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Polymorphic Sites (1236 and 3435) in Multi Drug Resistance Gene 1 Influencing Drug Response in Breast Cancer Patients

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Abstract: This investigation analyzed the occurrences of polymorphisms (SNPs) in MDR1 gene at exon 12 (C1236T) and 26 (C3435T) individually and in combination in breast cancer patients and determined their possible associations to adjuvant chemotherapy. The study group included hundred primary invasive ductal carcinoma patients who subsequently received chemotherapy (the regimen generally consisted of commonly used drugs like adriamycin, cyclophosphamide, 5-fluorouracil and docetaxal and their combinations). Blood samples from 100 healthy individuals used as controls were also genotyped for the MDR1 gene. This investigation revealed a statistically significant correlation with response to various regimens of adjuvant chemotherapy showing a low response to therapy with CT/TT genotype at (exon 12) 1236 codon ($p<0.001$) and favorable response to therapy with CT/TT genotype (exon 26) at 3435 codon ($p<0.001$). The combined effect of both the exons, i.e., mutant exon 12 and wildtype 26 gave poor response, whereas the combination of mutated exon 26 and wildtype exon 12 gave favorable response to chemotherapy ($p<0.0005$). These findings demonstrate for the first time that polymorphisms in exon 12 and 26 of the MDR1 gene greatly influence the variations in drug response in patients.

Key words: Breast cancer, multidrug resistance gene, C1236T and C3435T polymorphisms, SNPs, genotyping, chemotherapy, adriamycin, cyclophosphamide, docetaxel, 5-fluouracil

INTRODUCTION

The development of multidrug resistance in cancer patients presents a major obstacle to successful chemotherapy. Among the cellular mechanisms involved, a major one appears to be linked, is the over expression of a membrane transport protein termed as P-glycoprotein (P-gp) (Ambudkar *et al*., 1999). P-gp is encoded by one of the two members of mdr gene family in humans (MDR1 and MDR2). Only MDR1 in humans is involved in multidrug resistance as assessed by mdr cDNA transfections (Roninson, 1992). P-gp, the product of MDR1 gene actively transports a wide variety of chemically diverse compounds out of the cell, leading to decline in their intracellular concentrations and production of cytotoxic effects. This ATP-dependent drug efflux pump which has a tissue dependent expression pattern is not found in normal breast tissues, but P-gp expression has been detected in 48.5% of the breast cancer tissue by immunohistochemistry in various studies. Also there has been a potential increase in the expression level after chemotherapy (Bruce *et al*., 1997).

Mutational analyses of MDR1 gene reported that the gene is highly polymorphic and were extensively used to investigate P-gp structure-function relationships. More than 25 SNPs have been reported for the MDR1 gene, resulting up to 20 coding region variants (Kim *et al*., 2004; Nakamura *et al*., 2002; Pauli and Kroetz, 2004). Among the list of SNPs C1236T, G2677A/T and C3435T were reported in higher frequencies influencing various ethnic populations (Komoto *et al*., 2006). These SNPs have been found to produce altered pharmacokinetics of the drugs recognized by P-gp. Earlier we have demonstrated the role of some polymorphic genes in relation to disease susceptibility, overall survival and drug resistance (Reddy and Jamil, 2006; Suman *et al*., 2006; Kalyan and Jamil, 2006). It has been shown that a specific SNP at 1199 (G->A) in exon 11 of MDR1 gene provides a varied

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resistance to specific drug compared to the wild type genotype at the same codon as G1199A cells were reported to be more resistant to vinblastine and vincristine (Woodahl *et al*., 2004). Hence, it is seen that various SNPs in MDR1 gene not only result in altered pattern of expression of P-gp but also MDR1 genotypes play a crucial role in drug efficacy and drug response. In the present investigation we have determined the SNPs of MDR1 gene at codon 1236 (exon 12) and codon 3435 (exon 26) site and investigated the role of various haplotype combinations on the drug response pattern in breast cancer cases treated with adjuvant chemotherapy.

MATERIALS AND METHODS

Study group and study design: In this study, we included hundred breast cancer patients who were clinically and histopathologically diagnosed as Invasive Ductal Carcinoma (IDC) at three hospitals (Mahavir Hospital, Indo-American Cancer Research Institute and M.N.J. Cancer Hospital-Hyderabad, A.P. India) from June 2005-June 2006. All patients underwent complete staging procedures in the respective pathology laboratories. The study was approved by the Hospital Ethical-Committee Boards of both Bhagawan Mahavir Medical Research Centre (Hyderabad, A.P. India), (where the research was carried out) and Indo-American Cancer Research Institute. Written informed consent was obtained from all patients and control subjects included in this study.

Collection of demographic data: This demographic data was collected using a structured questionnaire, which contained the details of age, stage, disease history, family history and other relevant details as required.

Selection criteria: Hundred patients with primary breast cancer (invasive ductal carcinoma-IDC) were randomly enrolled in this study. These patients underwent surgery without prior neoadjuvant chemotherapy. During the selection of the patients feasibly progressiveness of the disease and body mass index were matched to remove the drug-dose as a confounder. Cases were not age matched, since, the drug-metabolizing enzyme polymorphisms were not related to age factor (Kurzawski *et al*., 2005). Hundred healthy females of similar age (50±5 years) as that of patients were voluntarily enrolled as controls and they were not receiving any prolonged medication, were not suffering from any genetic disorder nor had any family history of cancer. Both the study groups were drawn from Andhra region of Southern part of India.

Sample collection: Two milliliter of venous blood was drawn using sterile heparinized syringe from healthy individuals as control samples after clinical checkup. The biopsy samples of malignant cases were obtained after diagnosis from the pathology laboratory for genotyping using various protocols.

Clinicopathological diagnosis: Pathological tumor stages, types and status of nodal invasion were obtained from medical records. The reports of ER/PR statuses were not included because the correlation of these statuses was reported to be negligible with the MDR1 expression and function by some investigators (Bruce *et al*., 1997).

Chemotherapy regimens: The study group received mainly four different combinational regimens as adjuvant chemotherapy. The most common combination was of 5-fluorouracil, adriamycin and cyclophosphamide (FAC). The other combinational chemotherapy regimens were cyclophosphamide with adriamycin (CA); cyclophosphamide and adriamycin followed by docetaxel (CAD) and adriamycin with docetaxel (AD).

MDR1 genotyping

Extraction of DNA: The biopsy samples of cancer tissues were cut into small bits and homogenized in Phosphate Buffered Saline (PBS). Genomic DNA from homogenized tissue and from Control blood samples (300 μL) was extracted using protein salting out procedure followed by ethanol precipitation method (Miller *et al*., 1988).

Primer designing: PCR primers were designed to amplify the complete Exon 12 and Exon 26 of MDR1 based on the GenBank reference sequence (Ac. No. NT007933). Software tools as Primer and OligoAnalyzer (Version 3, IDT) had been used to select the most suited set of primers.

The set of primers designed for genotyping by PCR were:

Exon 12

Forward 5'CTCGCATGGGTCATCTCAC3'
Reverse 5'GTTTTTTTCTCACTCGTCCTGGTAG3'

Exon 26

Forward 5'GTTTATTTGAAGAGACTTAC3'
Reverse 5'CTGTGAACTCTTGTTTTCAG 3'

Polymerase Chain Reaction (PCR) of exon 12 and exon 26 of MDR1 gene: PCR was performed in a 50 μL reaction volume with conditions as initial denaturation at 94°C for 5 min followed by 35 cycles of 30 at 94°C; annealing of 30 sec at 58°C for exon 12 and at 51°C for exon 26; extension of 30 sec at 72°C and a final elongation at 72°C for 10 min. The PCR products were checked along with 100 bp Ladder in 2% Agarose gel.

Single Strand Confirmatory Polymorphism (SSCP) analysis: A 15% polyacrylamide gel was used for Single Strand Confirmatory Polymorphism (SSCP) analysis for determining the allelic variants in the exon 12 and exon 26 of MDR1 gene. Five microliter of PCR products were heat denaturated in an equal volume of deionized formamide and 0.01% xylene cyanol for 5 min. The denatured samples were snap cooled for 10 min and loaded on a SSCP gel which were run with continuous cooling at 4°C at 125 V for 3 h. SSCP bands were visualized using Silver Staining. The mobility shifts detected the presence of polymorphisms. For quality control, the genotyping analysis was repeated twice for every sample showing extra or shifted bands. To confirm the SNPs, PCR products showing the presence of polymorphisms were purified and examined by DNA sequencing (Sequencing India, MWG Biotech Ltd.).

Statistical analysis: All analyses were performed using Graph Pad Softwares (Online version) and Georgetown Linguistics (developed by Georgetown University). Fisher's Exact Test (FET) (GraphPad Softwares; Online version). Chi-Square for Trend with Mantel Extension Method (Epi Info Version6, developed by CDC, USA) was performed to examine significance of the association between haplotype combinations of both SNPs and chemotherapy response. Statistical significance of the genotype frequencies compared to the expected values and evaluated according to Hardy-Weinberg rule. The allelic variations among the cases and controls were analyzed using Chi-square test, Odd's ratio evaluation at 95% Confidence Interval and Fisher's exact test. A p-value less than 0.05 was considered to be statistically significant. Chi-square test was also used to evaluate the association between genotype variants individually and in combination with chemotherapy response.

RESULTS

Characteristics of patients: The average age of the randomly selected group of hundred invasive ductal carcinoma cases, with stage II (50%) and stage III (35%) disease was about 51.5 years. All the cases enrolled for the study showed metastatic disease with 60% of them showing nodal invasion. Fifty eight percent of the patients were pre-menopausal cases with an average age of 37.5 years. The average age of post-menopausal patients was found to be 61.2 years. The demographic details revealed early onset and late diagnosis of breast cancer in this scenario (Table 1).

Genotyping results: PCR amplification of MDR1 Gene (exon 12 and 26) in genomic DNA isolated from tumor tissues and blood samples; was carried out using the primers as stated above and the products were analyzed using SSCP-PAGE. The results of the distribution pattern of the denatured products are shown in Fig. 1. The PCR product of exon 12 was of 126 bp and that of exon 26 was 207 bp.

MDR1 genotype frequency: The frequency of wild type allele C at 1236 codon (exon 12) of MDR1 gene in the patients group was 0.79 and that of the mutant allele T was 0.21, where as that in controls C allele was in frequency of 96% and T allele was 4% only. Whereas, frequency of wild type C at 3435 codon in exon 26 was 0.75 in cases and 0.92 in controls and that of mutant allele T was 0.25 in cases and 0.08 in controls.

C->T transition at the 3435 codon (exon 26) was more prevalent compared to the C->T transition at the 1236 codon in exon 12 in cases as well as controls (Table 2).

The statistical significance of the haplotype distributions with regards to both exon 12 (1236) and 26

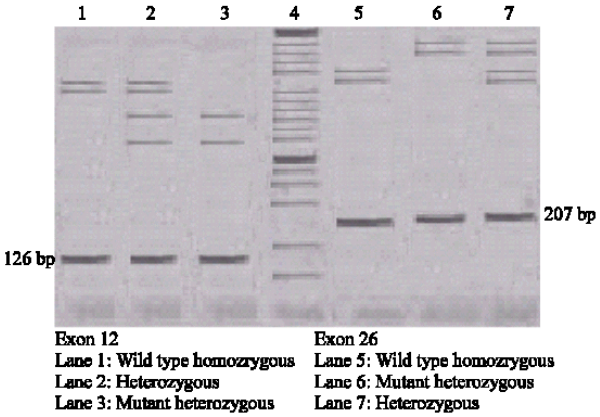

Fig. 1: Single Strand Confirmatory Polymorphisms (SSCP-PAGE) analyses of Exon 12 and Exon 26 of MDR1 gene and distributions of SNPs in the samples

Table 1: Characteristic of breast cancer patients (Invasive Ductal Carcinomas)

	Age group		Tumor stage				Nodal invasion			
Characteristics	Pre-menopausal (Avg. age -37.5 years)	Post-menopausal (Avg. age -61.2 years)	I	II	III	IV	N0	N1	N2	N3
No. of cases (n = 100)	58	42	13	50	35	02	40	10	34	16

Table 2: The genotype frequencies of MDR1 gene at Exon 12 and 26 (C1236T and C3435T)

Genotypes		Cases 100	Controls 100	Allelic frequency Case (100)	Allelic frequency Controls (100)	OR	(CI = 95%)	Chi-square (χ^2), df = 2 *p-value (two-sided)	Fisher's exact test p-value
Exon 12	1236 CC (WT)	73	93	C = 0.79	C = 0.96	0.08	0.0612 to 0.1167	15.548 (p = 0.001)	0.0003
	1236CT (HM1)	11	5						
	1236TT (HM2)	16	2	T = 0.21	T = 0.04				
Exon 26	3435CC (WT)	58	87	C = 0.75	C = 0.92	0.13	0.1026 to 0.1694	21.668 (p = 0.001)	0.0001
	3435CT (HM1)	34	9						
	3435TT (HM2)	8	4	T = 0.25	T = 0.08				

*: p<0.05 is considered as statistically significant. WT: Wild type; HM1: Heterozygous Mutant; HM 2: Homozygous Mutant

(3435) individually in cases and controls, were assessed using Fisher's Exact Test (FET) and chi-square test (CST). The mutant haplotypes (heterozygous-CT and homozygous-TT) more prevalent (27%) in cases when compared to controls (7%) at 1236 codon (exon 12) [FET-p<0.0003 and CST-p<0.001]. Similarly, the presence of mutant haplotypes was frequent in cancer cases (42%) when compared to healthy controls (13%) in a statistically significant manner in exon 26 at 3435 codon of MDR1 gene [FET: p<0.0001 and CST: p<0.0001].

The genotype distributions of both polymorphisms among the overall cases were in Hardy-Weinberg equilibrium.

Distribution of MDR1 haplotype with respect to age, stage and nodal invasion: There has been an interesting pattern of distribution of the haplotype at both sites (1236 and 3435) of MDR1 with respect to the various clinicopathological characteristics of the patients. 44.4% of patients showing the polymorphic haplotypes of exon 12 (1236 CT and TT) were in the age range of 50 to 59 years; whereas maximum numbers of patients with polymorphic haplotypes of exon 26 (3435 CT and TT) were in the range of 40 to 60 years. Almost 50% of the patients showing polymorphic haplotypes of each of exon 12 and exon 26 were having Stage II of the disease. Similarly more than 50% of the cases having either of polymorphic exon 12 or exon 26 had shown extensive nodal invasion (Table 3).

Type of response to chemotherapy versus individual haplotypes: The regimen of chemotherapy varied depending on the stage of the disease, tolerability of the patients and response, which ranged from 5-8 cycles. The mode of the drug administration was intravenous (Table 3). The response to chemotherapy was assessed after every fourth week according to WHO Criteria (WHO, 1979). The type of responses are defined as Complete Response (CR)-complete disappearance of all known disease for at least four weeks, Partial Response (PR)-estimated decrease in tumor size of 50% or more for at least four weeks and No Change (NC) no significant change for at least four weeks. This includes stable disease, estimated decrease of less than 50% and lesions with estimated increase of less than 25%, which is referred as poor response in the present investigation.

Table 3: No. of patients showing polymorphisms (ie 69 out of 100) in exon 12 (C1236T) and exon 26 (C3435T) of MDR1 gene categorized by age-range, stage and nodal invasion

Characteristics	C1236T in Exon 12 (n = 27) CT	C1236T in Exon 12 (n = 27) TT	C3435T in Exon 26 (n = 42) CT	C3435T in Exon 26 (n = 42) TT
Age range				
80-89	0	0	0	0
70-79	0	1	2	0
60-69	1	0	2	4
50-59	6	6	17	2
40-49	2	4	9	2
30-39	1	5	3	0
20-29	1	0	1	0
Tumor stage				
I	1	0	4	0
II	7	9	20	0
III	3	6	10	7
IV	0	1	0	1
Nodal invasion				
N1	3	4	6	0
N2	6	6	18	2
N3	2	6	10	6

Table 4: Patient's response (in %) in each category with individual variations in genotypes (C1236T and C3435T) of MDR1 gene to chemotherapy regimens

Response (Table 4)	1236TT	1236CT	1236CC	3435CC	3435CT	3435TT
Poor	66	24	3	39	3	0
Partial	34	76	72	52	67	14
Complete	0	0	25	9	30	86

The distributions of the MDR1 1236C>T (exonl2) and 3435C>T (exon26) genotypes in patients showing various stages of responses to chemotherapy are shown in Table 4.

TT haplotype at C1236T codon in exon 12 was found to be strongly linked with poor response (67%) where as only 23% of the cases with CT haplotype had shown poor response when compared to only 2% with wild type homozygous (CC) genotype at this site giving significantly poor response (p = 0.0001). On the contrary the TT haplotype at C3435T (exon26) codon in Exon26 of MDR1 gene was found to be associated with better

Table 5a: Correlation of response to chemotherapy Cyclophosphamide (1100 mg) and Adriamycin (110 mg) with the haplotypes of the C1236Tand C3435T polymorphisms of MDR1 gene

Response total patients-42	1236CC and 3435CC	1236CT and 3435CC	1236TT and 3435CC	1236CC and 3435CT	1236TT and 3435CT	1236CT and 3435CT	1236CC and 3435TT	1236CT and 3435TT	1236CT and 3435CT
Poor	15 [8 Stage II and 7 Stage III]	1 [Stage II]	1 [Stage II]	0	0	0	0	0	0
Partial	16 [9 Stage II and 8 Stage III]	1 [Stage II]	0	2 [1 Stage I and 1 Stage II]	0	1 [Stage I]	0	0	0
Complete	1 [Stage II]	0	0	1 [Stage II]	2 [Stage II]	1 [Stage II]	0	0	0

Table 5b: Correlation of response to chemotherapy Cyclophosphamide (1100 mg); Adriamycin (110 mg) and 5-Fluorouracil (700 mg) with the haplotypes of the C1236Tand C3435T polymorphisms of MDR1 gene

Response total patients-35	1236CC and 3435CC	1236CT and 3435CC	1236TT and 3435CC	1236CC and 3435CT	1236TT and 3435CT	1236CT and 3435CT	1236CC and 3435TT	1236CT and 3435TT	1236CT and 3435CT
Poor	7 [Stage II]	1 [Stage IV]	0	0	1 [Stage III]	0	0	0	0
Partial	6 [Stage III]	2 [1 Stage I and 1 Stage II]	1 [Stage III]	2 [1 Stage I	2 [Stage II] and 1 Stage II]	1 [Stage II]	0	0	0
Complete	1 [Stage III]	0	0	2 [Stage III]	0	2 [Stage II]	0	3 [Stage II]	1 [Stage III]

Table 5c: Correlation of response to chemotherapy Cyclophosphamide (1100 mg); Adriamycin (110 mg) and Docetaxel (130 mg) with the haplotypes of the C1236Tand C3435T polymorphisms of MDR1 gene

Response total patients-13	1236CC and 3435CC	1236CT and 3435CC	1236TT and 3435CC	1236CC and 3435CT	1236TT and 3435CT	1236CT and 3435CT	1236CC and 3435TT	1236CT and 3435TT	1236CT and 3435CT
Poor	3 [2 Stage II and 1 Stage III]	0	5 [3 Stage II and 2 Stage III]	0	1 [Stage II]	0	0	0	0
Partial	0	0	0	1 [Stage II]	0	1 [Stage III]	0	0	0
Complete	1 [Stage IV]	0	0	0	1 [Stage II]	0	0	0	0

Table 5d: Correlation of response to chemotherapy Adriamycin (90 mg) and Docetaxel (130 mg) with the haplotypes of the C1236Tand C3435T polymorphisms of MDR1 gene

Response total patients-10	1236CC and 3435CC	1236CT and 3435CC	1236TT and C3435C	1236CC and 3435CT	1236TT and 3435CT	1236CT and 3435CT	1236CC and 3435TT	1236CT and 3435TT	1236CT and 3435CT
Poor	1 [Stage III]	1 [Stage II]	0	0	0	0	0	0	0
Partial	1 [Stage II]	0	0	0	0	0	0	0	0
Complete	0	0	2 [Stage III]	0	1 [Stage II]	0	1 [Stage II]	2 [Stage III]	1 [Stage II]

pathological response towards chemotherapy treatment. 87% of the patients with T3435T (exon26) haplotype had shown complete response ($p = 0.0001$) whereas only 3.75% of the patients with wild type homozygous (CC) genotype had given complete response.

Response to chemotherapy combinations versus haplotype combinations: As mentioned earlier the 100 cases enrolled for the study received 4 different combinations of the drugs-adriamycin, cyclophosphamide, 5-fluorouracil and docetaxel.

For analyzing the genotyping data we have used the Epi-Info Version-6, developed by CDC, USA with adjustment for possible confounders such as age as a continuous variable and stage and chemotherapy combinations as nominal variable, the pattern of occurrence of response was found to be invariable with stage of the disease and chemotherapy combination. The distributions of various haplotype combinations among the responders (partial and complete responders) and non-responders (poor responders) with different chemotherapy combinations are shown in Table 5a-d. The distribution has been found to be statistically significant ($p<0.04$).

Collective responses to chemotherapy combinations versus the haplotype combinations: From the distributions it is evident that the number of responders increases towards the haplotype combinations with mutant MDR1 genotype at 3435codon (exon 26) and similarly the number of non-responders were high with haplotype combinations with mutant 1236 codon (exon 12) with wild type 3435 (exon 26), both irrespective of the stage of the disease and combination of drugs. Hence, distribution of the haplotype combination among the responders and non-responders among the overall cases in common is considered for further investigations (Table 6).

Table 6: Percentages (%) of patients in each category of response to chemotherapy with respect to their haplotype combinations of the 1236C>T and 3435C>T polymorphisms of MDR1 gene

Response	1236CC and 3435CC (*n = 50)	1236CT and 3435CC (n = 3)	1236TT and 3435CC (n = 4)	1236CC and 3435CT (n = 20)	1236CT and 3435CT (n = 6)	1236TT and 3435CT (n = 8)	1236CC and 3435TT (n = 1)	1236CT and 3435TT (n = 5)	1236TT and 3435TT (n = 2)
Poor	36	67	60	0	0	0	0	20	0
Partial	56	33	20	67	0	33	0	40	50
Complete	8	0	20	33	100	67	100	40	50

$p<0.0005$; *: n = Total number of patients with the specific haplotype combination

33.75% of the patients harboring the 1236CC (exon 12) with 3435CT (exon 26) and all the patients with haplotype combination of 1236CC (exon 12) and 3435TT (exon 26) had a significantly better response to chemotherapy compared than other haplotype combinations ($p<0.0005$).

DISCUSSION

In this study, haplotype combinations of the two polymorphic sites of MDRI gene of the patients were taken under consideration for correlation with drug response due to interaction with P-gp. The recognition site of P-gp is formed by two electron donor groups with a spatial separation of 2.5±0.3Å (Type I) or three electron donor groups with a spatial separation of the outer two groups of 4.6±0.6Å. It is reported that the binding of P-gp increases with the strength and the number of electron donor or hydrogen bonding acceptor groups forming the Type I and Type II units. Correspondingly, a high percentage of amino acids with hydrogen bonding donor side chains are found in the transmembrane sequences of P-gp relevant for substrate interaction. Molecules that minimally contain one Type II unit are predicted to be inducers of P-gp over expression. Drugs such as adriamycin and docetaxel which are used in the present investigation for chemotherapy are reported to be both type I and II substrate (Seeliig, 1998). Among the other two drugs (cyclophosphamide and 5-fluorouracil) 5-flourouracil had been reported to show an indirect effect on induction of MDR1 expression by influencing the amplification of Y-box binding protein-1, which in turn results in amplified transcription of the MDRI gene by binding onto the inverted CCAAT box of the promoter region (Stein *et al*., 2005; Bargou *et al*., 1997). The two substrate drugs (adriamycin and docetaxel) which act as P-gp inducers as described earlier, result in the over-expression of the protein and provide resistance to selective agent or cross-resistance to a broad spectrum of structurally and functionally dissimilar drugs in the wild type genotype of MDR1 gene.

The previous reports (Huizing *et al*., 1995; Huinink *et al*., 1993; Reichman *et al*., 1993; Seidman *et al*., 1995; Roy and Horowitz, 1985) stating the increase in P-gp expression in breast carcinoma with the heightened use of taxols and anthracyclines in the treatment of breast cancer supports our results indicating less number of patients with wild type exon 26 showing complete response (5%) in the study group. As described by Hoffmeyer *et al*. (2000) and other researchers (Komoto *et al*., 2006; Hoffmeyer *et al*., 2000), reduced efflux of the drugs by 3435TT genotype in the Exon 26 may have resulted in maximum responders with this genotype in our investigation.

Similarly, recent reports on polymorphisms at 3435 (CT) site has indicated a significant correlation with variable drug response in Small-Cell Lung cancer patients after chemotherapy (Sohn *et al*., 2006). In the TT genotype at 3435 of MDR1 gene it has been reported that the mRNA stability is reduced (Wang *et al*., 2005) and the gene expression and function is reduced by a factor of two compared to the CC (wild) genotype, hence, the very high percentages 86% of patients harboring the TT genotype had shown complete response in the present investigation. Our results indicated that patients with the TT genotype were more likely to respond to chemotherapy, support the hypothesis that basal expression and induction of MDR1 depends on the genotype at the polymorphic site C3435T. A similar correlation between final clinical outcome and MDR1 Exon 26 variants was recently reported for patients suffering from Acute Myeloid Leukemia (Krzysztof *et al*., 2004a) and Breast Carcinoma with neoadjuvant chemotherapy (Kafka *et al*., 2003).

Many authors suggested that impairment of function of the barrier in 3435TT subjects could render this genotype more susceptible to the development of the diseased condition (colon and renal cancers) (Siegsmund *et al*., 2002). The prevalence of T allele at 3435 codon of MDR1 gene in breast cancer patients in the present study depicts the allelotype as the susceptibility factor. Since, the cases under this investigation were those which did not receive neoadjuvent therapy, we believe that these mutations could be due to the tumor progression conditions.

In our observations breast cancer cases present linkage disequilibrium (19%) among the two SNPs. Though the SNP in exon 12 (C1236T) was not been

reported sufficiently to state its effect in altering drug response and efficacy but in a study on multiple myeloma, it was reported that C1236T (exon 12) was not only strongly linked with C3435T (exon 26), but also correlated to longer overall survival when compared to wild type genotype (Krzysztof *et al*., 2004b). Present results revealed a strong correlation of wild type exon 12 with poor response to the given chemotherapy treatment in the patients. An interesting observation was that the polymorphisms in exon 12 (1236CT and TT) occurred mostly in post-menopausal women showing a poor response to chemotherapy ($p = 0.0003$), whereas the SNP in exon 26 (C3435T) occurred in both pre and post menopausal cases and their response to chemotherapy was more favorable ($p = 0.0001$).

It is therefore seen that MDR1 1236C>T (exon 12) and 3435C>T (exon 26) polymorphisms and their haplotypes were associated with the response to chemotherapy consisting adriamycin and/or docetaxel (P-gp inducer) whereas response to cyclophosphamide is not influenced by these polymorphisms in primary IDC cases.

Hence, genotyping of the tumor samples with respect to these two SNPs may serve as an important clue for designing drug regime for forecasting better response and minimal adverse drug reactions in case of adjuvant chemotherapy in breast cancer patients.

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CONFLICT OF INTEREST

The authors declare that they have no competing interest.

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