Figs. 2 and 3).

The demographic, clinical, and laboratory data for the homozygous mutant, heterozygous, and wild genotype groups in the DR group are shown in Table 3 with no statistically significant differences in all groups for each variant.

Discussion

Diabetic retinopathy and diabetic macular edema are the results of chronic damage to retinal neurovascular structures affected by the duration of diabetes [19]. The pathophysiology of retinal damage remains uncertain but includes antioxidant imbalances and metabolic and neuroinflammatory insults [20]. Genetic variation in antioxidant genes such as *Mn-SOD* leads to defect in the enzymatic activity and alteration of ROS detoxification, which may increase the risk of disease [1].

In the meantime, the results of this study showed a significant association between A16V polymorphism and the development of diabetic macular edema DME among diabetic patients with DR; the VV genotype was significantly higher in patients with DME than those without DME (*P* 0.018). This indicates that A16V polymorphism is a susceptible genetic factor for DME [21]. This finding is consistent with Zhang, J. et al. [22] who, meanwhile, revealed the association of A16V polymorphism with DME in diabetic patients; they found that the A-allele frequency among DME patients was significantly lower than that of non-DME patients ($P < 0.05$) [22].

One possible explanation was that *A16V* polymorphism may be related to alteration of vascular permeability that causes DME rather than vascular occlusion related to DR, and A allele may protect against DME, and gene polymorphism was a susceptible genetic factor for DME [23]. The retina in particular is susceptible to oxidative stress due to its high proportion of polyunsaturated fatty acids, high consumption of oxygen, and exposure to visible light [1].

The results of the current study showed that there was no statistically significant difference between diabetic patients with retinopathy and diabetic without retinopathy for the different genotypes or allele distribution as regards *Mn-SOD A16V* polymorphism. (AA was 20% versus 28%, AV was 47% versus 50%, and VV was 33% versus 22%) The results also showed no significant difference for the wild A allele (47.6% versus 36.5%) and mutant V allele (52.4% versus 63.5%) for peripheral DR and non-peripheral DR groups respectively (*P* 0.125, OR 0.5, 95th CI 0.35–1.14). This finding was in agreement with another study, which previously revealed that *A16V* polymorphism in *Mn-SOD* was not associated with DR

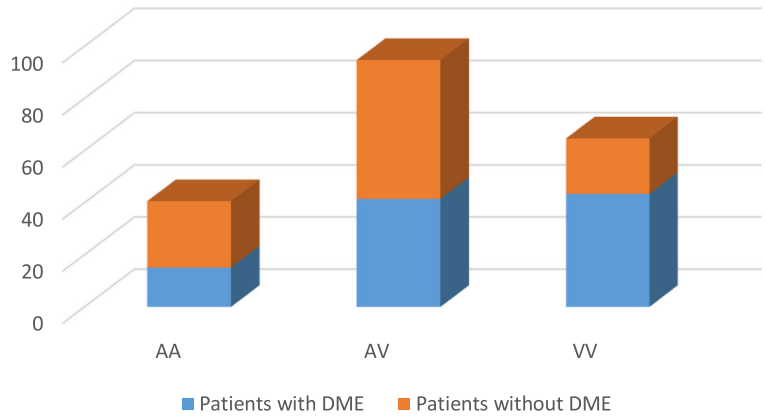

Fig. 1 Frequency distribution of different genotypes in DME and non-DME groups

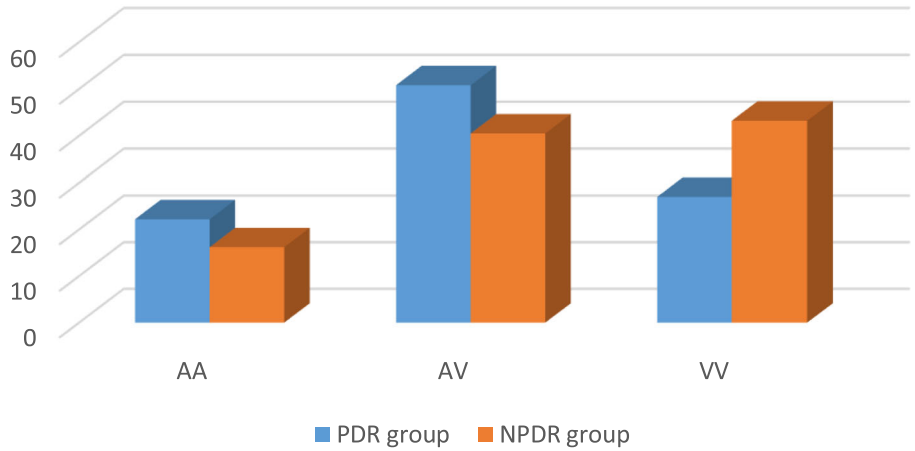

Fig. 2 Frequency distribution of different genotypes and alleles in peripheral DR and non-peripheral DR groups

but with a higher frequency of AA genotype in DR group (22.6% in cases versus 19.3% in controls); also, the allele frequency was not statistically significantly different between the cases and control groups (*P* 0.235) [24].

In contrast to the result of this study, Petrovic et al. revealed a significant association of *Mn SOD* polymorphism and DR as the VV polymorphic genotype was higher among the DR cases than in the control group 28.3% versus 16.1% respectively (*P* 0.006, OR 2.1, 95th CI1.2–3.4) [25]. Also Ye et al.'s study showed that the frequencies of VV genotype and V allele were significantly higher in the DR group than that in the diabetic without retinopathy group (*P* 0.001). Their study showed that the V allele was associated with a 1.96-fold higher risk of development of DR (OR 1.96, 95th CI 1.29–2.97) [26].

In contrast to the hypothesis that the V allele could be a high-risk allele, Tian et al. in their study found a higher frequency of AA genotypes (*P* 0.03) and A allele (*P* 0.04) in the DR group (type 1 or type 2) compared to diabetics without retinopathy in the DR group [27].

The discrepancy between the results of this study and the previous results could be explained by the heterogeneity of the genetic background among populations, which supports the need for replication studies among all ethnic groups, particularly those with a high degree of heterogeneity. There was also a lack of standardization of the DR phenotype in different studies (i.e., studies did not show that the cases included were either peripheral DR-only or peripheral DR-type and non-peripheral DR-type; mild or severe form and, concerning for the control group, some studies enrolling diabetics without retinopathy, while others enrolling those with mild retinopathy background).

There is a possibility of interaction between *Mn-SOD A16V* polymorphism, and other environmental factors not

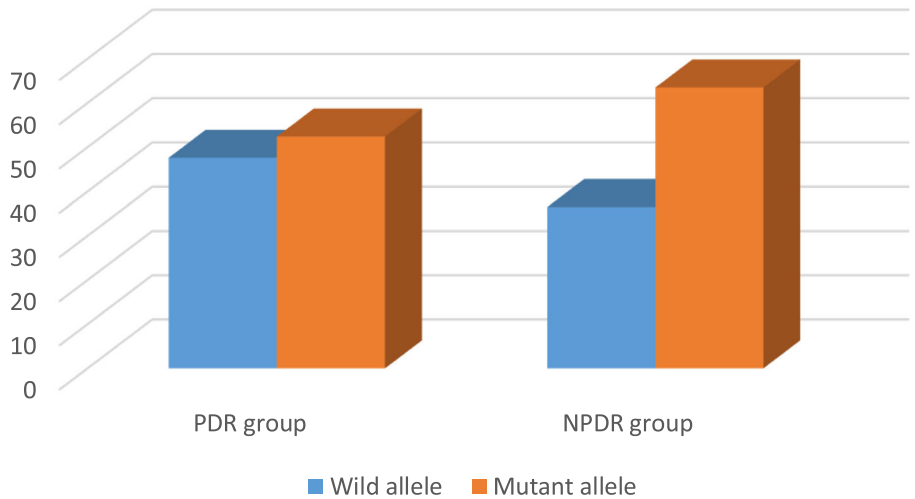

Fig. 3 Frequency distribution of different alleles in peripheral DR and non-peripheral DR groups

Table 3 The demographic, clinical, and laboratory data of the homozygous mutant, heterozygous, and wild genotype groups among the DR group

	(Homozygous wild AA) *N* = 20 (mean ± SD)	(Heterozygous AV) *N* = 47 (mean ± SD)	(Homozygous mutant VV) *N* = 33 (mean ± SD)	*P* value
Age*	57.8 ± 9.2	56.8 ± 9.2	60.1 ± 8.8	0.278
Sex				
Male *n* (%)	8 (40%)	21 (44.7%)	17 (51.5%)	0.695
Female *n* (%)	12 (60%)	26 (55.3%)	16 (48.5%)	
DM duration*	15.8 ± 7.1	18.4 ± 6.4	18.8 ± 7.1	0.254
Age of onset of diabetes*	42 ± 11.3	38.43 ± 10.2	41.33 ± 10.4	0.320
Blood pressure state				
Hypertensive	13 (65.0%)	21 (44.7%)	21 (63.6%)	0.148
Normotensive	7 (35.0%)	26 (55.3%)	12 (36.4%)	
Treatment				
Insulin *n* (%) oral	16 (80.0%)	39 (83.0%)	22 (66.7%)	0.219
Hypoglycemic *n* (%)	4 (20.0%)	8 (17.0%)	11 (33.3%)	
HbA1c (%)*	8.1 ± 1	8.8 ± 1.7	8.9 ± 1.7	0.199
+ ve family history of DR	4 (14.8%)	16 (59.3%)	7 (25.9%)	0.326
History of argon laser	16 (21.9%)	37 (50.7%)	20 (27.4%)	0.146
History of vitrectomy	6 (18.8%)	17 (53.1%)	9 (28.1%)	0.687

Data are presented as number and percentage or * mean and SD, *P* value < 0.05 is significant

taken into account in this study, such as glucose level, smoking status, and dietary and plasma antioxidant capacity. Results of some studies have shown that this antioxidant status can have an oxidative stress effect in some patients, so the antioxidant status associated with diabetes and related diseases still needs to be clarified [28].

Conclusion

Mn-SOD A16V polymorphism itself may not be associated with DR; there may be mutations in other genes that may alter the formation of ROS as advanced glycation end products through their receptors and therefore influence the production of growth factors and cytokines that could be the cause of DR.

Further studies with a larger sample size from different populations are therefore needed to better understand the genes associated with diabetic retinopathy. In this way, biochemical mechanisms for the disease can be identified that help to develop new tools for the identification of patients at risk.

Abbreviations

DM: Diabetes mellitus; DME: Diabetic macular edema; DR: Diabetic retinopathy; EDTA: Ethylenediaminetetraacetate; Mn-SOD: Manganese superoxide dismutase; mRNA: Messenger RNA; MTS: Mitochondrial targeting sequence; NPDR: Non-proliferative diabetic retinopathy; PDR: Proliferative diabetic retinopathy; ROS: Reactive oxygen species; SDS: Sequence Detection System; SNP: Single nucleotide polymorphism; TINI: Turbidimetric inhibition immunoassay

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Authors' contributions

DMA was responsible for manuscript writing. HAM was responsible for study design and protocol of practical work. NSM was responsible for patients' collection according to inclusion criteria. ME was responsible for samples' collection and preparation and follow-up of the practical work. SAE was responsible for doing the practical work. HAM and SME were responsible for revising the practical work, data analysis, and revising manuscript writing. All authors have read and approved the manuscript.

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Availability of data and materials

Available upon request

Ethics approval and consent to participate

All procedures performed in the study were under the ethical standards of Faculty of Medicine, Kasr Al-Ainy School of Medicine and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards [E. C. N-18- 2018], and a written informed consent was obtained from each participant after a full explanation of the study protocol.

Consent for publication

Not applicable

Competing interests

The authors declare no competing interests.

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