Uluslararası İleri Doğa Bilimleri ve Mühendislik Araştırmaları Dergisi
Sayı 8, S. 39-45, 8, 2024

Araştırma Makalesi

IJANSER

International Journal of Advanced Natural Sciences and Engineering Researches
Volume 8, pp. 39-45, 8, 2024

Research Article

https://as-proceeding.com/index.php/ijanser
ISSN: 2980-0811

Identification of a Common Mutation in the SOD Gene in a Sterile Patients in Iraq/Mosul

Mustafa Taha Al-Mashadani[1] and Owayes M. Hamed[1]

[1]*Biology dep. / College of Sciences Mosul University, Iraq.*

**e-mail : owsbio31@uomosul.edu.iq*

(Received: 03 September 2024, Accepted: 18 September 2024)

(3rd International Conference on Scientific and Innovative Studies ICSIS 2024, September 11-12, 2024)

ATIF/REFERENCE: Al-Mashadani, M. T. & Hamed, O. M. (2024). Identification of a Common Mutation in the SOD Gene in a Sterile Patients in Iraq/Mosul. *International Journal of Advanced Natural Sciences and Engineering Researches*, 8(8), 39-45.

Abstract- Infertility emerged as increased phenomenon around the world, and this became increased in Iraq due to environmental pollutants and lifestyle. This study aimed to assess the relationship between polymorphism in superoxide dismutase gene with infertility among Iraqi population in Mosul governate. The present study included 130 infertile men with 20 healthy subjects as a control, blood samples were collected from all persons under the study and DNA were extracted and subjected to conventional polymerase chain reaction followed by gel electrophoresis to show the polymorphisms in visualized bands. The results of the present study showed that there is polymorphism in SOD in multiple genotypes includes Val/Val, Val/Ala, and Ala/Ala, and the Val/Ala and Ala/Ala genotypes were at a highest percentage (36% for both) while Val/Val genotype was at 28%. This study concluded that there is a positive relationship between infertility and polymorphism in SOD gene.

Keywords: Sod, Genotypes, Infertility, Polymorphism, Iraq.

I. INTRODUCTION

Infertility is defined by the World Health Organization (WHO) as the inability of a couple to conceive following a year of consistent, unprotected sexual activity [1]. Male infertility, defined as a man's incapacity to conceive a female, affects about 7% of all males and accounts for 40–50% of human infertility cases [2].

Reactive oxygen species (ROS) show an important role in both function of sperm and fertilization. ROS physiologically and pathologically have role in fertility according to the previous studies, as the study of Yoon et al show that is the balance in the levels of ROS is vital for both physiological activity and antioxidants in order to avoid cellular oxidative injury that is essential in fertility, but high level of oxidative stress can lead to DNA damage, leading to infertility as well as recurrent pregnancy loss or genetic mutations that cause childhood diseases [3].

Purine and pyrimidine bases easily attacked by free radicals leading to damage of sperm DNA [4]. Damaging mechanisms by ROS may be either through single and double DNA breaks, cross-links, and chromosomal rearrangements (Lee et al., 2017), and in order to protect against this damage, about 85% of sperm genome is associated with the central nucleoprotamine [5].

Neutralization by such antioxidant defense system such as superoxide dismutase (SOD), and (NAD), and ((P)H dehydrogenase quinone (NQO). Since susceptibility to oxidative stress is determined in part by genetic background [6], there have been several studies investigating the association between functional genetic polymorphisms. In antioxidant pathways and risk of various diseases [7]. SOD Enzyme encoded by their own genes are widely involved in the cellular antioxidant response, GSH formation, and redox and methylation cycles during or involving spermatogenesis [8][9]. This gene is also on the ARE (antioxidant response element) motif in their inducible regions, facilitating the regulation of the NRF2 transcriptional pathway that activates oxidative stress [9]. This mechanism done by dissociation of the superoxide radical into ordinary molecular oxygen or hydrogen peroxide catalyzes by SOD [10]. In addition, there are several other roles of SOD, such as its correlation with sperm concentration and overall motility, while inversely correlated with sperm DNA fragmentation [11]. Variations in the superoxide dismutase genotype may potentially have an impact on reproductive outcomes. IVF conception rates and infertility are linked to the Ala16Val polymorphism in the SOD2 gene [12]. According to the case study, there was a markedly higher chance of male infertility in those who carried the Ala-MnSOD (rs4880) allele [13]. Superoxide dismutase activity was low in infertile males with SOD2 rs4880 CC variations [11]. According to studies by [11][14], the SOD2 mutation Val16Ala (rs4880) in Chinese people is linked to a markedly increased risk of male infertility as well as greater levels of sperm DNA fragmentation and lower levels of superoxide dismutase activity. Variants of antioxidant genes, PON1 Arg192Glu (rs662) and SOD2 Val16Ala (rs4880), have been linked to higher levels of sperm DNA fragmentation and a higher chance of male infertility [14]. Superoxide dismutase enzymes have been shown to potentially be relevant in mouse models. In spermatogenesis and testicular development [15]. Furthermore, low SOD1 enzyme levels under heat stress were shown to expedite the impairment of spasmogenic cells in mice; as a consequence, genetic disruption or functional polymorphism may cause both SOD1 and SOD2 to impair spermatogenesis [16].

This study aims to show the polymorphism in SOD in patients with infertility for first time in Mosul governate/Iraq.

II. MATERIALS AND METHODS

Case Study

This study included 150 Blood samples (130 infertility men, 20 healthy subjects).

Collection of Semen and Blood samples

Semen (5) ml of semen was withdrawn from all infertility subjects and divided into two Eppendorf tubes, the first one was used for physiological tests, and the second used for obtaining semen plasma and biochemical tests, in brief, semen centrifuged for 10 minutes at 3000 revolutions / min. Two ml of venous blood was collected in EDTA tubes and were used for DNA extraction.

DNA Extraction

Blood samples of patients and control in this study were used for DNA extraction according to the method used in the study of Iranpur and Esmailizadeh (2010) [17].

Determination of the purity and concentration of the extracted DNA

The concentration and purity of the DNA extracted from the study samples were estimated using the Bio drop device, and then the concentration of the extracted DNA was fixed at 25 ng/microliter.

Amplification-Refractory-Mutation-System (ARMS-PCR)

The concentration of DNA in the study samples was adjusted by dilution with a TE buffer solution to obtain the concentration required to perform the PCR reactions, and it was (25) ng/microliter for each sample. For each reaction, four primers (table 1) are added to the first and second initiator of the main band, the third primer of the normal allele, and the fourth primer of the mutant allele. The result is two or more packets that express the result of the interaction.

The master reaction mixture was prepared for each PCR reaction by mixing the DNA sample and the special primers (4 primers specially designed to determine the genetic variation of the gene in question, added together to a mixture for a reaction while determining the optimum temperature for the binding of the primers in the PCR reaction) for each mutation with the components of the Master -mix inside the 0.2-capacity Alpendorf tube supplied by the English company Biolaps. The reaction volume was fixed to 20 microliters with distilled water. The mixture was centrifuged in a Microfuge for 3-5 seconds to ensure that the reaction components were mixed. Then the reaction tubes were inserted into the PCR for the purpose of conducting the amplification reaction using the special program for each reaction (table 2), then the sample was loaded into the pits of the previously prepared agarose gel with a concentration of 2% with the addition of the volumetric guide DNA Ladder supplied by Biolaps Company in one of the pits, after that the samples are transferred by running the Electrophoresis device for a period of time. It ranges between (70-60) minutes, after which the gel is photographed using a UV trans-illumination device.

Determination of genetic variation of the SOD gene in situ (Val16Ala) using Mutiplex-ARMS-PCR technology.

The presence of the T⟶G mutation in situ (Ala-16-Val) was detected as 4 µl (100 nanogram) of template DNA and 1 µl (10 pmol) of each G20210A mutant primers supplied by the Korean company Macrogen were added to the contents of the master mix [18].

Table (1) shows the sequence of primers of the mutation of the SOD gene

Primer	Sequence
F- outer	5′-CACCAGCACTAGCAGCATGT-3
R- outer	5′-ACGCCTCCTGGTACTTCTCC-3
F- inner	5′-CCTGGAGCCCAGATACCCtAAAG-3
R- inner	5′-GCAGGCAGCTGGCTaCGGT-3

Then the reaction tubes were inserted into the thermocycler to conduct the amplification reaction using the special program for the reaction as shown in the table (2) [19].

Table (2): shows the program for the SOD gene mutation in loci (Ala-16-Val).

No.	Stage	Temperature	Time	Cycle number
1.	Initial denaturation	95	5 min.	1
2.	Denaturation	95	45 sec.	35
3.	Annealing	58	1 min.	
4.	Extension	72	1 min.	
5.	Final extension	72	7 min.	1
6.	Stop reaction	4	5 min.	1

Statistical analysis

Differences between groups were done using T-test in SPSS (V.23)

III. RESULTS AND DISCUSSION

The results, as shown in Figures (1) and (2) showed that there is a relationship between cases of male infertility and genetic mutations that affect the SOD gene in the Val16Ala locus. When observing Figure (2), it becomes clear that the result of the PCR reaction is 514, 366 and 189 bp, which indicates the appearance of genetic variation for the gene and for all genotypes Val/Val, Ala/Val, Ala/Ala and in different percentages as shown in the table (3).

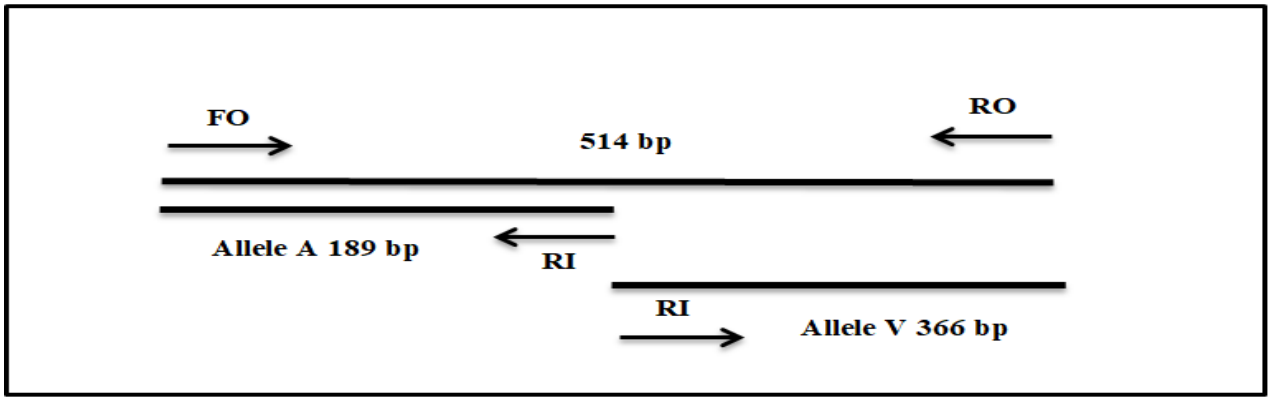

Figure (1): size of the bands generated by the ARMS-PCR reaction of the SOD gene. (FO=forward, RI=reverse)

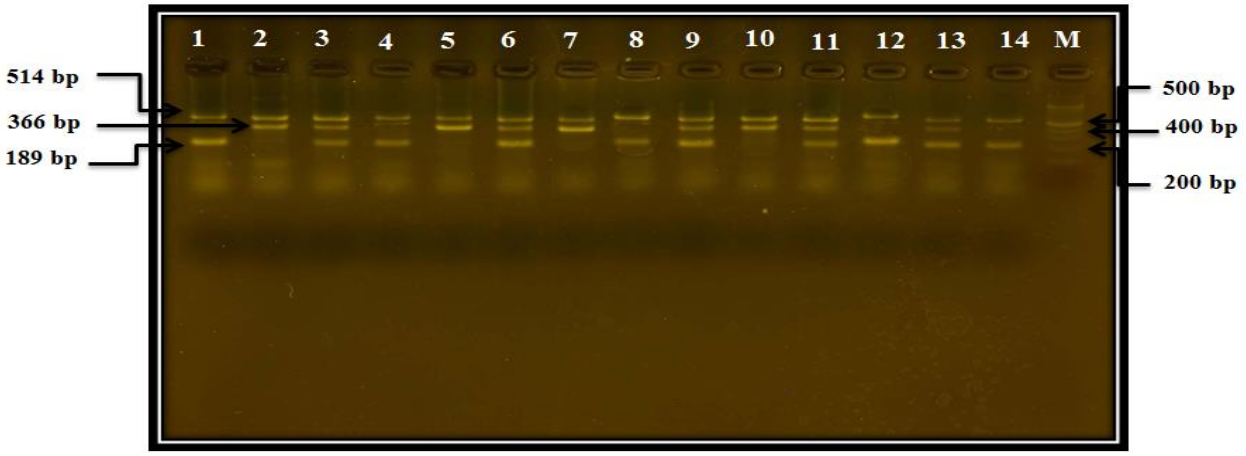

Figure (2): PCR product of SOD polymorphism. (M=ladder).

Figure (2): shows the result of the PCR reaction of the genetic variation of the SOD gene in Val16Ala that was migrated in a 2% agarose gel. As M represents the volumetric index and samples (2,5,7,10) represent samples carrying the healthy homozygous genotype ((Val/Val)) with the reaction product containing two bands of size 514 bp for the main gene and the band 366 bp represents the natural allele, while samples (3, 6,9,11,13) represent samples carrying the heterozygous genotype (Val/Ala) with the reaction product containing three bands of size 514 bp for the main gene and band 366 bp representing the normal allele and band 189 bp representing the mutant allele, while samples (1,4,8 12,14), so samples carrying the homozygous mutant (Ala/Ala) genotype represent the product of the PCR reaction, the product of the reaction contains two bands, the first with a size of 514 bp of the main gene and the band of 189 bp representing the mutant allele.

Table (3): distribution of the genotype and allelic level of SOD gene at the locus (Val16Ala) between the control group and the infertile men.

Genotypes	Patients		Control		P Value	OR	(95%Cl)
	NO.	%	NO.	%			
Val\Val	21	28	10	47	P = 0.0245	6.4286	1.2699 to 32.5421
Val\Ala	27	36	9	42			
Ala\Ala	27	36	2	11			
Alleles	NO.	%	NO.	%	P Value	OR	(95%Cl)
V	69	46	29	69	P = 0.0096	2.6187	1.2636 to 5.4273
A	81	54	13	31			

-V= normal allele, A=mutant allele.

Table (3) shows the level of allelic observation and the genotype of the SOD gene in the Val16Ala locus. The results of the study for the group of men suffering from undiagnosed infertility cases showed that the distribution of the mutated genotype (AA) was the highest (36%), while the percentage of The normal genotype (VV) was the lowest by (28%), while for the heterogeneous genotype (VA) only there was a rate of (36%) compared with the control group in which the proportion of the homozygous mutated genotype (AA) was the lowest by (11%) in While the normal genotype (VV) is the highest (47%), while the heterogeneous genotype is present (42%).

As for the level of allele viewing, the study showed that the mutant allele in the group of infertile men was the highest (54%) and the normal allele (46%) compared to the control group, where the percentage of viewing the mutant allele (31%) and the normal allele (69%).

It is worth noting that the occurrence of these genetic mutations in this gene (SOD) leads to a defect in the metabolic pathway of the SOD enzyme, and then the biological function of this enzyme will be lost, and the production of the enzyme SODs will be defective, which is one of the genetic causes of undiagnosed infertility in men. Studies have indicated Previously, the importance of SODs enzyme lies in protecting sperm from oxidation by superoxide dismutase (SOD) and hydrogen peroxide (H2O2) [20], as SODs enzyme catalyzes the decomposition of the superoxide radical into normal or molecular oxygen. Hydrogen peroxide, the results of the study also showed that the OR value was (6.4286) for the mutant genotype, and this in turn is a risk factor and cause of undiagnosed infertility cases in the study samples.

IV. CONCLUSION

This study concluded that there is a positive relationship between infertility and polymorphism in SOD gene.

REFERENCES

[1] Faraj, K., Dave, C., Bennett, R. C., & Vakharia, P. (2016). Male Infertility. eMedicine Specialities. Urology.
[2] Leslie, J. S., Rawlins, L. E., Chioza, B. A., Olubodun, O. R., Salter, C. G., Fasham, J., ... & Baple, E. L. (2020). MNS1 variant associated with situs inversus and male infertility. European Journal of Human Genetics, 28(1), 50-55.
[3] Yoon, C. J., Park, H. J., & Park, N. C. (2010). Reactive oxygen species in the internal spermatic and brachial veins of patients with varicocele-induced infertility. Korean Journal of Urology, 51(5), 348-353.
[4] Appasamy, M., Muttukrishna, S., Pizzey, A. R., Ozturk, O., Groome, N. P., Serhal, P., & Jauniaux, E. (2007). Relationship between male reproductive hormones, sperm DNA damage and markers of oxidative stress in infertility. Reproductive biomedicine online, 14(2), 159-165.
[5] Vessey, W., Perez-Miranda, A., Macfarquhar, R., Agarwal, A., & Homa, S. (2014). Reactive oxygen species in human semen: validation and qualification of a chemiluminescence assay. Fertility and sterility, 102(6), 1576-1583.
[6] Agarwal, A., Tvrda, E., & Sharma, R. (2014). Relationship amongst teratozoospermia, seminal oxidative stress and male infertility. Reproductive Biology and Endocrinology, 12, 1-8.
[7] Aitken, R. J., Jones, K. T., & Robertson, S. A. (2012). Reactive oxygen species and sperm function—in sickness and in health. Journal of andrology, 33(6), 1096-1106.
[8] Kensler, T. W., Wakabayashi, N., & Biswal, S. (2007). Cell survival responses to environmental stresses via the Keap1-Nrf2-ARE pathway. Annu. Rev. Pharmacol. Toxicol., 47(1), 89-116.
[9] Nakamura, B. N., Lawson, G., Chan, J. Y., Banuelos, J., Cortés, M. M., Hoang, Y. D., ... & Luderer, U. (2010). Knockout of the transcription factor NRF2 disrupts spermatogenesis in an age-dependent manner. Free Radical Biology and Medicine, 49(9), 1368-1379.
[10] Guerriero, G., Trocchia, S., Abdel-Gawad, F. K., & Ciarcia, G. (2014). Roles of reactive oxygen species in the spermatogenesis regulation. Frontiers in endocrinology, 5, 56.
[11] L. Yan, J. Liu, S.Wu, S. Zhang, G. Ji, and A. Gu, ―Seminal superoxide dismutase activity and its relationship with semen quality and SODgene polymorphism,‖ Journal of Assisted Reproduction and Genetics, vol. 31, no. 5, pp. 549–554, 2014.
[12] Ruiz-sanz et al. 2011 Ala16Val SOD2 polymorphism is associated with higher pregnancy rates in in vitro fertilization cycles, 39(7), 697-705.
[13] C. Faure, P. Leveille, C. Dupont et al., "Are superoxide dismutase 2 and nitric oxide synthase polymorphisms associated with idiopathic infertility?" Antioxidants and Redox Signaling, vol. 21, no. 4, pp. 565–569, 2014
[14] J. I. Ruiz-Sanz, I. Aurrekoetxea, R. Matorras, and M. B. Ruiz-Larrea, Ala16Val SOD2 polymorphism is associated with higher pregnancy rates in in vitro fertilization cycles,‖ Fertility and Sterility, vol. 95, no. 5, pp. 1601–1605, 2011.
[15] M. Tang, S. Wang, W. Wang et al., "The glutathione-S-transferase gene polymorphisms (GSTM1 and GSTT1) and idiopathic male infertility risk: a meta-analysis," Gene, vol. 511, no. 2, pp. 218–223, 2012.
[16] Ishii, T., Matsuki, S., Iuchi, Y., Okada, F., Toyosaki, S., Tomita, Y., ... & Fujii, J. (2005). Accelerated impairment of spermatogenic cells in SOD1-knockout mice under heat stress. Free Radical Research, 39(7), 697-705.
[17] Iranpur,V. and Esmailizadeh, A. (2010) Rapid Extraction of High Quality DNA from Whole Blood Stored at 4ºC For Long period. Department of Animal science, Faculty of Agriculture, Shahrekord University, Shahrekor, iron www.protocol-online.org.
[18] Mostafa T, Rashed LA, Osman I, Marawan M. Seminal plasma oxytocin and oxidative stress levels in infertile men with varicocele. Andrologia. 2015; 47:209–213.
[19] Abbasi et al., 2018 A. Reactive oxygen species impact on sperm DNA and its role in male infertility. Andrologia 2018, 50, e13012.
[20] Rima D, Shiv BK, Bhavna CH, Shilpa B, Saima KH.(2016). Oxidative stress induced damage to paternal genome and impact of meditation and yoga – Can it reduce incidence of childhood cancer? Asian Pac J Cancer Prev;17:4517-25