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Polymorphism in MnSOD Gene and Breast Cancer Risk in Kashmiri Patients: A Case Control Study

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Abstract

Reactive oxygen species (ROS) have been implicated in the etiology of many human diseases. Antioxidant enzymes such as MnSOD protects cells from oxidative stress and generation of ROS. A case control study, with aim to evaluate the relationship between MnSOD Ala-9Val polymorphism and breast cancer was carried out. The study included 522 subjects, including 255 cases and 267 controls, 12 samples were missing or yielded no results. Genotyping of samples were carried out using PCR-RFLP method. We observed that neither of two conditions heterozygous (MnSOD Val/Ala) or Variant/(MnSOD Ala/Ala) was significantly associated with overall breast cancer risk. The frequencies of Val and Ala allele was almost similar in cases, however, a significant association was seen in case of older women (above 45 years of age) (OR =1.98, CI=1.07-3.66, P=0.04).

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Also ORs were elevated in case of women using oral contraceptives carrying Val/Ala genotype. (OR=2.20; CI=0.54-8.96). In conclusion MnSOD Ala-9Val polymorphism may modify the risk for breast cancer development particularly in presence of age above 45years, oral contraceptives, and urban life style.

Keywords: MnSOD; breast cancer; polymorphism; case control; RFLP; Polymerase chain reaction.

1. Introduction

Women all over the world are affected by breast cancer, with an estimated 1.67 million new cases diagnosed in the year 2012, it is the most common cause of death among women in both the developed and developing countries [1]. India being the largest populous democracy, about 100,000 breast cancer cases are estimated to be diagnosed annually [2,3]. Over the past few years, Kashmir has also witnessed huge rush of patients affected with cancer, it has been reported that carcinoma of breast continues to be the second most common cancer among women and comprised of 14.6% of all female cancers [4]. Despite the common occurrence the exact etiology of breast cancer is still unknown [5], however, the lifetime exposure to exo and endogeneous estrogen appears to be closely associated to development and progression of disease. Also ROS may also contribute to the development of cancer, During normal cellular process Reactive oxygen species (ROS) are generated [6] from estrogen metabolism through catechol estrogen redox cycling [7,8].Since ROS generation is continuous process and low level of DNA repair mechanism and lack of histone protection to mitochondrial DNA exposes it to more oxidative damage [9]. Therefore, damaged mitochondrial DNA may play an important role in breast carcinogenesis [10, 11] in order to neutralize the effect of ROS, Superoxide dismutases play a crucial role in the detoxification of superoxide radicals (O^-) thereby protecting cells from damage induced by free radicals [12] and one such enzyme mitochondria manganese super oxide dismutase (MnSOD) provides major defence against damage by ROS, catalyzing their conversion into H_2O_2 and O_2 in mitochondria. It is encoded by single gene containing five exons, located on chromosome 6q25.3, synthesized in the cytosol and post transcriptionally modified for transport into the mitochondria [13]. A single nucleotide polymorphism, T to C substitution results in change of valine to alanine [14] the Ala variant forms α helical structure while val variant results in ß sheet conformation. The two forms differ in their transport rate within mitochondrial membrane, the Ala variant is easily transported and therefore has high activity inside as compared to val variant. The high activity of Ala variant could result in high production and therefore accumulation of ROS [15,16]It was observed that MnSOD is elevated in some cancers as compared to normal counters as in gastric esophageal, lung and colorectal cancer cells [17,18,19] While a variety of cancer cells including breast cancer [20,21] pancreatic cancer, ovarian cancer are known to express reduced level of antioxidant enzymes, especially MnSOD, when compared with their normal counter parts.

2. Materials and Methods

To investigate the associations between genetic factors and breast cancer risk in Kashmiri population a case control study was conducted. Since breast cancer is much more common in women than men therefore the population of the study specifically included women. The study was reviewed and approved by the ethics committee of Sheri kashmir institute of medical sciences (SKIMS) Soura, Srinagar. All women with suspicious

breast cancer lump or symptoms of breast cancer are referred to SKIMS for further examination from other hospitals. SKIMS being the central and largest hospital in valley with well-equipped regional cancer centre (RCC).All the pateints were ascertained from the RCC which operates as referral center and offers treatment and follow up of all breast cancer cases, diagnosed in the valley. Blood samples were collected from 522 subjects, 255 were female breast cancer patients diagnosed between age of 20-80yrs and 267 were control, however, 2%of samples either didn't yield results or were having incomplete information. Eligible control group included women who had no history of breast cancer or any other type of malignancy. All the study participants both patients and controls were from Kashmir. The participation rate of cases and controls was high covering around 98% of eligible cases and controls. In addition to blood samples a risk factors questionnaire which included extensive demographic epidemiologic and pathologic data was obtained from each participant through standardized interview. Blood samples of the patients and controls were collected into 5ml EDTA coated tubes and stored at -20c until use.

2.1.Genotyping

Genomic blood was obtained from whole blood, using phenol-chloroform method. Desired region of the genome was amplified using primer set (FORWARD 5'TAGACGGTCCCGCGGCGCTGA3') and (5'CCGTAGTCGTAGGGCAGGTCGGGGA3') that resulted in the amplification of 133bp fragment. PCR was carried out in a final volume of 25µl reaction mixture, 50-70ng DNA, 0.5µM Each primer in 10X PCR buffer containing 1.5mM $MgCl_2$ 200µM dNTPs (Fermentas) and 1Unit of Taq polymerase (Fermentas) after extensively standardizing all the prameters according to following conditions. The amplification started with an initial denaturation step at 95°C for 7 mins cycling parameters were 35 cycles of 95°C for 30 secs, annealing at 66°C for 30secs, extension at 72°C for 30 secs and final extension at 72°C for 7mins.After amplification, 10µl of 133bp PCR product was digested with 5Units BsaWI enzyme at 60°C. The Ala allele contains restriction site for the restriction enzyme, resulting in 69 and 64 bp. while Val allele remains uncut. All samples were subjected to two rounds of restriction digestion.

3. Results

Present study included a total of 250 cases and 260 controls Table 1 represents selective general characteristics of cases and controls that were included in the present study. Mean age calculated for cases was 49.5 years and that of controls was 45 years Since no significant differences were observed in cases and controls With respect to various characteristics ($p>0.05$), suggesting frequency matching was adequate**.**

Table 2 shows the allele and genotypic frequencies of different polymorphic variants among cases and controls resulting from SNP in MnSOD gene, the frequencies of Val and Ala alleles were almost same in cases (48.2, 51.8) and controls (48.8,51.1). among the cases prevalence of Val/Val , Val/Ala and Ala/Ala genotypes were (20.8, 54.8,24.4) while it was (24.23, 49.23, 26.53) among control respectively, compared with MnSOD Val/Val genotype ORs for MnSOD val/ala were(OR: 1.30; CI:0.83-2.01) and for Ala/Ala (OR:1.07;CI:0.64-1.77).

Table 1: General characteristics of a population

Characteristics		**Case n=250**	**Control n=260**	**P value**
Age	**<45years**	113	136	
	>45years	135	124	0.13
Menopausal status	**premenopausal**	85	112	
	postmenopausal	165	148	0.04
Family History	**No**	212	225	0.66
	Yes	38	35	
Use of oral contraceptives	**No**	225	222	
	Yes	25	38	0.14
Partiy	**Nulliparorus**	4	6	0.80
	parous	246	254	
Marital status	**Un married**	22	26	0.75
	married	228	234	
Smoking status	**Smoker**	10	14	
	Nonsmoker	240	246	0.60
Dwelling	**Rural**	170	156	0.07
	Urban	80	104	

Table 2: Representing genotypic frequency of genotypes and alleles

Genotype	**Case n=250**	**Control =260**	χ^2	**OR**	**CI**	**P**
Val/Val	52(20.8)	63(24.23)	-	Referent	-	-
Val/Ala	137(54.8)	128(49.23)	1.10	1.30	0.83-2.01	0.30
Ala/Ala	61 (24.4)	69(26.53)	0.02	1.07	0.64-1.77	0.88
Val/Ala+Ala/Ala	198(79.2)	197(75.76)	0.67	1.21	0.80-1.84	0.41
Val	241 (48.2)	254 (48.8)	-	Referent	-	-
Ala	259 (51.8)	266 (51.1)	0.02	1.02	0.80-1.31	0.88

When we combined the MnSOD (Val/Ala) and Val/Val genotype to compare these with Val/Val genotype (OR:

1.27; CI: 0.80-1.84). We found no association between genotype and breast cancer. Association between breast cancer and pautative risk factors by MnSOD genotypes are presented in table 3.there was significant evidence that older women (above 45years of age) carrying the genotype Val/Ala were at risk for breast, also ORs were elevated in case of women with Val/Ala genotype who used oral contraceptives and resided in urban areas. However, the presence of Ala alleles was not associated with other pautative risk factors for breast cancer such as menopausal status, marital status, smoking, parity, family history (Table 3).

Table3: Representing clinicopathological characteristics and MnSOD Val-9Ala polymorphism with respect to breast cancer

Risk factors		Genotype			
		Mnsod Val/Val	Mnsod Val/Ala	Mnsod Ala/Ala	Val/Val+Val/Ala
Menopausal status	Pre menopausal	1.0(ref) 22/33	1.27(0.64-2.48) 44/52	1.05(0.47-2.34) 19/27	1.20(0.63-2.25) 63/79
	Postmenopausal	1.0(ref) 30/30	1.22(0.67-2.20) 93/76	1.0(0.51-1.94) 42/42	1.14(0.65-2.01) 135/118
Age	<45Years	1.0(ref) 25/30	0.81(0.43-1.54) 51/75	1.43(0.70-2.92) 37/31	0.99(0.54-1.81) 88/106
	>45years	1.0(ref) 27/33	1.98(1.07-3.66) 86/53	0.77(0.35-1.58) 24/38	1.47(0.82-2.63) 110/91
Family history	No	1.0(ref) 42/52	1.32(0.82-2.14) 122/114	1.0(0.57-1.76) 48/59	1.21(0.77-1.92) 170/173
	yes	1.0(ref) 10/11	1.18(0.38-3.62 15/14	1.43(0.43-4.69) 13/10	1.28(0.46-3.54) 28/24
Use of oral contraceptives	No	1.0 (ref) 47/52	1.19(0.74-1.89) 128/119	1.08(0.62-1.89) 50/51	1.15(0.74-1.81) 178/170

	yes	1.0 05/11	2.20(0.54-8.96) 09/09	1.34(0.36-4.91) 11/18	1.63(0.48/5.44) 20/27
Parity	Nulliparous	1.0 01/01	0.66(0.02-18.07) 02/03	0.50(0.01-19.58) 01/02	0.60(0.02-13.59) 03/05
	Parous	1.0 51/62	1.31(0.84-2.04) 135/125	1.09(0.65-1.81) 60/67	1.23(0.81-1.88) 195/192
Marital status	Unmarried	1.0 05/06	0.80(0.19-3.35) 10/15	1.68(0.32-8.76) 07/05	1.02(0.26-3.94) 17/20
	Married	1.0 47/57	1.36(0.85-2.16) 127/113	1.02(0.60-1.73) 54/64	1.24(0.80-1.92) 181/177
Smoking status	Never	1.0 49/58	1.30(0.82-2.03) 133/122	1.04(0.62-1.74) 58/66	1.20(0.78-1.85) 191/188
	Ever	1.0 03/05	1.11(0.16-7.51) 04/06	1.66(0.19-14.27) 03/03	1.30(0.22-7.38) 07/09
Dwelling	Rural	1.0 37/32	0.91(0.53-1.60) 101/95	0.95(0.47-1.90) 32/29	0.92(0.54-1.58) 133/124
	urban	1.0 15/31	2.25(1.03-4.90) 36/33	1.50(0.68-3.27) 29/40	1.84(0.91-3.71) 65/73

4. Discussion

In order to evaluate the possible influence of the MnSOD Ala-9Val polymorphism on breast cancer risk, we carried out a case control study of 510 individuals (250 case and 260 controls) furthermore, we investigated

whether pautative risk factors influence breast cancer development. MnSOD polymorphic alleles are widely variable with ethinicity. The frequency of Ala allele is 12% among Japenese [22] and 14% among Chinese [23] where as it is more common in Caucasian population (41.55%). While as in our study, frequency of Ala allele was 50% which is comparable to that of Caucasian population. Similar finding was reported by [24] in Jordanian population. Our study yielded statistically positive association in case of older women. Also ORs were elevated in case women who used oral contraceptives with urban dwelling. These finding are in consistent with that reported in Finnish Caucasian population [16] and American population [25]. Previous epidemiological studies have shown conflicting. Previous epidemiological studies have shown conflicting result, numerous studies as carried by [16,26,27] reported positive association of this SNP with breast cancer risk, on the other hand some studies as performed by [28,25,29] didn't find any association between MnSOD polymorphism and breast cancer risk. These results were similar to those of our study. Futhermore no significant modification of ORs for MnSOD Ala/Ala genotype by smoking status was in consistent with the results as reported by [30 31], these apparently contradictory results could be analyzed considering biologically plausibility and the gene environment and gene gene interactions influence. Some studies reported possible gene-gene association such as performed by [32] that described interactions of MnSOD and glutathione peroxidase (GPX-I) gene polymorphism associated with breast cancer risk. These results postulate that the occurrence of substantial interaction with pro or anti oxidant properties exposures (from environment or genetic origin) could interfere in MnSOD polymorphism with breast cancer association.

5. Conclusion

In conclusion our study didn't find any significant association between MnSOD polymorphism and breast cancer, however, certain factors like age, oral contraceptives, lifestyle may modify the risk. Small sample size and focusing on only polymorphism were the main limitations of this study. In order to reach any substantive results, additional studies of MnSOD polymorphism with larger sample size, which can offer sufficient statistical power are needed to further validate the findings and which may help to find out the mechanisms to reduce risk of breast cancer, before any strict conclusions can be drawn on this issue.

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