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ORIGINAL ARTICLE

Impact of *ALDH1A1* and *NQO1* gene polymorphisms on the response and toxicity of chemotherapy in Bangladeshi breast cancer patients

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Abstract

Purpose Cyclophosphamide, Epirubicin/Doxorubicin, 5-fluorouracil (CEF or CAF) chemotherapy has long been a standard first-line treatment for breast cancer. The genetic variations of enzymes that are responsible for the metabolism of these drugs have been linked to altered treatment response and toxicity. Two drug-metabolizing enzymes ALDH1A1 and NQO1 are critically involved in the pathways of CEF/CAF metabolism. This study aimed to evaluate the effect of *ALDH1A1* (rs13959) and *NQO1* (rs1800566) polymorphisms on treatment response and toxicities caused by adjuvant (ACT) and neoadjuvant chemotherapy (NACT) where CEF/CAF combination was used to treat Bangladeshi breast cancer patients.

Methods A total of 330 patients were recruited from various hospitals, with 150 receiving neoadjuvant chemotherapy and 180 receiving adjuvant chemotherapy. To extract genomic DNA, a non-enzymatic simple salting out approach was adopted. The polymerase chain reaction-restriction fragment length polymorphism method was used to detect genetic polymorphisms. Unconditional logistic regression was used to derive odds ratios (ORs) with 95% confidence intervals (CIs) to study the association between genetic polymorphisms and clinical outcome and toxicity.

Results A statistically significant association was observed between *ALDH1A1* (rs13959) polymorphism and treatment response (TT vs. CC: aOR = 6.40, $p = 0.007$; recessive model: aOR = 6.38, $p = 0.002$; allele model: $p = 0.032$). Patients with the genotypes TT and CT + TT of the *NQO1* (rs1800566) polymorphism had a significantly higher risk of toxicities such as anemia (aOR = 0.34, $p = 0.006$ and aOR = 0.58, $p = 0.021$), neutropenia (aOR = 0.42, $p = 0.044$ and aOR = 0.57, $p = 0.027$), leukopenia (aOR = 0.33, $p = 0.010$ and aOR = 0.46, $p = 0.005$), and gastrointestinal toxicity (aOR = 0.30, $p = 0.02$ and aOR = 0.38, $p = 0.006$) when compared to the wild CC genotype, while patients with the genotype CT had a significant association with gastrointestinal toxicity (aOR = 0.42, $p = 0.02$) and leukopenia (aOR = 0.52, $p = 0.010$). The TT and CT + TT genotypes of rs13959 had a significantly higher risk of anemia (aOR = 2.00, $p = 0.037$ and aOR = 1.68, $p = 0.029$). There was no significant association between rs1800566 polymorphism and treatment response.

Conclusion Polymorphisms in *ALDH1A1* (rs13959) and *NQO1* (rs1800566) may be useful in predicting the probability of treatment response and adverse effects from CEF or CAF-based chemotherapy in breast cancer patients.

Keywords Polymorphism · Breast cancer · *ALDH1A1* · *NQO1* · Response · Toxicity

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Introduction

Breast cancer is now the most frequent and aggressive female malignancy in both developed and developing countries, and it is the most frequent cause of death in women worldwide [1, 2]. Throughout the world, 2.3 million women were diagnosed with breast cancer and 685,000 died in the year 2020 [3]. In Bangladesh, there is no different scenario. According to the Global Cancer Observatory report, 13,028 new female breast cancer cases were diagnosed in 2020 [4]. According to the International Agency for Cancer Research (IARC), over 7,000 Bangladeshi females die of breast cancer every year [5].

Breast cancer treatment is complicated, requiring a combination of local and systemic treatments. Surgery and radiotherapy are examples of local therapy, while hormone therapy, targeted therapy, and chemotherapy are examples of systemic therapy [6, 7]. The number of effective treatment choices for breast cancer is growing; nevertheless, the utility of particular therapy for specific patients is yet unclear, and adverse events associated with the therapy vary greatly from patient to patient. Variations in tumor and host variables are the cause of anticancer treatment therapy's inconsistency in terms of safety and efficacy [8, 9]. Slight changes in genotype can lead to varied protein expressions and, as a result, a diverse phenotype [10]. Pharmacogenetics is a recent branch of medicine that investigates how a person's genetic characteristics affect their drug response. These differences have an impact on therapy response, as well as potential side effects. A rising body of evidence suggests that genes that are responsible for carcinogen/drug metabolism or DNA repair may play a key role in predicting cancer susceptibility and treatment results in individuals. The finding of single-nucleotide polymorphisms (SNPs) in the drug-metabolizing enzyme may be used to predict chemotherapy toxicity and/or efficacy, which could have major clinical ramifications [11, 12].

The anticancer medication cyclophosphamide (CPA) is one of the most extensively utilized in treating solid tumors such as breast cancer, especially in the case of adjuvant settings [13, 14]. CPA is frequently used in combination with other chemotherapy medications like adriamycin/epirubicin and 5-fluorouracil (CAF/CEF) [13]. Anemia, leukopenia, neutropenia, thrombocytopenia, and gastrointestinal disorders are common adverse drug reactions (ADRs) associated with the CPA-based combination treatment for breast cancer [15].

NQO1 is a phase-1 drug-metabolizing enzyme. NAD(P)H quinone oxidoreductase-1 (NQO1) converts quinone-based anticancer drugs to hydroquinones, preventing oxidative stress, reactive oxygen species generation, and carcinogenesis [16, 17]. NQO1 is disabled by a homozygous common missense mutation (*NQO1**2 or rs1800566(T)), which has been linked to reduced enzyme activity and poor survival of women receiving adjuvant chemotherapy for breast cancer [18]. A study by Nagata et al. using AMR (Amrubicin hydrochloride) monotherapy for SCLC indicated that due to the *NQO1* C609T polymorphism, treatment was related to a substantial rate of bone marrow suppression and hematologic toxicity (grade 3/4) [19]. Akhtari et al. observed in their study that *NQO1* rs1800566 (TT genotype) SNP was associated with treatment response variation and erlotinib toxicity [20].

ALDH1A1, ALDH1A2, ALDH1A3, ALDH1B1, and ALDH1L1 are members of the ALDH1 (aldehyde dehydrogenase 1 family). ALDH1A1 is a relatively abundant cytosolic protein of the human body that belongs to a family of NAD(P)+-dependent enzymes and is principally engaged in the biotransformation of endogenous and exogenous primary alcohols to aldehydes and weak carboxylic acids, respectively [21, 22]. This enzyme participates in the metabolism of cyclophosphamide (CPA) and helps cancer cells retain their stemness [23]. The inter-individual response to cyclophosphamide therapy has been observed to be influenced by genetic diversity in ALDHs [24, 25]. Patients heterozygous for (*ALDH1A1**2 or rs615103) exhibited an elevated risk of liver damage in a limited study of breast cancer patients treated with high dose of cyclophosphamide [26]. Another study by Yao et al. discovered that SNP rs3764435 and rs63319 were linked to grade 3 and 4 hematological toxicity after AC (doxorubicin and cyclophosphamide) treatment in breast cancer patients [27]. However, studies regarding the influence of *ALDH1A1* polymorphism on the treatment response and toxicity of CAF/CEF-based chemotherapy were very few and inconclusive, and none of them have looked at the rs13959 SNP.

The purpose of this research was to determine the influence of genetic polymorphisms in the *ALDH1A1* (rs13959) and *NQO1* (rs1800566) genes on the treatment and toxicities caused by CAF/CEF-based adjuvant (ACT) and neoadjuvant (NACT) chemotherapy in Bangladeshi breast cancer patients.

Materials and methods

Study population and research settings

This research enrolled 330 participants between March 16, 2017, to December 31, 2019. They were all diagnosed with invasive breast cancer that was confirmed histologically. All of the participants were recruited from various hospitals in Bangladesh, including Dhaka Medical College Hospital, Bangabandhu Sheikh Mujib Medical University

Hospital, National Institute of Cancer Research and Hospital (NICRH), Ahsania Mission Cancer Hospital, and Delta Medical College Hospital. All of the patients got CEF/CAF-based chemotherapy, with 150 getting neoadjuvant treatment and 180 getting adjuvant treatment. Before providing a written agreement, patients were fully informed about the experimental methodology and the goal of the research. The research was conducted following Helsinki Declaration and its amendments (adopted by the 18th WMA general assembly, Helsinki, Finland, June 1964, and the last amendment in Seoul, South Korea, in October 2008). The genetic research was conducted in the Pharmacogenetic and Pharmacokinetics Laboratory of the Department of Clinical Pharmacy and Pharmacology, Faculty of Pharmacy, University of Dhaka, in collaboration with the Molecular Biology Research Laboratory, Southeast University, and QUEST Bangladesh Biomedical Research Centre.

Criteria for assessing responsiveness and toxicity

The influence of *NQO1* and *ALDH1A1* polymorphisms on chemotherapy treatment response was studied in 150 patients who received CEF/CAF-based NACT, whereas the toxicity effect was studied in patients (330) who received both CEF/CAF-based NACT and ACT. The Response Evaluation Criteria in Solid Tumors (RECIST): Revised RECIST guideline (version 1.1) was used to assess tumor response to treatment [28]. For data processing, two separate groups of patients were created—responder groups, including complete and partial responders and stable and progressing disease in case of non-responders. Chemotherapy toxicity was examined using the Common Terminology Criteria for Adverse Events (CTCAE v4.0) from 2009 [29]. The tumor-node-metastasis (TNM) staging method (Sixth edition) of the American Joint Committee on Cancer (AJCC) was used to evaluate both clinical stages before chemotherapy and the pathological response of the primary tumor and axillary lymph nodes following treatment. The treatment response was evaluated after 2 or 3 cycles of a planned 6-cycle regimen. Anemia, Neutropenia, and Thrombocytopenia were evaluated every 2 to 3 weeks, aligned with the chemotherapy cycles, especially 7–14 days after each cycle.

DNA extraction and genotyping

A 10 ml blood sample was taken from each patient and held at -20 °C in an EDTA-containing storage tube before DNA extraction. The simple non-enzymatic salting out approach was used to extract genomic DNA [30, 31]. Micro-volume Spectrophotometer (Genova Nano, Jenway) was used for the quantification of DNA, setting the absorbance ratio at 260/280 nm. The *ALDH1A1* (rs13959) and *NQO1* (rs1800566) polymorphisms were genotyped using the Polymerase Chain Reaction (PCR)-Restriction Fragment Length Polymorphism (RFLP) method.

Our research group with Primer Blast designed primers for ALDH1A1 rs13959 were 5'-TGTTGACAAGGCA GTGAAGG-3' (forward) and 5'-CAAACGCTGAATGCT TTTGA-3' (reverse), whereas for *NQO1* rs1800566 polymorphism, 5'-AAGCCCAGACCAACTTCT-3' (forward), 5'-ATTTGAATTCGGGCGTCTGCTG-3' (reverse) were used designed previously [32]. For the digestion of PCR products, two units of the appropriate enzymes (BamH*I* for rs13959 and HInf*I* for rs1800566) from New England Biolabs® of the United States were used. Digested products were processed with the help of electrophoresis using 1% agarose gel.

Statistical analysis

The chi-square test was used to discover the variety of different demographic and clinicopathological parameters among different genotypes containing patients having different treatment outcomes and toxicity. Unconditional logistic regression was used to derive odds ratios (ORs) with 95% confidence intervals (CIs) to study the association between genetic polymorphisms and clinical outcomes and toxicity, and ORs were stratified (aOR) with demographic and clinicopathological parameters. *p*-values less than 0.05 were used to determine statistical significance. All statistical analyses were performed with SPSS 16.0 for Windows (SPSS, Chicago, IL, USA).

Results

Patient's characteristics

Table 1 shows the relationship between *ALDH1A1* (rs13959) and *NQO1* (rs1800566) gene polymorphisms and clinicopathological factors such as age, histology, tumor grade, TNM stage, lymph node status, menstruation status, and hormone receptor status of patients. The characteristics of individuals with the *ALDH1A1* and *NQO1* polymorphisms (heterozygote and mutant homozygote) were compared to those of patients without the polymorphism (normal homozygote). Variations of these characteristics were not significantly associated with *ALDH1A1* and *NQO1* polymorphism ($p > 0.05$), according to statistical analysis using the value of *p* and OR (with 95% CI).

Table 1 Correlation of *ALDH1A1* and *NQO1* gene polymorphisms with clinicopathological characteristics

Characteristics	ALDH1A1 (rs13959)				NOQ1 (rs1800566)			
	Carriers ($n=205$)	Non-carriers ($n=125$)	OR (95% CI)	p-value	Carriers ($n=201$)	Non-carriers ($n=129$)	OR (95% CI)	p-value
Age								
< 45	90	60	Ref.	-	88	62	Ref	-
45–55	83	48	1.15 (0.71 to 1.86)	0.563	81	51	1.11 (0.69 to 1.80)	0.644
> 55	32	17	1.25 (0.64 to 2.45)	0.508	32	16	1.40 (0.71 to 2.78)	0.324
45–55 + > 55	115	65	1.17 (0.75 to 1.84)	0.468	113	67	1.18 (0.76 to 1.85)	0.446
Menstrual status								
Premenopausal	101	71	Ref	-	100	72	Ref	-
Perimenopausal	8	4	1.43 (0.41 to 4.94)	0.568	8	4	1.42 (0.41 to 4.89)	0.578
Postmenopausal	96	50	1.37 (0.87 to 2.17)	0.171	93	53	1.24 (0.79 to 1.96)	0.342
TNM stage (Clinical)								
I	55	38	Ref.	-	53	40	Ref.	-
II	71	46	1.06 (0.61 to 1.85)	0.820	70	47	1.12 (0.64 to 1.95)	0.678
III	65	35	1.28 (0.71 to 2.29)	0.401	64	36	1.34 (0.75to 2.39)	0.319
IV	14	6	1.61 (0.56 to 4.56)	0.369	14	6	1.76 (0.62 to 4.98)	0.286
Lymph node status								
No	57	41	Ref.	-	56	42	Ref.	-
N1	95	57	1.19 (0.71 to 2.01)	0.493	94	58	1.21 (0.72 to 2.03)	0.459
N2	40	21	1.37 (0.70 to 2.65)	0.352	38	23	1.23 (0.64 to 2.38)	0.520
N3	13	6	1.55 (0.54 to 4.44)	0.406	13	6	1.62 (0.57 to 4.62)	0.363
Histology								
Ductal	198	122	Ref.	-	195	125	Ref	-
Lobular	5	2	1.54 (0.29 to 8.06)	0.608	4	3	0.85 (0.18 to 3.88)	0.838
Mixed	2	1	1.23 (0.11 to 13.73)	0.865	2	1	1.28 (0.11 to 14.28)	0.839
Tumor grade								
Grade I	36	26	Ref.	-	35	27	Ref.	-
Grade II	99	65	1.10 (0.60 to 1.99)	0.753	97	67	1.11 (0.61 to 2.01)	0.713
Grade III	70	34	1.48 (0.77 to 2.84)	0.231	69	35	1.52 (0.79 to 2.90)	0.203
Hormone receptor status								
Estrogen Receptor (ER)								
Negative	85	50	Ref.	-	83	52	Ref.	-
Positive	120	75	0.94 (0.59 to 1.48)	0.793	118	77	0.96 (0.61 to 1.50)	0.859
Progesterone Receptor (PR)								
Negative	105	62	Ref.	-	104	63	Ref.	-
Positive	100	63	0.93 (0.60 to 1.46)	0.775	97	66	0.89 (0.57 to 1.38)	0.606
Her-2/neu status								
Negative	127	72	Ref.	-	125	74	Ref.	-
Positive	78	53	0.93 (0.62 to 1.42)	0.766	76	55	0.81 (0.52 to 1.28)	-

Distribution of genotypes and alleles of rs13959 and rs1800566

Table 2 shows the distribution of different genotypes of *ALDH1A1* (rs13959) and *NQO1* (rs1800566) polymorphisms. The calculation of Hardy-Weinberg equilibrium shows that both polymorphisms are in the state of genetic equilibrium between responders and non-responders (For rs13959: $\chi^2=1.87$, $p=0.171$ for responders and $\chi^2=3.41$, $p=0.065$ for non-responders, and for rs1800566: $\chi^2=1.38$, $p=0.241$ for responders and $\chi^2=1.48$, $p=0.224$ for non-responders). The minor allele frequencies (MAF) observed for rs13959 in responders was 43.75% and in non-responders was 31.45% and for rs1800566 in responders was 44.32% and in non-responders was 35.48%.

Association of rs13959 and rs1800566 with response of chemotherapy

Based on the RECIST criteria, 22 (14.60%) of 330 patients had a full response, 66 (44.00%) had a partial response, 60 (40.00%) had a stable state, and 2 (1.33%) had disease progression. Univariate chi-square analyses revealed that *ALDH1A1* (rs13959) polymorphism showed a significant

Table 2 Analysis of the hardy-weinberg equilibrium of *ALDH1A1* (rs13959) and *NQO1* (rs1800566) polymorphisms

Variables	Responders (CR + PR) ($n = 88$)	HWE		Non-responders (SD + PD) ($n = 62$)	HWE	
		χ^2	p		χ^2	p
ALDH1A1 (rs13959)						
CC	31 (35.23)	1.87	0.171	26 (41.93)	3.41	0.065
CT	37 (42.04)			33 (53.22)		
TT	20 (22.73)			3 (20.97)		
C allele	99 (56.25)			85 (68.55)		
T allele	77 (43.75)			39 (31.45)		
NQO1 (rs1800566)						
CC	30 (34.09)	1.38	0.241	28 (45.16)	1.48	0.224
CT	38 (43.18)			24 (38.71)		
TT	20 (22.73)			10 (16.13)		
C allele	98 (55.68)			80 (64.52)		
T allele	78 (44.32)			44 (35.48)		

Here, if $p > 0.05$ it is consistent with Hardy-Weinberg equilibrium

connection (p-value < 0.05) with the response of chemotherapy in terms of TT genotype, recessive model, and allele model ((TT vs. CC: aOR = 6.40, 95% CI = 1.60 to 25.57, $p = 0.007$; recessive model: aOR = 6.38, 95% CI = 1.70 to 23.93, $p = 0.002$; allele model: $p = 0.032$)), as shown in Table 3. The *NQO1* (rs1800566) polymorphism did not show any significant association with the treatment response.

Association of rs13959 and rs1800566 with toxicities of chemotherapy

The probability of *ALDH1A1* (rs13959) and *NQO1* (rs1800566) SNPs being associated with clinical toxicity resulting from ACT and NACT was also investigated in this study (Table 4). The CTCAE version 4.0 was used to assess the toxicity of chemotherapy in 330 patients. The results of unconditional logistic regression analysis revealed that the TT and CT + TT genotypes of *NQO1* (rs1800566) were associated with a lower frequency of toxicities such as anemia [aOR = 0.34, 95% CI = 0.18 to 0.67, $p = 0.006$ and aOR = 0.58, 95% CI = 0.36 to 0.92, $p = 0.021$]; neutropenia [aOR = 0.42, 95% CI = 0.21 to 0.87, $p = 0.044$ and aOR = 0.57, 95% CI = 0.35 to 0.94, $p = 0.027$]; leukopenia [aOR = 0.33, 95% CI = 0.15 to 0.75, $p = 0.010$ and aOR = 0.46, 95% CI = 0.26 to 0.79, $p = 0.005$]; and gastrointestinal toxicity [aOR = 0.30, 95% CI = 0.10 to 0.88, $p = 0.02$ and aOR = 0.38, 95% CI = 0.19 to 0.77, $p = 0.006$]. A significant association was also observed for CT genotype in term of gastrointestinal toxicity and leukopenia, respectively [aOR = 0.42, 95% CI = 0.20 to 0.90, $p = 0.02$ and aOR = 0.52, 95% CI = 0.29 to 0.93, $p = 0.010$]. In the case of thrombocytopenia, there was no strong association.

In patients with the *ALDH1A1* (rs13959) polymorphism, genotypes TT [aOR = 2.00, 95% CI = 1.09 to 3.67, $p = 0.037$] and CT + TT [aOR = 1.68, 95% CI = 1.05 to 2.69, $p = 0.029$] were more likely to show treatment-related anemia than those with the CC genotype. There was no significant link between this SNP and neutropenia, leukopenia, thrombocytopenia, or gastrointestinal toxicity.

Discussion

This study was performed with *ALDH1A1* (rs13959) and *NQO1* (rs1800566) polymorphisms in Bangladeshi breast cancer patients that looked at 330 patients who were given cyclophosphamide-based combined chemotherapy (CEF/CAF), 150 of them were given neoadjuvant chemotherapy and 180 were given adjuvant chemotherapy. This study observed a significant association of both *ALDH1A1* (rs13959) and *NQO1* (rs1800566) polymorphisms with

Table 3 Effects of *ALDH1A1* (rs13959) and *NQO1* (rs1800566) polymorphisms on the response of chemotherapy

Genetic polymorphisms	Genotypes	Responders (CR + PR) ($n = 88$)	Non-responders (SD + PD) ($n = 62$)	OR (95% CI)	aOR	[a]*p*-value
ALDH1A1 (rs13959)	CC (57)	31	26	Ref.	Ref	-
	CT (70)	37	33	0.94 (0.46 to 1.90)	0.85 (0.47–2.15)	0.864
	TT (23)	20	3	5.59 (1.49 to 20.94)	6.40 (1.60-25.57)	**0.007**
	Dominant model (CT + TT vs. CC)	31	26	Ref.		-
		57	36	1.33 (0.68 to 2.59)	1.45 (0.71-3.00)	0.310
	Recessive model (TT vs. CC + CT)	68	59	Ref.		-
		20	3	5.78 (1.64 to 20.44)	6.38 (1.70-23.93)	**0.002**
	Allele model (T vs. C)	99	85	Ref.		-
		77	39	1.69 (1.05 to 2.74)	-	**0.032**
NQO1 (rs1800566)	CC (58)	30	28	Ref.	Ref	-
	CT (62)	38	24	1.48 (0.71 to 3.05)	1.56 (0.71–3.43)	0.314
	TT (30)	20	10	1.87 (0.74 to 4.67)	1.58 (0.56–4.44)	0.490
	Dominant model (CT + TT vs. CC)	30	28	Ref.		-
		58	34	1.59 (0.82 to 3.10)	1.57 (0.75–3.27)	0.230
	Recessive model (TT vs. CC + CT)	20	10	Ref.		-
		68	52	0.65 (0.28 to 1.51)	1.22 (0.48–3.11)	0.670
	Allele model (T vs. C)	98	80	Ref.		-
		78	44	1.45 (0.90 to 2.32)	-	0.126

Here, $p < 0.05$ denotes statistically significant (bold)

treatment-associated toxicity, but a significant association in the case of treatment response was found for only *ALDH1A1* (rs13959). A previous study found a poor response for this SNP [33] (Table 5).

The SNPs *NQO1* (rs1800566) was found to be strongly linked to CEF/CAF chemotherapy-related adverse events like anemia, neutropenia, leukopenia, and gastrointestinal disorders, while *ALDH1A1* (rs13959) was linked to only anemia toxicity. Though the TT and CT + TT genotypes of *NQO1* (rs1800566) polymorphism were associated with a lower frequency of toxicities than the wild CC genotype; however, *ALDH1A1* (rs13959) polymorphism was related to the higher frequency of anemia toxicity. Besides, there was a significant correlation between treatment response and *ALDH1A1* (rs13959) polymorphism but not *NQO1* (rs1800566) polymorphism, which was inconsistent with other research findings [11, 19, 24]. A similar correlation was reported for overall toxicity in the case of rs1800566 [34]. This discrepancy may be because of the ethnic disparity and, more noteworthy hereditary admixture and variety in the number of inhabitants in Bangladesh. This could be attributed to the fact that the presence of multidrug resistance-associated protein 1 (MRP1), breast cancer resistance protein (BCRP) in cancer cells may cause efflux of the drug (e.g., cyclophosphamide,5-fluorouracil, adriamycin) by efflux transporter [35–37]. Also, highly effective nuclear DNA repair systems play a significant role in tumor cells' ability to tolerate the cytotoxic effects of anthracyclines (Doxorubicin, epirubicin) [36].

It is well known that the majority of drug-metabolizing enzymes involved in drug metabolism activation and detoxification pathways are extremely polymorphic [38]. Several studies have found that polymorphism in these genes was linked to the likelihood of chemotherapy toxicity, particularly in the case of cyclophosphamide-based (CPA) combination therapy. CPA is the most extensively utilized in the treatment of breast cancer [14]. It is commonly used in combination with other chemotherapeutic drugs such as adriamycin and 5-fluorouracil (CAF). Although the CPA-based combination treatment is beneficial for breast cancer, it has been linked to adverse drug reactions (ADRs) such as leukopenia, neutropenia, and gastrointestinal issues. Furthermore, anticancer medicines are well-known for having a limited therapeutic window; a higher plasma concentration in the body produces toxicity, while a lower quantity diminishes the drugs' efficacy. As a result, the function of pharmacogenomics, which aims to provide a prediction technique for severe drug toxicity, is crucial [39].

Table 4 Effects of *ALDH1A1* and *NQO1* polymorphisms on the toxicities induced by chemotherapy

Toxicity	ALDH1A1 (rs13959)				NQO1 (rs1800566)			
	CC (125)	CT (132)	TT (73)	CT (132) + TT (72)	CC (129)	CT (134)	TT (67)	CT (134) + TT (67)
Anemia								
Grade III (95) + Grade IV (50)	46	61	38	99	66	59	20	79
Grade ≤ II (185)	79	71	35	106	63	75	47	122
aOR (95% CI)	Ref.	1.53 (0.91–2.56)	2.00 (1.09–3.67)	1.68 (1.05–2.69)	Ref.	0.72 (0.44–1.19)	0.34 (0.18–0.67)	0.58 (0.36–0.92)
p-value	-	0.063	**0.037**	**0.029**	-	0.247	**0.006**	**0.021**
Neutropenia								
Grade III (57) + Grade IV (45)	32	44	26	70	48	39	15	54
Grade ≤ II (228)	93	88	47	135	81	95	52	147
aOR (95% CI)	Ref.	1.46 (0.83–2.54)	1.69 (0.89–3.22)	1.54 (0.92–2.56)	Ref.	0.65 (0.38–1.10)	0.42 (0.21–0.87)	0.57 (0.35–0.94)
p-value	-	0.175	0.22	0.094	-	0.163	**0.044**	**0.027**
Leukopenia								
Grade III (50) + Grade IV (33)	27	36	20	56	41	30	12	42
Grade ≤ II (247)	98	96	53	149	88	104	55	159
aOR (95% CI)	Ref.	1.39 (0.76–2.56)	1.50 (0.75–3.03)	1.43 (0.83–2.49)	Ref.	0.52 (0.29–0.93)	0.33 (0.15–0.75)	0.46 (0.26–0.79)
p-value	-	0.291	0.420	0.200	-	**0.010**	**0.010**	**0.005**
Thrombocytopenia								
Grade III (3) + Grade IV (1)	1	2	1	3	3	1	0	1
Grade ≤ II (326)	124	130	72	202	126	133	67	200
aOR (95% CI)	Ref.	3.85 (0.23–63.23)	2.17 (0.12–40.75)	2.91 (0.24–34.61)	Ref.	0.44 (0.04–5.21)	-	0.30 (0.03–3.30)
p-value	-	0.610	0.610	0.370	-	0.420	-	0.300
Gastrointestinal								
Grade III (39) + Grade IV (3)	13	18	11	29	24	13	5	18
Grade ≤ II (288)	112	114	62	176	105	121	62	183
aOR (95% CI)	Ref.	1.49 (0.67–3.29)	1.57 (0.64–3.81)	1.52 (0.74–3.11)	Ref.	0.42 (0.20–0.90)	0.30 (0.10–0.88)	0.38 (0.19–0.77)
p-value	-	0.510	0.510	0.250	-	**0.02**	**0.02**	**0.006**

Table 5 Previous findings of associations of chemotherapy outcomes with NQO1 rs1800566

Tumor type	Polymorphism	Treatment regimen	Sample size	Response	Toxicity	Reference
BC	NQO1 rs1800566	Doxorubicin (60 mg/m2), cyclophosphamide (600 mg/m2) IV, day1 of each 21 day cycle for a maximum of six cycles	227	Poor response	-	[33]
AML	NQO1 rs1800566	**For younger patients**: idarubicin 12 mg/m2 on days 1–3, cytarabine 200 mg/m2 on days 1–7 (3 + 7 schedule) **For patients > 65 years**: 12 mg/m2 on days 1–2 and cytarabine 200 mg/m2 on days 1–5.	225	-	Associated with mucositis, gastrointestinal toxicity and thrombocytopenia	[50]
BC	NQO1 rs1800566	FEC/FAC/FMC		No association	Association with overall toxicity	[49]

Myelosuppression is a common treatment-related complication in cancer patients [40]. It arises when cytotoxic chemotherapy disrupts the bone marrow's rapidly dividing hematopoietic stem and progenitor cells (HSPCs), which gives rise to blood cell lineages. Myelosuppression is depicted by anemia, lymphopenia, neutropenia, and thrombocytopenia and mostly occurs in breast cancer patients [41, 42]. It is worth noticing that due to quick metabolism or inactivation by various drug-metabolizing enzymes, several

clinically approved anti-cancer drugs have shown to be ineffective or resistant [43–45].

Our research revealed that *ALDH1A1* (rs13959) SNP was strongly linked with CAF-based chemotherapy-induced anemia. The rs13959 variant may result in lower ALDH enzyme activity, resulting in decreased 4-hydroxy-cyclophosphamide detoxification, causing drug accumulation inside the body and enhanced myelosuppression. This enzyme participates in CPA metabolism and genetic variation of *ALDH1A1* has been shown to alter inter-individual cyclophosphamide therapy response [24]. There were studies regarding ALDH expression in breast cancer, but the SNP study was limited. In different cancer cell lines, overexpression/increased activity of ALDHs has been found, and it increases the rate of cyclophosphamide metabolism and plasma clearance, resulting in poor treatment response or resistance to CPA [46] (Table 5). Our finding was supported by other previous studies. For instance, a study of gallbladder cancer discovered that *ALDH1A1* rs13959T > G was related to an elevated incidence of grade 3–4 hematological toxicity [47]. Another study conducted over 250 breast cancer patients in India found that *ALDH1A1* rs6151031 genotype *2/*2 was linked to higher drug toxicity in cyclophosphamide-treated breast cancer patients [24].

In this research, we found that *NQO1* (rs1800566) polymorphism was strongly associated with anemia, leukopenia, neutropenia, and gastrointestinal disorders. As part of detoxification, *NQO1* catalyzes the two-electron-mediated reduction of quinones to hydroquinones. The *NQO1* C609T polymorphism has been shown to have a well-established and significant impact on the enzymatic activity of the synthesized protein because the modified version of the enzyme is rapidly ubiquitinated and damaged by the proteasome [48]. As a result, the homozygous variant T allele had nearly total eradication of its enzymatic activity (2–4% of wild-type activity), whereas the heterozygous variant T allele had a threefold decrease in enzymatic activity relative to the homozygous wild-type allele [48]. Hence, the presence of the polymorphism causes reduction of the enzyme's ability to detoxify carcinogens and increases the likelihood of hematologic toxicity in susceptible individuals. Allele frequency of the *NQO1* C609T polymorphism varies greatly by geography and ethnicity. Database of HapMap showed that *NQO1* T allelic frequency ranges from 17.1 to 19.2% in Africa, 18.6% in Europe, and the highest 50% in Asian populations, and a large number of people possess CT and TT genotypes than the wild genome [49]. The association has also been studied in other studies and got a similar result to ours. A study conducted on acute myeloid leukemia patients treated with anthracycline showed that the genotypes of *NQO1* rs1800566 were associated with thrombocytopenia and GI toxicity [50] (Table 5). Another study by Fagerholm et al. discovered that *NQO1**2 homozygotes have low treatment-associated survival and a poor response to fluorouracil, epirubicin, and cyclophosphamide therapy [51].

Therefore, this study will help the researcher to estimate the frequency of polymorphism in this region and study further. These findings will help researchers to understand the association of *ALDH1A1* and *NQO1* better in breast cancer. Studies with a bigger sample size with more variants are required to determine the best therapies and get conclusive evidence.

Conclusion

This study revealed that the *ALDH1A1* 13,959 polymorphism is associated with response to chemotherapy. Besides, *NQO1* rs1800566 and *ALDH1A1* 13,959 polymorphisms are correlated to the toxicities, including anemia, neutropenia, leukopenia, and gastrointestinal toxicity in CEA/CFA-based neoadjuvant and adjuvant chemotherapy patients. This information will aid in the prediction of clinical toxicity associated with cyclophosphamide-based chemotherapy.

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Author contributions MSI – designed and supervised experimental studies; FA- performed the genetic analysis and wrote the first draft of the manuscript, SP – performed genetic analysis; MMR, MRR, MA – analyzed data; ASMM, MSI, MWA – collected samples. MSI, MAA – critically revised the manuscript. All authors contributed to manuscript revision and read and approved the submitted version.

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Data availability All the data connected with this manuscript will be avaible from the corresponding author on a valid request.

Declarations

Ethical approval This research protocol was reviewed and approved by the committee for ethical clearance and review, Southeast University, Bangladesh.

Conflict of interest The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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