Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 4, 2021, Pages. 13827 - 13832
Received 05 March 2021; Accepted 01 April 2021.

Lack of Association between Genetic Polymorphisms of Cu/Znsod (Rs2070424) and the Risk of Breast Cancer

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ABSTRACT

Oxidative stress is significant in numerous types of cancer. Superoxide dismutase (SOD) is an endogenous antioxidant enzyme that is critical in limiting the oxidative burden effectively. The purpose of this study was to investigate the effects of the Cu/Zn SOD (rs2070424) gene polymorphisms on the susceptibility to breast cancer. The present results revealed that no significant difference was observed in the rs2070424 SOD1 SNPs between the patients and controls. We found that the genotypic frequency of heterozygous A/G with homozygous A/A (OR=1.0000, 95% CI=0.2403 - 4.1607, P=1.00). and the genotypic frequency of homozygous variant G/G with the homozygous A/A (OR=0.4331,95% CI= 0.0084 - 22.3896 , P=0.6776). There were nosignificant differences in G allele frequencies in patients as compared to the controls inthe SOD1 A215G polymorphism (OR=1.00, 95% CI: 0.2497- 4.0055, P=1.00). Our findings showed that there was no significant association between SOD1 A215G polymorphism and Breast cancer susceptibility.

Keywords: Breast cancer; Antioxidant polymorphism; Cu/ZnSOD (rs2070424) , SOD1 A251G; PCR-RFLP.

Introduction

Breast cancer is the most commonly occurring cancer in women, comprising almost one third of all malignancies in females. It is second only to lung cancer as a cause of cancer mortality, and it is the leading cause of death for American women between the ages of 40 and 55 [1]. Oxidative stress plays an important role in carcinogenesis because of induction of DNA damage and its effects on intracellular signal transduction pathways [2]. ROS may also play a key role in cancer development by inducing and maintaining the oncogenic phenotypes of cancers [3]. Superoxide dismutase (SODs) are enzymes that catalyze the dismutation of superoxide into oxygen and hydrogen peroxide [4]. There are three major families of superoxide dismutase, depending on the metal cofactor: Cu/Zn (SOD1, OMIM number 147450), which binds copper and zinc; Fe and Mn types (SOD2, OMIM number 147460), which bind either iron or manganese; and the Ni type (SOD3, OMIM number 185490), which binds nickel [5]. Study on genetic polymorphisms, gain or loss of functions of several antioxidant enzymes such as SOD, CAT, GR, GPx and GST has become important way to understand the development of cancer and its therapies [6]. The SOD1 A215G polymorphism has been found to be associated with several diseases either as a risk factor [7] or as a protective factor [8]. Recent studies on antioxidant enzymes state that diseases like diabetes [9]., age related diseases [7] and cancers [10] are connected with defects in antioxidant pathways. Our findings showed that the G allele of the SOD1 A251G polymorphism has protective effects against the risk of gastric cancer [11] .

Material and Methods

Ethical statement

Every volunteer has informed written consent. The ethics committee of the MOH and MOHSER in Iraq's ethical approval for scientific research has accepted this research.

Study Population

The study subjects comprised from 70 patients selected from MerjanTeaching Hospital all were

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female as patients' group with age range (26-80 years). The control group study included 30 people apparentlyhealthy that also were females with age range (20–71) years. All subjects in this study were taken written consent before participation in this study.

DNA extraction and genotyping

DNA of blood was extracted and purified using extraction and purification kit from Geneaid company (UK). The genotyping of the study groups was performed using the PCR-RFLP technique after DNA extraction from blood samples.

The targeted sites of DNA were amplified using design specific primers which used to identify Cu/ZnSOD (rs2070424), obtained from Macrogen company/south Korea. Forward primer: 5'-AGTACTGTCAACCACTAGCA-3', and reverse primer: 5'- CCAGTGTGCGGCCAATGATG -3'.PCR was carried out in 20 µl reaction volumes containing 1 µl of each forward and reverse primer, 12.5 µl of Green Master Mix, 3 µl of Genomic DNA, and 2.5 µl of nuclease-free water to bring the reaction volume up to 20 µl . Amplification was conducted in a thermocycler (Biometra, Germany) with the following settings: 5 min pre-denaturation at 94°C; 30 cycles with denaturation for 30 seconds at 94°C, annealing for 30 seconds at 62°C, extending for 30 seconds at 72°C; and a final extension of 5 min [12].

PCR products were electrophoresed in 1 percent agarose at 75 V using gel electrophoresis (cleaver science – UK) and visualized with ethidium bromide. A gel documentation system (Cleaver Scientific –UK) was used to take photos.

polymerase chain reaction-restriction fragment length polymorphism method (PCR-RFLP).

The PCR product was digested with 2 units of one of the specific endonucleases (TaqI) for 1-4 h at 37˚C , according to manufacturer'sinstructions(Promega company. PCR and digestion products were analyzed by electrophoresis in 1 percent agarose at 75 V using gel electrophoresis (cleaver science – UK) and visualized with ethidium bromide.

Statistical analysis

All the statistical analyses were done with the SPSS statistical software (version 23; SPSS Inc., Chicago, IL), $p<0.05$ was considered statistically significant.

Results and Discussion

The genomic DNA (Fig.1) was extracted from the blood samples as a first step to amplify the target region of SOD1gene.

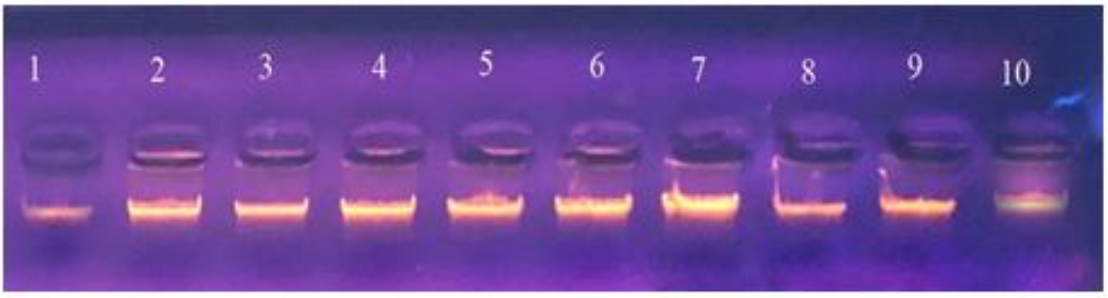


Figure 1. The electrophoresis pattern of gnomic DNA extracted from blood samples of breast cancer patients and healthy control groups.

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(Lane 1 -lane 10 refers to genomic DNA from blood samples; Electrophoresis conditions, 1% agarose, 75 V, 20 mA for 1h, stained with ethidium bromide).

Genotyping of Cu/ZnSOD (rs2070424) Gene Polymorphisms

The results revealed that the presence a single band (569bp) of the target sequence of Cu/ZnSOD (rs2070424) gene in agarose gel as in figure (2).

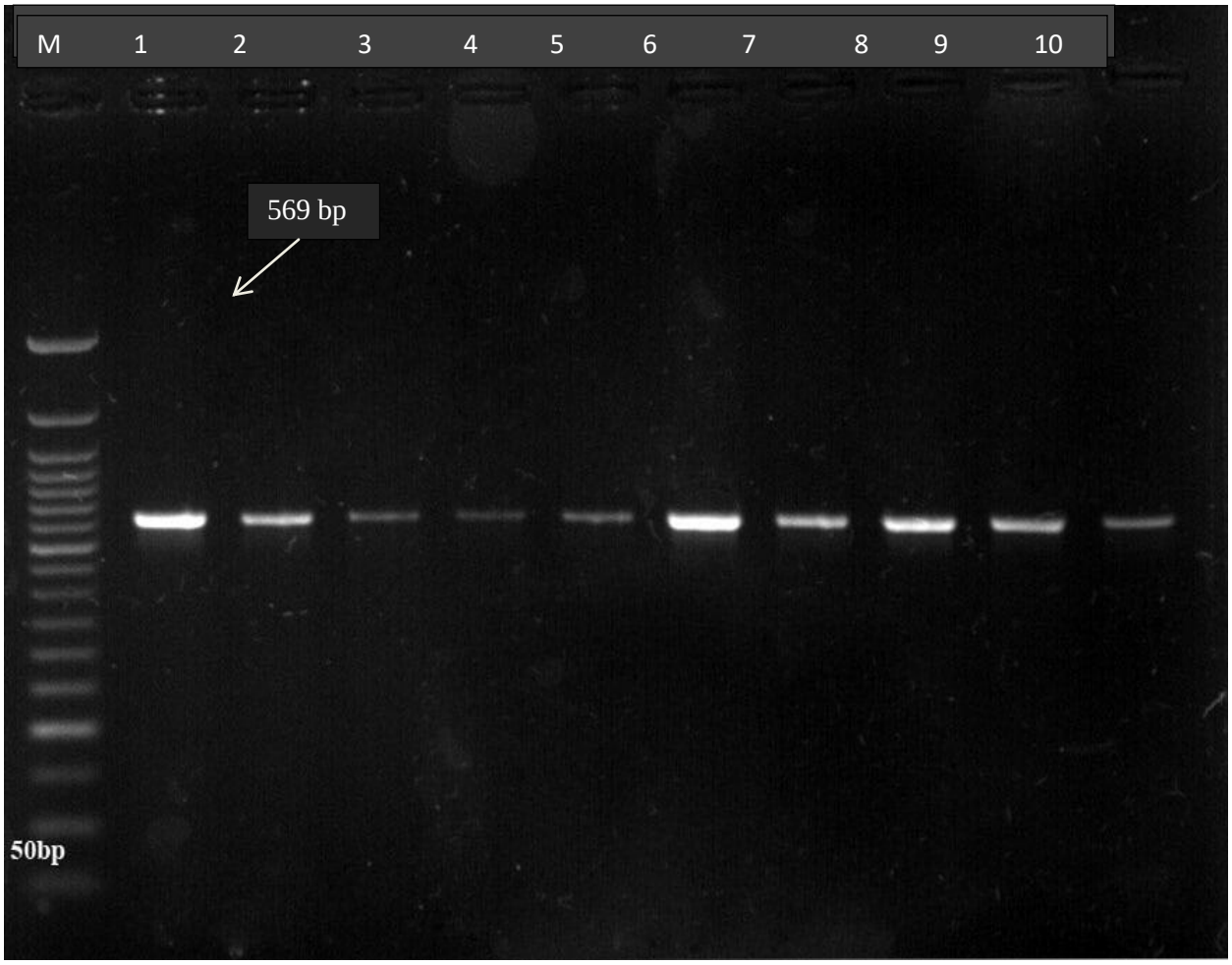


Figure 2. Agarose gel electrophoresis of an amplified product patterns of superoxide dismutase Cu/ZnSOD (rs2070424) with specific primer
(M: refers to DNA size marker (50); lanes 1 - 11 refer to PCR products of Cu/ZnSOD (rs2070424) (569bp) of breast cancer patients and healthy control groups. Electrophoresis conditions: 1% agarose concentration 1%; 75 V, 20 mA for 120 min. Staining method; precast ethidium bromide).

The results of PCR-RFLP showed that the presence of three different genotypes as in figure (3). the first A1/A1 (AA) homozygous, presents the expected 569bp fragment, the second A1/A2 (AG) demonstrated 569, 368 & 201 fragment.

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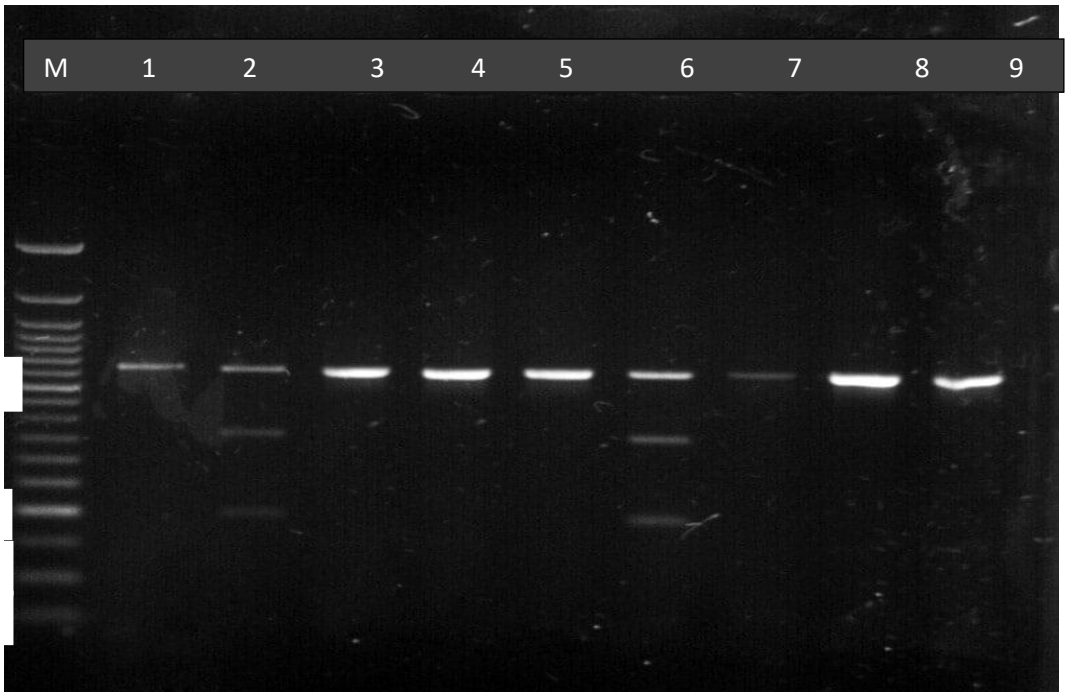


Figure 3. Electrophoresis patterns of allelotyping of Cu/ZnSOD (rs2070424) gene of breast cancer patients and healthy control groups using MspI enzyme by PCR-RFLP method.
(M: DNA ladder (50 bp); Lanes 1,3,4, 5,7,8, & 9 refer to a homozygous allele (AA) had a single band with 569 bp molecular size; Lanes 2&3 refer to a heterozygous allele (AG) had 3 bands with 569, 368 & 201 bp).

The Genotypes Distribution of Rs 2070424 Polymorphisms with Allele Frequency in Control and Case Groups.

The distribution observed in Cu/ZnSODRs 2070424 gene polymorphism in cases group and control group are showed in Table (1). The highest genotype in control group was AA homozygote genotype (90%) followed by heterozygote genotype AG (10%).in breast cancer disease,the highest genotype in control group was AA homozygote genotype (90%) followed by heterozygote genotype AG (10%). analyses showed no significant differences between cases and controls in the SOD1 A251G polymorphism (AG vs AA: OR=1.00, 95% CI0.240- 4.16, P=1.00). Analysis also showed no significant differences between cases and controls in the SOD1 A251G polymorphism (GG vs AA :OR=0.4331, 95% CI: 0.008-22.38 , P=0.6776).

Table1. Genotype distribution and odd ratio of Rs 2070424 polymorphisms between the patients vs healthy control

Genotype Rs2070424	**Patients No.(%)**	**Control No.(%)**	**Significance level**	**O.R**	**CI (95%)**

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AA [a]	63 (90%)	27 (90%)			
AG	7 (10.8%)	3 (10%)	1.00	1.00	0.240- 4.16
GG	0 (0%)	0 (0%)	0.6776	0.4331	0.008-22.38
Total No.	70	30			
Allele	Frequency	Frequency			
A	0.33	0.95			
G	0.67	0. 05	1.00	1.00	0.2497- 4.0055

$P \leq 0.05$; OR=(95%CI); [a] reference

The SOD1 A215G polymorphism has been found to be associated with several diseases either as a risk factor [7] or as a protective factor [8]. Among the antioxidant enzymes SOD & CAT plays a vital role in oxidant defence mechanism and can promote or suppress tumour formation in human gastric mucosa [13] . Other study showed no significant association of SOD1 (G allele of rs 2070424) with the development of gastrointestinal cancer in the rural population of south western Maharashtra from India ,When he compared the genotypic frequency of heterozygous A/G with homozygous A/A (OR: 0.86, CI: 0.59-1.23, p: 0.42) and the genotypic frequency of homozygous variant G/G with the homozygous A/A (OR: 0.16, CI: 0.215-1.32, p: 0.05) we did not find the association with development of the GI cancer [14]. .
We have found no evidence that common variations in SOD1, are associated with breast cancer. (OR: 0.91, CI: 0.76-1.09, p: 0.17) [15]. However, few studies from Iran have reported positive association between polymorphism in SOD1 rs2070424 gene and colorectal cancer [16]. while some studies reveal the negative association of SOD1 rs2070424 and Gastric cancer [11].Few Indian studies have proved association of SOD1 rs2070424 & SOD3 rs2536512 with cervical cancer risk [15] .

Conclusion

Based on the present statistical analysis, our study shows no significant association of SOD1 (G allele)of rs 2070424with the development of breast cancer .

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