RESEARCH ARTICLE

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The Effect of *CYP2C19*2* (*rs4244285*) and *CYP17* (*rs743572*) SNPs on Adriamycin and Paclitaxel based Chemotherapy Outcomes in Breast Cancer Patients

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Abstract

Background: Cytochrome P450 (CYP) are phase I metabolizing enzymes involved in detoxification of chemotherapeutic agents. Among the *CYP* gene family, including *CYP1A1, CYP1B1, CYP2C, CYP2D, CYP2E* and *CYP17*, their significance in cancer susceptibility is well established. However, there remains limited understanding regarding the polymorphisms of *CYP2C19*2* and *CYP17* and their potential correlation with chemotherapy-induced toxicity reactions in breast cancer (BC) patients. In this study we intended to identify the association of *CYP2C19*2* and *CYP17* gene polymorphisms on drug response as well as toxicity reactions in BC patients undergoing adriamycin/ paclitaxel based chemotherapy within Indian population. **Methods:** Two hundred BC patients receiving adriamycin and paclitaxel chemotherapy were enrolled in this study and chemotherapy induced hematological and non-hematological toxicity reactions were noted. The polymorphisms of *CYP2C19*2 (681G>A)* and *CYP17 (34T>C)* isoforms of cytochrome *p 450* gene was studied by PCR and RFLP analysis. **Results:** The univariate logistic regression analysis revealed significant associations between *CYP2C19*2 (681 G>A)* polymorphisms with hematological toxicities i.e., anemia (OR=9.77, 95% CI: 2.84-33.52; p=0.0003), neutropenia (OR=5.72, 95% CI: 1.75-18.68; p=0.003), febrile neutropenia (OR=4.29, 95% CI: 1.32-13.87; p=0.014) and thrombocytopenia (OR=5.86, 95% CI: 1.15-29.72); p=0.032) in BC patients. Additionally BC patients treated with adriamycin exhibited significant association between *CYP2C19*2* polymorphism with chemotherapy induced nausea and vomiting (CINV) (OR=99.73, 95% CI: 5.70-174.64); p=0.001), fatigue (OR=83.29, 95% CI: 4.77-145.69); p=0.002), bodyache (OR=4.44, 95% CI: 1.24-15.91); p=0.021) and peripheral neuropathy (OR=12.00, 95% CI: 1.80-79.89); p=0.010. Furthermore, the regression analysis indicated an association between *CYP17* with body ache (OR=2.77, 95% CI: 1.21-6.34; p=0.015) and peripheral neuropathy (OR=3.90, 95% CI: 1.59-9.53; p=0.002) in BC patients treated with paclitaxel chemotherapy. **Conclusion:** The findings obtained from this study illustrated significant association of *CYP2C9*2 (681G>A)* polymorphism with adreamicin based chemotherapy induced toxicities and *CYP17 (34T>C)* polymorphism with paclitaxel induced bodyache and peripheral neuropathy in BC patients.

Keywords: Breast cancer- genetic polymorphism- *CYP2C19- CYP17*- chemotherapy- toxicity

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Introduction

Breast cancer (BC) is the leading cause of cancer-related deaths among women, not only in developing countries but also in developed nations. The standard approach recommended for managing BC involves a combination of surgery followed by chemotherapy and radiotherapy. The systemic chemotherapy plays a significant role in BC treatment where combinations of anthracyclines, platinum agents and taxanes being widely adopted in the standard therapeutics of BC. Despite the development in standard chemotherapeutics, predicting treatment outcomes remains challenging due to deviations in patient to patient response towards chemotherapeutic drugs. While some patients can respond better to chemotherapy drugs, but others may experience toxic effects and adverse reactions. These diverse reactions can be attributed to genetic alterations in the drug metabolizing enzyme coding genes of patient. Hence, it is important to understand genetic diversity of each individual which determines the pharmacogenetic susceptibility of that individual. The inherited variations in drug metabolizing

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enzyme coding genes can determine the influence of chemotherapy drugs and impact of treatment outcomes [1]. It is important to note that most of the chemotherapeutic drugs exhibit toxicity to normal cells potentially leading to acute or chronic reactions in cancer patients. These acute toxicity reactions are grouped into hematological (anemia, thrombocytopenia, neutropenia and febrile neutropenia) or Non-hematologic (nausea, vomiting, fatigue, peripheral neuropathy) [2, 3]. Although research have been carried out to explore the role of genetic variants of different pathway genes involved in drug transporters and drug metabolism, there remained a scope to discover the role of these genetic determinants in drug toxicity and response.

Phase I and phase II metabolizing enzymes are the key determinants of detoxification and elimination of chemotherapeutic agents, with cytochrome P450 (CYP) enzymes comprising the family of phase I metabolizers for most chemotherapeutic drugs. The *CYP* genes are highly polymorphic in nature where number of isozymes such as *CYP1A1, CYP1B1, CYP2B6, CYP2C9, CYP2C19, CYP2D6, CYP2E1* and *CYP17* displaying functional polymorphisms and their importance in cancer susceptibility [4-7]. The genetic polymorphisms of *CYP* genes play a crucial role in determining clinical outcomes related to treatment response and toxicity. These variant genotypes encoding metabolizing enzymes can alter the activity of drug metabolizing enzymes potentially leading to abnormal drug metabolism [8-10]. It is also evident from earlier findings that polymorphisms of majority of *CYP* genes influence the clinical outcomes of chemotehepeutic drugs [11-13] and are associated with chemotherapy-induced toxicity reactions in BC patients [8]. The genetic polymorphisms in *CYP* genes have significant implications for the breast carcinogenesis risk where *CYP2C19* and *CYP17* polymorphisms have a crucial role in hormone metabolism [14]. The polymorphism of *CYP2C19* is known to influence the therapeutic response towards taxane based chemotherapy [11]; however there remains a gap in our understanding regarding the therapeutic effects of doxorubicin and paclitaxel- based chemotherapy. The *CYP17* polymorphism has been clinically correlated with treatment outcomes in response to hormone therapy in prostate cancer [15, 16]; however there is no evidence on other chemotherapeutic drugs and their clinical outcome with other cancers. Similarly, much interest have been developed to address the functional *polymorphism*s of *CYP17* with BC risk [17, 18] but others have failed to prove such association [19-21] and produced conflicting results. Consequently, there is need to further investigate the clinical significance of these polymorphisms in BC susceptibility and therapeutic response.

To the best of our current understanding, there is lack of studies on the relevance of *CYP* gene polymorphisms in chemotherapy-related toxicities associated with Adriamycin/Paclitaxel treatment for BC or any other cancer in India. Therefore, we decided to explore potential association between variant genotypes of *CYP2C19* and *CYP17* genes and their influence on treatment efficacy and clinical outcomes among BC patients undergoing chemotherapy with Adriamycin and paclitaxel. To address this gap, we specifically investigated the polymorphisms of *CYP2C19*2 (G>A, rs4244285)* and CYP17 (T>C, rs743572), and examined their association with chemotherapy- induced toxicity reactions in breast cancer patients.

Materials and Methods

Study subjects and Clinical data

A total of 200 women (Mean age: 50.24±10.93; range: 27-78 years) with morphologicaly confirmed and histologically diagnosed BC were enrolled in this study. BC patients seeking treatment at the Department of Oncology in a tertiary care hospital (Krishna hospital and Medical Research Centre) were selected for participation. Patients were enrolled based on predefined inclusion and exclusion criteria. The inclusion criteria were; histopathology confirmation, patients diagnosed with BC planned for standard chemotherapy (Adriamycin and Paclitaxel). Patients without a pathological diagnosis, incomplete treatment, incomplete followup, comorbidities, or abnormal liver or renal function tests were excuuded from the study. Detailed clinicopathological and demographic features along with follow-up data were recorded. Among these patients, 104 were treated primarily with adriamycin followed by paclitaxel while 96 patients received paclitaxel first followed by Adriamycin. Chemotherapeutic effects were assessed after each chemotherapy cycle through blood testing and physical and visual examinations following the National Cancer Institute- Common Toxicity Criteria (NCI-CTC) [22]. Informed consent was obtained from patients after explaining the purpose of their enrollment in the study. The study received ethics committee approval from Institutional Ethics Committee of Krihsna Institute of Medical Sciences before initiation.

Chemotherapy, Follow-up and Toxicity assessment

Once the patient was enrolled in the study, chemotherapy was planned based on the patient's stage and the reports of liver and renal function tests. Subsequently, patients received 4 cycles of combination chemotherapy with adriamycin and Cyclophsphamide, followed by 4 cycles of 3 weekly Paclitaxel. After completing the 1st cycle of chemotherapy in each schedule, patients were reevaluated between10th to 14th day after chemotherapy to assess chemotherapy-related toxicities. Patients were communicated about possible adverse effects and advised to report any serious side effects during scheduled follow-up. The details were meticulously recorded and graded according to NCI-CTC 4.03 criteria. During the course of chemotherapy, patients administered with chemotherapy were closely monitored for treatment response and acute toxicity. Routine blood and urine tests were conducted before each chemotherapy cycle to monitor overall health and detect chemotherapy-induced side effects. Hematological toxicities, including anemia, neutropenia, thrombocytopenia as well as non-hematological toxicities such as mucositis, chemotherapy induced nausea/vomiting (CINV), fatigue, bodyache, peripheral neuropathy carefully assessed and graded on a scale as 0, 1, 2, 3, 4.

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

Gene/ Genotype	rs number	Nucleotide change	Primer Sequence (Forward/Reverse)	PCR Conditions	PCR product size	Enzyme /Digestion conditions	Dominant (Wild type)	Heterozygous	Recessive (Mutant)
*CYP2C19*2 Ex-5 G681A*	rs4244285	(G>A)	FP: 5'-CCA GAG CTT GGC ATA TTG TA-3' RP: 5'-GAA GCA ATC AAT AAA GTC CCG A-3'	1 cycle of 95°C-10 minutes 35 Cycles 95°C -30 sec, 52°C-30 sec 72 °C- 30 Sec , 1 Cycle of 72°C-10 min	230 bp	1 Unit of SmaI Incubation at 37°C for 1h	121 bp 109 bp	NIL	230 bp
CYP17 T34C	rs743572	(T>C)	FP: 5'-CAT TCG CAC TCT GGA GTC-3' RP: 5'-GGC TCT TGG GGT ACT TG-3	1 cycle of 95°C-10 minutes 35 Cycles 95°C -30 sec, 53°C-45 sec 72 °C-30 Sec , 1 Cycle of 72°C-10 min	459 bp	1 Unit of MspA1 Incubation at 37°C for 1h	335 bp 124 bp	459 bp 335 bp 124 bp	459 bp

Genotyping assays

Five milliliters (mL) of whole blood were collected from each patient using sterile EDTA containing vacutainer after obtaining 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. The polymorphisms of *CYP2C19* and *CYP17* was studied by polymerase chain reaction- restriction fragment length polymorphisms (PCR-RFLP). The PCR amplification for confirmation of polymorphisms was carried out separately in 20 micro liter (µL) reaction mixtures containing 1X PCR buffer 0.2 mM each dNTP, 10 picomole (pmol) of each primers (IDT technologies), 1U Taq DNApolymerase (GeNei, Merck Bioscience) and 100 nanogram (ng) of purified genomic DNA. The primer sequence used to amplify *CYP2C19*2* (*G681A*) and *CYP17 (T34C)* along with the PCR amplification conditions are represented in Table 1. Thereafter, the amplified PCR products were subjected to restriction digestion using restriction enzymes with digestion conditions are detailed in Table 1. The resulting PCR products and restriction digestion reactions were checked by agarose gel electrophoresis in Tris-Acetate-EDTA (TAE) buffer thereafter stained with ethidium bromide (10 mg/mL) and visualized under UV-transilluminator and photographed in gel documentation system (BioRad Laboratories).

Statistical Analysis

A univariate logistic regression analysis was used to assess the association between *CYP2C19*2* and *CYP17* polymorphisms and the incidence of toxicity (0-1 grade vs. 2-4), expressing results as Odds Ratios (OR) and 95% confidence intervals (95% CIs). The OR was estimated to test whether any significant association existed between grade <1 toxicity caused by chemotherapy and the selected gene polymorphisms. Statistical significance was set at $p < 0.05$. All statistical analyses were carried out using SPSS (Version 21.0). Additionally, the association of each polymorphism with the severity of toxicities was compared to clinico-pathological and demographic information of the patients using chi-square test.

Results

*Genotype distribution of CYP2C19*2 (681 G>A) and CYP17 (34 T>C) polymorphisms and adriamycin/ paclitaxel based chemotherapy toxicity in BC patients*

A total of two hundred BC patients seeking chemotherapy treatment were observed for the adverse toxicity reactions in response to standard Adriamycin followed by paclitaxel and vice versa. Out of these 200 patients, 104 were first administered Adriamycin followed by paclitaxel chemotherapy, while 96 patients received paclitaxel first followed by Adriamycin. Univariate logistic regression analysis was used to explore the association between polymorphisms of *CYP2C19*2 (rs4244285)* and *CYP17 (rs743572)* with chemotherapy- induced acute toxicity reactions in BC patients. The chemotherapy-

Table 2. Univariate Analysis of Candidate SNPs of Cytochrome P450 (*CYP2C19*2, CYP17)* Gene and Risk of Adreamycin Chemotherapy Induced Severe Toxicity of Hematological Reactions in Breast Cancer Patients

Gene Name SNP	Genotype	Grade ≤1 (n=81)	Grade >1 (n=23)	OR (95% CI)	p value
		Anemia			
*CYP2C19*2*	G/G	76	14	1 (Reference)	
rs4244285	G/A+A/A	5	9	9.77 (2.84-33.52)	0.0003*
CYP17	T/T	37	11	1 (Reference)	
rs743572	T/C+C/C	44	12	0.91 (0.36-2.31)	0.855
		Neutropenia			
		(n=79)	(n=25)		
*CYP2C19*2*	G/G	73	17	1 (Reference)	
rs4244285	G/A+A/A	6	8	5.72 (1.75-18.68)	0.003*
CYP17	T/T	37	11	1 (Reference)	
rs743572	T/C+C/C	42	14	1.12 (0.45-2.77)	0.804
		Febrile Neutropenia			
		(n=80)	(n=24)		
*CYP2C19*2*	G/G	73	17	1 (Reference)	
rs4244285	G/A+A/A	7	7	4.29 (1.32-13.87)	0.014*
CYP17	T/T	37	11	1 (Reference)	
rs743572	T/C+C/C	43	13	1.01 (0.40-2.54)	0.971
		Thrombocytopenia			
		(n=97)	(n=7)		
*CYP2C19*2*	G/G	86	4	1 (reference)	
rs4244285	G/A+A/A	11	3	5.86 (1.15-29.72)	0.032*
CYP17	T/T	44	4	1 (reference)	
rs743572	T/C+C/C	53	3	0.62 (0.13-2.93)	0.549

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 $\chi 2$.

induced acute toxicity reactions were grouped into hematological and non-hematological toxicities, graded as grade ≤1 or >1 toxicities based on NCI-CTC criteria. Hematological toxicity reactions including anemia, neutropenia, febrile neutrepenia, thrombocytopeni were assessed through blood testing. Non-hematological toxicity reactions such as mucositis, CINV, fatigue, body ache and peripheral neuropathy were recorded after physical examinations. Among patients treated with Adriamycin followed by paclitaxel, out of 104 patients 23 patients exhibited severe toxicity (grade >1) in terms of anemia, 25 patients had severe neutropenia, 24 patients experienced febrile neutropenia and 7 patients faced thrombocytopenia. Severe non-hematological toxicities (grade >1) included mucositis in 16 patients, CINV in 34 patients, fatigue in 37 patients, body-ache in 15 patients and peripheral neuropathy in 5 patients after treatment with Adriamycin chemotherapy. When studying the association between genetic polymorphisms of *CYP2C19*2* and *CYP17* with severe hematological and non-hematological toxicity reactions in patients treated with Adriamycin and paclitaxel chemotherapy, the univariate logistic regression analysis of *CYP2C19*2* variant genotype showed significant association with hematological toxicities i.e., anemia (OR=9.77, 95% CI: 2.84-33.52; p=0.0003), neutropenia (OR=5.72, 95% CI: 1.75-18.68; p=0.003), febrile neutropenia (OR=4.29, 95% CI: 1.32-13.87; p=0.014) and thrombocytopenia (OR=5.86, 95% CI: 1.15-29.72); p=0.032). The regression analysis results of *CYP2C19*2* and *CYP17* and its association with hematological toxicities are presented in Table 2. When BC patients were administered primary with Adriamycin followed by paclitaxel, the *CYP2C19*2* polymorphism also demonstrated significant associations with non-hematological toxicity reactions such as chemotherapy induced nausea and vomiting (CINV) (OR=99.73, 95% CI: 5.70-174.64); p=0.001), fatigue (OR=83.29, 95% CI: 4.77-145.69); p=0.002), bodyache (OR=4.44, 95% CI: 1.24-15.91); p=0.021) and peripheral neuropathy (OR=12.00, 95% CI: 1.80-79.89); p=0.010). The results of interpretation are presented in Table 3. However, the *CYP17* polymorphism did not show any significant association with either hematological or non-hematological toxicities. The univariate analysis of polymorphisms of *CYP2C19*2* and *CYP17* when analyzed from the patients treated with paclitaxel chemotherapy, the results showed no association of *CYP2C19*2* and *CYP17* with hematological toxicities (Table 4). The univariate logistic regression analysis showed statistically significant association *CYP2C19*2 (G681A)* polymorphism with variant genotype in relation with severe non-hematological toxicity i.e, fatigue (OR=19.86, 95% CI: 4.03-97.83;

Table 3. Univariate Analysis of Candidate SNPs of Cytochrome *P450 (CYP2C1982, CYP17)* Gene and Risk of Adriamycin Chemotherapy Induced Severe Toxicity of Non-Hematological Reactions in Breast Cancer Patients.

		Mucositis			
Gene Name	Genotype	Grade ≤1	Grade >1	OR (95% CI)	p value
SNP		(n=88)	(n=16)		
*CYP2C19*2*	G/G	78	12	1 (Reference)	
rs4244285	G/A+A/A	10	4	2.60 (0.70-9.62)	0.152
CYP17	T/T	40	8	1 (Reference)	
rs743572	T/C+C/C	48	8	0.83 (0.28-2.41)	0.737
		CINV			
		(n=70)	(n=34)		
*CYP2C19*2*	G/G	70	20	1 (Reference)	
rs4244285	G/A+A/A	0	14	99.73 (5.70-174.64)	0.001*
CYP17	T/T	34	14	1 (Reference)	
rs743572	T/C+C/C	36	20	1.34 (0.58-3.08)	0.478
		Fatigue			
		(n=67)	(n=37)		
*CYP2C19*2*	G/G	67	23	1 (Reference)	
rs4244285	G/A+A/A	0	14	83.29 (4.77-145.69)	0.002*
CYP17	T/T	32	16	1 (Reference)	
rs743572	T/C+C/C	35	21	1.20 (0.53-2.69)	0.658
		Bodyache			
		(n=89)	(n=15)		
*CYP2C19*2*	G/G	80	10	1 (Reference)	
rs4244285	G/A+A/A	9	5	4.44 (1.24-15.91)	0.021*
CYP17	T/T	41	7	1 (Reference)	
rs743572	T/C+C/C	48	8	0.97 (0.32-2.92)	0.965
		Peripheral Neuropathy			
		(n=99)	(n=5)		
*CYP2C19*2*	G/G	88	2	1 (Reference)	
rs4244285	G/A+A/A	11	3	12.00 (1.80-79.89)	0.010*
CYP17	T/T	46	2	1 (Reference)	
rs743572	T/C+C/C	53	3	1.30 (0.20-8.13)	0.777

p=0.0002) in BC patients treated with paclitaxel based chemotherapy. The results of regression analysis signified association of CYP17 (T34C) with non-hematological toxicities such as bodyache (OR=2.77, 95% CI: 1.21-6.34; p=0.015) and peripheral neuropathy (OR=3.90, 95% CI: 1.59-9.53; p=0.002) in BC patients treated with paclitaxel based chemotherapy. The non-hematological toxicities in response to paclitaxel based chemotherapy and the distribution of *CYP2C19*2 (G681A)* and *CYP17 (T34C)* genotypes are represented in Table 5. Notably, these polymorphic *G681A* genotype of *CYP2C19*2* and *T34C* genotype of *CYP17* gene did not show any significant association with hematological toxicity conditions in response to paclitaxel based chemotherapy.

*Association of CYP2C19*2 (G681A) and CYP17 (T34C) polymorphisms with demographic and clinic-pathological factors of BC patients*

An association between genetic polymorphisms of metabolic *CYP2C19*2* and *CYP17* genes and the demographic and clinic-pathological characteristics of BC patients is presented in Table 6. When the demographic factors including age and body mass index of BC patients enrolled in this study are considered, the univariate logistic regression analysis showed significant negative association of variant genotype of CYP17 (T34C) with BMI >25 (OR=0.45, 95% CI: 0.25-0.80; p=0.007) and there was no association of both *CYP2C19*2* and *CYP17* with age of BC patients. The results of analysis in present study found no association *CYP2C19*2* and *CYP17* polymorphisms with histopathological TNM grade > stage II whereas clinical TNM stage >2 showed significant association with *CYP17 (T>C)* polymorphism (OR=1.88, 95% CI: 1.08-3.28; p=0.025). The univariate logistic regression analysis among genotype distribution of *CYP2C19*2 (G>A)* polymorphism showed significant association with ER/PR hormone receptor status of BC patients (OR=3.61, 95% CI: 1.30-9.98; p=0.013) whereas the genotype distribution of CYP17 (T>C) was negatively associated (OR=0.55, 95% CI: 0.31-0.98; p=0.044)

Table 4. Univariate Analysis of Candidate SNPs of Cytochrome *P450 (CYP2C19*2, CYP17)* Gene and Risk of Paclitaxel Chemotherapy Induced Severe Toxicity of Hematological Reactions in Breast Cancer Patients

		Anemia			
Gene Name	Genotype	Grade ≤1	Grade >1	OR (95% CI)	p value
SNP		(n=80)	(n=16)		
*CYP2C19*2*	G/G	69	14	1 (Reference)	
rs4244285	G/A+A/A	11	2	0.89 (0.17-4.49)	0.893
CYP17	T/T	37	9	1 (Reference)	
rs743572	T/C+C/C	43	7	0.66 (0.22-1.97)	0.466
		Neutropenia			
		(n=81)	(n=15)		
*CYP2C19*2*	G/G	71	12	1 (Reference)	
rs4244285	G/A+A/A	10	3	1.77 (0.42-7.40)	0.439
CYP17	T/T	40	6	1 (Reference)	
rs743572	T/C+C/C	41	9	1.46 (0.47-4.49)	0.505
		Febrile Neutropenia			
		(n=82)	(n=14)		
*CYP2C19*2*	G/G	71	12	1 (Reference)	
rs4244285	G/A+A/A	11	3	1.61 (0.39-6.64)	0.507
CYP17	T/T	40	6	1 (Reference)	
rs743572	T/C+C/C	42	8	1.26 (0.40-3.98)	0.682
		Thrombocytopenia			
		(n=94)	(n=2)		
*CYP2C19*2*	G/G	81	2	1 (Reference)	
rs4244285	G/A+A/A	13	0	1.28 (0.05-28.16)	0.875
CYP17	T/T	45	1	1 (Reference)	
rs743572	T/C+C/C	49	1	0.91 (0.05-15.12)	0.952

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 $\chi 2$.

with hormone receptors in BC patients. The genotype distribution of *CYP2C19*2* polymorphism showed significant negative correlation (OR=024, 95% CI: 0.10-0.60; p=0.002) with Her2 receptor status in BC patients enrolled in this study.

Discussion

The chemotherapeutic approach is commonly used for BC management which can effectively kill cancer cells but it also impacts normal cells leading to toxicities or adverse effects in patients. During chemotherapy treatment, approximately 60 to 70% of the chemotherapy drugs are metabolized by cytochrome P450 enzymes, including CYP1A2, CYP1B1, CYP2B6, CYP2C9, CYP2C19, CYP3A4, and CYP3A5. Among these, CYP2C19 exhibits the highest activity. However, individual patient responses to different chemotherapy drugs carry due to diverse genetic susceptibility. Several pharmacogenomic studies have clarified that numerous metabolic pathways can influence inter-individual variability in response to chemotherapy drugs. The genetic polymorphisms of metabolic genes of individuals can determine the chemotherapy treatment outcomes, where variant alleles control the variability of drug efficacy. Genetic alterations in drug detoxification genes lead to decreased enzyme activity, which may affect the efficiency of chemotherapeutic agents and can cause apparent adverse reactions in patients. Acute toxicities are crucial, as they directly affect treatment outcomes. The *CYP450* genes are highly polymorphic in nature, and common polymorphisms with a variety of isoforms, including *CYP1A1, CYP1B1, CYP2C, CYP2D* and *CYP2E*, have been associated with alterations inenzymatic activity. Genetic polymorphisms in drug metabolizing enzyme-coding *CYP* genes lead to inter-individual differences in drug responses, which affect chemotherapy treatment efficiency [23]. CYP2C19 is a member of the CYP450 enzyme family and plays an important role in the metabolism and elimination of a diverse range of chemotherapeutic drugs [24-26]. The CYP2C19*2 and CYP19*3 are the most commonly studied polymorphisms of *CYP2C19*. Several studies have reported an association of *CYP2C19* polymorphisms and therapeutic outcomes in response to different chemotherapeutic agents. The association of the *CYP2C19* polymorphism with hematological toxicities, including severe neutropenia, has been reported earlier in response to chemotherapeutic drugs in ovarian [27], breast [28], lung cancer [29] and non small cell lung carcinoma [30]. However, *CYP2C19* polymorphic variants were not assessed against Adriamycin and pclitaxel based-chemotherapy in BC patients. Very few studies have

Table 5. Univariate Analysis of Candidate SNPs of Cytochrome *P450 (CYP2C19*2, CYP17)* Gene and Risk of Paclitaxel Chemotherapy Induced Severe Toxicity of Non-Hematological Reactions in Breast Cancer Patients

		Mucositis			
Gene Name SNP	Genotype	Grade ≤1 (n=88)	Grade >1 (n=8)	OR (95% CI)	p value
*CYP2C19*2*	G/G	75	8	1 (Reference)	
rs4244285	G/A+A/A	13	0	0.32 (0.01-6.04)	0.454
CYP17	T/T	42	4	1 (Reference)	
rs743572	T/C+C/C	46	4	0.91 (0.21-3.88)	0.902
		CINV			
		(n=66)	(n=30)		
*CYP2C19*2*	G/G	60	23	1 (Reference)	
rs4244285	G/A+A/A	6	7	3.04 (0.92-10.02)	0.067
CYP17	T/T	36	10	1 (Reference)	
rs743572	T/C+C/C	30	20	2.40 (0.97-5.90)	0.056
		Fatigue			
		(n=67)	(n=29)		
*CYP2C19*2*	G/G	65	18	1 (Reference)	
rs4244285	G/A+A/A	2	11	19.86 (4.03-97-83)	0.0002*
CYP17	T/T	30	16	1 (Reference)	
rs743572	T/C+C/C	37	13	0.65 (0.27-1.58)	0.35
		Bodyache			
		(n=48)	(n=48)		
*CYP2C19*2*	G/G	42	41	1 (Reference)	
rs4244285	G/A+A/A	6	7	1.19 (0.37-3.85)	0.765
CYP17	T/T	30	18	1 (Reference)	
rs743572	T/C+C/C	18	30	2.77 (1.21-6.34)	0.015*
		Peripheral Neuropathy			
		(n=60)	(n=36)		
*CYP2C19*2*	G/G	53	30	1 (Reference)	
rs4244285	G/A+A/A	7	6	1.51 (0.46-4.92)	0.49
CYP17	G/G	36	10	1 (Reference)	
rs743572	G/T+T/T	24	26	3.90 (1.59-9.53)	0.002*

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 $\chi 2$.

investigated the significance of CYP17 in chemotherapy associated adverse toxicities. The clinical significance of chemotherapy-induced vomiting was noted when correlated with polymorphism of *CYP17* in North Indian women with BC [31]. However, no significant association of *CYP17* polymorphism was noted in prostate cancer patients in response to chemotherapy [32].

Accordingly, for the first time, we analyzed and correlated the association of *CYP17* and *CYP2C19* polymorphisms with adverse reactions in BC patients treated with Adriamycin /paclitaxel chemotherapy. The results of the present study indicated that the polymorphisms of drug metabolizing enzyme coding *CYP2C19* gene was significantly correlated with induction of chemotherapy induced toxicities in BC patients treated with Adreamicin treated chemotherapy. The logistic regression analysis signified that the variant heterozygous genotype of *CYP2C19*2* independently showed an association with hematological toxicity response against Adryamycin-based chemotherapy in BC patients. The frequency distribution of *CYP2C19*2* genotypes showed a significant deviation from Hardy-Weinberg equilibrium in BC patients treated with Adriamycin, which was in agreement with other investigated polymorphisms [12]. A significant association of *CYP2C19*2* was detected with Adriamycin-based chemotherapy resistance when analyzed for hematological and non-hematological toxicities. The *CYP2C19*2* polymorphism was significantly associated with anemia (p=0.0003), neutropenia (p=0.003) and febrile neutropenia (p=0.014) toxicities in BC patients administered with adriamycin chemotherapy. The polymorphism of *CYP17* did not show association with any of hematological adverse effects in BC patients treated with either Adreamicin or paclitaxel. The univariate regression analysis of *CYP17* polymorphisms showed a significant association with body-ache (OR=2.77, 95% CI: 1.21-6.34; p=0.015) and peripheral neuropathy (OR=3.90, 95% CI: 1.59-9.53; p=0.002) in BC patients treated with

Table 6. Polymorphisms of *CYP2C19*2, CYP17* Genes and Their association with Demographic and Clinic-Pathological Characteristics of Breast Cancer Patients

Characteristics	CYP2C19*2 (rs4244285)		CYP17 (rs743572)	
	G/G	G/A+A/A	T/T	T/C+C/C
	No (%)	No (%)	No (%)	No (%)
Age				
≤ 40	36 (18.00)	7 (3.50)	18 (9.00)	25 (12.50)
>40	137 (68.50)	20 (10.00)	76 (38.00)	81 (40.50)
OR (95% CI)	1 (Reference)	0.75 (0.29-1.93)	1 (Reference)	0.76 (0.38-1.51)
p value		0.548		0.446
BMI Kg/m^2				
≤ 25	102 (51.00)	20 (10.00)	48 (24.00)	74 (37.00)
>25	71 (35.50)	7 (3.50)	46 (23.00)	32 (16.00)
OR (95% CI)	1 (Reference)	0.50 (0.20-1.25)	1 (Reference)	0.45 (0.25-0.80)
p value		0.139		0.007*
Clinical TNM Grade				
≤ Stage II	90 (45.00)	12 (6.00)	57 (28.50)	45 (22.50)
> Stage II	83 (41.50)	15 (7.50)	41 (20.50)	61 (30.50)
OR (95% CI)	1 (Reference)	1.35 (0.59-3.06)	1 (Reference)	1.88 (1.08-3.28)
p value		0.464		0.025*
Histopathological TNM Grade				
≤ Stage II	77 (38.50)	13 (6.50)	43 (21.50)	47 (23.50)
> Stage II	96 (48.00)	14 (7.00)	51 (25.50)	59 (29.50)
OR (95% CI)	1 (Reference)	0.86 (0.38-1.94)	1 (Reference)	1.05 (0.60-1.84)
p value		0.723		0.842
Hormone Receptor Status				
ER/PR +ve	78 (39.00)	5 (2.50)	32 (16.00)	51 (25.50)
ER/PR -ve	95 (47.50)	22 (11.00)	62 (31.00)	55 (27.50)
OR (95% CI)	1 (Reference)	3.61 (1.30-9.98)	1 (Reference)	0.55 (0.31-0.98)
p value		0.013*		0.044*
Her2 +ve	22 (11.00)	10 (5.00)	16 (8.00)	16 (8.00)
Her2 -ve	151 (75.50)	17 (8.50)	78 (39.00)	90 (45.00)
OR (95% CI)	1 (Reference)	0.24 (0.10-0.60)	1 (Reference)	1.15 (0.54-2.45)
p value		0.002*		0.71

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

paclitaxel-based chemotherapy. A significant association between the *CYP17* variant genotypes and potential tumor related characteristics of BC patients including TNM grade > stage II (OR=1.88, 95% CI: 1.08-3.28; p=0.025) was noted in this study. Also, significant association of *CYP2C19*2* with hormone receptor status was observed in the studied BC patients.

In conclusion, the findings from this study highlight significant association of *CYP2C9*2* with hematological and non-hematological toxicities induced by Adriamycin-based chemotherapy in BC patients within selected population. The *CYP2C19*2* polymorphic variant genotype demonstrated significant association with anemia, neutropenia and thrombocytopenia in response to Adriamycin. Additionally, the *CYP17* polymorphism showed significant correlation with bodyache and peripheral neuropathy in response to paclitaxel-based chemotherapy. Notably, this study represents the first of its kind to analyze the influence of Adriamycin based chemotherapy on metabolic gene polymorphisms in BC patients.

Author Contribution Statement

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

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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

Abbreviations

BC: Breast Cancer
BMI: Body Mass Index
CYP: Cytochrome P450
PCR-RFLP: Polymerase Chain Reaction-Restriction Fragment Length Polymorphism
DNA: Deoxyribose Nucleic Acid
EDTA: Ethylenediamdie Tetra acetate
µl: Microliter
CINV: Chemotherapy Induced Nausea and Vomiting
ECOG: Estern Cooperative Oncology Group
NCI-CTC: National Cancer Institute-Common Toxicity Criteria
OR: Odds Ratio
CI: Confidence Interval
ER: Estrogen Receptor
PR: Progesterone receptor
Her2: Humen Epidermal Growth Factor Receptor

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