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RESEARCH ARTICLE

Study the effect of FGFR2 and FGFR4 gene variants on the response to cyclophosphamide doxorubicin-docetaxel based chemotherapy in locally advanced breast cancer patients

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ABSTRACT

Background: Breast cancer has significantly increased in Arab populations, accounting for 13-30% of the newly diagnosed women malignancies. Neoadjuvant chemotherapy, including regimens based on cyclophosphamide, doxorubicin, and docetaxel, is effective for early-stage operable and locally advanced cancer. However, chemoresistance remains a major challenge. **Aim**: We aimed to investigate FGFR2 (rs2981578) and FGFR4 (rs1966265) gene variants in predicting chemotherapy response in patients with locally advanced breast cancer. **Subjects and Methods**: This cross-sectional study involved 30 females with biopsy confirmed locally advanced primary breast cancer. Core biopsies were taken from patients received neoadjuvant cyclophosphamide-doxorubicin-docetaxel-based regimens. Histopathological diagnosis, and immunohistochemistry for HER2, PR, and ER were performed in the samples. Genotyping of FGFR2 and FGFR4 SNPs was performed using the TaqMan SNP genotyping assay. The RECIST criteria was used for assessment of treatment response. **Results**: Among the patients, only 66.7% of them responded to chemotherapy. Among the responded patients, premenopausal patients showed a significantly higher response rate as compared to menopausal patients (FEp=0.024). The distribution of FGFR2 and FGFR4 genotypes did not show significant difference between responders and non-responders (FEp=1.000). **Conclusion**: The G and A alleles of FGFR2 and FGFR4 variants were not associated with chemotherapy response. Further research with larger cohorts is needed to validate our study results and identify other variants that would guide tailored management.

Keywords: Breast Cancer**,** Neoadjuvant chemotherapy, FGFR2, FGFR4, gene variants

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BACKGROUND

Breast cancer (BC) stands as the most prevalent cancer in females. In 2020, GLOBOCAN reported that approximately 2.3 million women were affected, resulting in 685,000 deaths worldwide (Sung et al., 2021). The incidence rates have shown a consistent rise in Arab populations over the past decade, likely attributed to enhanced data collection from cancer registries and improved accessibility to screening and diagnostic programs for patients. In Egypt alone, in the year 2018, 23,081 women were diagnosed with breast cancer (World Health Organization (WHO), 2020).

Despite randomized trials having established neoadjuvant chemotherapy (NCT) as the standard therapy for inflammatory and inoperable breast cancer, it is now adopted as a primary treatment modality for early breast cancer as well. NCT reduces tumor recurrence and mortality while enhancing disease-free and overall survival rates. Additionally, it promotes assessment of tumors' response to therapy, making it a highly effective method for assessing novel predictive and prognostic

factors. Clinical experience demonstrated that anthracyclines, such as doxorubicin (DOXO) and taxanes (T) are among the most powerful chemotherapeutic agents for integrated breast cancer management (Ejlertsen, 2016; Marmé et al., 2012). However, the variability in toxicity levels and life-threatening side effects that impair quality of life present significant challenges for patients undergoing neoadjuvant chemotherapy. Moreover, chemoresistance remains a major challenge in treating breast cancer, necessitating comprehensive understanding of its underlying mechanisms. There is an urgent need for identification of molecular biomarkers that can predict response to therapy and allow early detection of drug resistance. This would enable timely interventions and personalized management plans. (Tufail et al., 2022).

Fibroblast growth factors constitute a diverse family of polypeptide growth factors present in organisms spanning from nematodes to humans. Members of the FGF family exhibit a size range of 17 to 34 KDa and display high conservation in both gene structure and amino acid sequence. Fibroblast Growth Factor Receptors (FGFRs) belong to the receptor tyrosine kinases (RTKs) subfamily. Mutations and overexpression of FGFRs are associated with growth and progression of multiple tumors, including ovarian, breast, and renal cell cancers (Liang et al., 2012; Powers et al., 2000; Sheu et al., 2015; Terzuoli et al., 2018). Downstream Signaling of FGF/FGFR is essential in moderating essential cellular processes, including proliferation, differentiation, and apoptosis. These processes are fundamental to embryogenesis, organ development, and maintaining homeostasis in adult tissues. (Szymczyk et al., 2021).

FGFR2 (fibroblast growth factor receptor 2 gene) encodes the FGFR2 protein, a receptor tyrosine kinase which plays essential roles in developmental processes along with other FGFR tyrosine kinases. In cancer, these receptors are known to undergo deregulation through different mechanisms. FGFR2 has been reported to be activated or amplified in breast and gastric cancers while FGFR2 mutations were identified in endometrial and breast cancer (Dutt et al., 2008; Jain and Turner, 2012; Reintjes et al., 2013; Turner and Grose, 2010).

The most common FGFR2 alterations include mutations (1.49%) and amplification (0.37%). FGFR2 variation is detected in 2.39% of patients with breast carcinoma. FGFR2 is recognized as a breast cancer susceptibility gene. Certain variants significantly enhance breast tumorigenicity by maintaining tumor-initiating cells and supporting self-renewal (Kim et al., 2013) , thereby increasing the risk of breast carcinoma (Cui et al., 2016). Reference sequence (Rs2981578) of FGFR2 modifies the DNA binding affinity of transcription factors such as Oct-1, Runx2, and C/EBPb, resulting in elevated expression of FGFR2 mRNA in breast tumors with homozygous patients of the risk allele which leads to increased cell growth, angiogenesis and invasiveness (Meyer et al., 2008).

Genome-wide analysis studies have detected various single nucleotide polymorphisms (SNPs) within intron 2 of the FGFR2 gene; (rs7895676, rs2981578 and rs2981582), that were hypothesized to be linked with a higher breast cancer susceptibility (Easton et al., 2007; Hunter et al., 2007). Remarkably, FGFR2 inhibitors showed a high efficiency in induction of tumor growth arrest (André et al., 2013; Ye et al., 2014). FGFR2 amplification was suggested as a new therapeutic target through FGFR2 specific inhibitors, especially in triple negative BC, which is resistant to currently available therapies (Turner et al., 2010).

While Fibroblast growth factor receptor 4 (FGFR4) is a gene encoding a protein characterized by a tyrosine kinase domain in addition to three immunoglobulin-like domains. This protein is involved in processes such as mitogenesis and differentiation. It exhibits high expression levels in several tumors including breast cancer cells, endometrial cancer, intestinal cancer, and skin cancer. Overall, FGFR4 is altered in approximately 1.86% of breast carcinoma cases (My Cancer Genome, 2017). These alterations may have implications for understanding the biology of breast carcinoma and developing targeted treatments (Levine et al., 2020).

In essence, FGFR4, with its high expression in certain breast cancer cells, it appears to play an essential role in PI3K/AKT mediated cell survival

(Tiong et al., 2016). The rs1966265 variant is a missense variant that is linked to increased risk of breast cancer under both co-dominant and dominant models. (Jiang et al., 2015).

The expression of FGF and FGFR in cancer cells was found to correlate with unfavorable patient prognosis and resistance to multiplex cytotoxic agents, including paclitaxel, cisplatin, etoposide, 5-fluorouracil, doxorubicin, and others, across different tumor types. (Szymczyk et al., 2021). Few FGFR genetic variants had been found to be linked with clinical outcomes in cyclophosphamide, doxorubicin and docetaxel-based chemotherapy of breast cancer especially in Caucasians.

Our aim is to evaluate the possible correlations between the selected 2 SNPs (rs2981578 of FGFR2 and rs1966265 of FGFR4) and the efficacy of cyclophosphamide/doxorubicin/ docetaxel based NCT (neoadjuvant chemotherapy) and the association between these genetic variants and clinicopathological features in Egyptian females with breast cancer.

SUBJECTS AND METHODS

Study design and participants

A minimum sample size of thirty patients was considered acceptable for conducting this study with a power of 80% and at a significance level of 0.05. This cross-sectional study involved 30 females with histopathological-proven invasive breast cancer who attended the oncology clinic in the Medical Research Institute hospital, Alexandria, Egypt from September 2022 to November 2023. The study was approved by the research ethics committee of the Medical Research Institute (IORG0008812, IRB00010526). Written informed Consents were obtained from all patients who agreed to have their samples tested for research before participating in the study.

Inclusion and exclusion criteria

Patients diagnosed as locally advanced primary breast cancer with histologically proven breast cancer with a pathological stage IIA-IIIC disease and were indicated for neoadjuvant cyclophosphamide-doxorubicin-docetaxel based regimens and agreed to participate were considered eligible. The chemotherapy regimen was received as 4 cycles of AC (Cyclophosphamide 600mg/m², Doxorubicin (Adriamycin) 60mg/m² every 21 days), followed by docetaxel 75mg/m^2 every 21 days for 12 weeks. After assessment by the end of cyclophosphamide-doxorubicin-docetaxel based regimen, The studied patients were divided into: **Responders**: patients who achieved complete/partial response to cyclophosphamide-doxorubicin-docetaxel based regimen. **Non-responders**: patients who had a stable or progressive disease course on cyclophosphamide-doxorubicin-docetaxel based regimen. Meanwhile patients with metastatic breast cancer, concurrently receiving anti HER2 therapy or previous chemotherapy/radiotherapy, any concomitant cancer disorder were excluded.

Data Collection

The study included the following data: demographic information, medical history including menstrual history and family history of similar condition, results of physical examination, radiological investigation including, mammogram, CT for chest, abdomen and pelvis, bone scan and echocardiogram, laboratory tests including complete blood count (CBC), liver and kidney functions, and tumor markers including CA15.3.Additionally, Core biopsies of breast tissue were obtained for histopathological diagnosis of breast carcinoma. Immunohistochemistry for HER2 /PR/ER was also done. The tumors were classified histologically based on the WHO criteria, and the histological grade was assessed using the modified Bloom–Richardson system. Immunohistochemistry (IHC) was used to analyze the estrogen receptor (ER) and progesterone receptor (PR) expressions according to the Allred scoring method by summation of proportion (0-5) and intensity (0-3). A positive ER and PR staining were considered if at least 1% of the nuclei stained. HER2 by IHC is given a score (+1) in case of incomplete faint membrane attaining in >10%, (2+) weak to moderate complete membrane attaining in >10% and (3+) in case of complete intense membrane staining appreciated by low power. HER2 staining with an IHC score of 2+ and no gene amplification was confirmed using fluorescence in situ hybridization (FISH). Patients were categorized into four molecular

subtypes: luminal A, luminal B, HER2 overexpressing, and basal-like (triple negative).

Laboratory investigations

Five milliliters (mLs) of venous blood were sampled using sterile vacutainer from every participant before the initiation of neoadjuvant chemotherapy regimen. Two mLs were placed in a serum separator tube which was centrifuged to obtain the serum for routine laboratory tests. Three mLs were withdrawn in an EDTA vacutainer tube for CBC using the counter Cell (Abbott, Germany) while the remaining blood was stored in a separate sterile vacutainer K2 EDTA tube at-20 °C for SNP genotyping. Routine biochemical tests involved (urea, creatinine, AST, ALT) using the Beckman Coulter AU480 (clinical chemistry auto-analyzer) and Cobas e801 for the CA 15.3 tumor marker.

Genomic DNA Extraction

Two mLs of whole blood were sampled using the K2 EDTA vacutainer tubes and stored at -20c for further analysis. DNA was extracted from the entire peripheral blood mononuclear cells (250 µL) using the QIAamp DNA blood mini kit (Qiagen, Germany, cat. No51304) following the manufacturer's instructions. Both the quantity and purity of the extracted nucleic acid were assessed by the NanoDrop 2000 (Thermo Scientific, USA) at 260 and 280 nm.

Genotyping of FGFR2 G > A (rs 2981578) SNP and FGFR4 G > A (rs 1966265) SNP

The FGFR2 (rs2981578) and FGFR4 (rs1966265) SNPs genotyping was carried out through the TaqMan SNP genotyping assay. The assay uses sequence-specific forward and reverse primers to amplify the target sequence, along with two TaqMan minor groove binder (MGB) probes with nonfluorescent quenchers: a VIC-labeled probe to detect the allele 1 sequence and a FAM-labeled probe to detect the allele 2 sequence. According to manufacturers' instructions, the PCR reaction mix contained 20 ng DNA/reaction. 10 µL TaqMan® Universal PCR Master Mix (Applied biosystems, USA, Cat. no: 4371353) were added to 1 µL of TaqMan® SNP Genotyping Assay 20x (rs 2981578, Id:C_2917323_20-rs 1966265, Id:C_11317464_20) while genomic DNA was diluted with DNAase free water to achieve the desired DNA concentration in 20µL (final volume). Thermal cycling profile was carried out by the StepOne Real-time PCR system (Applied Biosystems, USA) included an initial pre-heating step to activate AmpliTaq Gold enzyme at 95 °C for 10 min. The thermal cycling included 40 cycles. Each cycle consisted of a denaturation step at 95°C for 15 seconds followed by annealing and extension at 60°C for 1 minute.

Response evaluation criteria

Clinical and radiological assessments were repeated by the end of NAC regimen to evaluate the treatment response. Patients' response were evaluated based on the Response Evaluation Criteria in Solid Tumors (RECIST) (Gruber-Rouh et al., 2017). The patients' response was classified into CR which involves disappearance of all tumor lesions, while PR involves more than 30% reduction in the total tumor size. Progressive disease (PD) includes growth >20% or occurrence of new lesions. TNM Staging of patients was performed at presentation based on the American Joint Committee on Cancer (AJCC) 8th manual criteria of cancer staging. For data analysis, the breast cancer patients were categorized into responder group (complete + partial response) and non-responder group (stable + progressive disease). The FGFR gene variants' effect on the response and its association with clinicopathological features was evaluated.

Statistical analysis

Data was analyzed using IBM SPSS software package version 20.0**.** (Armonk, NY: IBM Corp). The normality of distribution was checked using the Shapiro-Wilk test. Qualitative data was presented as number and percent. Quantitative data was presented as median and range or mean and standard deviation. Distributions of the genotype were compared with those expected for samples from populations in Hardy–Weinberg equilibrium using the Chi-square ($\chi 2$) test to compare the differences in the genotype frequencies between patients with different treatment outcomes. Additionally, chi-square ($\chi 2$) test was also used to analyze associations between an individual SNP and clinicopathological parameters. Fisher's Exact correction for chi-square was

used when more than 20% of cells have expected frequencies less than 5. In addition, Student t-test was used to compare between two studied groups regarding the normally distributed quantitative variables. P-value at 0.05 or less was considered statistically significant.

RESULTS

This cross-sectional study involved 30 biopsy-proven primary breast cancer patients who received cyclophosphamide-doxorubicin-docetaxel neoadjuvant regimen. Response to the chemotherapy regimen was assessed at the end of regimen. According to the RECIST criteria, BC patients were classified into 20 (66.7%) responders and 10 (33.3%) non-responders. Concerning the clinicopathological parameters, our studied patients' age ranged from 27 to 68 years. Concerning family history, only 3 patients (10%) reported positive family history. In terms of the menopausal status, 18 patients (60%) were menopausal while, 12 patients (40%) were premenopausal. Interestingly, response was significantly higher in premenopausal patients compared to menopausal females (FEp=0.024*) while age did not show any significant difference between responders and non-responders (Table 1).

Histopathologically, most patients (93.3%) were diagnosed as invasive ductal carcinoma (IDC) while only (6.6%) of patients were diagnosed as invasive lobular carcinoma (ILC) and mucoid type (Figure 1 and 2). According to the tumor grade, 24 patients out of 30 (80%) were grade II while 6 patients out of 30 (20%) were grade III (Table 1). Regarding the TNM classification, tumor, most of patients were classified as T2 (50%). For lymph node involvement, most of patients (68%) were positive while (32%) of patients were negative. For the tumor stage, the most common stage was stage IIIA (40%) followed by stage IIB (36.7%), stage IIIB (13.3%), stage IIA (6.7%) and stage IIIC (3.3%) (Table 1). No statistically significant differences were found in tumor grade and stage between both groups. Concerning the biological tissue markers, most of patients were positive for ER (76.7%) and PR (73.3%) while only (20 %) of patients were positive for HER2. Regarding molecular type, most of patients were luminal A type (63.3%) followed by TNBC type (16.7%) of patients (Figures 3 and 4). Responders did not show any significant difference in the biological tissue markers ER, PR, HER2 compared to non-responders (P=0.372, P=0.682, P= 1.0) respectively (Table 1).

Our study revealed a lack of association between the studied FGFR variants and the response of breast cancer patients to cyclophosphamide, doxorubicin, and docetaxel based- neoadjuvant chemotherapy. (FEp =1 for both FGFR2 rs2981578 and FGFR4 rs1966265) (Table 2). The genotype distribution patterns of SNP rs 2981578 of FGFR2 gene among the BC patients were in agreement with Hardy-Weinberg equilibrium (GG=17,AG=13,AA=0,p=0.130 for all BC patients), (GG=11, AG=9,AA=0,p=0.194 for responders) (GG=6, AG= 4, AA= 0 and p=0.429 for all non-responders) (Table 3). While the genotype distribution patterns of SNP rs 1966265 of FGFR4 gene among BC patients were in agreement with Hardy-Weinberg equilibrium (GG=24,AG=6,AA=0,p=0.543),while (GG=16, AG=4,AA=0,p=0.619 for responders),(GG=8, AG= 2, AA=0 and p=0.725 for non-responders) (Table 3).

The frequency of allele G and allele A did not show significant difference between responders and non-responders for FGFR2 rs2981578 (P=1.0) and FGFR4 rs 1966265 (P=1.0) (Table 2). The genotype pattern of FGFR2 rs2981578 was distributed as follows; the (GG) genotype was more frequent than the (A/G) genotype in both responders and non-responders (55% and 60%) respectively. For FGFR4 rs 1966265, the genotype (GG) was more frequent in both responders and non-responders (80%) compared to the heterozygous (A/G) genotype (20%). The genotype distribution of both variants did not show any significant difference between responders and non-responders. (^{FE}p=1.000) (Table 2). When the dominant model of inheritance was tested, we did not detect any statistically significant difference in the frequency of exposed genotypes ''GA and AA'' between the non-responder group and the responder group for both studied gene variants. (p >0.05) (Table 4). Additionally, testing of the recessive model was not feasible due to the absence of AA genotypes; also, the odds ratio

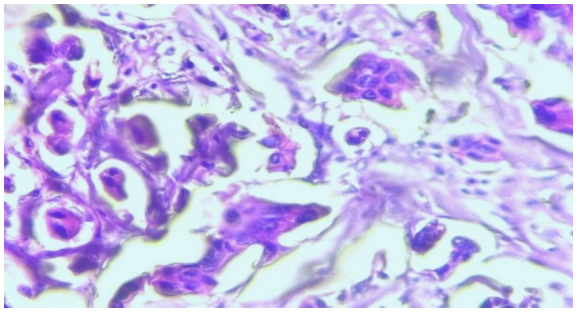

Figure 1. Invasive ductal carcinoma grade II with nests and tubules of malignant ductal cells (H&EX200).

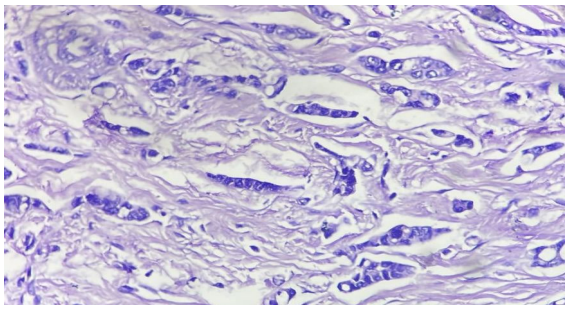

Figure 2. A case of invasive lobular carcinoma with Indian file pattern of tumor cells (H&EX200).

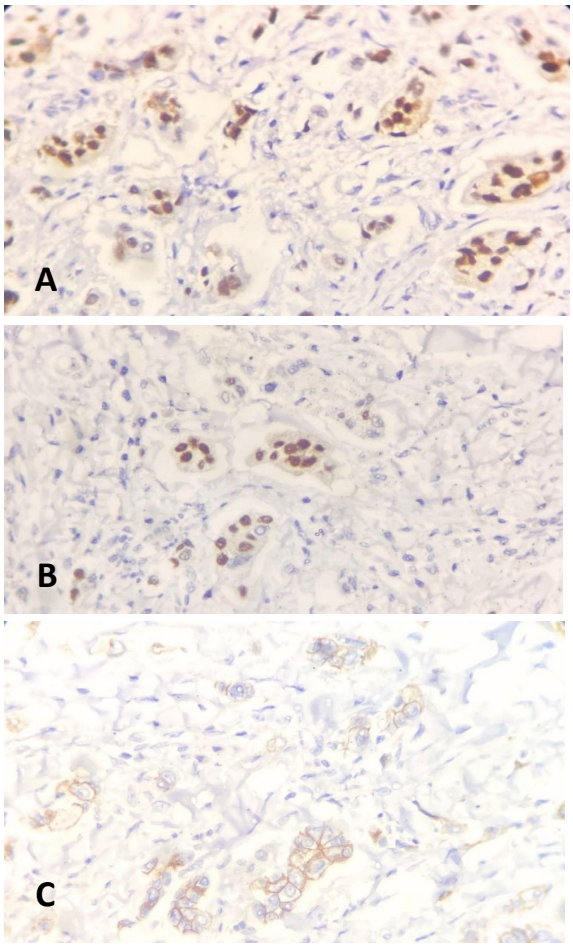

Figure 3. A case of luminal A breast cancer with positive nuclear ER (A) and PR (B)and +1 membranous her2 (C) (IHCX200).

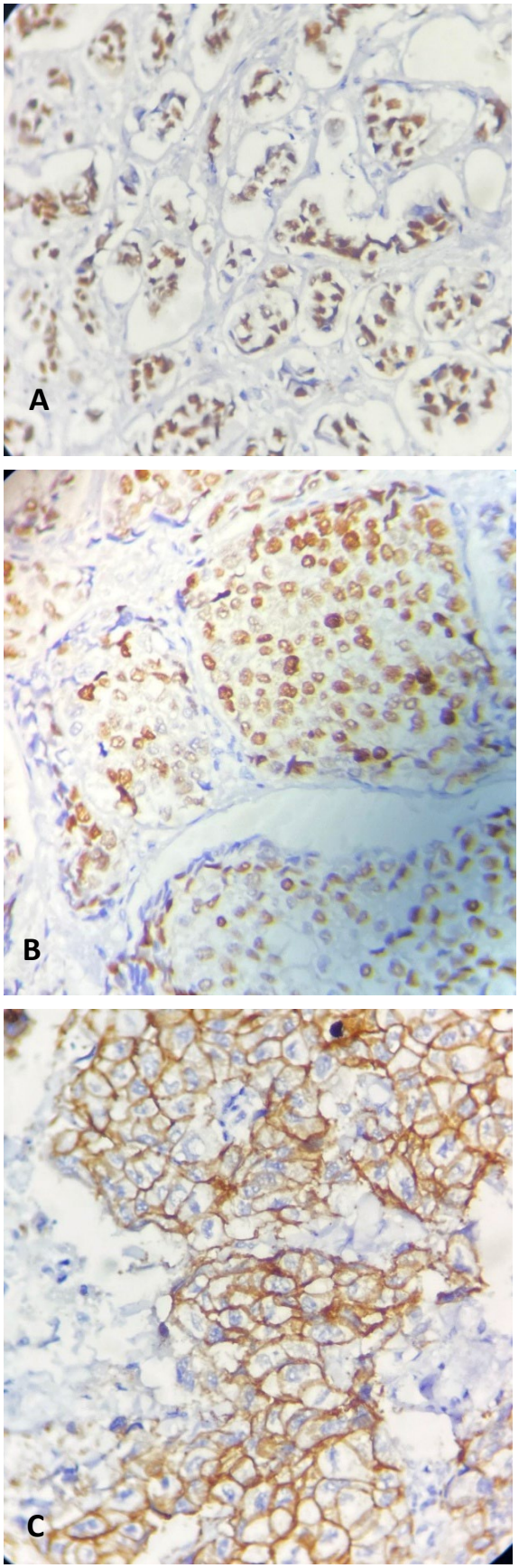

Figure 4. A case of luminal B breast cancer showing positive nuclear ER (A) and PR staining(B) and positive her2 membranous staining (+3) (C) (IHCX200).

Table 1. Comparison between the two studied groups according to some clinicopathological variables.

	Total (n = 30)		Response				Test of Sig.	P
			Non-responder (n = 10)		Responder (n = 20)			
	No.	%	No.	%	No.	%		
Menopause								
Pre menopause	12	40.0	1	10.0	11	55.0	χ^2= 5.625*	^{FE}p=0.024*
Menopausal	18	60.0	9	90.0	9	45.0		
Age (years)								
Min. – Max.	27.0 – 68.0		42.0 – 58.0		27.0 – 68.0		t= 0.626	0.537
Mean ± SD.	50.0 ± 10.92		51.40 ± 5.42		49.30 ± 12.90			
≤50	14	46.7	3	30.0	11	55.0	χ^2= 1.674	^{FE}p=0.260
>50	16	53.3	7	70.0	9	45.0		
Grade II	24	80.0	9	90.0	15	75.0	χ^2= 0.938	^{FE}p= 0.633
Grade III	6	20.0	1	10.0	5	25.0		
Stage IIA	2	6.7	1	10.0	1	5.0	χ^2= 0.268	^{FE}p= 1.000
IIB	11	36.7	4	40.0	7	35.0	χ^2= 0.072	^{FE}p= 1.000
IIIA	12	40.0	5	50.0	7	35.0	χ^2= 0.625	^{FE}p= 0.461
IIIB	4	13.3	0	0.0	4	20.0	χ^2= 2.308	^{FE}p= 0.272
IIIC	1	3.3	0	0.0	1	5.0	χ^2= 0.517	^{FE}p= 1.000
ER Negative	7	23.3	1	10.0	6	30.0	χ^2= 1.491	^{FE}p= 0.372
ER Positive	23	76.7	9	90.0	14	70.0		
PR Negative	8	26.7	2	20.0	6	30.0	χ^2= 0.341	^{FE}p= 0.682
PR Positive	22	73.3	8	80.0	14	70.0		
HER2 Negative	24	80.0	8	80.0	16	80.0	χ^2= 0.000	^{FE}p= 1.000
HER2 Positive	6	20.0	2	20.0	4	20.0		
Molecular type								
Luminal A	19	63.3	8	80.0	11	55.0	χ^2= 1.794	^{FE}p= 0.246
Luminal B	4	13.3	1	10.0	3	15.0	χ^2= 0.144	^{FE}p= 1.000
HER2 enriched	2	6.7	1	10.0	1	5.0	χ^2= 0.268	^{FE}p= 1.000
TNBC	5	16.7	0	0.0	5	25.0	χ^2= 3.000	^{FE}p= 0.140

Table 2. Comparison of genotype and allele frequency between the studied groups

	Responders (n=20)		Non-responders (n=10)		Statistical analysis	
	Genotype/ Allele count	Genotype/ Allele %	Genotype /Allele count	Genotype /Allele %	χ^2	P
Genotype Frequency						
FGFR2 (rs2981578)					0.068	^{FE}p=1.000
GG®	11	55.0	6	60.0		
AG	9	45.0	4	40.0		
FGFR4 (rs1966265)					0.000	^{FE}p=1.000
GG®	16	80.0	8	80.0		
AG	4	20.0	2	20.0		
Allele Frequency						
FGFR2 (rs2981578)					0.049	^{FE}p=1.0
G Allele	31	77.5	16	80		
A Allele	9	22.2	4	20		
FGFR4 (rs1966265)					0.0	^{FE}p=1.0
G Allele	36	90	18	90		
A Allele	4	10	2	10		

®: Reference group Based on Fisher's exact-test results. χ^2: Chi square test, FE: Fisher Exact, p: p value for comparing between Non-responder and Responder, *: Statistically significant at p ≤ 0.05

Table 3. Comparison of FGFR2 (rs 2981578) and FGFR4 (rs1966265) genotypes between the studied groups and their agreement with Hardy-Weinberg (H.W) equilibrium.

	Observed frequency		H.W. Expected frequency		Statistical analysis (Observed vs H.W. expected	
	Genotype count	Genotype %	Genotype count	Genotype %	χ^2	P
FGFR2 (rs 2981578) genotype						
Responders (n=20)					1.686	0.194
GG®	11	55	12.01	60.06		
AG	9	45	6.98	34.88		
AA	0	0	1.01	5.06		
Non-Responders (n=10)					0.625	0.429
GG®	6	60	6.4	64		
AG	4	40	3.2	32		
AA	0	0	0.4	4		
Total					2.295	0.130
GG®	17	56.6				
AG	13	43.3				
AA	0	0				
FGFR4 (rs1966265) genotype						
Responders (n=20)					0.247	0.619
GG®	16	80	16.2	81		
AG	4	20	3.6	18		
AA	0	0	0.2	1		
Non-Responders (n=10)					0.123	0.725
GG®	8	80	8.1	81		
AG	2	20	1.8	18		
AA	0	0	0.1	1		
Total					0.370	0.543
GG®	24	80				
AG	6	20				
AA	0	0				

χ^2: Chi square test, HW: Hardy-Weinberg equilibrium, p: p value for Chi square for goodness of fit, If P < 0.05 - not consistent with HWE

Table 4. Assessment of the response according to SNP genotypes in different models of inheritance.

Assumed model of inheritance	Model (unexposed vs exposed)	Genotype	Responders (n=20)	Non-responders (n=10)	Exact p-value	Odd's ratio (95% CI)
FGFR2 (rs2981578)						
Recessive	(GG and GA) vs AA	GG and GA AA	20 (100%) 0 (0%)	10 (100%) 0 (0%)	-	-
Dominant	GG vs (GA and AA)	GG GA and AA	11(55%) 9(45%)	6 (60%) 4 (40%)	0.795	0.815(0.174 -3.807)
FGFR4 (rs1966265)						
Recessive	(GG and GA) vs AA	GG and GA AA	20 (100%) 0 (0%)	10(100%) 0(0%)	-	-
Dominant	GG vs (GA and AA)	GG GA and AA	16 (80%) 4 (20%)	8 (80%) 2 (20%)	1.000	1.000 (0.150 – 6.671)

p: p value for Odd`s ratio for comparing between the studied groups, C.I: Confidence interval

cannot be estimated because of the absence of AA genotype among both responders and non-responders (Table 4).

DISCUSSION

Neoadjuvant chemotherapy (NAC) has been shown to be advantageous for early-stage operable breast cancer patients, as well as those with locally advanced or inoperable breast cancer. However, the response to breast cancer (BC) therapy can vary widely based on the intrinsic subtype of the tumor (Asaoka et al., 2020). The effectiveness of neoadjuvant chemotherapy serves as a crucial factor in determining outcomes, with the response acting as a valid surrogate marker for survival. Patients whose tumors completely regress in response to neoadjuvant chemotherapy experience significantly better survival compared to those with other response types (Tse et al., 2021). However, drug resistance remains a major challenge, being a main reason for treatment failure and contributing to the difficulty of breast cancer treatment. The variability in therapeutic response among individuals may be attributed to differences in pharmacokinetics and pharmacodynamics (Chan et al., 2019). Changes in the genes responsible for coding receptor metabolizing and transporter proteins can impact their functionality, leading to diverse responses to therapy (Chan et al., 2019). Estrogen receptor (ER) status serves as a predictor for the response to hormonal treatments. Unfortunately, there is still a lack of biomarkers to predict NAC therapy response in breast cancer patients.

Despite the significant progress in our understanding of the genetic architecture of the susceptibility to breast cancer provided by numerous GWAS, efforts to identify novel variants linked to breast cancer prognosis remain limited (Zhu et al., 2022). Analysis of the genes that contain SNPs is crucial for both the diagnosis of early cases and tailored management of cancer patients (Zhang and Hao, 2018). The importance of FGFR signaling in breast cancer is well-established. Various FGFR alterations with diverse biological and oncogenic properties have been recently recognized. Importantly, the presence of compound mutations within FGFRs has the potential to modify oncogenic characteristics and influence the responsiveness to FGFR inhibitors. Commonly observed is the activation of the PI3K pathway, which could also impact the effectiveness of FGFR inhibitors (Santolla and Maggiolini, 2020). Notably, recent findings from multiple studies have highlighted the emergence of acquired mutations in receptor tyrosine kinases following targeted drug treatments. Consequently, assessing the significance of FGFR mutations has gained increasing importance, encompassing minor mutations, amplifications combined with mutations, and compound mutations. Recently, significant attention has linked growth factors and their associated receptors with different drug resistance mechanisms. This is due to the association between their increased secretion and cancer progression. (Nakamura et al., 2021).

Since, cyclophosphamide-doxorubicin-docetaxel are among the most effective chemotherapeutic agents for comprehensive breast cancer management. Therefore, we were interested to study the association between FGFR2 rs 2981578 and FGFR4 rs 1966265 gene variants and the response to cyclophosphamide-doxorubicin-docetaxel regimen in primary locally advanced BC patients. The FGFR gene variants were detected using TaqMan genotyping RT PCR on 30 biopsy-proven breast cancer patients who attended the oncology clinic for neoadjuvant chemotherapy. The RECIST criteria was adopted to classify the patients' response into responders and non-responders. Our patients' mean age is 50 years. A similar trend has been observed in the Arab population 49–52 years which is relatively younger than presenting age in developed countries 63 years. The relationship between age and the risk and outcome of breast cancer is intricate. Although the exact nature of this relationship remains unclear, women diagnosed before the age of 50 generally experience poorer outcomes compared to those diagnosed later in life (Ahn et al., 2007; Anders et al., 2008).

New findings indicate that tumors that occur at a younger age exhibit increased expression of mammary stem cells genes and alteration of the mitogen-activated protein kinase (MAPK) and

phosphoinositide 3-kinase (PI3K) related pathways. These alterations may explain hormonal therapy resistance and enhanced sensitivity to chemotherapy in ER-positive tumors (Ahn et al., 2007; Anders et al., 2008). Following the RECIST criteria, the response was evaluated on 30 patients who underwent NCT. It was noted that 20 patients (66.7%) showed a response, while 10 patients (33.3%) did not. These observed response rates are slightly elevated compared to previous studies (Silva et al., 2019) which could be explained by the difference in ethnicity and sample size.

Our results showed that premenopausal females represent 55% of responders and 40% of non-responders. Interestingly, premenopausal females showed higher response compared to post-menopausal (P=0.024). Our finding agreed with Silva et al. (2019) who stated that premenopausal status was an independent predictor of clinical complete response in luminal type breast cancer regardless the expression of HER2. Histopathologically, most patients (93.3%) were diagnosed with IDC while only (6.6%) of patients were diagnosed as ILC and mucoid type. In terms of tumor grade, 75% of responders were grade II compared to 90% in non-responders. Stage IIIA was the most common presenting stage in our study (40%) followed by stage IIB (36.7%). According to the TNM staging, most responders and non-responders were classified as T2 (45% and 60%) respectively. While lymph node involvement, N1 represents 70% of both responders and non-responders.

Regarding biological tissue markers, 70% of responders were positive for both ER and PR compared to 90% and 80% in non-responders respectively. Concerning HER2 status, only (20%) of both responders and non-responders were positive. (Table 1). No significant differences in biological tissue markers were observed between responders and non-responders. (P=0.372, P=0.682, P= 1.0) for ER, PR and HER2 respectively. The genotype distribution patterns of SNP rs 2981578 of FGFR2 gene among the BC patients were in agreement with Hardy-Weinberg equilibrium (GG=17,AG=13,AA=0,p=0.130 for all BC patients), (GG=11, AG=9,AA=0,p=0.194 for responders) (GG=6, AG= 4, AA= 0 and p=0.429 for all non-responders), (Table 3). While the genotype distribution patterns of SNP rs 1966265 of FGFR4 gene among BC patients were in agreement with Hardy-Weinberg equilibrium (GG=24,AG=6,AA=0,p=0.543),while (GG=16, AG=4,AA=0,p=0.619 for responders),(GG=8, AG= 2, AA=0 and p=0.725 for non-responders) (Table 3).

Additionally, we did not find any statistically significant difference in the genotype distribution of both SNPs between responders and non-responders (^{FE}p=1.000). When the allele frequency of rs1966265 was analyzed, no significant difference was detected between responders and non-responders. Similarly, when the allele frequency of rs2981578 was analyzed, no significant difference was detected between responders and non-responders (^{FE}p=1.000). Although, the (A) allele was more frequent in responders (22.5%) compared to non-responders (20%) for rs2981578, however, it fails to reach the statistically significant level which could be explained by the small sample size. Unlike our results, Chen et al. (2018) documented a significant correlation between the AA genotype and A allele of FGFR4 rs1966265, as well as the A alleles of FGFR2 rs2981578, with an enhanced chemosensitivity in breast cancer patients undergoing neoadjuvant chemotherapy based on cyclophosphamide, doxorubicin, and docetaxel (NCT).

Numerous case–control studies were carried out in different ethnic groups including Jordanian Arab women to determine the correlation between FGFR2 gene variants and breast cancer vulnerability (Al-Eitan et al., 2020; Arif et al., 2021; Boyarskikh et al., 2009; Hosseini et al., 2018; Jara et al., 2013; Michailidou et al., 2013). They found that FGFR2 (rs2981582 C > T, rs1219648 A > G, and rs2420946 C > T) genetic variants were linked to breast cancer's risk. However, none of them have studied the relation between the suspected gene variants and response to chemotherapy. Notably, our study has certain limitations that warrant acknowledgment. First, we have only studied two SNPs of the FGFR2, FGFR4 genes while there are still other SNPs that need further exploration. Second, our study has a relatively small sample size of participants. Finally, our

study has a cross-sectional design, therefore, the causal relationship between the studied variants and the treatment response could not be evaluated. Validation of our study findings in other multi-centric studies with prospective design in different ethnic populations is highly recommended.

CONCLUSION

The G and A alleles of FGFR2 and FGFR4 variants were not found to be linked with the response to cyclophosphamide-doxorubicin-docetaxel based neoadjuvant regimen. Understanding the molecular, genetic, and clinical profiles will help guide decision-making for delivering more effective patient-centered care.

Author Contribution Statement:

Omneya Ahmed Ibrahim Abdelkarem: Selected the research idea and research design, participated in the performance of laboratory tests and the wet lab molecular Taqman SNP genotyping assays, and interpreted the results, wrote the first draft of the manuscript, checked for plagiarism and was responsible for the publishing process.

Amr Hussein: Participated in patient selection and participated in the writing and reference gathering of the paper.

Ayman Farouk: Participated in the research design , recruited the patients and participated in the writing and reference gathering of the paper.

Heba Elsheredy: Participated in the research design, recruited the patients, collected the clinical data and participated in the writing and reference gathering of the paper.
Mai Mamdouh Sayed abdel Aziz: Participated in the performance of histopathological diagnosis and Immunohistochemistry analysis of the receptor status. Participated in the writing and reference gathering of the paper.

Sahar Mohammed Omer: Selected the research idea and research design, participated in the literature review, wrote and gathered references of the paper, participated in the performance of laboratory tests and the wet lab molecular Taqman SNP genotyping assays,and interpreted the results.

All authors reviewed and edited the manuscript and approved the final version of the manuscript.

Conflict of interest

The authors declare that there is no conflict of interest.

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Data Availability

All data are supplied in the provided tables

Ethical Approval

The study was approved by the local ethics committee of the Medical Research Institute, in accordance with the Code of Ethics of the World Medical Association (Declaration of Helsinki) (reference number: IORG0008812, IRB00010526), for research involving humans.

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