Original Article

Association of manganese superoxide dismutase Ala16Val polymorphism in the incidence of acute myocardial infarction in the Egyptians

Lobna M. Abdelrauf [a], Mohamed F. Abdel Rahman [b], Sahar M. Abdel-Maksoud [a], Nabil M. Farag [c], Ingy M. Hashad [a],*

[a] Clinical Biochemistry Unit, Faculty of Pharmacy and Biotechnology, The German University in Cairo, Egypt
[b] Department of Biochemistry, Faculty of Pharmacy, October University for Modern Sciences and Arts, Giza, Egypt
[c] Department of Cardiology, Faculty of Medicine, Ain Shams University, Cairo, Egypt

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ABSTRACT

Background: Oxidative stress has been implicated in various diseases including atherosclerosis; the most common pathologic process underlying acute myocardial infarction (AMI). The manganese superoxide dismutase (MnSOD) antioxidant enzyme affords the major defense against reactive oxygen species (ROS) within the mitochondria. MnSOD Alanine16Valine (A16V) single nucleotide polymorphism (SNP) has been shown to decrease MnSOD detoxification activity. **Aim:** A case-control study was conducted to investigate the association between MnSOD A16V polymorphism and the incidence of AMI in the Egyptians, investigate the contribution of oxidative stress represented by hexanoyl lysine adduct (HEL), an oxidative stress biomarker, in the pathogenesis of AMI and finally correlate the MnSOD genotypes with HEL serum levels. **Methods:** A total of 200 Egyptian subjects were recruited for the study; 100 AMI patients and 100 control subjects. Genotypes of the MnSOD A16V polymorphism were determined using a polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). Serum HEL was measured by ELISA. **Results:** A significant difference in the distribution of the MnSOD A16V genotypes was observed; VV genotype was significantly higher in AMI than controls ($p \leq 0.0001$). Also, studying the allele frequencies revealed that Val allele was significantly higher in AMI than controls ($p \leq 0.0001$). Serum analysis showed higher levels of HEL in AMI patients ($p = 0.0142$). Furthermore, HEL levels were found to be significantly higher in VV genotype in AMI ($p = 0.0273$). **Conclusions:** Our study suggests that MnSOD A16V polymorphism is associated with increased risk of developing AMI in the Egyptians. Moreover, the VV genotype is associated with higher HEL levels.

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1. Introduction

Oxidative stress is linked to atherosclerosis. Reactive oxygen species (ROS) disturb the function of vascular wall cells. ROS also stimulate lipid peroxidation. In addition, oxidized low-density lipoprotein (oxLDL) promotes atherogenesis and destabilizes plaque via several pathways. ROS are involved in inflammatory cell recruitment as well [1,2].

MnSOD is an endogenous antioxidant enzyme synthesized in the cytosol and is post-transcriptionally transported into mitochondria, which protects cells from oxidative damage by catalyzing dismutation of superoxide radicals, producing hydrogen peroxide and molecular oxygen [3].

Several SNPs in *sod2* gene, MnSOD encoding gene, have been described. Ala16Val SNP has been demonstrated to have a functional significance which arises from the substitution of cytosine (C) by thymine (T) resulting in the substitution of an alanine (GCT) for a valine (GTT) at the 16th residue of the signal peptide in the mitochondrial-targeting sequence [4].

This substitution is thought to alter the conformational structure of the mitochondrial targeting sequence of MnSOD affecting

its mitochondrial import and thus its efficacy in fighting oxidative damage [5].

Hexanoyl lysine adduct (HEL) is a lipid hydroperoxide-protein adduct, which is formed upon the oxidative modification of omega-6 (ω-6) fatty acids such as linoleic acid and arachidonic acid and their interaction with lysine residue in proteins. HEL moiety has been reported to exist in oxLDL and human atherosclerotic lesions. HEL is considered a novel lipid peroxidation biomarker [6,7].

Several studies tried to link between MnSOD polymorphism and oxidative stress-related disorders including atherosclerosis. However, results were somehow controversial. The aim of the current study was to investigate MnSOD Ala16Val polymorphism and its association with the incidence of AMI in the Egyptian population and to correlate between genotypes and the serum levels of HEL.

2. Subjects and Methods

2.1. Subjects

200 subjects were involved in this study. They were 100 AMI patients (55 males with age range 44–60 years and 45 females with age range 45–60 years) recruited from the intensive care unit of three different hospitals; the National Heart Institute, Imbaba, Giza, El Demerdash Hospital, Ain Shams, Cairo and Egypt Air Hospital, Almazah, Cairo, Egypt. Patients were included in the study if they were diagnosed with AMI by clinical presentation, ECG changes and/or elevated biochemical markers. Exclusion criteria for AMI patients included age above 60 years, any concomitant acute or chronic severe diseases such as diabetes, renal failure, hepatic insufficiency, cardiovascular disease other than MI and smoking.

Control group consisted of 100 healthy unrelated subjects recruited for the study from volunteers attending the blood bank at Children Cancer Hospital (57357), Cairo, Egypt. They comprised of 60 males (age range 42–58 years) and 40 females (age range 41–56 years). Exclusion criteria for control subjects included age above 60 years, chronic diseases (diabetes, hypertension, renal failure, hepatic insufficiency or cardiovascular diseases) and smoking. The study was conducted after taking the approval of the ethical committees of the German University in Cairo and the three participating hospitals. In addition, an informed written consent was obtained from all participating subjects.

The sample size of the study was calculated for a matched case control study with a power of 80%, ratio of cases to controls 1:1; exposure in controls 50%; expected odds ratio of 2.7 and alpha error of 5%.

2.2. Methods

2.2.1. Blood sampling and DNA purification

Five mls of blood were drawn from each participant and divided on two different vacutainers; one containing EDTA K3 and a second plain vacutainer for whole blood and serum collection, respectively. DNA was extracted and purified from blood leukocytes using Thermo Scientific DNA extraction kit (Lithuania, EU). Serum was separated and kept stored at −80 °C till analysis.

2.2.2. Polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) assay

Primers suitable for amplification of the gene region containing A16V polymorphism were used; forward primer (5′ GCTGTGCTTTCTCGTCTTCAG 3′) and reverse primer (5′ TGGTACTTCTCCTCGGTGACG 3′). The substitution of A with V at

16th amino acid genetic code creates a recognition site for the restriction enzyme. PCR was performed to amplify the gene region containing the target SNP, using 2 μL of each primer (supplied by Invitrogen, Thermo Fisher Scientific, Inc.), 1 μL DMSO, 12 μL of the purified DNA (50 ng), 8 μL nuclease free water and 25 μL master mix (Thermo Scientific, Lithuania, EU) to have a final reaction volume of 50 μL. The prepared PCR mixture was placed in thermocycler (Biometra T) were the following program was run: predenaturation step 1 for 5 min at 94 °C. Then three steps repeated for 38 cycles; denaturation for 30 s at 94 °C, annealing for 30 s at 60 °C and extension for 30 s at 72 °C. This was followed by a final extension step for 10 min at 72 °C. The produced PCR product was 207 bp. Restriction enzyme *Bsaw* I (New England biolabs, 5000 units/ml) was used to digest the PCR product to determine the genotype. 1 μL of *Bsaw* I was added to 4 μL buffer and 15 μL PCR product. The final volume was adjusted to 30 μL using 10 μL nuclease free water. The restriction product was incubated for 1 h at 37 °C. The product was loaded to a 4% agarose gel for analysis. *Bsaw*I restriction enzyme digested the PCR product into two fragments 167 bp and 40 bp if T allele is present. While in case of C allele DNA was kept undigested as shown in Fig. 1.

#### 2.2.3. Lipid profile for participating subjects

Serum was used to measure triglycerides (TG) and total cholesterol (TC) by an enzymatic colorimetric method using kits provided by Diamond diagnostics, Egypt.

2.2.4. Measurement of serum HEL levels

Serum levels of HEL were measured using a commercially available ELISA kit provided by JaICA, Japan.

3. Statistical analysis

Statistical analysis was performed using the statistical program GraphPad prism. Data are represented as mean ± SEM. To compare differences between groups, odds ratio, nonparametric student *t*-test (Mann-Whitney) and nonparametric one-way ANOVA
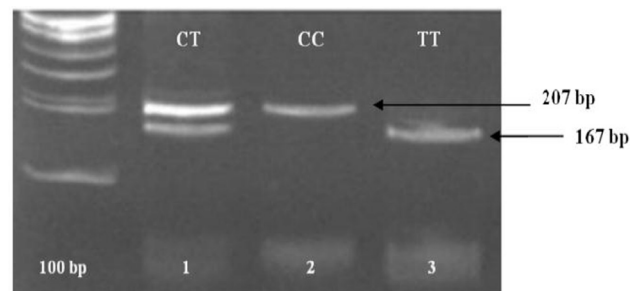
Fig. 1. Representative 4% agarose gel electrophoresis of *Bsaw* I restriction digestion of MnSOD A16V SNP in AMI subjects. Lane 1 is an example of CT genotype, lane 2 is CC and lane 3 is TT.

Table 1
General characteristics of participants.

	AMI	Control
Number (M/F)	100 (55/45)	100 (60/40)
Age range	Males: 44–60	Males: 42–58
	Females: 45–60	Females: 41–56
TC	217.7 ± 8.28[*]	184.4 ± 4.22
TG	152.5 ± 7.82[***]	120.3 ± 6.37

Values are expressed as mean±SEM, TC; Total cholesterol, TG; Triglycerides.
[*] Significant difference at $p = 0.0205$.
[***] Highly significant difference at $p = 0.0006$.

Table 2
Genotype distribution of MnSOD A16V in AMI patients and controls.

Genotypes Distribution (%)	AMI	Control
• AA genotype	20%	37%
• AV genotype	31%	42%
• VV genotype	49%	21%

(Kruskal-Wallis) were used. In all statistical tests two-tailed p value ≤0.05 was considered statistically significant. Data were also tested for fitting in Hardy-Weinberg equilibrium (HWE), *p* value higher than 0.05 was counted as a compatible result.

4. Results

4.1. General characteristics of patients and controls

General characteristics of patients and controls are displayed in Table 1.

4.2. Association between MnSOD A16V polymorphism and AMI in Egyptians

Genotypes of controls were not deviated from Hardy-Weinberg equilibrium (*p* = 0.168). The genotype distribution pattern was significantly different between study groups (Table 2). It is noticed that the VV genotype was significantly higher in AMI than controls ($p \leq 0.0001$).

Furthermore, studying the odds ratio (OR) between AMI and control subjects showed 2.5 folds higher risk for individuals carrying Val allele compared to those carrying the Ala allele. The dominant model of genotypes showed 2.3 folds higher risk among AV +VV genotypes compared to AA genotype while the recessive model showed that homozygous VV individuals are at 3.6 folds higher risk than individuals having other genotypes (AA+AV) (Table 3).
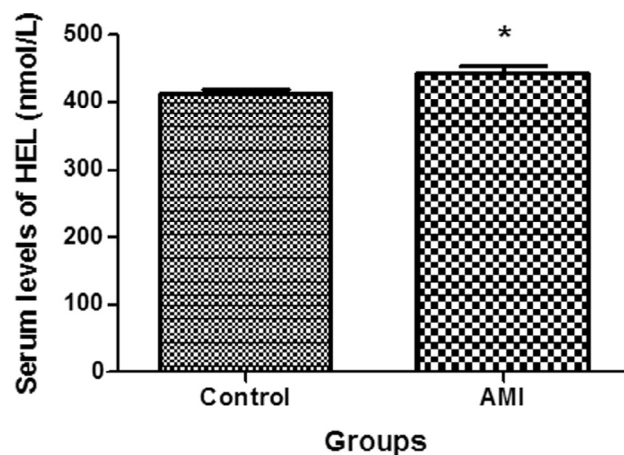
4.3. HEL levels in AMI and controls

AMI group showed a significant increase in serum HEL levels when compared to control (*p* = 0.0142) (Fig. 2).

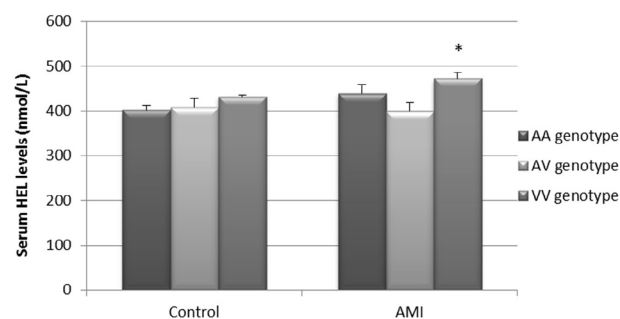### 4.4. Correlation between MnSOD A16V polymorphisms and HEL serum levels

Association of MnSOD genotypes with HEL levels was illustrated in Fig. 3. For the control group, there was no significant difference in serum HEL serum levels among the different MnSOD genotypes. While Serum HEL concentrations are significantly different among the various MnSOD genotypes in AMI patients; where HEL concentration is highest in VV genotype compared with AV and AA genotypes (*p* = 0.0273).

Fig. 2. Serum levels of HEL in AMI and controls. Results are expressed as mean ± SEM. *Significant difference at *p* = 0.0142. Serum levels of HEL were significantly higher in AMI than control (*p* = 0.0142).

Fig. 3. Correlation between HEL serum levels and MnSOD genotypes in study groups. In AMI patients, HEL concentration is highest in VV genotype compared to other genotypes (*p* = 0.0273). For the control group, there was no significant difference in HEL serum levels among the different MnSOD genotypes. While in AMI patients, HEL serum levels were significantly higher in VV genotype compared to AA and AV genotypes (p = 0.0273).

5. Discussion

Several studies had discussed the association of MnSOD A16V polymorphism with CAD in different populations; however, results were not consistent.

Our current study results showed that the Val allele and the VV genotype are associated with the incidence of AMI in Egyptians. These results are consistent with a study conducted on Japanese and linked Val allele to the incidence of AMI [8]. Same results were obtained from studies on Italian, Chinese, Caucasian and Danish populations [9–12].

In contrast to all the above studies, studies on Slovene showed no correlation between the A16V polymorphism of MnSOD gene and the risk of MI [13].

Table 3
Odds ratio (OR) and 95% confidence intervals (CI) for MnSOD genotype distribution and allele frequencies in study groups.

A16V		AMI patients (n = 100)	Control subjects (n = 100)	OR (95% CI)	*p*-value
Genotypes					
Dominant Model	AV+VV	80	63	2.349 (1.243–4.440)	*p* = 0.0077
	AA	20	37		
Recessive Model	VV	49	21	3.614 (1.943–6.725)	*p* < 0.0001
	AV+AA	51	79		
Alleles					
Val allele		129	84	2.509 (1.676–3.756)	*p* < 0.0001
Ala allele		71	116		

Several studies have suggested that the MnSOD A16V polymorphism is associated with various oxidative stress-dependent pathologies. Disease risk is linked to the Val allele or VV genotype in diabetes microvascular complications, obesity, hypercholesterolemia and metastatic potential of breast cancer [14–17]. Interestingly, in few studies, the disease risk was associated with the Ala allele or AA genotype for example, in prostate cancer, breast cancer and other cancers [18,19].

This finding was explained by the disruption of the protein by the change of alanine to valine. This α-helix structure is important for the translocation of the enzyme to the mitochondrial matrix where it exerts its function. Disruption by valine causes the protein to be retained at the level of the mitochondrial inner membrane and has been associated with increased susceptibility to oxidative stress. Therefore, Val allele was thought to be a risk factor for atherosclerosis [9,20].

Regarding HEL, the current study showed a significantly higher serum levels in AMI patients compared to controls ($p = 0.00142$). Several studies showed similar results suggesting that HEL serum levels increase in various oxidative stress dependent pathologies [7,21–23].

It is proposed that oxidized lipid components of oxLDL may play a key role in the atherogenic process by inducing the transcription of inflammatory genes in vascular smooth muscle cells and augmenting the recruitment as well as the retention of monocytes in the subendothelial space [24].

Studying the association of HEL levels with genotype distribution of MnSOD A16V SNP showed a significant increase in the serum levels of HEL in the VV genotype when compared to the AV and AA genotypes in the AMI patients ($p = 0.0273$). This confirms the findings that suggest that the Val allele results in decreased formation of active MnSOD within the mitochondrial matrix, leaving mitochondria inadequately protected against superoxide radicals thus increasing the risk of AMI [25].

Hence, we conclude that A16V polymorphism is associated with increased risk of developing AMI in Egyptians. The study also showed that the VV genotype of AMI patients was accompanied with higher HEL levels.

Acknowledgment

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