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

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Abstract

Background: The present study was planned to investigate the possible association between polymorphisms of superoxide dismutase, SOD1 (Cu,Zn-SOD), SOD2(Mn-SOD), SOD3(EC-SOD) and catalase genes and the risk of cervical cancer in rural population of Maharashtra.

Methods: A case control study included 350 proven cervical cancer cases and 400 healthy age matched control women. Genotyping of isoforms of SOD and CAT were done by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method.

Results: When we studied genotype frequencies of codon 326 and 262 region of promoter of *CAT* gene, there was no significant association between A to T transition at codon 326 (p=0.45) and *CAT* C262T polymorphism (p=0.30) with susceptibility to cervical cancer. The variant G allele of SOD1 at codon 251 significantly increased in cervical cancer patients as compared with the control women (OR=3.02, 95% CI: 1.72-5.31; p<0.0001).

Conclusion: Findings from this investigation confirms conceivable association of A251G of SOD1 and G172A of SOD3 genes with risk of cervical cancer in women of Maharashtra.

Keywords: *Genetic polymorphism, Superoxide dismutase, Catalase, Cervical cancer.*

Introduction

Cervical cancer (CC) is 4th most common cancer in women worldwide representing 570,000 new cases and 311,000 deaths in 2018 where 90 % of deaths from CC occurred in low or middle income and developing nations [1]. CC is the 3rd leading public health crisis in India where, the burden of CC is increasing with alarming rate accounting 97,000 new cases and 60,000 deaths occurred in women between age ranges (15 to 69yrs) because of this dreaded disease in 2018 which represent one-fourth of global burden of cervical cancers. It is surprisingly noted that the women residing at rural regions of India are at higher risk of developing CC as compared to their urban counterparts[2].The rate of CC causing deaths in women was highest in southern states of India which was followed by Maharashtra[3]. The predominant risk factors causing CC remained unnoticeable, but the epidemiological factors contributing to development

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and progression of CC in underprivileged areas are early marriage age, sex at early age, poor genital hygiene, use of oral contraceptives, Human Papillomavius (HPV) are considered. Apart from these factors the interactions between genetic and environmental factors have been shown to be associated with cervical carcinogenesis[4]. However, the exact mechanism of cervical carcinogenesis has not been fully elucidated till date. Growing studies evidenced that the reactive oxygen species (ROS) produced through inevitable consequences of oxidative stress may damage to cellular macromolecules and contribute to promotion of carcinogenesis [5]. To control the deleterious effects of ROS, the cellular machinery have antioxidant enzymes such as superoxide dismutase (SOD) and catalase (CAT) to brace against oxidative stress.

A single nucleotide polymorphism (SNP) in SOD or CAT genes may affect the stability and activity of enzymes, ultimately leading to altered expression of functional protein important for ROS detoxification. Several SNPs have been identified in SOD isoforms including, transition of A to G at 251 position of codon 251 in exon 10 of SOD1, Ile58Thr polymorphism at codon 58 in exon 3 of SOD2, transition mutation of G to A at position 172 in alanine to threonine substitution (Ala40Thr) in SOD3 gene associated with different types of cancers [6-10]. Similarly several studies have reported CAT gene polymorphism including A to T point mutation in codon 326 of exon 7 in the promoter region of CAT gene and C262T polymorphism in 262 region of promoter of CAT gene in multiple cancer risk [11-16]. However, the results remain inconsistent and inconclusive. Limited number of epidemiologic studies from India reported association of polymorphisms in SOD and CAT gene with series of cancers [8,17], but studies on SNPs in either SOD or CAT gene and their association with cervical cancer risk in any of Indian population are missing. Therefore in this study we hypothesized that the polymorphism in isoforms of SOD i.e, SOD1 (Cu, Zn-SOD), SOD2 (Mn-SOD), SOD3 (EC-SOD) and CAT genes which may contribute to etiology of CC in rural women of Maharashtra.

Materials and Method

Study subjects

350 women with proven cervical carcinoma and 400 healthy and age matched women as controls were included in this hospital based case-control study. All cases ranged in age from 20-80 years (yr) (Mean ± SD) (48.67 ±13.78) were enlisted immediately after being diagnosed during the year 2013-2018. Informed consent was obtained from all participants.

Genomic DNA isolation from whole blood

Genomic DNA was isolated from blood samples of 350 cases and 400 controls by the standard method describer earlier by Datkhile et al 2019 [18].

Genotyping assays.

Genotyping of SOD and CAT gene was performed by PCR-RFLP. The primers selected to amplify the specific SNPs of interest were; Forward primer (FP); 5'-AGTACTGTCAACCACTAGCA-3'Reverse primer (RP); 5'-CCAGTGTGCGGCCAATGATG-3' for codon 251of SOD1, FP ;5'-AGCTGGTCCCATTATCTAATAG-3'RP ;5'TCAGTGCAGGCTGAAGAGAT-3' for codon 399 of SDO2, FP; 5'-GACATGTACGCCAAGGTCAC-RP; 5'-AACTGGTGCACGTGGATG-3' for codon 272 of SOD3 gene. The primers used for identification of SNPs of catalase gene were; FP: 5'-AATCAGAAGGCAGTCCTCCC-RP: 5'-TCGGGGAGCACAGAGTGTAC -3' for codon 326 of CAT gene and FP: 5'-AGAGCCTCGCCCCGCCGGACCG-3'RP: 5'-TAAGAGCTGAGAAAGCATAGCT-3' for promoter region of CAT gene. The PCR reactions performed seperately in 20 µL reaction volume containing 100 nanogram of genomic DNA, 5 picomoles of each above mentioned primers, 200 micromolar each dNTPs, 10 mili molar (mM) Tris-HCl (pH 9.0), 50 mM KCl 1.5 mM MgCl2 and 1U of Taq DNA polymerase. After confirmation of amplification of specific fragments by agarose gel electrophoresis, each PCR products were allowed for restriction digestion with specific restriction enzymes at 37°C. 1 unit of MspI, EcoRV and BssHII restriction enzymes were used respectively for digestion of SOD1, SOD2 and SOD3 gene. Similarly HinFI and SmaI enzymes were used to digest codon 326 and 262 region of promoter of CAT gene. The restriction products were resolved on 2 or 3 % agarose gels according to the fragment sizes thereafter documented with UV transilluminator system.

Statistical Analysis

The association between the SOD, CAT genotypes and risk of developing CC were studied by logistic

regression model which was used to calculate the Odds ratio (OR) and 95% confidence intervals (CI).

Results

Total number of 350 cases (20-80 yr; median age of 50 yr) were included in this study. Clinically, most of the cases were in stage III well differentiated squamous cell carcinoma, enrolled in the study before receiving any of the cancer treatment. It was observed that CC occurred in patients (78.90 %) who were married at younger age and pregnant soon (15-20 yr) which showed significant association when compared to the controls (p=0.01). Surprisingly, age of cervical cancer occurrence in cases were below 50 years (61.40%; p=0.03) in rural population.

SOD genotyping:

The frequency distribution of SOD1 A251G, SOD2 C399T and SOD3G172A genotypes were determined in CC cases and control groups are shown in Table 1. When we observed the genotypic frequencies of SOD1 A251G polymorphism we found AA genotype (52.3%) in cases whereas controls with 63.00%. But, variant GG genotype frequency in cases was 12.60% which was significantly deviated from expected frequencies of controls which was not more than 5% which revealed that variant GG genotypes (OR=3.02, 95% CI: 1.72-5.31; p=0.0001) heterozygous AG (OR=1.55, 95% CI: 1.16-2.08; p=0.003) increased the risk of CC. The results of genotype frequency of SOD2 C399T showed C allele frequency of cases (4.9%) and that of control (1.2%) whereas variant T allele of cases (31%) and controls (33.2% which did not show significant difference as expected frequencies on Hardy-Weinberg equilibrium.

CAT genotyping:

We also studied the genotype frequencies of codon 326 and 262 region of promoter of *CAT* gene summarized in Table1. When we analyzed frequency distribution of A326T genotypes in codon 326 of exon 7, the A allele of cases (52 %) and controls (49.2%) where T allele in cases (7.4%) and control group (10.5%) which showed no association of either TT genotype or a combination of AT+TT genotype with CC risk. When we studied C/T and T/T genotypes of 262 promoter of CAT gene, we observed that T/T genotypes of cases (33.7%) and controls (28.5%) and C/T +T/T genotypes of cases (90.6%) and controls 89.5%) which also showed no association of C262T polymorphism with CC risk. However, there was no significant association between the CAT A326T polymorphism and susceptibility to CC, (T allele vs A allele, OR=0.67, 95% CI=0.39-1.13, p=0.13; AT+TT vs AA, OR=0.89, 95% CI=0.67-1.19, p=0.45).

Table 1: The genotype frequencies of *SOD and CAT* gene variants and their association with cervical cancer in untreated CC patients and healthy controls.

GENE	Genotype	CASES (n= 350) (%)	CONTROL (n = 400) (%)	Odds' Ratio (95% CI)	p value	Adjusted Odds Ratio (95% CI)	P value
SOD1 Cu,Zn-SOD codon 251 exon-10 (rs2070424)	AA/AA	183(52.3%)	252(63%)	1(Reference)		1(Reference)	
	AA/GG	123(35.1%)	128(32%)	1.3(0.96-1.82)	0.07	1.34(0.97-1.85)	0.07
	GG/GG	44(12.6%)	20(5%)	3.02(1.72-5.31)	0.0001*	2.85(1.60-5.06)	0.001
	AA/GG+GG/GG	167(47.7%)	148(37%)	1.55(1.16-2.08)	0.003	1.53(1.14-2.05)	0.005
SOD2 C399T Codon399 exon-3 (rs1141718)	CC/CC	17(4.9%)	5(1.2%)	1(Reference)		1(Reference)	
	CC/TT	224(64%)	262(65.5%)	0.25(0.09-0.69)	0.007	0.28(0.09-0.80)	0.017
	TT/TT	109(31.1%)	133(33.2%)	0.24(0.08-0.67)	0.0001	0.28(0.09-0.82)	0.021
	CC/TT +TT/TT	333(95.1%)	395(98.8%)	0.24(0.09-0.67)	0.006	0.27(0.10-0.75)	0.013

Cont ... **Table 1: The genotype frequencies of *SOD and CAT* gene variants and their association with cervical cancer in untreated CC patients and healthy controls.**

SOD3 EC-SOD exon3 (rs2536512)	GG/GG	47(13.4%)	74(18.5%)	1(Reference)		1(Reference)	
	GG/AA	151(43.1%)	208(52%)	1.14(0.75-1.74)	0.42	1.11(0.72-1.71)	0.623
	AA/AA	152(43.4%)	118(29.5%)	2.02(1.30-3.14)	0.001*	1.94(1.24-3.04)	0.004
	GG/AA + AA/AA	303(86.6%)	326(81.5%)	1.46(0.98-2.17)	0.06	1.40(0.938-2.095)	0.09
Catalase codon326 exon-7 (rs7943316)	AA/AA	182(52%)	197(49.2%)	1(Reference)		1(Reference)	
	AA/TT	142(40.6)	161(40.2%)	0.95(0.70-1.29)	0.76	0.93(0.68-1.28)	0.683
	TT/ TT	26(7.4%)	42(10.5%)	0.67(0.39-1.13)	0.13	0.58(0.33-1.02)	0.059
	AA/TT + TT/ TT	168(48%)	203(50.8%)	0.89(0.67-1.19)	0.45	0.88(0.65-1.18)	0.412
Catalase C262T Promoter (rs1001179)	CC/CC	33(9.4%)	42(10.5%)	1(Reference)		1(Reference)	
	CC/TT	199(56.9%)	244(61%)	1.03(0.63-1.69)	0.88	1.09(0.60-1.6)	0.972
	TT/TT	118(33.7%)	114(28.5%)	1.31(0.78-2.22)	0.30	1.37(0.78-2.39)	0.263
	CC/TT +TT/ TT	317(90.6%)	358 (89.5%)	1.12 (0.69-1.82)	0.62	1.18(0.72-1.95)	0.495

Significance $p < 0.005$

Discussion

Cervical cancer is a major public health problem differentially distributed across different regions particularly abundant in rural parts of India. Comprehensive case-control study was attempted to discover the relationship of SNPs of SOD and catalase genes which could manipulate the risk of CC in the women representing rural population of Maharashtra. When we studied association of polymorphisms in isoforms of SOD and catalase genes with the help of logistic regression analysis, we observed that amongst three SNPs of superoxide dismutase genes (*rs*2070424, *rs*1141718, *rs*2536512), women carrying G/G genotype in SOD1 and A/A genotype in SOD3 were at higher risk of developing cervical cancer but there was no association of SOD2 C399T polymorphism with CC risk. Similarly, we assessed two SNPs in catalase gene (*rs7943316, rs1001179)* and found no association of polymorphism in this gene with risk of CC in rural women population of Maharashtra. Limited literature available on polymorphisms in oxidative stress associated antioxidant genes and their role in cancer development. SOD and catalase genes are briefly studied for their association with certain pathological disorders along with cancer. Up till now several studies reported positive association of polymorphism in SOD and catalase genes with cancer risk but with inconclusive results[11, 19]. The polymorphism in SOD as well as catalase genes have found to be associated positively with increased risk of breast cancer[6] but, in contrast no association was found in bladder[20], oesophagus[21] and colorectal cancer [22].

Studies with limited information on association of polymorphism in either SOD or catalase gene and risk of cervical cancer is available in literature. The association of SOD[23] and catalase polymorphism[24] with cervical cancer susceptibility was revealed in some studies however, no association of SOD with cervical cancer

was reported by Attatippaholkun and Wikainapakul 2013[25]. Earlier studies discovered the association of SOD2 gene polymorphism with multiple cancer risk[7, 26] but, surprisingly our results interpreted that SOD1 and SOD3 polymorphisms are associated with CC risk. Till date none of the Indian studies reported involvement of polymorphism in SOD and catalase with the risk of cervical cancer. To the best of our knowledge, present study is first to investigate the relationship of SOD and catalase gene with CC risk, where we found no association of SOD2 and catalase gene with risk of cervical cancer. Thus our results revealed for the first time that the SNPs rs2070424 of SOD1 and rs2536512 of SOD3 could increases the susceptibility of women towards CC risk from a pool of unexplored rural population of Maharashtra.

Conclusion

In conclusion, present study for the first time provides the evidence that SOD1 (G allele of *rs*2070424) and SOD3 (A allele of *rs*2536512) polymorphisms may express genetic susceptibility of CC in women of rural Maharashtra but the *rs*1141718 polymorphism of SOD2 and *rs7943316, rs1001179* polymorphisms of catalase gene did not show association with cervical cancer development.

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Conflict of Interest: None declared.

Ethical Clearance: The study protocol was approved by Institutional Ethics Committee of Krishna Institute of Medical Sciences. (**Reference:** KIMSDU/IEC/06/2019)

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