

CYP2D6 and CYP2E1 Gene Polymorphisms and their Association with Cervical Cancer Susceptibility: A Hospital Based Case-Control Study from South-Western Maharashtra

Kailas D Datkhile^{1*}, Pratik P Durgawale¹, Rashmi A Gudur², Anand K Gudur², Satish R Patil¹

Abstract

Background: In last few years several studies all over the world discovered the genetic polymorphisms in different cytochrome P450 genes associated with risk of various cancers, but contradictory outcomes were evidenced in case of cervical cancer risk. In this case-control study we aimed to see whether the polymorphism of *CYP2D6* or *CYP2E1* genes may or may not be associated with cervical cancer risk in women of rural Maharashtra. **Methods:** In this case-control study, the association of *CYP2D6* and *CYP2E1* gene polymorphism with cervical cancer risk was studied by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method. The study was conducted with 350 clinically confirmed cervical cancer patients and 350 healthy women in a population of South-Western Maharashtra. The Odds ratio (OR) with 95% confidence interval and p-value were evaluated, where $p \leq 0.005$ was considered as statistically significant. **Results:** After the analysis of SNP (rs389209) of *CYP2D6* and SNPs (rs2031920, rs6413432, rs6413420) of *CYP2E1*, we noticed that variant allele A of *CYP2E1**6 showed significant increase in cervical cancer cases (OR=4.81; 95% CI: 1.57- 14.77; $p=0.005$). The genotypic distribution of heterozygote G/A genotype of *CYP2D6**4 showed negative association with cervical cancer development when age of cancer occurrence (OR=0.41; 95% CI: 0.27- 0.61; $p<0.0001$) and tobacco history (OR=0.35; 95% CI: 0.20- 0.59; $p=0.0001$) was considered. **Conclusion:** The findings from this study supported that rs6413432 SNP of *CYP2E1**6 increased cervical cancer risk in the studied rural women population.

Keywords: Cervical cancer- *CYP2D6*- *CYP2E1*- genetic polymorphism- PCR-RFLP

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Introduction

In last two decades cervical cancer (CC) constituted nearly 10 % of all female cancers worldwide, ranking third among women accounting 604,127 new cases in year 2020 (GLOBOCAN, 2022). As per global cancer observatory report, CC is the second largest cause of cancer causing deaths in Indian women where 123,907 (9.4%) of new CC cases were reported and 77,348 women were died in 2020 because of CC accounting 9.1% of total cancer deaths in the country (GLOBOCAN, 2020). The incidence of CC in rural India is likely to be high where the majority of women are either illiterate or less educated and unaware about the hazards of CC (Shrivastava et al., 2018). Exposure to oral contraceptives, tobacco habits, age, low income household, earlier sex age, multiple sex partners, sexually transmitted diseases like genital herpes, human immunodeficiency virus (HIV) and human papillomavirus (HPV) have been intensively described

as risk factors for development of CC. Besides, host genetic factors also contribute to susceptibility of cervical carcinogenesis however; exact mechanism of these factors in CC development is not fully understood. Cytochrome P450 is an important enzyme coded by *cytochrome P450* gene (*CYP*) involved in metabolism of exogenous and endogenous substances in the human body. Genetic variations in *CYP* genes may lead to defective metabolizing enzymes with increased susceptibility to certain toxic agents which have been reported to be associated with risk of developing cancer. It has been demonstrated that several *CYP* enzyme genes are polymorphic which result into single nucleotide polymorphisms (SNPs). Amongst these cytochrome P450D6 and cytochrome P450E1 are the phase I enzymes encoded by *CYP2D6* and *CYP2E1* genes respectively and are important in xenobiotics metabolism in human body. A number of studies have been demonstrated functional association of polymorphisms in *CYP2D6* and *CYP2E1* genes with

¹Department of Molecular Biology & Genetics, Krishna Institute of Medical Sciences, "Deemed to be University", Taluka-Karad, Dist- Satara, Pin-415 539, (Maharashtra) India. ²Department of Oncology Krishna Institute of Medical Sciences "Deemed to be University" Taluka-Karad, Dist- Satara, Pin-415 539, Maharashtra, India. *For Correspondence: hodgeneticslab@kimskarad.in

increased or decreased susceptibility to several cancers including esophagus, lung and colorectal cancer (Leng et al., 2012; Shahriary et al., 2012; Jiang et al., 2013; Shen et al., 2015; Zeng et al., 2017) and more recently breast (Lu et al., 2017), bladder (Yin et al., 2018) and head and neck cancer (Farhat et al., 2020; Aday et al., 2021). Few Indian studies also noted association of CYP2D6 polymorphism with breast cancer risk in South Indian population (Surekha et al., 2010). The role of CYP2D6 polymorphism was also reported in determining lung cancer risk in North Indian population (Sobti et al., 2003). There was a single study from Mumbai; Maharashtra reported significant association of CYP2D6 with head and neck cancer (Shukla et al., 2012). Similarly polymorphism in CYP2E1 isoforms was also illustrated in colorectal cancer risk in Kashmiri population (Sameer et al., 2011), gastric cancer risk in West Bengal population (Ghosh et al., 2017) and head and neck cancer risk in North Indian population (Gupta et al., 2014). However, other studies reported no contribution of CYP2E1 gene polymorphism with oral cancer susceptibility in South Indians (Balaji et al., 2011). Likewise, no direct association between the CYP2E1 polymorphism with risk of stomach cancer was reported in North-Eastern region of India (Malakar et al., 2014).

It was thought that the CYP2D6 and CYP2E1 polymorphisms involved in metabolization of several carcinogens are considered to be associated with CC development however; the results were not conclusive where some studies reported that CYP2E1 gene polymorphisms may increase the risk of CC but not others. Cervical cancer is the most prominent death causing disease in women of rural India. When we looked into the literature, we found lack of studies on the association of the polymorphisms in carcinogen metabolizing genes with CC risk in rural Indian women. We believed that the SNPs of CYP2D6 (rs3892097) and CYP2E1 (rs2031920, rs6413432, rs6413420) may or may not contribute to cervical carcinogenesis in rural population. Therefore, in this hospital based case-control study we studied polymorphisms in CYP2D6 and CYP2E1 genes to see their effect on risk of CC development in a population of South-Western Maharashtra of India.

Materials and Methods

Selection of study subjects

This hospital based case-control study was conducted on 350 newly diagnosed CC patients and equal number of healthy, disease free, age matched female controls. Controls were randomly selected from a group of healthy women visiting to a tertiary care hospital for other purposes including blood donation. Also the volunteers from hospital staff were included as controls. All cases ranged in age from 20-80 years (Mean \pm SD) (48.67 \pm 13.78) were sequentially enrolled immediately after diagnosis during the year 2015-2019. The cases already receiving treatment for malignancy were excluded. Trained interviewers used a structured questionnaire to collect demographic and clinical data from the participants. The demographic information of both controls and cases

includes socioeconomic status, dietary habits, education, menopausal status, age at first pregnancy, family history, history of tobacco and alcohol consumption. The study protocol was approved by Institutional Ethics Committee for the utilization of human subjects in the research.

Genomic DNA isolation from whole blood

Five milliliter (mL) of intravenous blood from CC patients and normal controls was collected in sterile EDTA containing vacutainer after receiving their written informed consent. Genomic DNA extraction was carried out from the blood samples by a modified method where red blood cells are processed with red cell lysis buffer (10mM Tris-HCl pH 7.6, 320 mM sucrose, 5mM MgCl₂, 1% Triton X-100, pH 7.6), thereafter treated with nucleic lysis buffer (10mM Tris-HCl, 11.4 mM sodium citrate, 1 mM EDTA, 1 % SDS, pH 8.0). After treatment with 100 μ g/mL concentration of proteinase K at 55°C and subsequently RNase A (100 μ g/mL) at 37°C, precipitated and purified DNA was checked on 1% agarose gel for its quality as well as quantity. The pure DNA was used for genotyping by polymerase chain reaction (PCR) and Restriction Fragment Length Polymorphism (RFLP).

Genotyping Assays

The genotyping of CYP2D6 and CYP2E1 isoforms was studied by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). A total of 20 microliter (μ L) of PCR reaction mixture consisted of 0.2 μ g of genomic 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 each forward and reverse primers (CYP2D6*4) forward primer (FP): 5'-GCTTCGCAACCACTCCG-3' and reverse primer: 5'-AAATCCTGCTCTTCCGAGGC-3'; (CYP2E1*5B) FP: 5'-ACCCCAATGGGTGTCTGTC-3', RP: 5'-TCATTCTGTCTTCTAACTGGCAAT-3';(CYP2E1*6) FP: 5'-AGGCTCGTCAGTTCCTGAAA-3', RP: 5'-AAGGCAGGAGATGACTTGA-3';(CYP2E1*7B) FP: 5'-CTGGAGTTCCCGTTGTCTA-3', RP: 5'-GGGTGAAGACTTGGGAA- TA -3'. The PCR amplification of CYP2D6 and CYP2E1 genes were performed in a Master Cycler Gradient PCR machine (Eppendorf India Limited). The PCR condition for amplification of 334bp fragment of CYP2D6: Initial denaturation at 95°C for 5 minutes (min) followed by 30 cycles of 95°C - 20 seconds (sec) , 57°C - 20 sec, 72°C - 20 sec and final extension at 72°C for 5 min. The PCR conditions for 576 bp of CYP2E1*5B: (95°C for 5 min; 30 cycles of 95°C - 30 sec, 54°C - 45 sec, 72°C - 30 sec; 72°C - 5 min); CYP2E1*6 (685bp): (95°C for 5 min; 30 cycles of 95°C - 30 sec, 62°C - 45 sec, 72°C - 30 sec; 72°C - 5 min); CYP2E1*7B (547bp): (95°C for 5 min; 30 cycles of 95°C - 30 sec, 54°C - 45 sec, 72°C - 30 sec; 72°C - 5 min). After confirmation of PCR amplification on agarose gel electrophoresis, the RFLP analysis for the studied alleles of (CYP2D6*4, CYP2E1*5B, CYP2E1*6, CYP2E1*7B) were carried out with the help of 1 unit of BstO1, PstI, DraI and DdeI restriction enzymes respectively. Following the restriction digestion

of PCR products of CYP2D6 at 60°C and CYP2E1*5B, CYP2E1*6, CYP2E1*7B at 37°C, the digested products were separated on 1-3% low EEO agarose (GeNei, Merck Biosciences) gel, stained with ethidium bromide and photographed with gel documentation system. The variant and wild type genotypes were analyzed based on their restriction digestion pattern.

Statistical Analysis

The association between the CYP2D6, CYP2E1 genotypes and risk of developing CC were studied by Odds ratio (OR). Logistic regression model was used to calculate the OR and 95% confidence intervals (CI) with adjustment of variables to determine the CC risk associated with genotypes. All P values were two-sided and differences were considered statistically significant for $p \leq 0.005$. All statistical analyses were performed with SPSS (Version 11.0) software.

Results

CYP2D6 (rs3892097), CYP2E1*5B (rs2031920), CYP2E1*6 (rs6413432) and CYP2E1*7B (rs6413420) polymorphisms were analyzed by restriction digestion with BstO1, PstI, DraI and DdeI enzymes in 350 cases of CC and equal number of control women from a tertiary care hospital in Karad city of Maharashtra state. The

patients were ranged from 20 to 80 years age (Median age, 47 yr) and healthy female controls ranged from 20-75 yr (Median age, 42 yr). The demographic variables of the study participants including age at cancer occurrence, age at first pregnancy, diet, tobacco habit status, education and economic status were recorded. There was no significant difference with mean age of patients (48.68 ± 13.78) and controls (41.68 ± 14.04). There was no significant difference in diet (OR 1.08; 95%CI, 0.61-1.20; $p=0.61$), education (OR0.85; 95%CI, 0.60-1.20; $p=0.82$) and socioeconomic status(OR 1.45; 95%CI, 1.07-1.97; $p=0.01$) observed between cases and controls but long term and heavy tobacco habits significantly increased the susceptibility of CC (OR: 2.63; 95%CI, 1.93-3.58; $p=0.001$) in rural women. Surprisingly, it was observed that CC occurred in patients (78.57 %) who got married at early age and conceive soon (15-20 yrs).

Comparative analysis of genotypic polymorphism of CYP2D6 gene in cervical cancer cases and controls

In the cytochrome P450 family, CYP2D6 is an important toxicant metabolizing enzyme gene. The genotypic frequency distribution of CYP2D6 determined in CC cases and healthy control females is summarized in Table 1. The frequency of wild type rapid metabolizer (WRM), heterozygous rapid metabolizer (HRM) and mutant poor metabolizer (MPM) genotypes in CC cases

Table 1. The Genotype and Allele Frequencies of CYP2D6*4, CYP2E1*5B, CYP2E1*6, CYP2E1*7B, Gene Polymorphisms in Untreated Cervical Cancer Cases and Healthy Controls.

Gene	Genotype/Allele	Cases (n= 350) (%)	Control (n =350)(%)	OR (95% CI)	P value	Adjusted OR (95% CI)	P value
CYP2D6*4	GG/GG	276 (78.86)	216 (61.71)	1 (Reference)		1 (Reference)	
G1846A	GG/AA	71 (20.28)	129 (36.86)	0.43 (0.30-0.60)	<0.0001	0.42 (0.30-0.60)	<0.0001
rs3892097	AA/AA	3 (0.86)	5 (1.43)	0.46 (0.11-1.98)	0.3	0.60 (0.13-2.74)	0.6
	GG/AA+AA/AA	74 (21.14)	134 (38.29)	0.43 (0.30-0.60)	<0.0001	0.42 (0.296-0.58)	<0.0001
	G allele	311 (88.86)	280 (80.00)	1 (Reference)		1 (Reference)	
	A allele	39 (11.14)	70 (20.00)	0.50 (0.32-0.76)	0.001	0.49 (0.32-0.76)	0.001
CYP2E1*5B	GG/GG	330 (94.29)	335 (95.71)	1 (Reference)		1 (Reference)	
G1293C	GG/CC	13 (3.71)	10 (2.86)	1.31 (0.57-3.05)	0.51	1.09 (0.46-2.58)	0.84
rs2031920	CC/CC	7 (2.00)	5 (1.43)	1.42 (0.44-4.52)	0.55	1.36 (0.40-4.57)	0.61
	GG/CC+CC/CC	20 (5.71)	15 (4.29)	1.35 (0.68-2.68)	0.38	1.17 (0.58-2.37)	0.65
	G allele	336 (96.00)	340 (97.14)	1 (Reference)		1 (Reference)	
	C allele	14 (4.00)	10 (2.86)	1.41 (0.62-3.23)	0.4	1.19 (0.51-2.77)	0.68
CYP2E1*6	TT/TT	127 (36.29)	153 (43.71)	1 (Reference)		1 (Reference)	
T7632A	TT /AA	207 (59.14)	193 (55.14)	1.29 (0.95-1.75)	0.1	1.32 (0.96-1.81)	0.08
rs6413432	AA/AA	16 (4.57)	4 (1.14)	4.81 (1.57-14.77)	0.005	4.15 (1.31-13.17)	0.001
	TT/TA+ AA/AA	223 (63.71)	197 (56.29)	1.36 (1.06-1.84)	0.04	1.38 (1.01-1.90)	0.001
	T allele	230 (65.71)	249 (71.14)	1 (Reference)		1 (Reference)	
	A allele	120 (34.29)	101 (29.86)	1.28 (0.93-1.77)	0.12	1.28 (0.92-1.77)	0.01
CYP2E1*7B	GG/GG	308 (88.00)	272 (77.71)	1 (Reference)		1 (Reference)	
G-71T	GG/TT	36 (10.29)	73 (20.86)	0.43 (0.28-0.67)	0.0002	0.41 (0.26-0.64)	0.0001
rs6413420	TT/TT	6 (1.71)	5 (1.43)	1.05 (0.31-3.14)	0.93	0.88 (0.26-2.89)	0.84
	GG/TT+TT/TT	42 (12.00)	78 (22.29)	0.47 (0.31-0.71)	0.0004	0.45 (0.29-0.67)	0.0001
	G allele	326 (93.14)	308 (88.00)	1 (Reference)		1 (Reference)	
	T allele	24 (6.86)	42 (12.00)	0.53 (0.31-0.91)	0.02	0.55 (0.33-0.90)	0.02

Significance $p < 0.005$; OR, Odds ratio; CI, Confidence Interval

Table 2. Association of *CYP2D6*4*, *CYP2E1*5B*, *CYP2E1*6*, *CYP2E1*7B*, Gene Variants with Demographic Variables Including Age of Cancer Occurrence, Age at First Pregnancy, and Tobacco Smoking in Cervical Cancer Cases and Control Group from Population of Maharashtra.

Gene	Genotype	Age (yrs)		Age (yrs) @ 1 st pregnancy		Tobacco status	
		(Cases/Control)		(Cases/Control)		(Cases/Control)	
		≤ 50 N=215/255	> 50 N=135/95	15-20 N=275/155	21-35 N=75/195	Users N=189/108	Non-Users N=161/242
<i>CYP2D6*4</i>	GG/GG	167/150	109/66	216/93	60/123	150/64	126/152
<i>G1846A</i>	GG/AA+AA/AA	48 /105	26/29	59/62	15/72	39/44	35/90
<i>rs3892097</i>	OR	0.41	0.54	0.54	0.42	0.35	0.46
	(95% CI)	(0.27-0.61)	(0.29-1.004)	(0.35-0.82)	(0.22-0.80)	(0.20-0.59)	(0.29-0.74)
	P value	<0.0001	0.05	0.004	0.008	0.0001	0.001
	GG/GG	202/242	128/93	258/152	72/183	175/101	155/234
<i>CYP2E1*5B</i>	GG/CC+CC/CC	13/13	2-Jul	17/3	12-Mar	14/7	8-Jun
<i>G1293C</i>	OR	1.19	2.54	3.33	0.63	1.15	1.13
<i>rs2031920</i>	(95% CI)	(0.54-2.64)	(0.51-12.52)	(0.96-11.57)	(0.17-2.31)	(0.45-2.95)	(0.38-3.32)
	P value	0.65	0.25	0.05	0.49	0.76	0.82
<i>CYP2E1*6</i>	TT/TT	74/106	53/47	101/61	26/92	68/46	59/107
<i>T7632A</i>	TT/TA+ AA/AA	141/149	82/48	174/94	49/103	121/62	102/135
<i>rs6413432</i>	OR	1.35	0.48	1.11	1.68	1.32	1.37
	(95% CI)	(0.93-1.97)	(0.24-0.95)	(0.74-1.67)	(0.97-2.92)	(0.81-2.14)	(0.91-2.06)
	P value	0.11	0.03	0.54	0.06	0.26	0.13
<i>CYP2E1*7B</i>	GG/GG	191/200	117/72	246/112	62/160	165/84	143/188
<i>G-71T</i>	GG/TT+TT/TT	24/55	18/23	29/43	13/35	24/24	18/54
<i>rs6413420</i>	OR	0.45	0.48	0.3	0.95	0.5	0.43
	(95% CI)	(0.27-0.76)	(0.24-0.95)	(0.18-0.51)	(0.47-1.93)	(0.27-0.95)	(0.24-0.77)
	P value	0.003	0.03	<0.0001	0.9	0.03	0.005

Significance p, 0.0001; OR, Odds ratio; CI, Confidence Interval.

were 78.86%, 20.28% and 0.86% respectively whereas the frequency of WRM, HRM and MPM genotypes in controls were 61.71%, 36.86% and 1.43% respectively. In this study, we noted lowered frequency of heterozygous rapid metabolizer of *CYP2D6* in cases than in control female. The overall frequency of mutant poor metabolizer for *CYP2D6* was not significantly different between cases and controls. The heterozygote (HRM) genotype was higher in controls than the cases (OR 0.43; 95%CI, 0.30-0.60; p=0.0001) which showed negative association of HRM genotype of *CYP2D6* with CC in the studied population.

Comparative analysis of genotypic polymorphism of *CYP2E1* gene in cervical cancer cases and controls

The genotypic frequency distribution of *CYP2E1*5B*, *CYP2E1*6* and *CYP2E1*7B* determined in CC cases and healthy age and sex matched controls is summarized in Table 1. The frequency of G/G, G/C and C/C genotypes of *CYP2E1*5B* in CC cases was 94.29, 3.71 and 2.0 % and that of controls were 95.71, 2.86 and 1.43% in healthy controls which showed no difference. When we considered the frequency distribution of *CYP2E1*6* (rs6413432), we observed that AA variant genotype increased in CC cases as compared to the controls (OR 4.81; 95%CI, 1.57 - 14.77; p=0.005) which signified the probable involvement of AA genotype in CC development. The genotype frequencies in 350 cases were

determined as 36.29% for homozygous wild genotype (T/T), 59.14% for heterozygous genotype (T/A) and 4.57% for homozygous variant (A/A) genotype. The corresponding allele frequency of this polymorphism in studied population were 68.75% for wild type *1A (T) allele and 31.43% for mutated variant *6(A) allele. The combined effect of T/A + A/A genotypes showed association (OR= 1.36; 95% CI=1.06-1.84, p<0.04) with CC among rural women of South-Western Maharashtra. Investigation of *CYP2E1*7B* polymorphism yielded the genotype frequencies of 88% for G/G genotype, 10.29% for G/T and 1.71% for T/T genotype in cases and 77.81% for G/G, 20.86% G/T and 1.43% T/T genotypes in controls. The allele frequencies of *CYP2E1*7B* were 90.57% and 9.43% for G and T alleles respectively. The heterozygote G/T genotype showed negative association (OR 0.43; 95%CI, 0.28-0.67; p=0.0002). The combined effect of heterozygote and mutant genotypes also showed no relationship with development of CC (OR 0.47; 95%CI, 0.31-0.71; p=0.0004).

Correlation of *CYP2D6* and *CYP2E1* gene polymorphisms with confounding factors associated with cervical cancer risk

The interactions between demographic variables such as age of cancer occurrence, age of the first pregnancy, tobacco exposure, economic status, dietary habits with the genotype frequencies of *CYP2D6*4*, *CYP2E1*5B*,

CYP2E1*6 and CYP2E1*7B were analyzed among the cases and control women of the rural population. The results of correlation of CYP2D6 and CYP2E1 genotypes among the cases and controls and their interactions with CC risk are summarized in Table 2. When we stratified the genotypic distribution according to the age of cancer occurrence, we observed no change in homozygous wild genotype and heterozygous or homozygous variant genotypes in *CYP2E1* genes. According to the results, the logistic regression analysis showed that the SNP rs3892097 of CYP2D6*4 was negatively associated with risk of CC in G/A genotype status (OR=0.41; 95% CI: 0.27- 0.61; p<0.0001). When the genotype distribution data was stratified with regard to the age of the first pregnancy, we observed negative association of heterozygote G/T genotype of CYP2E1*7B with the range of 15-20yr age for first pregnancy (OR=0.30;95% CI= 0.18-0.51; p<0.0001). When we studied the correlation of SNPs of CYP2D6 and CYP2E1 with tobacco history, the variant genotype of CYP2D6*4 SNP rs3892097 showed negative correlation with CC development (OR=0.35; 95% CI: 0.20- 0.59; p=0.0001). None of the SNPs of CYP2E1 showed significant change in variant genotype frequency.

Discussion

India is a multiethnic and linguistically diverse country where majority of the population (65%) reside in rural areas. Cervical cancer constitutes a major cause of death in women living in rural pockets with low socioeconomic status. It has been proven that the rural population lives in hilly and agriculture zone of Maharashtra and is exposed to environmental and dietary risk factors including tobacco, which have been shown to contain carcinogens. Similarly, early marriage age and early pregnancies in girls from rural areas accelerate disturbance in metabolic pathways. It has been postulated that polymorphism in metabolizing enzyme genes may increase the human susceptibility to different toxicants which are associated with carcinogenesis. Amongst these CYP2D6 and CYP2E1 are most common metabolic genes which are highly polymorphic, resulted into hundreds of SNPs in human body. In present case-control study, we demonstrated the SNPs of CYP2D6 and CYP2E1 in the rural population of Maharashtra for the first time, as the role of these polymorphisms in relation to the risk of CC or any other cancer has not been reported earlier from this part of world. The association of polymorphisms in the selected genes with risk of CC in rural women was studied by crude and adjusted Odds ratio with their 95% confidential intervals calculated using both homozygous and heterozygous genotypes. The rs3892097 SNP of CYP2D6 is not much studied however; CYP2E1 is studied for its SNPs (rs2031920, rs6413432 and rs6413420) and their association with variety of cancers, but deficit in the literature for their role in cervical carcinogenesis. When we studied the polymorphism of CYP2D6*4, the results indicated that no functional polymorphism in CYP2D6*4 with either of mutant poor metabolizer or heterozygote rapid metaobolizer were associated with susceptibility to the CC. These results were consistent with other

studies of (Wajid et al., (2007) and Markulla et al.,(2014) in cervical and breast cancers patients. However, the heterozygote genotype (heterozygote rapid metabolizer) in CC cases showed negative association (OR=0.43; 95% CI: 0.30 – 0.60; p=0<0001) with CC development. The previously published records on association of CYP2E1 (rs2031920, rs6413432 and rs6413420) polymorphisms with any other cancer risk remained controversial. When we considered CYP2E1 polymorphisms, the results from our study presented the involvement of variant A or *6 allele of CYP2E1*6 in the susceptibility of CC (OR=4.81; 95% CI: 1.57- 14.77; p=0.005) in the rural population of South-Western Maharashtra, which is not reported earlier by any of the researcher. Our results are consistent with other reports where role of CYP2E1*6 has been discussed with susceptibility to other cancers (Chong et al., 2016; Lu et al., 2017). The results from this study also signified that CYP2E1*5B and CYP2E1*7B polymorphisms were not associated with CC risk in studied rural population however, heterozygous G/T genotype of CYP2E1*7B showed negative association with CC development (OR=0.43; 95% CI: 0.28 - 0.67; p=0.0002). Other studies demonstrated the candidate SNPs of CYP2D6 and CYP2E1 conferred a risk of developing liver (Shen et al., 2015), lung (Sobti et al., 2003) breast (Surekha et al., 2010; Lu et al., 2017) head and neck (Gupta et al., 2014; Farhat et al.,2020) and gastric (Ghosh et al., 2017) cancers. Although, number of studies have been carried out around the world on association between CYP2E1 polymorphism and cancer risk, the findings have been inconsistent whereas others revealed no association of CYP2E1 with colorectal (Gao et al., 2007), gastric (Malakar et al., 2014; Zhang et al., 2016), urinary (Fang et al., 2017) and head and neck cancer risk (Balaji et al., 2011). Similarly, genetic polymorphisms in other metabolizing enzyme coding genes and their susceptibility to risk of CC have been studied earlier (Gutman et al., 2009; Abbas et al., 2014; Ding et al., 2018), but with controversial remarks by others (Kim et al., 2000; Sierra-Torres et al., 2003; Sugawara et al., 2003; Wajid et al., 2007). In our earlier studies, we attempted studies on the association of other *CYP* genes with CC risk and evidenced the increased susceptibility of CC in rural women of South-Western Maharashtra with *CYP17* gene polymorphism (Datkhile et al., 2021). Similarly, we assumed that CYP2D6 and CYP2E1 polymorphisms may involved in cervical carcinogenesis in the same population. The results obtained in this study declared that the functional SNP rs6413432 of CYP2E1*6 may be involved in CC development in rural population of South-Western Maharashtra, which was not reported earlier.

In conclusion, it was noted that CYP2E1*6 rs6413432 genotype may contribute to CC susceptibility. To the best of our knowledge, we for the first time revealed the polymorphism of *CYP2D6* and *CYP2E1* genes and their association with CC risk, as their role in CC or any other cancer has not been reported earlier from this part of world.

Abbreviations

CC: Cervical Cancer

CYP2D6: Cytochrome *P450D6* gene

CYP2E1: Cytochrome *P450E1* gene
PCR-RFLP: Polymerase Chain Reaction-Restriction
Fragment Length Polymorphism.
SNP: Single Nucleotide Polymorphism
OR: Odds Ratio
CI: Confidence Interval
μL: Microliter
μg: Microgram
DNA: Deoxyribose Nucleic Acid
EDTA: Ethylene Diamine Tetra Acetate
SDS: Sodium Dodecyl Sulphate
Min: minutes
Sec: Seconds
Pmole: Picomole

Author Contribution Statement

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

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Ethics Committee Approval

The study protocol (KIMSDU/IEC/3/2013) was approved by Institutional Ethics Committee of Krishna Institute of Medical Sciences ‘Deemed to be University’, Karad.

Declaration of competing interest

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

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