RESEARCH ARTICLE

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Superoxide Dismutase (*rs2070424*, *rs4880*, *rs2536512*) and Catalase (*rs794316*, *rs1001179*) SNPs and their Association with Breast Cancer Risk: Findings from a Hospital Based Case-Control Study

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Abstract

Background: The antioxidant enzymes are important cellular components involved in detoxification of reactive oxygen species (ROS) and protect cells from ROS induced oxidative damage. Single nucleotide polymorphisms (SNPs) of antioxidant enzyme coding genes such as superoxide dismutase (SOD) and catalase (CAT) may alter the enzyme activity which can influence susceptibility towards carcinogenesis. Therefore, the present study was planned to investigate possible SNPs of SOD (SOD1 (Cu,Zn-SOD), SOD2(Mn-SOD), SOD3(EC-SOD) and CAT genes and their possible association with breast cancer risk in rural Indian women. **Methods:** In this case-control study, the association of SOD and CAT gene polymorphism was studied by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method. The study was conducted among 400 clinically breast cancer patients and 400 healthy women in a population of South-Western Maharashtra. The logistic regression analysis was carried out to calculate Odds ratio (OR) with 95% confidence interval and p-value, where $p \leq 0.05$ was considered as statistically significant. **Results:** The results of analysis of genotype frequency distribution showed significant association of *rs4880* SNP of Mn-SOD with BC risk at homozygous variant (CC/CC) genotype (OR 2.46; 95%CI, 1.61-3.75; $p<0.0001$) and corresponding frequency of variant (C) allele (OR 1.53; 95%CI, 1.25-1.86; $p<0.0001$). In CAT gene polymorphisms the variant (T/T) was increased significantly in BC cases as compared to controls (OR 3.45; 95%CI, 2.17-5.50; $p<0.0001$) along with its variant (T) allele (OR 2.01; 95%CI, 1.63-2.48; $p<0.0001$). **Conclusions:** The results implied that, C/C genotype of SOD2-1183T/C polymorphism and T/T genotype of CAT-262 C/T polymorphism may be associated with an increased breast cancer risk. However, SOD1-251 A/G and SOD3-172 G/A polymorphisms did not show any significant difference in variant homozygous genotypes of patients compared to controls.

Keywords: Breast Cancer- Superoxide dismutase- Catalase- Single nucleotide polymorphism- PCR-RFLP

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Introduction

Breast cancer (BC) prognosis is very poor in low and middle income countries which led to alarming increase of BC incidence in developing countries. The prevalence of BC in rural India is comparatively high where lack of knowledge, high use of tobacco, illiteracy unhealthy diet, limited resources for early detection and treatment are intensively described as responsible factors for BC carcinogenesis. Breast cancer genetics is heterogeneous and more complex where several genetic factors are linked with increased risk of carcinogenesis however, there remained challenge to identify more precise mechanism involved in BC development. Genetic alterations in brca1 and brca 2 genes are commonly studied for their association with BC. Along with, there are number of other host genetic factors associated with increased risk of BC as well as other types of cancer. Genetic polymorphisms of DNA damage/repair and tumor suppressor pathway genes are commonly studied for their association with cancer risk. Single nucleotide polymorphisms (SNPs) are the most common genetic variations in human genome located in different region of genes. These SNPs can regulate

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oxidative stress, DNA repair, cell cycle, metabolism and regulate individual's genetic susceptibility towards cancer [1-5]. Reactive oxygen species (ROS) are formed as a consequence of oxidative stress which alter normal cell function and harm cellular machinery which ultimately promote carcinogenesis [6]. In normal cells antioxidative defense system is activated to overcome the deleterious effect of ROS. An antioxidant enzyme system such as super oxide dismutase (SOD) and catalase (*CAT*) plays an important role in limiting the adverse effects of oxidative stress. The SOD and *CAT* genes have been considered as candidate genes for cancer susceptibility because inactivated enzymes may alter efficient radical scavenging activity and ROS detoxification which are harmful to the cells. Genetic variations including SNPs of antioxidant enzyme coding genes may contribute to cancer development [7-10]. Different studies evaluated association of SNPs of both SOD and *CAT* genes with different cancers, however the results were inconclusive and warranted further research for validation with larger populations [11, 12].

Limited studies from India determined association of SNPs of antioxidant enzyme genes with cancer risk [7, 13, 14] but the reports on SNPs of either SOD or *CAT* genes are missing to prove their association with BC risk. Therefore we designed a study to explore the association of polymorphisms of SOD i.e, SOD1 (Cu, Zn-SOD), SOD2 (Mn-SOD), SOD3 (EC-SOD) and *CAT* genes with development of BC in rural women of Maharashtra along with other clinical factors. We genotyped three SNPs of SOD gene; (A251G at codon 251 of the exon 10 of SOD1 (*rs2070424*), T1183C at codon 1183 of exon 2 of SOD2 (*rs4880*) and G172A of the exon 3 of SOD3 (*rs2536512*) and two SNPs of *CAT* gene (A326T at codon 326 of exon 7 (*rs7943316*) and C262T at 262 region of promoter of *CAT* gene (*rs1001179*) from 400 BC patients and 400 healthy control women to evaluate their association with BC risk in women from rural areas of South-Western Maharashtra of India.

Materials and Methods

Selection of study subjects

A hospital based case-control study was conducted with 400 histopathologically confirmed breast cancer patients at Department of Oncology of Krishna Hospital & Medical Research Centre (KH&MRC), Karad, Maharashtra. Equal number of healthy, disease free, age matched female controls were randomly selected from a group of women visiting to a tertiary care hospital for other purposes. All cases ranged in age from 23-85 years (Mean ± SD; 52.43 ±12.37) were sequentially enrolled immediately after diagnosis during the year 2016-2020. Inclusion critera: Patients with 23 to 85 years age, histopathologically confirmed, no metastasis at diagnosis were included in this study. Exclusion criteria: No pathological diagnosis, relapsed disease or metastasis, severe co-morbidities, missing or incomplete data, cases already receiving treatment for malignancy were excluded from this study. The patients were communicated regarding the purpose of their involvement in the study protocol. Informed consent was obtained from the participants for their participation in the study. Trained interviewers used a predefined proforma to collect demographic and clinicopathological data from the participants along with examination findings. The study protocol was approved by Institutional Ethics Committee of Krishna Vishwa Vidyapeeth (Deemed to be University) for utilization of human subjects in the research.

Sample collection and Genomic DNA isolation from whole blood

Five milliliter (mL) of whole blood samples from 400 patients and 400 controls was collected in sterile EDTA containing vacutainer after receiving informed consent. Genomic DNA extraction was carried out from the peripheral blood sample using HipurA®Blood genomic DNA miniprep purification kit. (Cat no. MB504-250PR) (HiMedia Laboratories) following the manufacturer's instructions. This pure genomic DNA was used for genotyping assays by polymerase chain reaction (PCR) and Restriction fragment Length Polymorphism (RFLP).

Genotyping assays

Genotyping of SOD and *CAT* gene was performed by PCR-RFLP. A total of 20 microliter (µL) of PCR reaction mixture consisted of 0.2 µg of genmic DNA, 1X PCR buffer containing Tris HCl (pH.8), KCL, EDTA, DTT, 25mM MgCl2, 0.2 mM each dNTPs, 1U of Taq DNA polymerase (Bangalore GeNei) and 10 picomole of appropriate primer sets presented in Table 1. The PCR amplification of SOD and *CAT* genes were performed in a Master Cycler Gradient PCR machine (Eppendorf India Limited) with PCR conditions for amplification as prescribed bellow: The amplification conditions for PCR of SOD1 codon 251 of 570 bp, (denaturation at 95^0C- 5 min, 30 cycles of 95^0C- 30 sec, 58^0C- 30 sec, 72^0C- 30 sec and final extension at 72^0C- 5 min), SOD2 codon 16 of 208 bp, 95^0C- 5 min, 30 cycles of 95^0C- 20 sec, 57^0C- 30 sec, 72^0C- 20 sec, 72^0C- 5 min and SOD3 codon 172 of 245 bp (95^0C- 5 min, 35 cycles of 95^0C- 30 sec, 65^0C- 30 sec, 72^0C- 45 sec and 72^0C- 5 min). The PCR conditions for amplification of 250 bp of codon 326 of *CAT* gene; (95^0C- 5 min, 35 cycles of 95^0C- 30 sec, 55^0C- 30 sec, 72^0C- 30 sec and 72^0C- 5 min) and 185 bp promoter region of *CAT* gene (95^0C- 5 min, 30 cycles of 95^0C- 20 sec, 66^0C- 20 sec, 72^0C- 25 sec and 72^0C- 5 min). After confirmation of PCR amplification on agarose gel electrophoresis, the RFLP analysis for the studied alleles of (*SOD1, SOD2, SOD3, CAT1* and *CAT2*) were carried out with the help of MspI, BsaW1, BssHII, HinFI and SmaI restriction enzymes respectively. Following the restriction digestion of PCR products, the restriction products were separated on 2-3% low EEO agarose (GeNei, Merck Biosciences) gel according to the fragment sizes, stained with ethidium bromide and photographed with gel documentation system (BioRad Laboratories). The variant and wild type genotypes were analyzed based on their restriction digestion pattern (Table 1).

Statistical analysis

The association between the SOD, *CAT* genotypes

Table 1. The List of Candidate Genes Selected in the Present Study with Details of PCR and RFLP Procedures Including Primers Restriction Enzymes and Expected Products of Selected Genes.

Gene	Genotype	rs number	Nucleotide change	Primer Sequence Forward/Reverse	PCR product size	Enzyme / Digestion conditions	Dominant (Wild type)	Heterozygous	Recessive (Mutant)
SOD1	codon-251 exon-10 (A251G)	rs2070424	(A>G)	FP: 5'-AGT ACT GTC AAC CAC TAG CA-3' RP: 5'-CCA GTG TGC GGC CAA TGA TG-3'	570 bp	MspI 37°C for 16h	570 bp	570 bp, 369 bp, 201 bp	369 bp, 201 bp
SOD2	codon-16 exon-2 (T1183C)	rs4880	(T>C)	FP: 5'- GCT GTG CTT TCT CGT CTT CAG-3'; RP: 5'-TGG TAC TTC TCC TCG GTG ACG-3'	208 bp	BsaW1 60°C for 16h	167 bp, 41 bp	208 bp 167 bp, 41 bp	208 bp
SOD3	codon-40 exon-3 (G172A)	rs2536512	(G>A)	FP: 5'-GAC ATG TAC GCC AAG GTC AC-3' RP: 5'-AAC TGG TGC ACG TGG ATG-3'	245 bp	BssHII 37°C for 16h	183 bp, 62 bp,	245 bp, 183 bp, 62 bp,	245 bp
Catalase	codon-326 exon-7 (A21T)	rs7943316	(A>T)	FP: 5'-AAT CAG AAG GCA GTC CTC CC-3' RP: 5'-TCG GGG AGC ACA GAG TGT AC-3'	250 bp	HinFI 37°C for 16h	177 bp, 73 bp	250 bp, 177 bp, 73 bp	250 bp
Catalase	262 region of promoter (C262T)	rs1001179	(C>T)	FP: 5'-AGA GCC TCG CCC CGC CGG ACC G-3' RP: 5'-' TAA GAG CTG AGA AAG CAT AGC T-3'	185 bp	SmaI 37°C for 16h	155 bp, 30 bp	185 bp, 155 bp, 30 bp	185 bp

and risk of developing BC were studied by logistic regression analysis model which was used to calculate the Odds ratio (OR) and 95% confidence intervals (CI) with adjustment of variables to determine the BC risk associated with genotypes. All p values were two-sided and differences were considered statistically significant for $p \leq 0.05$. All statistical analyses were performed with SPSS (Version 11.0) software.

Results

Comparison of selected demographic and clinicopathological characteristics of study subjects

The age of BC patients ranged from 23-85 years (Mean ± SD: 52.43 ±12.37; Median age, 50yrs) with majority of patients (322) were >40 years age group, whereas healthy control females ranged from 24-81 yrs (Mean ± SD: 42.37 ±13.90; Median age 40yrs). The occurrence of BC frequency was high among the individuals more than 40 years of age (80.50%) than those of patients less than 40 years (19.50%). When age of the first pregnancy was compared, we interestingly observed higher incidence of BC (54.50%) in age of ≤20 years. 93.25% women were from poor economic status from rural communities with 79% women with mixed (vegetarian and non-vegetarian) diet. The studied population with 10.25 % women showed family history of cancer. When we confirmed the status of tobacco chewing habit for their involvement in cancer association we noticed significant correlation (OR 3.07; 95%CI, 2.29-4.12; p<0.0001) with BC development in women of rural population. Along with demographic characteristics, clinicopathological features including hormone receptor status, histological subtypes, histological grade, tumor localization, tumor size were recorded for BC patients.

Comparative analysis genotypic distribution of superoxide dismutase and catalase genes in breast cancer cases and controls

The genotypic distribution of antioxidative pathway genes including superoxide dismutase and catalase were determined from 400 cases and equal number of controls is summarized in Table 2. The SNPs of SOD with Cu, Zn-SOD, Mn-SOD and EC-SOD isoforms was studied and the results of genotype distribution showed significant association of *rs4880* SNP of Mn-SOD with BC risk at both homozygous CC/CC genotype (OR 2.46; 95%CI, 1.61-3.75; p<0.0001) and heterozygous TT/CC genotypes (OR 2.13; 95%CI, 1.51-3.75; p<0.0001). The corresponding allele frequency of variant (C) allele showed significant correlation with BC risk (OR 1.53; 95%CI, 1.25-1.86; p<0.0001). The combined TT/CC+CC/CC genotypes also showed significant association with BC risk (OR 2.21; 95%CI, 1.60-3.07; p<0.0001). Similarly combined GG/AA+AA/AA genotype of EC-SOD (*rs2536512*) showed association (OR 1.56; 95%CI, 1.14-2.14; p=0.005) with BC risk in the studied population. When genotypic polymorphism of *CAT* gene with two common SNPs (*rs7943316*, *rs1001179*) was studied, we noted that variant (T/T) genotype of *CAT* (*rs1001179*) was increased significantly

Table 2. Distribution of Genotype and Allele Frequencies of SOD and CAT Gene Polymorphisms in Untreated Breast Cancer Cases and Healthy Controlsa

Gene name (SNP)	Genotype/ Allele	Cases (n= 400) (%)	Control (n =400) (%)	OR (95% CI)	P value
SOD1	AA / AA	262 (65.50)	255 (63.75)	1 (Reference)	
Cu,Zn-SOD	AA / GG	118 (29.50)	125 (31.25)	0.91 (0.67-1.24)	0.586
(A251G)	GG/ GG	20 (5.00)	20 (5.00)	0.97 (0.51-1.85)	0.934
exon-10	AA /GG+GG/GG	138 (34.50)	145 (36.25)	0.92 (0.69-1.23)	0.604
rs2070424	A allele	642 (80.25)	635 (79.38)	1 (Reference)	
	G allele	158 (19.75)	165 (20.62)	0.91 (0.71-1.17)	0.494
SOD2	TT / TT	74 (18.50)	134 (33.50)	1 (Reference)	
Mn-SOD	TT / CC	232 (58.00)	197 (49.25)	2.13 (1.51-3.75)	<0.0001*
(T1183C)	CC / CC	94 (23.50)	69 (17.25)	2.46 (1.61-3.75)	<0.0001*
exon-2	TT /CC+CC /CC	326 (81.50)	266 (66.50)	2.21 (1.60-3.07)	<0.0001*
rs4880	T allele	380 (47.50)	465 (58.12	1 (Reference)	
	A allele	420 (52.25)	335 (41.88)	1.53 (1.25-1.86)	<0.0001*
SOD3	GG / GG	90 (22.50)	125 (31.25)	1 (Reference)	
EC-SOD	GG / AA	239 (59.75)	201 (50.25)	1.65 (1.18-2.29)	0.002*
(G172A)	AA / AA	71 (17.75)	74 (18.50)	1.33 (0.87-2.03)	0.184
exon-3	GG /AA+AA / AA	310 (77.50)	275 (68.75)	1.56 (1.14-2.14)	0.005*
rs2536512	G allele	419 (52.38)	451 (56.38)	1 (Reference)	
	A allele	381 (47.62)	349 (43.62)	1.17 (0.96-1.43)	0.018
Catalase	AA/AA	128 (32.00)	153 (38.25)	1 (Reference)	
(A21T)	AA/TT	229 (57.25)	194 (48.50)	1.41 (1.04-1.91)	0.025*
exon-7 rs7943316	TT/TT	43 (10.75)	53 (13.25)	0.96 (0.60-1.54)	0.897
	AA/TT + TT/TT	272 (68.00)	247 (61.75)	1.31 (0.98-1.76)	0.064
	A Allele	485 (60.62)	500 (62.50)	1 (Reference)	
	T Allele	315 (39.38)	300 (37.50)	1.08 (0.88-1.32)	0.44
Catalase	CC/CC	113 (28.25)	210 (52.50)	1 (Reference)	
(C262T)	CC/TT	220 (55.00)	154 (38.50)	2.65 (1.95-3.61)	<0.0001*
rs1001179	TT/TT	67 (16.75)	36 (9.00)	3.45 (2.17-5.50)	<0.0001*
	CC/TT + TT/TT	287 (71.75)	190 (47.50)	2.80 (2.09-3.76)	<0.0001*
	C Allele	446 (55.75)	574 (71.75)	1 (Reference)	
	T Allele	354 (44.25)	226 (28.25)	2.01 (1.63-2.48)	<0.0001*

SNP, Single nucleotide polymorphism; OR, Odds ratio, CI, Confidence interval; Significance p< 0.05; *, Indicates significant Odds Ratio (p<0.05), p value determined based on $\chi 2$

in BC cases as compared to controls (OR 3.45; 95%CI, 2.17-5.50; p<0.0001) which indicated significant involvement of (T) allele in BC risk (OR 2.01; 95%CI, 1.63-2.48; p<0.0001). The combined C/T +T/T genotype presented significant association with BC risk (OR 2.80; 95%CI, 2.09-3.76; p<0.0001) among rural women of South-Western Maharashtra. When the polymorphism of variant genotypes of both SOD and *CAT* genes with BC risk in the recessive genotype model, we noted significant correlation of Mn-SOD (*rs4880*), (OR 1.47; 95%CI, 1.04-2.08; p=0.028 and *CAT* (*rs1001179*), (OR 2.03; 95%CI, 1.32-3.13; p=0.001) with BC risk (Table 3). The dominant model also showed significant association of Mn-SOD (*rs4880*), (OR 2.21; 95%CI, 1.60-3.07; p<0.0001) and EC-SOD (*rs2536512*), (OR 1.56; 95%CI, 1.14-2.14; p=0.005) with BC risk. Similarly *rs1001179* SNP of *CAT* gene also showed significant with BC risk (OR 2.80; 95%CI, 2.09-3.76; p<0.0001) as per dominant model (Table 4).

Correlation between superoxide dismutase and catalase genotypes and clinicopathologic characteristics among breast cancer cases

When we analyzed correlation of both SOD and *CAT* genotypes with clinicopathologic characterstics among 400 BC cases, we observed that there were no significant correlations between any of the SOD isoforms except Mn-SOD. When we investigated association of geneotypes of SOD and *CAT* genes with expression of hormore receptors in BC cases, we observed significant association of Mn –SOD (*rs4880*) with levels of Her2 in BC cases with $X^2 = 4.38$; p=0.036. We observed no significant differences between any of the genotypes of *CAT* genes and hormone receptor status in BC patients (Table 5).

Table 3. Association between Breast Cancer Risk and the Single Nucleotide Polymorphism Variant of SOD and CAT Genes in the Recessive Model

Genes SNP	Genotype	Cases (n= 400) (%)	Control (n =400) (%)	OR (95% CI)	P value
SOD1 Cu, Zn-SOD	AA / AA + AA / GG	380 (95.00)	380(95.00)	1 (Reference)	
rs2070424	GG/ GG	20 (5.00)	20 (5.00)	1.00 (0.52-1.88)	1
SOD2	TT / TT + TT / CC	306 (76.50)	331 (82.75)	1 (Reference)	
Mn-SOD					
rs4880	CC / CC	94 (23.50)	69 (17.25)	1.47 (1.04-2.08)	0.028*
SOD3	GG / GG + GG / AA	329 (82.25)	326 (81.50)	1 (Reference)	
EC-SOD					
rs2536512	AA / AA	71 (17.75)	74 (18.50)	0.95 (0.66-1.36)	0.783
Catalase	AA/AA + AA/TT	357 (89.25)	347 (86.75)	1 (Reference)	
rs7943316	TT/TT	43 (10.75)	53 (13.25)	0.78 (0.51-1.21)	0.277
Catalase	CC/CC + CC/TT	333 (83.25)	364 (91.00)	1 (Reference)	
rs1001179	TT/TT	67 (16.75)	36 (9.00)	2.03 (1.32-3.13)	0.001*

SNP, Single nucleotide polymorphism; OR, Odds ratio, CI, Confidence interval; Significance p< 0.05; *, Indicates significant Odds Ratio (p<0.05), p value determined based on $\chi 2$

Table 4. Association between Breast Cancer Risk and the Single Nucleotide Polymorphism Variant of SOD and CAT Genes in the Dominant Model

Genes SNP	Genotype	Cases (n= 400) (%)	Control (n =400) (%)	OR (95% CI)	P value
SOD1 Cu,Zn-SOD	AA / AA	262 (65.50)	255 (63.75)	1 (Reference	
rs2070424	AA / GG +GG/ GG	138 (34.50)	145 (36.25)	0.92 (0.69-1.23)	0.604
SOD2	TT / TT	74(18.50)	134 (33.50)	1 (Reference)	
Mn-SOD					
rs4880	TT /CC+CC /CC	326 (81.50)	266 (66.50)	2.21 (1.60-3.07)	<0.0001*
SOD3	GG / GG	90 (22.50)	125 (31.25)	1 (Reference)	
EC-SOD					
rs2536512	GG /AA+AA / AA	310 (77.50)	275 (68.75)	1.56 (1.14-2.14)	0.005*
Catalase	AA/AA	128 (32.00)	153 (38.25)	1 (Reference)	
rs7943316	AA/TT + TT/TT	272 (68.00)	247 (61.75)	1.31 (0.98-1.76)	0.064
Catalase	CC/CC	113 (28.25)	210 (52.50)	1 (Reference)	
rs1001179	CC/TT + TT/TT	287 (71.75)	190 (47.50)	2.80 (2.09-3.76)	<0.0001*

SNP, Single nucleotide polymorphism; OR, Odds ratio, CI, Confidence interval; Significance p< 0.05; *, Indicates significant Odds Ratio (p<0.05), p value determined based on $\chi 2$

Correlation of superoxide dismutase and catalase gene polymorphisms with confounding factors associated with breast cancer risk

In order to identify an interaction of demographic variables including age of cancer occurrence, age of first pregnancy and tobacco smoking habits, the genotypic distribution of antioxidant pathway SOD and *CAT* genes were studied among 400 BC cases and 400 controls. The results of stratification of confounding factors and their correlation with antioxidant gene polymorphisms among BC cases and controls are summarized in Table 6. When we stratified the genotypic distribution according to the age of cancer occurrence, we observed significant correlation of heterozygoys AA/GG + GG/GG genotype of *rs4880* SNP of Mn-SOD with BC risk in cases with age of ≤ 40 years (OR=2.81; 95% CI: 1.50- 5.26; p=0.001). Similarly both *rs7943316* and *rs1001179* SNPs of *CAT* genes showed significant association with BC risk in cases with ≤ 40 years age of cancer occurrence. The *rs1001179* SNP of *CAT* gene also showed significant correlation with BC risk in the patients with >40 years age. According to the results of logistic regression analysis the *rs2070424* SNP of Cu, Zn-SOD was negatively associated with risk of BC in A/G genotype status in the subjects stratified with ≤ 20 age of first pregnancy (OR=0.56; 95% CI: 0.37- 0.84; p=0.005) whereas *rs4880* (OR=3.41; 95% CI: 2.18- 5.35; p<0.0001) and *rs2536512* (OR=2.34; 95% CI: 1.51- 3.64; p<0.0001) SNPs showed significant association with BC development in subjects with ≤ 20 age of first pregnancy. The *rs1001179* SNP of *CAT* gene was significantly associated with BC risk in cases (OR=6.88; 95% CI: 4.25- 10.88; p<0.0001) with >20 years age of first pregnancy. When we studied correlation of SNPs of SOD and *CAT* genes with tobacco

Table 5. Association between SOD, CAT Genotypes and Clinicopathologic Characteristics Including Hormone Receptor Status among Breast Cancer Cases

Variables	Total (n)	Genotypes			
SOD1 (rs2070424)		AA/AA (262)	AA/GG (118)	GG/GG (20)	X2 (p value)
ER Status					
Positive	200	131 (50.00)	59 (50.00)	10 (50.00)	0.023
Negative	166	110 (41.98)	50 (42.38)	6 (30.00)	(0.877)
Unknown	34	21 (8.02)	9 (7.62)	4 (20.00)	
PR Status					
Positive	181	117 (44.66)	52 (44.07)	12 (60.00)	0.108
Negative	185	125 (47.71)	56 (47.46)	4 (20.00)	(0.742)
Unknown	34	20 (7.63)	10 (8.47)	4 (20.00)	
Her2 Status					
Positive	56	40 (15.27)	15 (12.71)	1 (5.00)	1.012
Negative	310	199 (75.95)	93 (78.81)	18 (90.00)	(0.314)
Unknown	34	23 (8.78)	10 (8.48)	1 (5.00)	
SOD2 (rs4880)		TT/TT (74)	TT/CC (232)	CC/CC (94)	X2 (p value)
ER Status					
Positive	200	35 (47.30)	119 (51.30)	46 (48.94)	0.0001
Negative	166	29 (39.19)	91 (39.22)	46 (48.94)	(0.991)
Unknown	34	10 (13.51)	22 (9.48)	2 (2.12)	
PR Status					
Positive	181	33 (44.60)	108 (46.55)	40(42.56)	0.015
Negative	185	31 (41.89)	102 (43.97)	52 (55.32)	(0.901)
Unknown	34	10 (13.51)	22(9.48)	2 (2.12)	
Her2 Status					
Positive	56	16 (21.62)	25 (10.78)	15 (15.96)	4.38
Negative	310	49 (66.22)	185 (79.74)	76 (80.85)	(0.036)*
Unknown	34	9 (12.16)	22 (9.48)	3 (3.19)	
SOD3 (rs2536512)		GG/GG (90)	GG/AA (239)	AA/AA (71)	
ER Status					
Positive	200	46 (51.11)	120 (50.21)	34 (47.89)	0.057
Negative	166	34 (37.78)	101 (42.26)	31 (43.66)	(0.81)
Unknown	34	10 (11.11)	18 (7.53)	6 (8.45)	
PR Status					
Positive	181	42 (46.67)	106 (44.36)	33 (46.48)	0.094
Negative	185	38 (42.22)	115 (48.11)	32 (45.07)	(0.759)
Unknown	34	10 (11.11)	18 (7.53)	6 (8.45)	
Her2 Status					
Positive	56	10 (11.11)	39 (16.32)	7 (9.86)	0.805
Negative	310	72 (80.00)	180 (75.31)	58 (81.69)	(0.369)
Unknown	34	8 (8.89)	20 (8.37)	6 (8.45)	
Catalase (rs7943316)		AA/AA (128)	AA/TT (229)	TT/TT (43)	X2 (p value)
ER Status					
Positive	200	64 (50.00)	113 (49.35)	23 (53.49)	2.658
Negative	166	55 (42.97)	95 (41.48)	16 (37.21)	(0.103)
Unknown	34	9 (7.03)	21 (9.17)	4 (9.30)	
PR Status					
Positive	181	57 (44.53)	100 (43.67)	24 (55.81)	0.244
Negative	185	62 (48.44)	108 (47.16)	15 (34.89)	(0.621)
Unknown	34	9 (7.03)	21 (9.17)	4 (9.30)	

Table 5. Continued

Variables	Total (n)	Genotypes			
Catalase (rs7943316)		AA/AA (128)	AA/TT (229)	TT/TT (43)	X2 (p value)
Her2 Status					
Positive	56	21 (16.41)	29 (12.66)	6 (13.96)	0.649
Negative	310	97 (75.78)	180 (78.60)	33 (76.74)	(0.42)
Unknown	34	10 (7.81)	20 (8.73)	4 (9.30)	
Catalase (rs1001179)		CC/CC (113)	CC/TT (220)	TT/TT (67)	X2 (p value)
ER Status					
Positive	200	57 (50.43)	111 (50.45)	32 (47.76)	0.012
Negative	166	45 (44.25)	92 (41.82)	29 (43.28)	(0.911)
Unknown	34	11 (9.73)	17 (7.73)	6 (8.96)	
PR Status					
Positive	181	52 (46.02)	102 (46.36)	27 (40.30)	0.037
Negative	185	50 (44.25)	101 (45.91)	34 (50.75)	(0.846)
Unknown	34	11(9.73)	17 (7.73)	6 (8.95)	
Her2 Status					
Positive	56	18 (15.93)	32 (14.45)	6 (8.96)	0.141
Negative	310	84 (74.34)	172 (78.18)	54 (80.60)	(0.706)
Unknown	34	11 (9.73)	16 (7.27)	7 (10.44)	

ER, Estrogen Receptor; PR, Progesterone Receptor; Her2, Human epidermal growth factor receptor 2, Significance p< 0.05; p value determined based on $\chi 2$

smoking habit, the heterozygous and variant genotype combination of Mn-SOD (*rs4880*) (OR=1.83; 95% CI: 1.09- 3.08; p=0.021) and *rs1001179* SNP of *CAT* gene (OR=2.13; 95% CI: 1.32- 3.42; p=0.001) showed strong association with BC development in tobacco smokers.

Discussion

Breast cancer is the most common cancer in women comprising one third of all cancer types occurring in females which remained a challenge for researchers all over the world. Oxidative stress is one of the most important factors considered in breast carcinogenesis because the reactive oxygen species (ROS) released from it causes intracellular DNA damage which may lead to cancer development. The antioxidant enzyme SOD and *CAT* genes play an important role in scavenging of ROS and protect the cells against its deleterious effects. The genetic alterations in antioxidant enzyme genes may lead to gain or loss of functions of antioxidant enzymes such as SOD and *CAT* which has become important to understand the development of cancer [9, 10]. The genetic polymorphisms in SOD and *CAT* genes have been studied earlier for their association with several diseases [15-17]. According to various research, several studies evidenced an association of polymorphisms of SOD and *CAT* genes with increased susceptibility of various type of cancers including prostate [18, 19], cervical [20, 14], lung [9] and colorectal cancer [21, 22]. However, other epidemiological studies reported conflicting results stating no association of polymorphism in these genes with cancer development in different population [23-26]. Some researchers reported association of polymorphisms of SOD and *CAT* genes with BC risk in different ethnic groups [27-29], whereas others with contradictory opinions with no association between either of SOD or *CAT* gene polymorphisms with risk of BC [30, 31]. Studies on association of polymorphisms of antioxidant enzyme coding genes with BC susceptibility are lacking from India. Therefore, in present study we attempted to investigate polymorphisms of SOD and *CAT* genes and to identify their possible association with BC risk in women residing to the rural areas of Maharashtra.

The selected study population was investigated earlier for relationship of different SNPs of various pathway genes with cancer risk, we observed significant association of rs1801270 SNP of p21 gene with BC risk [32], similarly, rs25489 and rs25487 SNPs of *XRCC1*; rs1056836 SNP of metabolic CYP1B1*3 and rs6413432 SNP of CYP2E1*6 genes were associated with cervical cancer risk [33-35] and rs743572 SNP of CYP17 was associated with increased risk of cervical cancer in the rural women population [36]. The same women population was screened for polymorphisms of SOD and *CAT* genes and their correlation with BC risk. We studied the polymorphisms of superoxide dismutase (SOD1: codon-251 of exon 10; SOD2: codon-16 of exon 2; SOD3: codon-40 of exon 3) and codon-326 of exon 7 and C>T transition occurring at 262 region of the promoter of *CAT* gene. The polymorphism studies resulted into an association of 1183CC homozygous variant genotype of Mn-SOD and 262TT genotype of *CAT* gene with BC susceptibility. The *rs4880* SNP of Mn-SOD with homozygous variant allele (OR=1.53; 95% CI: 1.25-1.86, p<0.0001) and *rs1001179* SNP of *CAT* gene with variant allele (OR=2.01; 95% CI: 1.63-2.48, p<0.0001) were significantly associated with BC risk when studied with dominant and recessive model. Our findings are in accordance with previous reports where variant genotype

Table 6. Association of SOD and CAT Gene Variants with Demographic Variables Including age of Cancer Occurrence, Age at First Pregnancy and Tobacco Smoking in Breast Cancer Cases and Control Group from Population of Maharashtra.

Gene SNP	Genotype	Age (yrs) (Cases/Control)		Age (yrs) @ 1st pregnancy (Cases/Control)		Tobacco status (Cases/Control)	
		≤ 40	> 40	≤ 20	> 20	Users	Non-Users N=181/287
		N=78/207	N=322/193	N=218/181	N=182/219	N=219/113	
SOD1 exon-10 (A251G) rs2070424	AA/AA	55/121	207/134	140/91	122/164	139/77	123/178
	AA/AA + GG/GG	23/86	115/59	78/90	60/55	80/36	58/109
	OR (95% CI)	0.58 (0.33-1.02)	1.26 (0.86-1.84)	0.56 (0.37-0.84)	1.46 (0.94-2.26)	1.23 (0.76-1.99)	0.77 (0.51-1.14)
	p value	0.063	0.232	0.005*	0.084	0.398	0.192
SOD2 exon-2 (T1183C) rs4880	TT/TT	15/83	59/51	41/80	33/54	43/35	31/99
	TT/TT + CC/CC	63/124	263/142	177/101	149/165	176/78	150/188
	OR (95% CI)	2.81 (1.50-5.26)	1.60 (1.04-2.45)	3.41 (2.18-5.35)	1.47 (0.90-2.40)	1.83 (1.09-3.08)	2.54 (1.61-4.02)
	p value	0.001*	0.030*	<0.0001*	0.115	0.021*	0.0001*
SOD3 exon-3 (G172A) rs2536512	GG/GG	25/74	65/51	47/71	43/54	51/35	39/90
	GG/GG+AA/AA	53/133	257/142	171/110	139/165	168/78	142/197
	OR (95% CI)	1.17 (0.67-2.05)	1.42 (0.93-2.16)	2.34 (1.51-3.64)	1.05 (0.66-1.67)	1.47 (0.89-2.45)	1.66 (1.07-2.56)
	p value	0.559	0.101	0.0001*	0.81	0.131	0.021*
Catalase exon-7 (A21T) rs7943316	AA/AA	Aug-78	120/75	79/83	49/70	71/38	57/115
	AA/AA + TT/TT	70/129	202/118	139/98	133/149	148/75	124/172
	OR (95% CI)	5.29 (2.41-11.58)	1.06 (0.74-1.54)	1.49 (0.99-2.22)	1.27 (0.82-1.96)	1.05 (0.65-1.71)	1.45 (0.98-2.15)
	pvalue	<0.0001*	0.718	0.051	0.271	0.824	0.061
Catalase 262 region of promoter (C262T) rs1001179	CC/CC	19/104	94/106	78/74	35/136	61/51	52/159
	CC/CC + TT/TT	59/103	228/87	140/107	147/83	158/62	129/128
	OR (95% CI)	3.13 (1.74-5.62)	2.95 (2.03-4.28)	1.24 (0.82-1.86)	6.88 (4.25-10.88)	2.13 (1.32-3.42)	3.08 (2.07-4.58)
	P value	0.0001*	<0.0001*	0.296	<0.0001*	0.001*	<0.0001*

SNP, Single nucleotide polymorphism; OR, Odds ratio, CI, Confidence interval; Significance p< 0.05; *, Indicates significant Odds Ratio (p<0.05), p value determined based on $\chi 2$

of *rs4880* SNP of Mn-SOD was associated with BC susceptibility [37]. We observed lack of association between polymorphism of Cu, Zn-SOD (*rs2070424*) and EC-SOD (*rs2536512*) with BC risk in the women of rural Maharashtra. This is the first study to investigate the SNPs (*rs2070424*, *rs4880*, *rs2536512*) of SOD and (*rs7943316*, *rs1001179*) *CAT* genes and their role in BC susceptibility in rural Indian women. The results of frequency of T/C, C/C, T/C+C/C (dominant model) genotypes and C allele of *rs4880* variant of Mn-SOD and C/T, T/T, C/T+T/T genotypes and T allele of *rs1001179* variant of *CAT* genes were significantly different in BC patients than controls (p<0.0001) and showed significant association with risk of developing BC in the studied population.

In conclusion, the consequent results obtained from this study revealed functional association of *rs4880* SNP of MnSOD and *rs1001179* SNP of *CAT* gene with BC risk in the studied rural population of south-western Maharashtra which is derived for the first time from this population. The findings obtained from this study also indicated that tobacco smoking habits was responsible for their association with Mn-SOD and *CAT* gene polymorphisms with BC risk among studied population.

Abbreviations

BC: Breast Cancer; PCR-RFLP: Polymerase Chain Reaction-Restriction Fragment Length Polymorphism; SNP: Single Nucleotide Polymorphism; ROS: Reactive Oxygen Species; SOD: Superoxide dismutase; *CAT*: Catalase; OR: Odds Ratio; CI: Confidence Interval; SD: Standard deviation; µL: Microliter; µg: Microgram; DNA: Deoxyribose Nucleic acid; EDTA: Ethelyne Diamine Tetra Acetate

Author Contribution Statement

KDD, SJB, KDD, RAG, Design: KDD, SJB, AKG, Experimental Studies: PPD, NLJ, ALM Clinical studies: AKG, RAG, Data analysis: KDD, AKG, Statistical analysis: KDD, PDD Manuscript preparation: KDD, SJB, AKG, RAG. All authors read and approved the final manuscript.

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approval

The study protocol was approved by protocol committee of Krishna Vishwa Vidyapeeth (Deemed to be University)

Declaration of Conflict of interest

The authors declare that they have no competing financial or any other conflict of interests that could have appeared to influence the work reported in this paper.

Ethics Committee Approval

The study protocol was approved by Institutional Ethics Committee of Krishna Vishwa Vidyapeeth 'Deemed to be University', Karad.

Availability of data

Not applicable

References

1. Deng N, Zhou H, Fan H, Yuan Y. Single nucleotide polymorphisms and cancer susceptibility. Oncotarget. 2017;8(66):110635-49. https://doi.org/10.18632/oncotarget.22372.
2. Ye ZM, Li LJ, Luo MB, Qing HY, Zheng JH, Zhang C, et al. A systematic review and network meta-analysis of single nucleotide polymorphisms associated with pancreatic cancer risk. Aging (Albany NY). 2020;12(24):25256-74. https://doi.org/10.18632/aging.104128.
3. Hoxhaj I, Vukovic V, Boccia S, Pastorino R. Single nucleotide polymorphisms and the risk of developing a second primary cancer among head and neck cancer patients: A systematic literature review and meta-analysis. BMC Cancer. 2021;21(1):660. https://doi.org/10.1186/s12885-021-08335-0.
4. Wang G, Heij LR, Liu D, Dahl E, Lang SA, Ulmer TF, et al. The role of single-nucleotide polymorphisms in cholangiocarcinoma: A systematic review. Cancers (Basel). 2022;14(23). https://doi.org/10.3390/cancers14235969.
5. Das AP, Chaudhary N, Tyagi S, Agarwal SM. Meta-analysis of 49 snps covering 25,446 cases and 41,106 controls identifies polymorphisms in hormone regulation and DNA repair genes associated with increased endometrial cancer risk. Genes (Basel). 2023;14(3). https://doi.org/10.3390/genes14030741.
6. Nishikawa M. Reactive oxygen species in tumor metastasis. Cancer Lett. 2008;266(1):53-9. https://doi.org/10.1016/j.canlet.2008.02.031.
7. Bag A, Bag N. Target sequence polymorphism of human manganese superoxide dismutase gene and its association with cancer risk: A review. Cancer Epidemiol Biomarkers Prev. 2008;17(12):3298-305. https://doi.org/10.1158/1055-9965.Epi-08-0235.
8. Geybels MS, van den Brandt PA, van Schooten FJ, Verhage BA. Oxidative stress-related genetic variants, pro- and antioxidant intake and status, and advanced prostate cancer risk. Cancer Epidemiol Biomarkers Prev. 2015;24(1):178-86. https://doi.org/10.1158/1055-9965.Epi-14-0968.
9. Kang SW. Superoxide dismutase 2 gene and cancer risk: Evidence from an updated meta-analysis. Int J Clin Exp Med. 2015;8(9):14647-55.
10. Shen Y, Li D, Tian P, Shen K, Zhu J, Feng M, et al. The catalase c-262t gene polymorphism and cancer risk: A systematic review and meta-analysis. Medicine (Baltimore). 2015;94(13):e679. https://doi.org/10.1097/md.0000000000000679.
11. Choi JY, Neuhouser ML, Barnett M, Hudson M, Kristal AR, Thornquist M, et al. Polymorphisms in oxidative stress-related genes are not associated with prostate cancer risk in heavy smokers. Cancer Epidemiol Biomarkers Prev. 2007;16(6):1115-20. https://doi.org/10.1158/1055-9965.Epi-07-0040.
12. Abe M, Xie W, Regan MM, King IB, Stampfer MJ, Kantoff PW, et al. Single-nucleotide polymorphisms within the antioxidant defence system and associations with aggressive prostate cancer. BJU Int. 2011;107(1):126-34. https://doi.org/10.1111/j.1464-410X.2010.09344.x.
13. Bag A, Jyala NS, Bag N. Indian studies on genetic polymorphisms and cancer risk. Indian J Cancer. 2012;49(1):144-62. https://doi.org/10.4103/0019-509x.98941.
14. Datkhile K, Gudur A, Gudur RA, al e. Polymorphism in superoxide dismutase, catalase genes and their role in cervical cancer susceptibility among rural population of maharashtra: Findings from a hospital based case control study. Indian Journal of Forensic Medicine & Toxicology. 2020;14(3):366-71. https://doi.org/10.37506/ijfmt.v14i3.10386.
15. Flekac M, Skrha J, Hilgertova J, Lacinova Z, Jarolimkova M. Gene polymorphisms of superoxide dismutases and catalase in diabetes mellitus. BMC Med Genet. 2008;9:30. https://doi.org/10.1186/1471-2350-9-30.
16. Zhang Y, Zhang L, Sun D, Li Z, Wang L, Liu P. Genetic polymorphisms of superoxide dismutases, catalase, and glutathione peroxidase in age-related cataract. Mol Vis. 2011;17:2325-32.
17. Crawford A, Fassett RG, Geraghty DP, Kunde DA, Ball MJ, Robertson IK, et al. Relationships between single nucleotide polymorphisms of antioxidant enzymes and disease. Gene. 2012;501(2):89-103. https://doi.org/10.1016/j.gene.2012.04.011.
18. Mao C, Qiu LX, Zhan P, Xue K, Ding H, Du FB, et al. Mnsod val16ala polymorphism and prostate cancer susceptibility: A meta-analysis involving 8,962 subjects. J Cancer Res Clin Oncol. 2010;136(7):975-9. https://doi.org/10.1007/s00432-009-0742-x.
19. Li X, Shen M, Cai H, Liu K, Liu Y, Huang Z, et al. Association between manganese superoxide dismutase (mnsod) polymorphism and prostate cancer susceptibility: A meta-analysis. Int J Biol Markers. 2016;31(4):e422-e30. https://doi.org/10.5301/jbm.5000188.
20. Castaldo SA, da Silva AP, Matos A, Inácio Â, Bicho M, Medeiros R, et al. The role of cyba (p22phox) and catalase genetic polymorphisms and their possible epistatic interaction in cervical cancer. Tumour Biol. 2015;36(2):909-14. https://doi.org/10.1007/s13277-014-2714-2.
21. Ebrahimpour S, Saadat I. Association of cat c-262t and sod1 a251g single nucleotide polymorphisms susceptible to gastric cancer. Mol Biol Res Commun. 2014;3(4):223-9.
22. Jamhiri I, Saadat I, Omidvari S. Genetic polymorphisms of superoxide dismutase-1 a251g and catalase c-262t with the risk of colorectal cancer. Mol Biol Res Commun. 2017;6(2):85-90.
23. Martin RC, Lan Q, Hughes K, Doll MA, Martini BD, Lissowska J, et al. No apparent association between genetic polymorphisms (-102 c>t) and (-9 t>c) in the human manganese superoxide dismutase gene and gastric cancer(1). J Surg Res. 2005;124(1):92-7. https://doi.org/10.1016/j.jss.2004.09.009.
24. Funke S, Hoffmeister M, Brenner H, Chang-Claude J. Effect modification by smoking on the association between genetic polymorphisms in oxidative stress genes and colorectal cancer risk. Cancer Epidemiol Biomarkers Prev. 2009;18(8):2336-8. https://doi.org/10.1158/1055-9965.Epi-09-0507.
25. Sun L, König IR, Homann N. Manganese superoxide dismutase (mnsod) polymorphism, alcohol, cigarette smoking and risk of oesophageal cancer. Alcohol Alcohol. 2009;44(4):353-7. https://doi.org/10.1093/alcalc/agp025.
26. Tefik T, Kucukgergin C, Sanli O, Oktar T, Seckin S, Ozsoy C. Manganese superoxide dismutase ile58thr, catalase c-262t and myeloperoxidase g-463a gene polymorphisms in patients with prostate cancer: Relation to advanced and metastatic disease. BJU Int. 2013;112(4):E406-14. https://doi.org/10.1111/bju.12176.
27. Tsai SM, Wu SH, Hou MF, Chen YL, Ma H, Tsai LY. Oxidative stress-related enzyme gene polymorphisms and susceptibility to breast cancer in non-smoking, non-alcohol-consuming taiwanese women: A case-control study. Ann Clin Biochem. 2012;49(Pt 2):152-8. https://doi.org/10.1258/

acb.2011.011098.
28. Liu K, Liu X, Wang M, Wang X, Kang H, Lin S, et al. Two common functional catalase gene polymorphisms (*rs1001179* and *rs794316*) and cancer susceptibility: Evidence from 14,942 cancer cases and 43,285 controls. Oncotarget. 2016;7(39):62954-65. https://doi.org/10.18632/oncotarget.10617.
29. Kakkoura MG, Demetriou CA, Loizidou MA, Loucaides G, Neophytou I, Malas S, et al. Mnsod and cat polymorphisms modulate the effect of the mediterranean diet on breast cancer risk among greek-cypriot women. Eur J Nutr. 2016;55(4):1535-44. https://doi.org/10.1007/s00394-015-0971-5.
30. Qiu LX, Yao L, Mao C, Chen B, Zhan P, Yuan H, et al. Lack of association between mnsod val16ala polymorphism and breast cancer risk: A meta-analysis involving 58,448 subjects. Breast Cancer Res Treat. 2010;123(2):543-7. https://doi.org/10.1007/s10549-010-0777-3.
31. Wang C, Liu Y, Zhou J, Ye L, Chen N, Zhu M, et al. There is no relationship between sod2 val-16ala polymorphism and breast cancer risk or survival. Mol Clin Oncol. 2017;7(4):579-90. https://doi.org/10.3892/mco.2017.1376.
32. Datkhile KD, Bhosale SJ, Durgawale PP, Jagdale NJ, More AL, Gudur RA, et al. Tp53 (rs1042522, rs28934571) and tp21 (rs1801270, rs1059234) polymorphisms and risk of breast cancer among rural women of maharashtra: Findings from a hospital based case- control study. Asian Pac J Cancer Prev. 2023;24(5):1611-9. https://doi.org/10.31557/apjcp.2023.24.5.1611.
33. Datkhile KD, Durgawale PP, Patil MN, Gudur RA, Gudur AK, Patil SR. Impact of polymorphism in base excision repair and nucleotide excision repair genes and risk of cervical cancer: A case-control study. Asian Pac J Cancer Prev. 2022;23(4):1291-300. https://doi.org/10.31557/apjcp.2022.23.4.1291.
34. Datkhile KD, Durgawale PP, Gudur RA, Gudur AK, Patil SR. Cyp2d6 and cyp2e1 gene polymorphisms and their association with cervical cancer susceptibility: A hospital based case-control study from south-western maharashtra. Asian Pac J Cancer Prev. 2022;23(8):2591-7. https://doi.org/10.31557/apjcp.2022.23.8.2591.
35. Datkhile K, Patil S, Patil M, Durgawale P, Jagdale N, Deshmukh V, et al. Genetic polymorphisms of cyp1a, cyp1b, cyp2c and risk of cervical cancer among rural population of maharashtra findings from a hospital-based case-control study. J Cancer Res Ther. 2023;19:1925-30. https://doi.org/10.4103/jcrt.jcrt_292_21.
36. Datkhile K, Gudur R, Gudur A, Patil M, Durgawale P, Jagdale N, et al. Cytochrome p450 17 (cyp17) gene polymorphism (rs743572) and cervical cancer risk in women of rural maharashtra. Gene Rep. 2021;24:101219. https://doi.org/10.1016/j.genrep.2021.101219.
37. Gallegos-Arreola MP, Ramírez-Patiño R, Sánchez-López JY, Zúñiga-González GM, Figuera LE, Delgado-Saucedo JI, et al. Sod2 gene variants (*rs4880* and rs5746136) and their association with breast cancer risk. Curr Issues Mol Biol. 2022;44(11):5221-33. https://doi.org/10.3390/cimb44110355.