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Genetic Polymorphism of Manganese Superoxide Dismutase (MnSOD) Among Breast Cancer and Benign Breast Patients in Jordan: A Preliminary Study

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Abstract

This study investigated the association of MnSOD gene polymorphism with risk of both breast cancer and benign breast disorders among Jordanian females. MnSOD genotyping assay was conducted among 81 Jordanian participants including: 44 breast cancer patients, 19 benign breast patients and 18 healthy controls. In the breast cancer group, MnSOD allele frequency for Ala and Val alleles was 41% and 59%, respectively, which is similar to the Caucasian population. The most common MnSOD genotype among all study groups is Ala/Val genotype, while the Ala/Ala genotype was rare in benign breast patients and controls and was completely absent in breast cancer patients. Women with Ala/Val genotype were at highly significant increased risk for breast cancer (OR=10, 95% CI=2.51-39.83), while Val/Val genotype was correlated to a significantly decreased risk for breast cancer (OR=0.125, 95% CI=0.03-0.5). On the other hand, the similar risks for benign breast diseases were nonsignificant. Larger sample is needed to support that MnSOD polymorphism may be involved in breast cancer development and progression.

الملخص

لقد تم تقيم التعداد الشكلي لجين (MnSOD) لدى إناث أردنيات مصابات بسرطان الثدي الخبيث و اخريات مصابات بسرطان الثدي الحميد, بالاضافه الى دراسة إرتباط التعداد الشكلي لجين (MnSOD) مع قابلية الاصابه بسرطان الثدي الخبيث والحميد على حد سواء. تم تحديد الطراز الجيني للأنزيم (MnSOD) ل 81 عينة ضمت 44 مريضه بسرطان الثدي الخبيث, 19 مريضه بسرطان الثدي الحميد و 18 من الإناث الاصحاء. كانت نسبة تكرار الأليلين الخاصين بالأنزيم (MnSOD) ألانين و فالين في عينات الدراسه مجتمعة هي 41% و 59% على التوالي, والتي هي اكثر قربا من الشعب القوقازي. كان الطراز الجيني ألانين/فالين هو الاكثر شيوعا بين مجموعات الدراسه, بينما كان الطراز الجيني ألانين/ألانين نادر الانتشار لدى الإناث الأصحاء و الإناث المصابات بسرطان الثدي الحميد وغائبا تماما لدي المصابات بسرطان الثدي الخبيث. لقد وجد ان السيدات اللاتي يحملن الطراز الجيني ألانين/فالين هن الأكثر عرضة للاصابه بسرطان الثدي الخبيث بينما السيدات اللاتي يحملن الطراز الجيني فالين/فالين هن الأقل عرضة للإصابة بسرطان الثدي الخبيث. أما قابلية الاصابه بسرطان الثدي الحميد المرتبطة بالطراز الجيني لأنزيم (MnSOD) كانت نوعا ما قريبة من احتمالية الاصابة بسرطان الثدي الخبيث إلا انها لم تكن ذات ارتباط معنوي مثلها. استخلصت هذه الدراسه الى تأييد احتمالية ضلوع التعداد الشكلي لجين (MnSOD) بتطور سرطان الثدي الخبيث.

Keywords: Breast Cancer; Benign Breast Tumor; Manganese Superoxide Dismutase (MnSOD); Polymorphism; Reactive Oxygen Species (ROS).

1. Introduction

Breast cancer is the second most common type of cancer after lung cancer and the fifth most common cause of cancer death among both sexes worldwide (World Health Organization, 2006). Among women, breast cancer is the leader incident cancer and the second cause of death (Jemal *et al.,* 2005). In Jordan, breast cancer amounts to 32 % of the total number of cancer patients, with an average of 500 females and 5 males diagnosed with breast cancer every year, which is relatively high when compared to the overall size of the population (Al-Khatib, 2007).

Although benign breast conditions are not life-threatening as breast cancer, benign lesions of the breast are far more frequent than cancerous ones and can cause serious physical symptoms, a financial burden for health services and emotional problems for patients and families since certain benign conditions are linked with an increased risk of developing breast cancer (Guray and Sahin, 2006).

The etiology of breast cancer is multifactorial, both hormonal genetic and environmental factors are implicated in the pathogenesis of breast cancer (Russo *et al*., 2000). One potential mechanistic basis for these factors is through reactive oxygen species (ROS)-induced oxidative damage (Wang *et al.,* 2001; Mitrunen *et al.,* 2001; Millikan *et al.,* 2004; Cai *et al.,* 2004; Mukhopadhyay *et al.,* 2004 ; Waris and Ahsan, 2006).

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The mitochondrial respiratory chain constitutes the main intracellular source of ROS in most tissues (Julio, 2003), that makes the mitochondrial antioxidant defense "manganese-containing superoxide dismutase" liable to maintain the steady-state concentration of ROS at non-toxic levels. Therefore, it would be advantageous to pinpoint the biological role of manganese superoxide dismutase (MnSOD) and its signification in cancer development since there were many studies considered it as a tumor suppressor gene (Mukhopadhyay *et al.,* 2004).

MnSOD is encoded by a single gene, containing five exons and located on chromosomal region 6q25.3. Recently, a genetic variant of MnSOD was identified, a Thymine (T) to Cytosine (C) substitution in the mitochondrial targeting sequence, which changes the code of the amino acid Valine from (GTT) to that of Alanine (GCT), leading to structural alteration in the enzyme conformation (Shimoda-Matsubayashi *et al.,* 1996). This alteration may affect the cellular allocation of MnSOD into mitochondria; therefore the enzyme could leave mitochondria without full defense against superoxide radicals (Rosenblum *et al.,* 1996). Sutton *et al.* (2003) supported that Alanine Ala allele of MnSOD allows more efficient transport into mitochondrial matrix while MnSOD of Valine Val allele will be partially arrested in the inner mitochondrial membrane.

To date, several studies have examined the correlation between MnSOD polymorphism and breast cancer risk with inconsistent results. Ambrosone *et al*. (1999), and Mitrunen *et al.* (2001) and Cai *et al.* (2004) reported that having an Ala allele increased the risk of breast cancer. In contrast, Egan *et al*. (2003), Knight *et al.* (2004) and Gaudet *et al*. (2005) did not support such association. Millikan *et al.* (2004) and Tamimi *et al.* (2004) also did not support an overall association but they suggested that MnSOD polymorphism may modify breast cancer risk. But there were no reports evaluated the association between MnSOD polymorphism and benign breast disorders risks. So, the aim of this study is to screen MnSOD gene polymorphism among Jordanian breast cancer patients, benign breast patients and healthy women and investigate the association between MnSOD polymorphism and risk of both breast cancer and benign breast disorders.

2. Materials and Methods

2.1. Study Samples

A total of 81 individuals were included in this study. Informed consent form was explained and provided to each individual participated in this study for interview and samples collection. All samples were collected from King Hussein Medical City (KHMC) in Amman, in the period between June 2007 and May 2008.

Control group included 18 blood samples drawn from healthy Jordanian females without any history of breast cancer or previous benign breast problems, which were collected in the routine laboratory of KHMC. The age of these females ranged from 24 to 62 years (mean=40.1 years).

Test group included 44 blood samples (cancer group) collected from Jordanian female patients histopathologically diagnosed with breast cancer, the age of these patients ranged from 25 years to 75 years (mean=52.3 years). Another 19 blood samples (benign group) were optioned from Jordanian females who were histopathologicaly diagnosed with benign breast diseases, the age of these females ranged from 17 to 70 years (mean=41.79 years).

2.2. MnSOD Genotyping

Genomic DNA was extracted from whole blood samples, using Wizard Genomic DNA Purification Kit (Promega, USA). DNA extraction was performed in accordance with the manufacturer's instructions.

MnSOD genotyping was conducted using polymerase chain reaction-restriction fragment length polymorphism PCR-RFLP as described in (Cai *et al.,* (2004) but with some modification. PCR amplification was carried out in a total volume of 25 µl containing: 2 µl of genomic DNA, 0.8 µM of each primer (5'-ACCAGCAGGCAGCTGGCGCCGG-3' and 5'-GCGTTGATGTGAGGTTCCAG-3') (Alpha DNA, Montreal), 8.5 µl of nuclease-free water and 12.5 µl of 2X PCR Master Mix (Fermentas, USA) containing: 0.05 units/µl *Taq* DNA polymerase supplied in reaction buffer, 4 mM of $MgCl_2$ and 0.4 mM of each dNTPs [dATP, dCTP, dGTP, dTTP]. PCR was performed in thermal cycler (BIO-RAD, USA). The thermal profile involved initial denaturation at 95 ˚C for 15 min, followed by 35 cycles of denaturation (94 ˚C for 30s), annealing (58˚C for 30s), and extension (72 ˚C for 30s), and completed with a final extension at 72˚C for 7 min.

RFLP technique was accomplished to detect MnSOD polymorphism using a restriction endonuclease enzyme *Ngo*MIV (Promega, USA) which recognizes a short specific DNA sequence to cleave double-stranded DNA at specific site within the recognition sequence. Restriction reaction was performed in a total volume of 20 µl containing 11.8 µl nuclease-free water, 2 µl 10X restriction enzyme buffer, 0.2 µl acetylated bovine serum albumin, 4 µl PCR product DNA and 2 µl (1 unit) of *Ngo*MIV restriction enzyme. After mixing, the reaction mixture was incubated at 37 ˚C for 4 hrs. Restricted products were electrophoresed on 4% agarose gel stained with ethidium bromide at 100 v for 45 min and visualized under UV. Fragment patterns specific for MnSOD genotypes were: Ala/Val (18 bp, 89 bp and 107 bp) bands, Val/Val (107 bp) band and Ala/Ala (18 bp and 89 bp) which were no table in Figure (1).

2.3. Statistical Analysis

Statistical analysis was carried out using the Statistical Package for Social Sciences (SPSS 10.05; SPSS Inc, Chicago, IL, USA, 1999). Chi-square test was used to evaluate case-control differences for MnSOD genotype distribution among different groups where statistical significance was set at $P < 0.05$. The association between MnSOD genotypes and the development of both malignant and benign breast disorders was examined by calculating the odds ratios (OR) and 95% confidence intervals (CI).

3. Results

As shown in Table (1), Ala/Ala genotype of MnSOD was completely absent only in breast cancer group, and there was a large difference between the frequency of Val/Val and Ala/Val genotypes (9.1% and 90.9%), respectively. While in control group, Ala/Ala genotype accounts for 5.6% of control samples, and the difference between Val/Val and Ala/Val genotypes frequency was relatively small (44.4% and 50%), respectively. The genotype distribution was statistically significant when the cancer and control groups were compared.

In benign group, the frequency of Ala/Val genotype (73.7%) was higher than in control group (50%). Approximately, the frequency of Val/Val genotype in benign group (21%) was half the frequency in control group (44.4%), and the frequency of Ala/Ala genotype was equal in both groups. Differences of genotype distributions between benign and control groups did not reach statistical significance (P=0.302) as shown in Table (1).

In addition, cancer group was compared to benign group, most of cancer patients (90.9%) and benign patients (73.7%) appeared to have the Ala/Val genotype, while the Val/Val was found in 9.1% of cancer patients and 21% of benign patients. Although the Ala/Ala genotype was found in 5.3% of benign patients and was absent in cancer group, the distribution of genotypes was not significantly different between cancer and benign groups (P=0.118).

MnSOD allele frequencies among each study group and among whole individuals participated in this study were calculated using the formula described by Campbell (1996). Val allele was the dominant allele among all groups, the prevalence of Val allele was 55% among cancer group, 58% among benign group, and 69% among control group. The frequency of Val allele in the whole study participants was 59% (Figure 2).

The risk of breast cancer associated with MnSOD genotypes was evaluated in this study. Females with Ala/Val genotype had a 10-fold significant increase risk for breast cancer development (OR=10, 95% CI=2.51-39.83), while a highly significant decreased risk for breast cancer was associated with Val/Val genotype (OR=0.125, 95% CI=0.03-0.5). The risk for breast cancer development associated with Ala/Ala genotype could not be determined because the Ala/Ala genotype was not found in any breast cancer females. But using Chi-square test, there was no significant difference (P=0.115) between cancer patients and healthy controls with Ala/Ala genotype (Table 2).

On the other hand, non significant 2.8-fold increase risk for benign breast diseases was associated with Ala/Val genotype (OR=2.8, 95% CI=0.71-11.1). Also, there was no significant risk of benign breast diseases associated with neither Val/Val genotype (OR=0.33, 95% CI=0.08-1.41) nor Ala/Ala genotype (OR=0.94, 95% CI=0.055-16.33) (Table 3).

4. Discussion

MnSOD polymorphic alleles are widely variable with ethnicity, the frequency for Ala allele is 12% among Japanese (Shimoda-Matsubayashi *et al.,* 1996) and 14% among Chinese (Cai *et al.,* 2004) , whereas it is more common (41-55%) in the Caucasian population (Ambrosone *et al.,* 1999). In this study, MnSOD genotyping assay was conducted on 81 Jordanian participants including: 44 breast cancer patients, 19 benign breast patients and 18 healthy controls, the frequency of Ala allele was 41% (Figure 2) which is comparable to that in Caucasian population.

MnSOD genotyping revealed that Ala/Val genotype had the highest frequency distribution for cancer group, benign group and control group (90.9%, 73.7% and 50%), respectively (Table 1). Frequency of MnSOD genotypes was found to be significantly different between breast cancer patient and controls, where the Ala/Ala genotype was completely absent among breast cancer patient that could be due to ethnic variation. However, more investigations and large sample will be needed to shed additional light on the role of Ala/Ala genotype in the etiology of breast cancer.

Due to the absence of Ala/Ala genotype in breast cancer group, this study could not estimate the association between Ala/Ala genotype and breast cancer risk. However, the most remarkable breast cancer risk was associated with Ala/Val genotype compared with Val/Val genotype. A 10-fold significantly increased risk of breast cancer was found among women having the Ala/Val genotype, while women with the Val/Val genotype have a significant decreased risk of developing breast cancer (Table 2). This study also evaluated the risk of benign breast diseases associated with MnSOD polymorphism, 2.8- fold increased risk of benign breast diseases was associated with Ala/Val genotype, a decreased risk was associated to Val/Val genotype and no association was observed with Ala/Ala genotype, although none of these risks was significant (Table 3).

Our results were in agreement with the findings of Ambrosone *et al.* (1999), Mitrunen *et al.* (2001) and Cai *et al.,* (2004), which all based on that the Ala-containing MnSOD is transported more efficiently through the mitochondrial membrane (Sutton *et al.,* 2003), were explained by the possible highly active Ala-containing MnSOD that could cause accumulation of H_2O_2 in mitochondria at least in the absence of H_2O_2- scavenging enzymes such as glutathione peroxidase and catalase, which could lead to oxidative damage and consequently cancer. Thus, it will be interesting to evaluate MnSOD genotype-activity relationship in order to explain such results.

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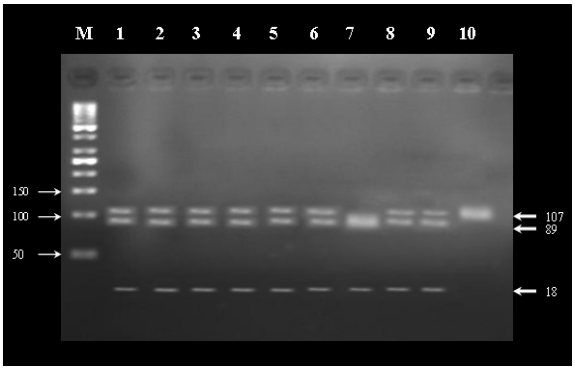

Figure 1. 4% agarose gel electrophoresis of selected genotyping assays. Lane M: 50 bp molecular weight ladder. Lanes 1,2,3,4,5,6,8 and 9 partially-digested samples showing three bands (107bp, 89bp and 18bp) representing Ala/Val genotype. Lane 7: a fully-digested sample showing two bands (89 bp and 18 bp) representing Ala/Ala genotype. Lane 10: undigested sample showing one 107 bp band representing Val/Val genotype.

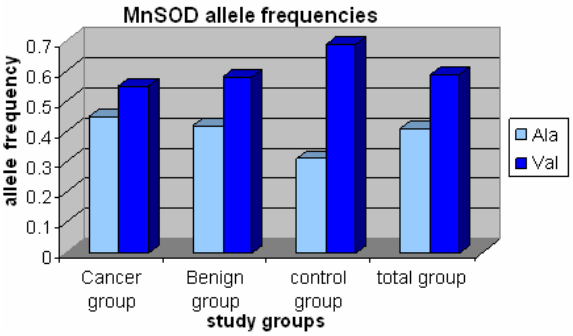

Figure 2. *MnSOD* allele frequencies among all study groups.

Table 1. Distribution of MnSOD genotypes among breast cancer patients, benign breast patients and control group.

MnSOD genotypes	Cancer group n = 44	Benign group n = 19	control group n =18
Ala/Ala	0	1 (5.3 %)	1 (5.6 %)
Val/Val	4 (9.1 %) *	4 (21.0 %)	8 (44.4 %)
Ala/Val	40 (90.9 %) *	14 (73.7 %)	9 (50.0 %)

Data presented as numbers of cases (percentage)
*: *P* value < 0.05 for cancer patients versus control differences

Table 2. Risk of breast cancer associated with MnSOD genotypes.

MnSOD genotypes	P value	odds ratios	95% confidence intervals
Ala/Ala	0.115		
Val/Val	0.001	0.125	0.03 - 0.50
Ala/Val	0.000	10	2.50 - 39.83

*: *P* value < 0.05 for gene polymorphism

Table 3. Risk of benign breast disease associated with MnSOD genotypes.

MnSOD genotypes	P *value*	*odds ratios*	*95% confidence intervals*
Ala/Ala	*0.969*	*0.94*	*0.055 - 16.33*
Val/Val	*0.129*	*0.33*	*0.08 - 1.41*
Ala/Val	*0.138*	*2.80*	*0.71 - 11.10*

*: *P* value < 0.05 for gene polymorphism

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