ASSOCIATION OF SUPEROXIDE DISMUTASE 2 GENE POLYMORPHISM RS4880 WITH POLYCYSTIC OVARY SYNDROME AMONG IRAQI WOMEN

Luay Q. Abdulhameed
University of Diyala, College of Education for Pure Sciences, Department of biology, Diyala – Iraq
ORCID No: 0000-0003-3414-4961

Mohanad W. Mahdi Alzubaidy
University of Diyala, College of Education for Pure Sciences, Department of biology, Diyala – Iraq
ORCID No: 0000-0002-4609-1265

Aseel J. Kadim
University of Diyala, College of Education for Pure Sciences, Department of biology, Diyala – Iraq
ORCID No: 0000-0001-5246-4411

Saja Mohammed Mohsen
Middle Technical University, Baquba Technical Institute, Medical laboratory Techniques Department, Diyala, Iraq
ORCID No: 0000-0001-5552-538X

ABSTRACT

Aimed of this paper to determine the impact of SNP rs4880 of gene SOD2 in Iraqi women with polycystic ovarian syndrome. Methods. Samples of blood were collected from Iraqi women with polycystic ovarian syndrome attending Maternity Teaching Hospital (Al-Batool) in Baquba City / Diyala Governorate, Iraq, and their ages ranged (28-54 years). Genotyping was performed by PCR/SNP(specific primers), risk score for polycystic ovarian syndrome disease was determined by Hardy-Weinberg equilibrium (HWE). Results. (HWE) was analyzed in polycystic ovarian syndrome patients and healthy participants, and it was discovered that the SOD2 (rs4880) genotypes were in agreement with the equilibrium, with no clear differences (p >1.120, 0.796) between the observed and expected genotype frequencies. When rs4880 genotype and allele frequencies were compared in polycystic ovarian syndrome patients and healthy participants, it was shown that there were no significant differences in these frequencies. In addition, the common CT genotype of rs4880 scored high in patients and healthy participants and was considered a preventive fraction (RR = 0.49). While, CC and TT genotypes were considered the etiological fraction and associated with polycystic ovarian syndrome (RR = 1.28, 1.87). Even that according to allele analysis C allele may preventive, while T allele could be etiological for disease. Conclusions. The results indicated that CC, TT genotype and T allele is a risk factor with polycystic ovarian syndrome for rs4880 and might have a role in the etiopathogenic mechanism in Iraqi women with polycystic ovarian syndrome. However, more researchs with bigger sample sizes is needed are necessary to verify our findings.

Keyword: PCOS, SOD2, rs4880, polymorphism

INTRODUCTION

The Polycystic Ovarian Syndrome (PCOS) is one of the most common problems women face during their reproductive years. It is characterized by hyperandrogenism, less frequent ovulation in enlarged ovaries including multiple follicular cysts [1].

Characterized Symptoms of PCOS include obesity, irregular cycles, menstrual irregularities, hirsutism, infertility, acne and endocrine abnormalities (including hyperandrogenemia, high LH/FSH ratio, and blood hyperinsulinemia) [2]. PCOS affects 6 to 14 percent of women of childbearing age, 30 to 75 percent of obese women with PCOS, and as many as 28 percent of obese and overweight women with PCOS [3]. Medical studies using ultrasound have found that one in four women has polycystic ovaries, But most of them do not suffer from any other symptoms associated with PCOS syndrome or do not show them [4]. Even though PCOS has been studied for a long times, its etiology and pathophysiology are still largely unknown . inflammatory state, endothelial injury, oxidative stress, and genetic mechanisms all play important role in PCOS [5]. It has been shown that PCOS is associated with oxidative stress Women with PCOS have abnormal levels of circulating markers of oxidative stress, according to Murri and colleagues' meta-analysis. This suggests that oxidative stress may be involved in PCOS' pathophysiology [6]. Oxidative stress is characterized by an imbalance between oxidants and antioxidants and the production of excessive amounts of reactive oxygen species (ROS) [7]. A class of molecules known as antioxidants is capable of reducing free radical damage. It is either enzymatic (superoxide dismutase (SOD), catalase, glutathione peroxide (GPx), and glutathione reductase (GR)) or non-enzymatic (glutathione (GSH), -tocopherol (vitamin E), ascorbate (vitamin C), and -carotene) or both. Female fertility and the pathogenesis of female infertility have been linked to these antioxidants [8,9]. One of the antioxidant is SOD, which catalyzes the conversion of superoxide to elemental oxygen and hydrogen peroxide. Convert of two molecules of superoxide anions into molecular oxygen and hydrogen peroxide[10]. There are three types of SOD is present in humans in three different forms: SOD1 is a dimer consists of two units and present in the cytoplasm, SOD2 and SOD3 are tetramers (four subunits), SOD2 is present in the mitochondria, and SOD3 is present extracellular. SOD1 and SOD3 are composed of copper and zinc, while SOD2 is composed of manganese in its reactive center [11]. In antioxidant reactions against reactive oxygen species, SOD dismutates the superoxide anion into hydrogen peroxide and is consider the first line of defense against ROS [12]. Regarding the molecular aspect, SOD genes regulation is critical for maintaining in balancing the of level of ROS [13]. This is due to the forms of SODs and their control at both expression and activity levels. These enzymes help to prevent the occurrence of oxidative stress, by inhibiting the generation and propagation of endogenous free radicals produced by the cell's metabolism [14].So, SNPs of antioxidant enzymes such as superoxide dismutase (SOD2, rs4880) have been identified in the coding which affect the net activity of the enzymes [15]. The purpose of this study is to determine the impact of SNP rs4880 of SOD2 in Iraqi women with polycystic ovarian syndrome.

Materials and Methods

Participants

58 PCOS-affected women were enrolled in the study, regardless of their marital status, with group consists of 27 healthy women without any symptoms considered a control group and both group ranged age (28-54) years. This study was conducted at the Maternity Teaching Hospital (Al-Batool) in Baquba City / Diyala Governorate, Iraq, between February and August of 2021 year, according to the study. Informed consent was obtained from each participant after they were informed of the study and given a special questionnaire to collect information. All patients underwent an ultrasound. Patients were diagnosed by obstetric and gynecologist specialists. Blood samples were kept in tubes EDTA and the samples were saved frozen at 20-25C until used for each isolate to extract DNA.

Preparation and extraction of DNA

In accordance with the manufacturer's instructions, total DNA was extracted from 2mL of peripheral blood from each patient using the Relia Prep DNA extraction kit from Promega.

Preparation PCR mixture and PCR Conditions

Using Primer3plus software designed the forward and reverse primers for rs4880, and Macrogen/Korea provided them. The forward primer sequence 5′CCAACGCCTCCTGGTACTTC3′ and the revers: 5′GTGCTTTCTCGTCTTCAGCAC3′. Polymerase chain reactions were performed using thermocycler device (USA). The PCR products were successfully amplified using a reaction mixture consisting of 12.5µL of Go Tag Green Master Mix/ Promega, 1,5 µL of each primer, 6.5 µL of D.W and 3 µL of patient`s DNA, with final volume 25 µL. Amplification conditions were for final thermocycling program was as follows: the initial denaturation was single cycle for 95 ºC at 7 min , 35 cycles each one includes denaturation at 95 ºC for 30s, annealing at 58ºC for 30s and extensions at 72 ºC for 30s, then final cycle extension at 72 ºC for 4 min. The size of PCR product is 215bp.

Statistical analysis

Alleles and genotype frequencies were analyzed using chi-square tests, while Hardy-Weinberg Equation was used to determine the populations studied were in genetic equilibrium from site https://wpcalc.com/en/equilibrium-hardy-weinberg. The calculation of the differences in genotypical and allelic frequency between studied groups was based on WINPEPI Computer program (version 11.63), to calculate

Fisher's exact test, calculate the relative risk (RR), Preventive or Etiological fraction (PF or EF) with a 95% confidence interval (CI). $P < 0.05$ was considered to indicate a statistically significant difference [16].

Results

Three genotypes (CC, CT, and TT) and two alleles (C and T) were presented for the rs4880 (C/T; Chromosome6). The genotypes in Iraqi women with PCOS and healthy participants were found to be in agreement with the equilibrium, with no significant differences (p >1.120-0.796) between the observed and expected genotype frequencies (Table 1).

Discussion

According to the results of this study, the SOD2 gene - rs4880 resulted in the emergence of three genotypes (CC, CT, and TT) that correspond to two alleles (Table 1). (C and T). These genetic patterns were found that consistent with the Hardy–Weinberg equation (HWE) in Table2.

Table 1: Numbers and percentage frequencies (observed and expected) of SOD2 gene (rs4880) genotypes and their (HWE) in women with PCOS and control individuals

Groups			***CRP* gene rs4880 -Genotypes or alleles**					**H-W*X*2 P ≤**
			CC	**CT**	**TT**	**C**	**T**	**1.120**
women with PCOS (No. =58)	Observed	No.	8	22	28	38	78	
		%	13.79	37.93	48.28	32.76	67.24	
	Expected	No.	6.22	25.56	26.22	Not estimated		
		%	10.72	44.07	45.21			
Controls (No. =27)	Observed	No.	3	15	9	21	33	**0.796**
		%	11.11	55.56	33.33	38.89	61.11	
	Expected	No.	4.08	12.84	10.08	Not Estimated		
		%	15.11	47.56	37.33			

Using Fisher’s exact test, the rs4880 polymorphism analysis showed that frequency of all genotypes was non-significantly higher in the patients group than in the control group. The genotype CC and C allele recorded lower ratio in both the patients (13.79vs 32.76%) and control groups (11.11 vs 38.89% respectively) with Probability Fisher (0.869vs 0.440), making it the least frequent genotype in the Iraqi population. On the other hand, the frequency of TT genotype and T allele (48.28vs 67.24%respectively) was significantly elevated in patients compared to the control group (33.33vs 61.11% respectively) with Probability Fisher (0.202vs 0.440). Depending on the values of RR (1.28, 1.87), both CC, TT genotypes and T allele

(1.31) were considered the etiological fraction and associated with PCOS. While, the frequencies of CT genotype was high significant in control compared to patients (55.56vs. 37.93% respectively), with values of RR (0.49). Beside that, CT genotype and C allele (0.77) were considered the Preventive fraction and no associated with PCOS.

Table 2: Statistical analysis of association between genotypes and alleles of SOD2 gene (rs4880) for women with PCOS versus Controls

Type of comparison	Statistical Evaluation			Fisher's Exact Probability	95%Confidence Intervals
	rs4880 Genotype or Allele	Relative Risk (RR)	Preventive or Etiological fraction (PF or EF)		
women with PCOS versus Controls	CC	1.28	21.9%	0.869	0.32-6.41
	CT	0.49	28.4%	0.131	0.19-1.25
	TT	1.87	22.4%	0.202	0.72-4.99
	C	0.77	23.4%	0.440	0.39-1.51
	T	1.31	23.4%	0.440	0.66-2.56

Genetic polymorphisms play an important role in PCOS susceptibility, and they have a significant impact on its clinical manifestations [17]. We found that at 16 sites in mitochondrial targeting sequence (MTS) of the SOD2 precursor, occurring substitution alanine to valine and this produces rs4880 is a single-nucleotide polymorphism (SNP), which has been shown to affect SOD2 activity in mitochondria [18]. Furthermore, this genotypic variability may also be linked to increased risks of diabetes and its complications, cardiovascular diseases, obesity, breast cancer, prostate cancer, and pulmonary arterial hypertension [19]. Results in presented study were consistent with the results [20] demonstrates that the SOD2 rs4880 genetic polymorphism is associated with the risk of PCOS in Chinese women and may be related to endocrine abnormalities of the reproductive axis and the impairment of antioxidant function and thus may contribute to the pathogenesis of PCOS in patients. Other studies indicate that SNP rs4880 is associated with an increased 2-fold-risk of PCOS when TT genotype and T allele are present [21]. Moreover, an association between PCOS and the CC genotype was found in a previous study [20]. This corresponds to our current study results.

Researchers found that genetic variation in genes for antioxidative enzymes and inflammatory pathway mediators not only modifies PCOS risk, but also influences PCOS patients' metabolic characteristics [22]. It has been shown that conformational changes in polymorphism rs4880 can affect the efficiency of transport of SOD2 into mitochondria as well as to alterations in SOD2 structure, Sod2 has also been linked to proinflammatory states and metabolic changes such as levels higher of cholesterol, glucose and leptin [23]. So far, Genetic studies of a large scale improve our understanding of PCOS pathogenesis, and they could identify polymorphisms that

could be used as a predictive biomarker for evaluating the risk of developing PCOS and for predicting treatment response in an individual patient [24, 25].

In conclusion, this is the first publication to describe the genotype distribution of the SOD2 rs4880 genetic polymorphism in Iraqi women with polycystic ovarian syndrome and to show related between SOD2 gene rs4880 genotypes and polycystic ovarian syndrome disease.

Acknowledgments

The authors would like to thank the physicians and nurses at the Maternity Teaching Hospital (Al-Batool) for their valuable assistance during this study. The authors also extend their gratitude to all of the women with PCOS who participated in the study and wish them good health in their personal lives.

Ethical approval

The local ethical committee at Council College of Education for Pure Sciences/ the University of Diyala approved the project.

Funding

Neither the public, commercial, nor non-profit sectors provided any funding for this research.

Conflicts of Interest

None.

Reference

1- Hasan, H. A., Selman, M. O., & Jwad, M. A. (2020). Effect of Body Mass Index on Serum CA125 Level in Females with PCOS. Iraqi Journal of Embryos and Infertility Researches, 10(1), 101-118.

2- Al-Hashimy, D. H., Al-Rikaby, H. R., & Al-Khayaat, E. S. (2019). Study of some hormonal disorders associated with polycystic ovarian syndrome in women in Thi Qar Governorate. Journal of Education for Pure Science, 9(2).

3- Murri, M., Luque-Ramírez, M., Insenser, M., Ojeda-Ojeda, M., & Escobar-Morreale, H. F. (2013). Circulating markers of oxidative stress and polycystic ovary syndrome (PCOS): a systematic review and meta-analysis. Human reproduction update, 19(3), 268-288.

4- Marbut, M. M., Awwad, N. Y., Yousif, M. N., & Ahmed, M. S. (2019). Hormonal assessment in women with polycystic ovary syndrome in Tikrit city. Journal of Madenat Alelem University College, 11(1), 1-9.

5- Mohammadi, M. (2019). Oxidative stress and polycystic ovary syndrome: a brief review. International journal of preventive medicine, 10, 86.

6- Sulaiman, M. A., Al-Farsi, Y. M., Al-Khaduri, M. M., Saleh, J., & Waly, M. I. (2018). Polycystic ovarian syndrome is linked to increased oxidative stress in Omani women. International journal of women's health, 10, 763-771.

7- Pizzino, G., Irrera, N., Cucinotta, M., Pallio, G., Mannino, F., Arcoraci, V., Squadrito F., Altavilla D., Bitto, A. (2017). Oxidative Stress: Harms and Benefits for Human Health. Oxidative Medicine and Cellular Longevity, 2017, 8416763: Obtenido de Oxidative Stress: Harms and Benefits for Human Health. Oxidative.

8- Zuo, T., Zhu, M., & Xu, W. (2016). Roles of oxidative stress in polycystic ovary syndrome and cancers. Oxidative medicine and cellular longevity, 2016.

9- Sabuncu, T., Vural, H., Harma, M., & Harma, M. (2001). Oxidative stress in polycystic ovary syndrome and its contribution to the risk of cardiovascular disease☆. Clinical biochemistry, 34(5), 407-413.

10- Hadi, Z. K., & Al-Maamori, J. A. I. (2020). Evaluation some oxidants-Enzymatic endogenous antioxidants status among women patients with polycystic ovary syndrome. Paper presented at the AIP Conference Proceedings.

11- Seleem, A. K., El Refaeey, A. A., Shaalan, D., Sherbiny, Y., & Badawy, A. (2014). Superoxide dismutase in polycystic ovary syndrome patients undergoing intracytoplasmic sperm injection. Journal of assisted reproduction and genetics, 31(4), 499-504.

12- Najah, A., Salman, S. A., Akram, H. M., Ahmed, M. A.-A., Ameer, L. A. A., & Khairi, A. W. A. (2017). Measurement of serum Superoxide dismutase levels in women with polycystic ovarian syndrome and chronic periodontitis. Journal of Baghdad College of Dentistry, 29(4), 76-81.

13- Miao, L., & Clair, D. K. S. (2009). Regulation of superoxide dismutase genes: implications in disease. Free Radical Biology and Medicine, 47(4), 344-356.

14- Lei, X. G., Zhu, J.-H., Cheng, W.-H., Bao, Y., Ho, Y.-S., Reddi, A. R., . . . Arnér, E. S. (2016). Paradoxical roles of antioxidant enzymes: basic mechanisms and health implications. Physiological Reviews, 96(1), 307-364.

15- Hernández-Guerrero, C., Parra-Carriedo, A., Ruiz-de-Santiago, D., Galicia-Castillo, O., Buenrostro-Jáuregui, M., & Díaz-Gutiérrez, C. (2018). Genetic polymorphisms of antioxidant enzymes CAT and SOD affect the outcome of clinical, biochemical, and anthropometric variables in people with obesity under a dietary intervention. Genes & nutrition, 13(1), 1-10.

16- Alzubadiy, M. W. M., Almohaidi, A. M. S., Sultan, A. A., & Abdulhameed, L. Q. (2019). Evaluation of E-selectin rs 5367 C/T Polymorphism in Iraqi Diabetic Foot patients. Paper presented at the Journal of Physics: Conference Series.

17- Branavan, U., Muneeswaran, K., Wijesundera, S., Jayakody, S., Chandrasekharan, V., & Wijeyaratne, C. (2018). Identification of selected genetic polymorphisms in polycystic ovary syndrome in Sri Lankan women using low cost genotyping techniques. Plos one, 13(12).

18- Sutton, A., Khoury, H., Prip-Buus, C., Cepanec, C., Pessayre, D., & Degoul, F. (2003). The Ala16Val genetic dimorphism modulates the import of human manganese superoxide dismutase into rat liver mitochondria. Pharmacogenetics and Genomics, 13(3), 145-157.

19- Lewandowski, Ł., Urbanowicz, I., Kepinska, M., & Milnerowicz, H. (2021). Concentration/activity of superoxide dismutase isozymes and the pro-/antioxidative status, in context of type 2 diabetes and selected single nucleotide polymorphisms (genes: INS, SOD1, SOD2, SOD3)–Preliminary findings. Biomedicine & Pharmacotherapy, 137, 111396.

20- Liu, Q., Liu, H., Bai, H., Huang, W., Zhang, R., Tan, J., . . . Fan, P. (2019). Association of SOD2 A16V and PON2 S311C polymorphisms with polycystic ovary syndrome in Chinese women. Journal of endocrinological investigation, 42(8), 909-921.

21- Polat, S., & Şimşek, Y. (2020). Five variants of the superoxide dismutase genes in Turkish women with polycystic ovary syndrome. Free Radical Research, 54(6), 467-476.

22- Herman, R., Jensterle, M., Janež, A., Goričar, K., & Dolžan, V. (2020). Genetic Variability in Antioxidative and Inflammatory Pathways Modifies the Risk for PCOS and Influences Metabolic Profile of the Syndrome. Metabolites, 10(11), 439.

23- de Oliveira Paludo, F. J., de Bittencourt Pasquali, M. A., de Vargas, A. R., de Oliveira, I. B., Gonçalves, L. V. B., Gelain, D. P., & Moreira, J. C. F. (2021). Influences of the polymorphisms of the Sod2 gene (rs4880) on the motility and vigor of X-and Y-bearing sperm at different pH values. Biomedicine & Pharmacotherapy, 142, 111993.

24- Sagvekar, P., Dadachanji, R., Patil, K., & Mukherjee, S. (2018). Pathomechanisms of polycystic ovary syndrome: multidimensional approaches. Front Biosci (Elite Ed), 10(3), 384-422.

25- Dapas, M., Lin, F. T., Nadkarni, G. N., Sisk, R., Legro, R. S., Urbanek, M., . . . Dunaif, A. (2020). Distinct subtypes of polycystic ovary syndrome with novel genetic associations: An unsupervised, phenotypic clustering analysis. PLoS medicine, 17(6), e1003132.