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Original Article

Polymorphism variant of *MnSOD* A16V and risk of female infertility in northern Iran

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ABSTRACT

Objective: Infertility is a disease of the reproductive system defined by inability to conceive after having regular unprotected intercourse. Both environmental and genetic factors can be involved in female infertility. Manganese superoxide dismutase (MnSOD) is a crucial mitochondrial antioxidant enzyme that has a key role in cellular defense against agents that induce oxidative stress. The present study was aimed to evaluate the *MnSOD* A16V gene polymorphism in female infertility in northern Iran.
Materials and methods: Samples were obtained from 150 patients diagnosed with female infertility and 150 controls and genotyped by the polymerase chain reaction-restriction fragment length polymorphism method.
Results: The *MnSOD* genotype frequencies amongst the 150 cases were A/A = 27.3%, A/V = 69.4%, and V/V = 3.3%; the A and V allele frequencies were 62% and 38%, respectively. The *MnSOD* genotype frequencies amongst the 150 controls were A/A = 33.3%, A/V = 48.0%, and V/V = 18.7%; the A and V allele were 57% and 43%, respectively. We observed a significant difference in genotype distributions of *MnSOD* A16V polymorphism between patients and controls ($p = 0.0001$).
Conclusion: It is suggested that the *MnSOD* A16V polymorphism may be associated with a risk of female infertility in northern Iran. More studies should be considered with a larger number of patients and controls to confirm our results.

Introduction

Infertility is defined as the lack of conception in a couple after 1 year of unprotected intercourse, and it is a major health problem that can impact the quality of life, especially in women [1]. Of all infertile couples, 40–50% are attributed to female infertility, whereas in 30–40% of infertile couples, male infertility is the cause, and the remaining 10–30% is attributed to both male and female infertility [2]. In Iran, approximately 20% of couples experience an episode of infertility during their reproductive lives. It has been shown that both environmental and genetic factors can be involved in infertility [1]. Genetic factors play a crucial role in female infertility. Therefore, there is an expanding interest in the role of genetic factors like manganese superoxide dismutase (*MnSOD*) polymorphic variants in female infertility [3].

Endogenous antioxidant enzymes like catalase, glutathione peroxidase (Gpx1), peroxiredoxins, and MnSOD play an important role in the cell defense against reactive oxygen species (ROS) [4]. MnSOD is an essential enzyme in cell defense against mitochondrial ROS that converts ROS to hydrogen peroxide (H_2O_2), then catalase and Gpx1 neutralizes H_2O_2 to O_2 and H_2O [5].

The MnSOD gene is located on chromosome 6q25.3 and includes five exons and four introns [6]. The most extensively studied polymorphism of *MnSOD* is A16V (C47T) single nucleotide polymorphism (SNP) in codon 16 of 24-amino acid mitochondrial targeting sequence (MTS domain) that changes (C) alanine to (T) valine amino acid [7]. It causes a conformational change of the secondary structure from an α-helix to a β-sheet [8]. The import of alanine protein (A form) is

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30–40% more efficient in comparison to the valine protein (V form), and it causes overproduction of ROS [9]. In women, additional generation of ROS causes cellular ovarian injury and leads to apoptosis. Hence, through a disruption of endogenous antioxidant enzymes, this may contribute to the defects in oocyte maturation, fertilization, and embryo development [3].

The aim of the present study was to determine whether *MnSOD* A16V polymorphism is associated with female infertility in northern Iran.

Materials and methods

Participants

The current study included a total of 150 women with infertility and 150 women with at least one child. Controls and patients were selected from the same population living in the Guilan province, in the north of Iran, who were recruited between 2014 and 2015. Data on patient characteristics at the study entry for each individual were collected from the Mehr Infertility Remedial Centre in Rasht, Iran. In the present study, we tried to select women in the 28–40 year age range, because strong evidence suggests that couples who are trying to get pregnant become less fertile as they get older. The control group was comprised of unrelated healthy individuals matched in age with the patients. Patients had an infertility history of at least 2 years with their consorts with confirmed normal urological assessment. Women with unexplained infertility were enrolled in this study. Infertile patients of known cause, such as hormonal, structural, immunological, and coagulation abnormalities, were excluded.

Each participant donated 2 mL blood drawn into EDTA-coated tubes (Venoject, Terumo, Leuven, Belgium), which was used for genomic DNA extraction. This project was approved by the local licensing committee, and informed consent was obtained from all individuals and was performed according to the Helsinki Declaration of 1975, as revised in 1983.

Genotyping

Genomic DNA was extracted from whole blood samples using the Gpp solution kit (Dana Gene Pajouh, Tehran, Iran) according to the recommended protocol. DNA purity and concentration were determined by a spectrophotometer at 260 nm and 280 nm. Each DNA sample was stored in TE buffer (5 μM Tris, HCl, 0.1 μM EDTA, pH 8.5) at −20°C until analysis. Polymorphism spanning fragments were amplified by the polymerase chain reaction (PCR) and performed using PCR-restriction fragment length polymorphism (PCR-RFLP). The region of *MnSOD* including the A16V variation was amplified using primers: (F: 5′ CGGGCTGTGCTTTCTCGTC 3′ and R: 5′ TCAGC CTGGAAC CTACC CTT- 3′). PCR products were subsequently digested with restriction enzyme *BsawI*. The PCR conditions were as follows: initial denaturation at 95°C for 5 minutes, 35 cycles of denaturation at 95°C for 45 seconds, annealing at 55°C for 40 seconds, with a final step at 72°C for 5 minutes to allow a complete extension of PCR fragment. Enzyme digestion products were separated on 2% agarose gel electrophoresis and visualized by ethidium bromide staining. Genotype results were regularly confirmed by randomly selecting 20% of the samples that were regenotyped by another laboratory member to improve the quality of genotyping and its validity, and no discrepancy in genotyping was found.

Statistical analysis

Data management and analysis were performed using MedCalc software (version 12.1, MedCalc, Mariakerke, Belgium). Genotype frequencies between cases and controls were compared by the χ^2 test. To estimate the association between the *MnSOD* A16V variant and the risk of female infertility, odds ratios (OR) and 95% confidence intervals (95% CI) were calculated by logistic regression and differences in demographic variables [including smoking history, family history of infertility, and body mass index (BMI)] between patients and controls were compared by the χ^2 test. The association between the *MnSOD* A16V variant and risk of female infertility was investigated by treating the three genotypes (major allele homozygous, heterozygous, and variant allele homozygous) as ordinal variables in the analysis. The homozygosity with the more frequent allele among controls was set as the references group. A value of $p < 0.05$ was considered statistically significant.

Results

In the present study, 300 participants, including 150 female infertile patients and 150 infertility-free controls were assessed. The mean age of the patients was 36.2 ± 2.1 years and the mean age of the controls was 34.5 ± 2.3 years. No significant difference in age was seen between infertile patients and controls ($p > 0.05$). Genotyping of A16V was done by the PCR-RFLP method (Figure 1). The main characteristics of the patients are presented in Table 1. Analysis suggested that age, smoking status, family history of infertility, and BMI were not significantly different between cases and controls. The prevalence of genotype frequencies for AA, AV, and VV was 33.3%, 48.0%, and 18.7% in controls, and 27.3%, 69.4%, and 3.3% in patients, respectively. Significant differences were found in allele and genotype distributions of *MnSOD* A16V between infertility cases and controls ($p = 0.0001$); in the subgroup with AV genotypes, the results suggested that the association was more apparent among others ($p = 0.029$, OR = 1.76, 95% CI = 1.05–2.93), but not in allele frequencies ($p = 0.2$). All information about allele and genotype frequencies and associated ORs (95% CI) for cases and controls are summarized in Table 2.

Discussion

In this case–control study, we evaluated the role of *MnSOD* A16V polymorphism in 150 female infertile patients and 150 controls. Our results suggest that there is significant association in genotype distribution between cases and controls ($p = 0.0001$), and the individuals with AV genotypes were associated with increased risk of female infertility ($p = 0.029$, OR = 1.76, 95% CI = 1.05–2.93).

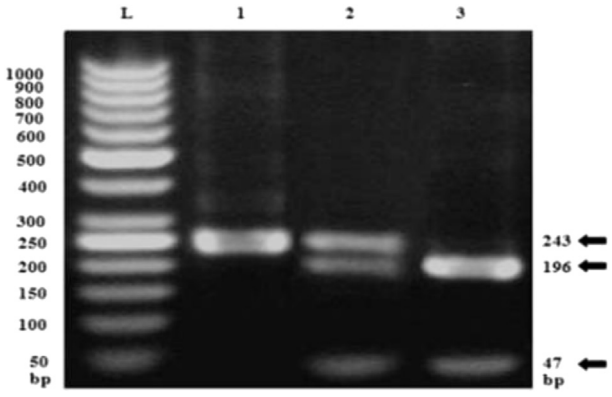

Figure 1. Restriction fragment length polymorphism (RFLP) analysis of manganese superoxide dismutase (*MnSOD*) A16V polymorphism. The 47 bp fragment had low clearness because of being small. L = DNA ladder; 1 = AA homozygote; 2 = AV heterozygote; 3 = VV homozygote.

Table 1
Characteristics of female infertility patients and controls enrolled in the study.

Variable	Controls ($n = 150$)	Cases ($n = 150$)	p
Age (y)	34.5 ± 2.3	36.2 ± 2.1	0.385
< 30	14 (9.3)	9 (6.0)	
≥ 30	136 (90.6)	141 (94.0)	
Smoking status			0.035
Never	90 (60.0)	75 (50.0)	
Former	19 (12.6)	36 (24.0)	
Current	41 (27.4)	39 (26.0)	
Family history of infertility			0.174
No	120 (80.0)	109 (72.6)	
Yes	30 (20.0)	41 (27.4)	
BMI (kg/m^2)	22 ± 3.6	24 ± 5.2	0.001

Data are presented as n (%) or mean ± standard deviation.
BMI = body mass index.

Table 2
Allele and genotype frequencies of manganese superoxide dismutase (*MnSOD*) A16V polymorphism among cases and controls and the associations with risk of female infertility.

	Controls ($n = 150$)	Patients ($n = 150$)			
	n (%)	n (%)	OR (95% CI)	p[a]	p[b]
Alleles					
A	172 (57.0)	186 (62.0)	1.00 (reference)	0.279	–
V	128 (43.0)	114 (38.0)	0.93 (0.66–1.30)		0.244
Genotypes					
AA	50 (33.3)	41 (27.3)	1.00 (reference)	0.0001	–
AV	72 (48.0)	104 (69.4)	1.76 (1.05–2.93)		0.029
VV	28 (18.7)	5 (3.3)	0.21 (0.07–0.61)		0.004

CI = confidence interval; OR = odds ratio.
[a] Allele and genotype frequencies in cases and controls were compared using χ^2 test.
[b] Significance level for allele and genotype frequencies in cases and controls.

Female infertility is one of the main social problems that impacts the quality of life in infertile women [1,2]. ROS can have an effect on reproductive function. In women, ROS overproduction may affect the ovarian physiology because it controls several functions like steroidogenesis, ovulation, follicular development, and luteolysis [3]. Hence, through a disruption of endogenous antioxidant enzymes, ROS may contribute to the defects in oocyte maturation, fertilization, and embryo development. Gpx1, peroxiredoxins, and MnSOD are three kinds of endogenous antioxidant enzymes in cell defense against mitochondrial ROS [10].

MnSOD is the essential enzyme in cell defense, and it is shown to be involved with male and female infertility [11]. The A16V SNP site in *MnSOD* gene is in the mitochondrial targeting sequence in which the A variant of the enzyme is 30–40% more active and has more efficient import into the mitochondrial matrix in comparison with the V variant [12]. The decrease of importing of MnSOD results in decrease in the enzyme concentration in the matrix and causes excessive ROS products [13]. Overproduction of ROS links hyperglycemia and increasing level of visceral fat [14]. The clinical characteristics of the infertile women in the current study showed a significantly higher BMI compared with the control group [3]. It is believed that there is a relationship between higher BMI and risk of female infertility. Obesity cannot be defined just by BMI, and several factors like percentage of fat mass and distribution should be considered [11]. The results of the present study are similar to those of Faure et al [3] who demonstrated an association between *MnSOD* A16V polymorphism and female infertility. To our knowledge, our study is the first genetic variation study on the association between *MnSOD* A16V polymorphism and the risk of female infertility in the Middle East.

Finally, some important limitations need to be considered. We only evaluated one SNP in the *MnSOD* gene, which was inadequate to assess female infertility risk for the gene studies. In addition, these data must be interpreted with caution, because our population that was studied was not large enough.

In conclusion, our results suggested that *MnSOD A16V* polymorphism may be associated with a risk of female infertility in northern Iran. However, more studies should be done with larger numbers of patients and controls to confirm our results.

Conflicts of interest

The authors declare no conflict of interest.

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