IOSR Journal of Dental and Medical Sciences (IOSR-JDMS)
e-ISSN: 2279-0853, p-ISSN: 2279-0861.Volume 24, Issue 1 Ser. 6 (January. 2025), PP 14-23
www.iosrjournals.org

Effect of ABCB1/MDR1 Gene Polymorphism at 3435C > T (rs1045642) on Multidrug Resistance Expression in Breast Cancer Patients

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Abstract:
Background*: Breast carcinoma is the most common malignancy and the leading cause of cancer-associated mortality among women. The relationship between the multidrug resistance 1 (MDR1) C3435T polymorphism and breast cancer risk remains poorly defined. The current study aims to determine the MDR1 C3435T polymorphism frequency and the possible association with the clinicopathological parameters, P-glycoprotein (P-gp) protein expression, and breast cancer risk.*
Materials and Methods*: PCR-RFLP was performed on 426 breast cancer patients and 300 healthy female controls to determine MDR1 C3435T polymorphism. RT-PCR determined the MDR1 expression for gene expression and Immunohistochemistry for protein P-gp expression.*
Results*: The genotype distribution in both the patient and control groups was consistent with the Hardy-Weinberg equilibrium ($\chi 2 = 0.00$, $P > 0.05$ for cases and $\chi 2 = 0.03$, $P = 0.85$ for controls). There was no significant difference in the allelic and genotypic distribution between the control and patient groups ($P = 0.888$). The MDR1 C3435T CC genotype was significantly associated with clinicopathological parameters such as advanced disease stage, high histopathological grade, and positive lymph node status ($P < 0.01$). Also, patients' MDR1 expression levels and polymorphism were significantly associated ($P< 0.001$).*
Conclusion: *MDR1 C3435T polymorphism was not found to be a risk factor for breast cancer. However, the wild CC genotype may induce resistance to chemotherapy by elevating the MDR1 gene and P-gp protein expression.*
Key Word: *Breast cancer; C3435T polymorphism; Multidrug-resistant gene; P-glycoprotein.*

Date of Submission: 13-01-2025 Date of Acceptance: 23-01-2025

I. Introduction

Breast cancer is the second most common malignancy, with an incidence of 2,310,051 new cases globally at a mortality rate of 17.1 per 100000 cases[1]. As per Global Cancer Observatory (GLOBOCAN 2022), carcinoma of the breast is the leading cancer in India, accounting for 26.6% of all cancers in India, with 1.9 million new cases in 2022.[1] Globally, India ranked third highest in breast cancer incidence after China (3.6 million new cases) and the United States of America (2.8 million new cases)[2]. According to the projected breast cancer burden in India, the Disability-adjusted life years (DALYs) were found to be 72,071 in 2026 owing to an increase in population size and aging[3]. A higher incidence of breast cancer in high-income countries (571/100 000) than in low-income countries (95/100 000) significantly explains the rise in the incidence in the Indian subcontinent owing to rapid urbanization, industrialization and population growth[4]. In developed countries, delayed age of marriage, late first childbirth, and menopause are major risk factors associated with breast cancer development[5-7]. In addition, the COVID-19 pandemic globally hindered the health system and screening programs, further 1 delaying diagnosis and increasing detection at advanced stages and mortality[8].

Breast cancer constitutes a heterogeneous class of disease, classified into different subtypes based on the presence of estrogen receptor alpha (ER), progesterone receptor (PR), human epidermal growth receptor 2 (HER2/neu), or the absence of these markers as triple-negative breast cancer (TNBC)[9]. Each subtype has a different treatment strategy. Patients with positive hormone receptor status are often treated with endocrine therapy in combination with systematic adjuvant therapy. Traditional cytotoxic chemotherapeutics, such as 5-fluorouracil, epirubicin or doxorubicin, and cyclophosphamide (FEC/FAC), are the first-line therapies for TNBC patients[10].

ATP-binding cassette B1 (ABCB1), also known as multidrug-resistant gene-1 (MDR-1), located on chromosomal region 7q21, utilizes the energy from ATP hydrolysis for active transport of substrates from the cell interior and membrane to outside protecting cells from the effect of toxic substances or metabolites. P-glycoprotein (P-gp), a 170 KDa transmembrane transport protein encoded by the MDR1 gene, is expressed in

various human tissues, mediating the cells to eliminate lipophilic substrates. The commonly used chemotherapeutic drugs anthracyclines and taxanes are also substrates to P-gp[11-13]. The enhanced efflux of drugs, genetic factors, and excess xenobiotic metabolism cause the reduced efficacy of chemotherapy drugs[14,15]. Drug resistance can be present ab initio or acquired during treatment. Various mechanisms, such as the ATP-dependent efflux pumps, may mediate drug resistance, which reduces intracellular drug concentrations[16]. ABCB1/MDR1, a broad-spectrum multidrug efflux pump, eliminates toxins and carcinogenic substances as well as some of the common anti-cancer drugs such as vinblastine, vincristine, doxorubicin, daunorubicin, etoposide and paclitaxel out of the cell[17].

The MDR1 gene has more than 100 SNPs identified to date; one among these is a synonymous single nucleotide polymorphism located in exon26 3435C > T (rs1045642), often associated with altered mRNA expression, protein levels, and substrate specificity[18,19]. Various researchers have assessed the relationship between MDR1 C3435T polymorphism and breast cancer risk in divergent ethnic groups. However, the end conclusions are erratic, and the clinical significance of the polymorphism remains confounding[20-23]. Hoffmeyer et al. first explained a C3435T single base polymorphism in exon 26 and described that this may influence P-glycoprotein expression[23].

Our present study was designed to evaluate the relevance of MDR1 C3435T polymorphism with breast cancer risk in a South Indian population and its association with clinicopathological parameters. Additionally, we assessed the possible association between C3435T polymorphism and MDR1 expression levels.

II. Material And Methods

Study population:

The study cohort consisted of 300 age-matched healthy females and 426 histopathologically confirmed invasive ductal breast cancer cases visited at the Regional Cancer Center, Bangalore, India, between 1st Jan 2014 to 31st Dec 2015. Clinicopathological features collected from the case files included age at diagnosis, menstrual status, histopathological type, tumor size, pathological stage, Ellis and Elston's modification of the Scarff-Bloom Richardson (SBR) grade, axillary lymph nodes status, hormone receptor status such as estrogen receptor (ER) and progesterone receptor (PR), and HER2/neu receptor status.

To study polymorphism, 5 mL of blood was collected from all subjects before treatment in heparin vacutainers and immediately processed for DNA extraction. Tumor and grey-white tissue at least 5 cm away from the tumor as control were collected from patients whenever they underwent a modified radical mastectomy (MRM). Patients who received neoadjuvant chemotherapy were excluded from the study. The study was approved by the Scientific Review Board (SRB) and Medical Ethical Committee of the Institute, and written informed consent was collected from all the participants. The patient cohort is as reported in our previously published article[24].

Inclusion criteria:

- Female patients with primary invasive ducal carcinoma (IDC) breast
- ≥ 18 years of age

Exclusion criteria:

- Male breast cancer patients
- Any breast cancer patient other than IDC such as ductal carcinoma in situ (DCIS), inflammatory breast cancer, metastatic breast cancer, Paget's disease of the breast, Invasive lobular carcinoma (ILC), Phyllodes tumor, Tubular carcinoma
- Patients with prior therapy such as lumpectomy, neo-adjuvant chemotherapy, breast conservative surgery (BCS)

Genotyping:

The DNA extraction and genotyping were conducted as per a previous publication by Karuvaje et al. [24.] Briefly, Genomic DNA (gDNA) was isolated from leukocytes in peripheral blood using the conventional phenol-chloroform method, and concentration was determined by optical density measurement at a wavelength of 260 nm on Eppendorf Biospectrophotometer Kinetics™ (Eppendorf, Hamburg, Germany). The MDR1 C3435T genotypes were determined by polymerase chain reaction and restriction fragment length polymorphism (PCR-RFLP) assay using the primer sequences 5'-GCTGGTCCTGAAGTTGATCTGTGAAC-3' as forward and 5'-ACATTAGGCAGTGACTCGATGAAGGCA-3' as reverse primer[21]. A 30 µl PCR reaction contained 100 ng of gDNA, 1X buffer, 0.8 mM dNTP mix, 1 µM of respective primers (Sigma Aldrich), 2 U Taq DNA polymerase, and 1.25 mM MgCl2(Merck).

The PCR was performed on a Bio-Rad Thermal cycler S1000 (Bio-Rad Laboratories, USA) with initial denaturation at 94°C for 5 min followed by 35 cycles of denaturation at 95°C for 30 s, annealing at 63°C for 30

s, extension at 72 °C for 30 s, and a final extension at 72 °C for 7 min. Amplification was verified by running the PCR product on a 2% ethidium bromide-stained agarose gel and visualized under an ultraviolet transilluminator.

The 248 bp PCR product was digested overnight at 37 °C with 2 units of *Mbo* I restriction enzyme (New England Biolabs, Inc. UK). The digested products were separated in a 3% ethidium bromide agarose gel and visualized under an ultraviolet transilluminator. The digested products were 170 bp and 70 bp for the wild-type genotype CC, 238 bp, 170 bp and 70 bp for the heterozygous genotype CT, and 238 bp for the homozygous mutant TT genotype.

MDR1 gene expression analysis:

RT-PCR method was followed as mentioned in a previous publication[24]. We determined the MDR gene expression in 154 tumors and adjacent normal grey-white tissues of breast cancer patients by the RT-PCR method. Total RNA was extracted from 20 mg tissue samples stored in RNAlater® (Sigma-Aldrich, USA) using TRI Reagent (Sigma-Aldrich, USA) as specified by the manufacturer. The RNA obtained was treated with DNAse I (New England Biolabs, Inc. UK) to remove gDNA contamination. The integrity and purity of isolated RNA was electrophoretically examined on a 1% agarose gel and concentration was determined on Eppendorf Biospectrophotometer Kinetics™ (Eppendorf, Hamburg, Germany). 2 µg of DNAse-treated RNA was reverse transcribed into complementary DNA (cDNA) in a 20-µL reaction volume using High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA, USA) the following the manufacturer's protocol.

MDR1 gene expression was evaluated by quantitative real-time PCR using an Eppendorf Mastercycler® ep realplex (2S; Eppendorf, Hamburg, Germany). The following primer sequence was used to assess the MDR1 expression: MDR1 Forward 5'-TGATTGCATTTGGAGAGACAA-3'; MDR1 Reverse 5'-CCAGAAGGCCAGAGCATAAG-3'. Beta-2-microglobulin (β2M) housekeeping gene served as an internal control to measure the accuracy of RNA extraction and cDNA synthesis during the RT-PCR performance. β2M primer sequence: Forward 5'-GAGTATGCCTGCCGTGTG-3'; Reverse 5'-AATCCAAATGCGGCATCT-3'.

A 20-µL reaction volume consisting of 10 µL SYBR FAST master mix, 1 µL template cDNA, 1 µL of forward and reverse primer mix, and 8 µL double distilled water (ddH2O). KAPA SYBR FAST qPCR Master Mix served as the dye to bind to the amplified DNA and emit fluorescence during the reaction. All experiments were performed in triplicate, and the values are expressed as fold changes in mRNA expression.

MDR1 protein expression analysis:

MDR1 protein expression was determined by immunohistochemistry on 134 formalin-fixed paraffin-embedded primary breast cancer tissue blocks. Representative tumor blocks marked by the pathologist were used to construct tissue microarray (TMA) blocks. Cylindrical tissue cores with a diameter of 0.6 mm spaced 0.8 mm apart from each other were constructed using a tissue arraying instrument (Beecher Instruments, Sun Prairie, WI, USA). Thirty-two tissue cores were arranged into a single recipient paraffin block, each core representing an individual sample (duplicates). A section of 3-4 microns was cut from the TMA block, and H& E was stained to confirm the adequacy of tumor tissue.

3-micron tissue sections were mounted on 2% (3-aminopropyl) triethoxysilane-coated glass slides, deparaffinized, followed by steam antigen retrieval in Tris EDTA buffer (pH 9.0) at 145◦C for 20 minutes in a pressure cooker. Endogenous peroxidase activity was blocked by treating the sections with 3% H2O2 for 20 min followed by 30 min incubation with 3% skimmed milk to block non-specific binding sites. The sections were incubated with primary anti-human MDR1 (UIC2) mouse monoclonal antibody (1:20 dilution, sc-73354, Santa Cruz Biotechnology, Inc. USA) for 2 hours at room temperature. Super Sensitive™ Polymer-HRP IHC Detection Kit utilizing a non-biotin polymeric technique consisting of a Super enhancer and Poly-HRP reagents were used as a detection system (Biogenex Laboratories Inc., Fremont, CA, USA). 3,3`-diaminobenzidine (DAB) was used as a chromogen for color development. Haemotoxylin was used as a counterstain. A normal stomach tissue section stained with MDR1 antibody was used as the positive control (as suggested by the manufacturer). In contrast, a section with no primary antibody added was used as a negative control.

MDR1 scoring was assessed based on the intensity of staining and the percentage of tumor cells exhibiting staining using the Allred and Quick scoring methods. The staining was depicted by adding the intensity and percentage of cell score (for each factor) to arrive at a consensual score. The final score was ranked on a scale of 1–6, with 1–2 denoting mild positivity, 3–4 denoting moderate positivity, and 5–6 denoting strong positivity.

Statistical analysis:

We performed statistical analysis using IBM SPSS software for Windows Version 22. The Chi-square ($\chi 2$) test assessed the Hardy -Weinberg equilibrium in genotype distribution. Logistic regression analysis evaluated the association between MDR1 C3435T polymorphism and breast cancer risk. We applied the logistic regression analysis Chi-square test ($\chi 2$) to assess the association between the studied polymorphism and the clinicopathological parameters.

A non-parametric analysis was conducted since the MDR1 gene expression was skewed and did not follow a normal distribution. Mann-Whitney U test assessed the association between the gene expression level and clinicopathological parameters. Pearson's Chi-square ($\chi 2$) test evaluated the association between MDR1 protein expression and clinicopathological parameters. A two-way contingency table calculated the odds ratio (OR) with a 95% confidence interval (CI). Kruskal Wallis test with Dunn's multiple correction was performed to assess the MDR1 gene and MDR1 protein expression distribution across the C3435T genotypes. A P value < 0.05 was considered statistically significant.

III. Result

The clinicopathological characteristics of patients with primary invasive ductal carcinoma are summarized in Table 1. There was no significant difference in the median age of cases (46 years (range 56-39)) and controls (49.5 years (range 62-35)) groups (P> 0.05). In the study population, 69% (295/426) of the patients were above 40 years of age at diagnosis, and 55% of the patients were reported to be post-menopausal. The pathological review shows 80% of patients as histopathological grade 3 and lymph node-positive (71%), with 52% of the patients ER-positive, 45% PR-positive, and 34% HER2/neu cases.

Table no 1: **Demographic Details of breast cancer patients and controls**

CHARACTERISTICS	**N (%)**	**CHARACTERISTICS**	**N (%)**
ALL PATIENTS	426 (100)	**ALL CONTROLS**	300 (100)
AGE OF THE PATIENTS		**AGE OF THE CONTROLS**	
≤ 40 years	131 (30.8)	≤ 40 years	98 (32.67)
> 40 years	295 (69.2)	> 40 years	202 (67.3)
MENOPAUSAL STATUS		**MENOPAUSAL STATUS OF CONTROLS**	
Pre-menopausal	190 (44.6)	Pre-menopausal	128 (42.67)
Post-menopausal	236 (55.4)	Post-menopausal	172 (57.3)
TUMOR SIZE		NA	
≤ 5 cm	228		
>5 cm	198		
PATHOLOGICAL STAGE			
Early	171 (40.1)		
Late	255 (59.9)		
NODAL STATUS			
N-negative	123 (28.9)		
N-positive	303 (71.1)		
HISTOPATHOLOGICAL GRADE			
Grade 2	83 (19.5)		
Grade 3	343 (80.5)		
ESTROGEN RECEPTOR STATUS			
Negative	201 (47.2)		
Positive	225 (52.8)		
PROGESTRONE RECEPTOR STATUS			
Negative	233 (54.7)		
Positive	193 (45.3)		
HER2/Neu RECEPTOR STATUS			
Negative	279 (65.5)		
Positive	147 (34.5)		

NA: Not applicable

C3435T SNP analysis:

The genotypic and allelic frequencies of MDR1 C3435T polymorphism in breast cancer patients and controls are encapsulated in Table 2. The genotype distributions in both patient and control groups were consistent with Hardy-Weinberg equilibrium ($\chi 2 = 0.001$, $P > 0.05$ for cases and $\chi 2 = 0.03$, $P = 0.85$ for controls), and the minor allele frequency was 0.56 and 0.58 for cases and controls, respectively, as tested by the Court lab calculator. In the patient group, the wild-type homozygous genotype (CC) was found in 81 (19%) cases, the heterozygous

genotype (CT) was found in 209 (49.1%), and the mutant homozygous genotype (TT) was found in 136 (33%). Comparatively, the frequencies of genotypes were 53 (17.7%) for CC, 148 (49.3%) for CT, and 99 (33%) for TT in the control group. There was no significant difference in the genotypic frequencies between the control and patient groups (P = 0.888). The allele frequencies were 43.5 and 42.3 % for the C allele and 56.5 and 57.7 % for the T allele in cases and controls, respectively.

Table 2: ABCB1 C3435T polymorphism genotypic and allelic distribution among cases and controls

Variable	Cases (%)	Controls (%)	P value
Genotype			
CC	81 (19)	53 (17.7)	0.888
CT	209 (49.1)	148 (49.3)	
TT	136 (31.9)	99 (33)	
Alleles			
C	371 (43.5)	254 (42.3)	0.646
T	481 (56.5)	346 (57.7)	
Dominant Model			
CC	81 (16.57)	53 (17.66)	0.645
CT+TT	345 (83.43)	247 (82.34)	
Recessive Model			
CC+CT	290 (68.15)	201 (67)	0.761
TT	136 (31.85)	99 (33)	

The clinicopathological parameters of patients with primary invasive ductal carcinoma were grouped according to the MDR1 C3435T genotypes (Table 3). Our data suggests that there is a strong association between MDR1 CC genotype and tumor size greater than > 5 cm (P = 0.001), late pathological stage (P = 0.002), histopathological grade 3 (P = 0.006), positive axillary lymph node status (P = 0.041) and negative estrogen receptor status (P = 0.01). The age at diagnosis, menopausal status, PR status, and HER2/neu status were not found to be significant (P > 0.05).

Table 3: Association between Clinicopathological parameters and MDR1 C3435T polymorphism

Variable	Total	C3435T polymorphism			P Value
Age at diagnosis	**426**	CC (%)	CT (%)	TT (%)	
≤ 40	131	23(17.6)	68(51.9)	40 (30.5)	0.727
>40	295	58(19.7)	141(47.8)	96 (32.5)	
Menopausal status	**426**				
Premenopausal	190	31 (16.3)	97 (51.1)	62 (32.6)	0.655
Postmenopausal	236	45 (19.1)	111 (47)	80 (33.9)	
Tumor Size	**426**				
2 cm	90	9 (10)	40 (44.4)	41 (45.6)	**0.001***
2-5 cm	138	20 (14.5)	73 (52.9)	45 (32.6)	
5 cm	198	52 (26.3)	96 (48.5)	50 (25.3)	
Pathological Stage	**426**				
Early	171	20 (11.7)	85 (49.7)	66 (38.6)	**0.002***
Late	255	61 (23.9)	124 (48.6)	70 (27.5)	
Histological Grade (SBR)	**426**				
Grade 2	83	8 (9.6)	38 (45.8)	37 (44.6)	**0.006***
Grade 3	343	73 (21.3)	171 (49.9)	99 (28.9)	
Axillary Lymph node status	**426**				
Node-Positive	123	15 (12.2)	61 (49.6)	47 (38.2)	**0.041***
Node Negative	303	66 (21.8)	148 (48.8)	89 (29.4)	
Estrogen Receptor (ER) Status	**426**				
ER Negative	201	45 (22.4)	83 (41.3)	73 (36.3)	**0.01***
ER Positive	225	36 (16)	126 (56)	63 (28)	
Progesterone Receptor (PR) status	**426**				
PR Negative	233	49 (21)	105 (45.1)	79 (33.9)	0.182
PR Positive	193	32 (16.6)	104 (53.9)	57 (29.5)	
HER2/neu status	**426**				

Negative	279	61 (21.9)	134 (48)	84 (30.1)	0.107
Positive	147	20 (13.6)	75 (51)	52 (35.4)	

HER-2/neu -Human Epidermal Receptor-2/neu;
*P<0.05 is significant

MDR1 expression:

The MDR1 gene expression was evaluated in tumors and adjacent normal tissues. The Ct value of each sample was normalized to β2M and calibrated to the normal sample using the comparative Ct method (2-ΔΔCt). The RT-PCR analysis revealed upregulated expression of the MDR1 gene in tumor tissues in comparison to the adjacent normal tissue (P < 0.001) (Figure 1).

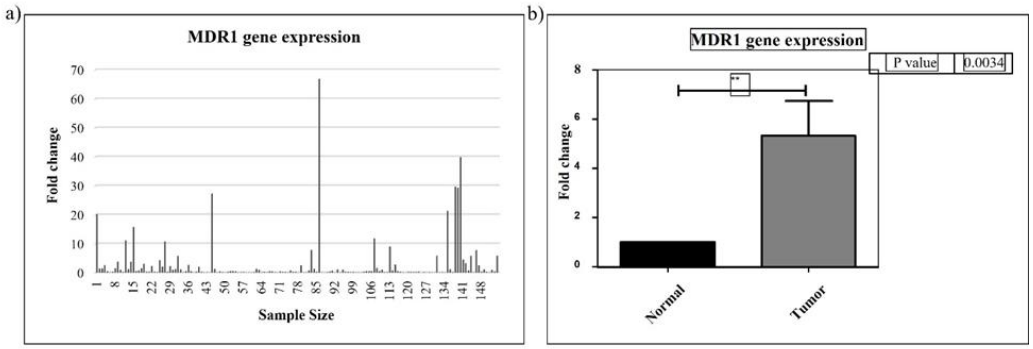

Figure-1: **a)** The fold change in MDR1 gene expression across patients measured by Ct method ($2^{-\Delta\Delta Ct}$). **b)** The MDR1 gene expression was significantly higher in the tumor tissues compared to the adjacent normal tissue.

51.9% of patients demonstrated high MDR1 expression, which is also significantly associated with larger tumor size (P = 0.001), late stage of disease (pathological stage) (P < 0.001), positive axillary lymph node status (P = 0.003) and histopathological grade 3 (P = 0.006). However, the gene expression did not show any association with age, menopausal status, or hormone receptor status (P > 0.05) (Table 4 and Figure 2). The MDR1 gene expression was grouped high and low based on a 2-fold increase in expression compared to the adjacent normal tissue.

Table 4: Association between MDR1 gene expression and clinicopathological parameters

Factor	Group	MDR1 Expression		P value	OR (95% CI)
		High	Low		
Age	≤ 40	47	59	0.223	0.62 (0.29-1.30)
	> 40	27	21		
Menopausal status	Positive	36	46	0.332	0.70 (0.35-1.39)
	Negative	38	34		
Tumor Size	≤ 5 cm	48	31	**0.001 ***	2.89 (1.44-5.93)
	> 5 cm	26	49		
Pathological Stage	Late	51	32	**< 0.001***	3.29 (1.62- 6.84)
	Early	23	48		
Axillary lymph node status	Positive	61	48	**0.002***	3.13 (1.40-7.19)
	Negative	13	32		
Histopathological Grade	3	65	55	**0.006***	3.25 (1.33-8.63)
	2	9	25		
ER	Positive	39	45	0.746	0.87 (0.43-1.71)
	Negative	35	35		
PR	Positive	35	39	0.873	0.94 (0.47-1.86)
	Negative	39	41		
HER2	Positive	25	33	0.406	0.73 (0.35-1.47)
	Negative	47	49		

*P<0.05 is significant

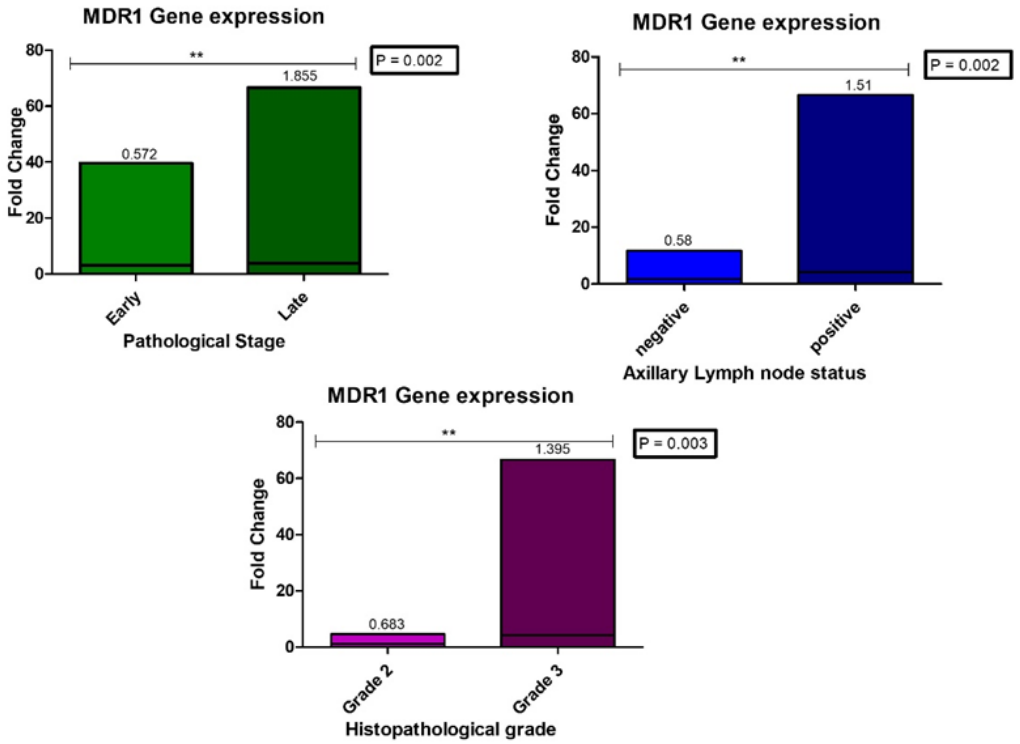

Figure 2: Gene expression analysis of MDR1 gene by RT-PCR. Expression was measured in terms of fold change. The MDR1 expression is significantly associated with a) late pathological stage, b) positive lymph node status, and c) high histopathological grade.

The tumor cells displayed both membranous and cytoplasmic staining patterns for the MDR1 marker on IHC. The MDR1 protein expression was upregulated in about 60% of the cases with moderate to high expression levels (Figure 3). The protein expression displayed concordant results concerning association with clinicopathological parameters. The subsequent analysis of the potential gene and protein expression relationship revealed a positive correlation between the variables (rs= 0.769; P <0.001). (Figure 4)

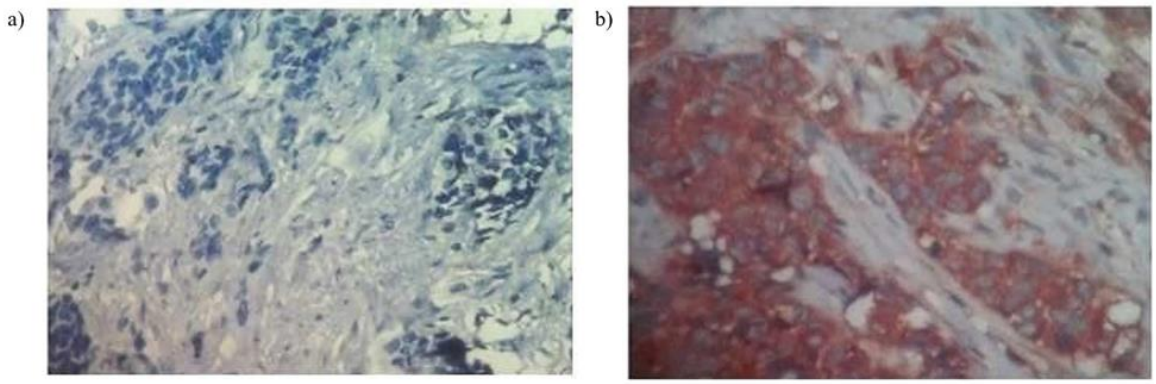

Figure 3: Immunohistochemistry staining for ABCB1 (MDR1/P-glycoprotein) on invasive ductal carcinoma breast tissues displaying cytoplasmic and membranous staining pattern: a) negatively stained cells; b) positively stained cells

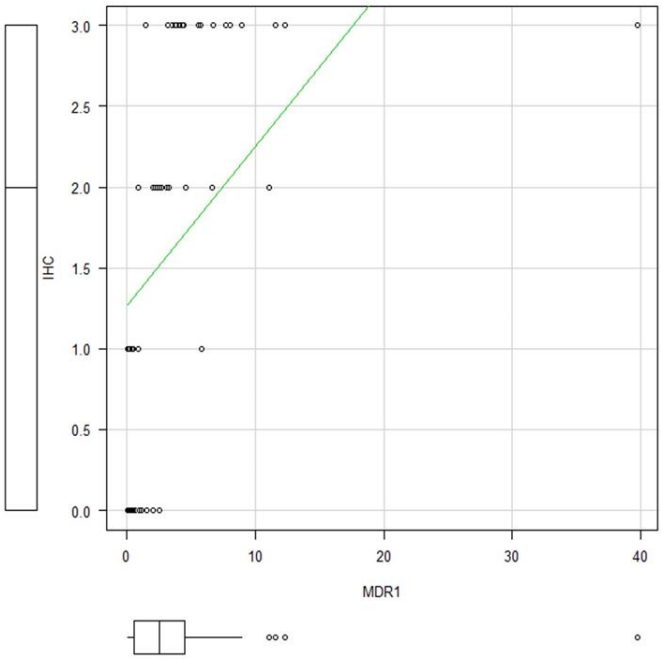

Figure 4: The Spearmen Rank correlation coefficient shows a positive correlation between the MDR gene expression and protein expression

C3435T Polymorphism and MDR1 tissue expression:

The influence of MDR1 C3435T polymorphism on the gene expression is depicted in Table 5. There was a significant association between C3435T polymorphs and MDR1 gene expression ($P < 0.001$). It was also observed that gene expression significantly differed in all three polymorphs with high expression in the CC genotypes. Similarly, the MDR1 protein expression is also significantly associated with the C3435T genotypes. The MDR1 protein expression significantly differed in the CC and TT genotypes, while the CC-CT group did not differ in expression ($P = 0.585$).

Table 5: Association between MDR1 gene expression and C3435T polymorphism

Variable	C	3435T genotypes		P value	Multiple comparisons	P value
	CC Median (Range)	CT Median (Range)	TT Median (Range)			
MDR1	5.76 (0.09-66.7)	1.42 (0.06-21.2)	0.275 (0.008-7.7)	**< 0.001**	CC-CT CC-TT CT-TT	0.001 < 0.001 < 0.001

IV. Discussion

Generally, most breast cancers respond well to initial chemotherapy, but many soon develop resistance to various anti-cancer agents. This phenomenon of multidrug resistance acts as the major hurdle in the successful treatment of breast cancer. 90% of cancer deaths occurred due to drug resistance. ATP-binding cassette transporters (ABC-transporters) are involved in the transmembrane transfer of various metabolites and toxins utilizing the energy driven by ATP hydrolysis.

In the present study, we evaluated the effect of MDR1 C3435T gene polymorphism on the risk of breast cancer and their role in modulating the gene and protein expression. The C3435T polymorphism results have conflicted, possibly due to ethnicity, study design, and sample size differences. Most of the studies reveal an association of mutant TT genotype and T allele with breast cancer risk. In a study by Abouhalima et al. [21] on Jordanian females with breast cancer, a significant association was noted between the C3435T CC genotype and an increased risk of breast cancer ($P < 0.001$). In contrast, in another study on Jordanian subjects, the T allelic frequency was higher than the C allele frequency [22]. A group of researchers [18,23,24] revealed a higher prevalence of T allele and TT genotype in breast cancer patients compared with healthy control ($P = 0.013$, $P = 0.019$, and $P = 0.025$, respectively). In agreement with various previous reported studies, our study results reject

an association between MDR1 C3435T polymorphism and breast cancer risk with no significant difference between the patient and control groups.

Further, the association between MDR1 C3435T polymorphism and clinicopathological parameters was determined. Our study observed a significant association between the polymorph and the pathological stage, high histopathological grade (grade 3), and positive lymph node status. Although there was no significant difference in the expression of CC and TT across patient groups, it was observed that patients with advanced stage, high grade, and positive lymph node status had a higher frequency of CC genotype. Similarly, in a study on 221 Caucasian females with breast cancer, a lower frequency of the T allele was observed in stage IV patients and patients with high histopathological grade [23]. A study on 248 Mexican women with early-stage breast cancer did not observe any statistical difference in the C3435T genotypic distribution among cases and controls. However, the study reports a 2-fold increased risk of breast cancer premenopausal patients with T allele [25]. Other studies did not report any association between the ABCB1 gene polymorphism and the clinical and pathological parameters [17,26-28].

The comparison between the MDR1 C3435T polymorphism and its influence on expression level revealed a significant association between the CC genotype and an increase in both gene and P-gp expression ($P < 0.001$), similar to that observed in an Iranian population [28]. In another study on a Caucasian population involving 21 healthy volunteers and patients, the P-gp duodenal expression correlated with C3435T polymorphism, and it was observed that individuals with the CC genotype had a 2-fold higher P-gp expression than those of the TT genotype [20]. Breast cancer patients with positive-ER status are treated with endocrine therapy with or without combination with systemic chemotherapeutic agents. In contrast, those with negative receptor status rely entirely on chemo-drugs. The association of MDR1 gene expression with negative ER status in our study suggests a more aggressive nature of tumors with drug resistance and poor prognosis.

Different sample sizes for polymorphism and tissue expression analysis are significant limitations of our study, as a few sub-sets of patients did not undergo surgery after presentation. A further evaluation of MDR1 expression and treatment outcome of the patient group would provide a better insight into the possible effect of the polymorphism. Due to the loss of more than 70% of follow-up cases, we could not relate the impact of polymorphism on treatment outcomes.

V. Conclusion

MDR1 C3435T polymorphism was not found to be a risk factor for breast cancer. However, the wild CC genotype may induce resistance to chemotherapy by elevating the MDR1 gene and P-gp protein expression.

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