BRCA1 and EGFR as prognostic biomarkers in triple negative metastatic breast cancer patients treated with cisplatin plus docetaxel

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Abstract

Background: The triple negative (TN) metastatic breast cancer (MBC) patients are known to have worse prognosis, shorter progressive free survival (PFS), and overall survival (OS), that mandates using aggressive chemotherapy regimens.
Aim: This phase II study aimed at investigating the efficacy and safety of using Cisplatin and Docetaxel in patients with triple negative metastatic breast cancer, and the possibility of using breast cancer susceptibility gene1 (BRCA1) expression as a predictive marker of chemotherapy response, and epidermal growth factor receptor (EGFR) as prognostic marker.
Patients and Method: Between January 2006 and March 2009, 40 eligible patients with TN MBC were included in the study; we examined BRCA1 expression and EGFR protein in their specimens using immunohistochemistry.
The patients were treated with cisplatin 75 mg/m^2 and docetaxel 75 mg/m^2 every 3 weeks, TN measurable MBC patients previously treated with anthracycline in their adjuvant or neo adjuvant settings were included in the study.
Results: The median age of the treated patients was 43.5 years. Nearly half of the patients had an ECOG performance status of 0 or 1, and about third of them had one metastatic site. These metastatic sites were predominantly visceral in 80% of the patients. Fifty five percent of TNMBC stained positive for BRCA1 and sixty five percent for EGFR. Positivity for both markers was significantly associated with grade III tumors *(p=0 .004)*, OS, and PFS *(p= 0.001 and 0.009)* respectively.
Overall, the regimen was well tolerated as GIII vomiting and neurological side effects were observed in 20% of the patients. Other toxicities were generally mild and medically manageable; with no treatment mortality was recorded. The overall disease control rate (ODCR) was 60 %; the median PFS was 8 months, with a median overall OS of 17.5 months; while the median OS among responders was 23 months (95% CI 21.35 to 25.32).
The patients with negative EGFR had a significantly better OR, PFS, and OS than EGFR positive cases. There was no significant difference concerning OR, PFS, and OS, between positive and negative BRCA1 cases, which could be attributed to the better efficacy of cisplatin in the positive BRCA1 cases.
Conclusion: This chemotherapy regimen is effective with tolerable toxicity profile, our results point out the importance of BRCA1 expression as predictive marker of chemotherapy response, and EGFR as prognostic marker, which could identify a certain group of patients with more aggressive disease who might benefit from using anti EGFR targeted therapy plus cisplatin.

Introduction

Breast cancers are heterogeneous group of tumors with diverse behavior, outcome, and sensitivity to therapy, so, we need to identify and characterize tumors with poor prognosis in order to reduce mortality **[1].**
In recent years, the term triple negative (TN) breast cancer has emerged to describe those cancers which do not express oestrogen (ER), progesterone (PR) receptors, or Her 2 **[2].** Many studies had estimated that TN cases represents between 12-20% of all breast cancers **[3, 4]**. Those TN cases constitute one of the most challenging breast cancer groups, with only systemic chemotherapy is currently available for their treatment **[5].**
Most cases of breast cancer are sporadic and do not result from a hereditary genetic predisposition, but about 5-10% of all cases are caused by a single gene mutation that increases the susceptibility to develop breast cancer **[6]**. The identification of these breast cancer susceptibility genes has contributed to major modifications on the treatment of women with inherited predisposition to breast cancer.
BRCA1 is a cancer susceptibility gene located on the long (q) arm of the chromosome 17. The normal gene plays a role in repairing breaks in DNA but when is mutated this repair function may become disabled thus leading to more DN-replication errors and cancerous growth **[7, 8]**. The temporal patterns of BRCA1 expression in human fetuses imply a role for BRCA1 in the morphogenesis and differentiation of human mammary gland **[9].**
BRCA1 protein normally functions as a negative regulator of the cell cycle [**10, 11, 12],** also, BRCA1-positive tumors encompass a heterogeneous group of tumors that show distinctive pathological and clinical features. BRCA1-associated cancers are typically high-grade invasive duct carcinoma and are mostly triple negative **[13, 14].**
The majority of triple negative cancers exhibit the basal phenotype, i.e. they also expressed basal type cytokeratins (Keratin 5 or 6) which could be an independent prognostic factor from size, grade, lymph node status **[15],** or EGFR (80-90% of the TN cancers are of the basal phenotype). The proportion of BRCA1 associated cancers with the basal phenotype was estimated to be 57% **[14],** to 88% **[13].**
Epidermal growth factor receptor (EGFR) is more frequently expressed in TN BCs than in non TN cases **[16, 17],** So, EGFR is now considered a perfect candidate for the targeted treatment of the TNBC. It was shown that high rates of objective response to neoadjuvant anthracycline plus taxane **[18].**
The phenotypic and molecular similarity of the TN BCs to BRCA1-associated

BCs might be of use in designing their treatment protocol **[19].**

There is increasing evidence that the DNA repair defects that are characteristic of BRCA-1 related cancers may provide sensitivity to certain systemic agents to treat TN MBC patients such as the bifunctional alkylating agents and platinum drugs **[20, 21].** The higher response to docetaxel as part of the TAC regimen in the treatment of the TNBC patients was addressed in the past few years **[22, 23],** with no clinical evidence to prove a decreased sensitivity to taxanes in TNBC versus non-TNBC **[24].**

In the view of the regained interest in the use of platinum salts in patients with TNBC, many studies are ongoing for the past few years to test the efficacy of cisplatin added to docetaxel in such group of patients **[4].**

To date, there have been a few studies on immunohistochemical analyses of BRCA1 protein in sporadic breast carcinomas, and occasional studies have investigated the role of platinum chemotherapy added to taxanes in the subgroup of BRCA1 mutation carriers. The objective of this study was to assess the efficacy and safety profile of cisplatin and docetaxel in TN metastatic breast cancer patients in correlation to BRCA1 and EGFR expression as predictive and prognostic markers to the clinical outcome.

Material and Methods

a) Study design and legibility criteria

Patients who are between 18 and 65 years old, having performance status score of 0-2 according to the ECOG scale, with previous pathological diagnosis of breast cancer and who received anthracyclins as part of their adjuvant or neoadjuvant treatment were included in the study. All patients had pathological confirmation of being TN (at time of diagnosis of the primary tumor or through examining the tissue paraffin blocks), the metastatic cases with one or more measurable or assessable sites of metastasis (visceral and/or non visceral), having normal renal and hepatic functions, and adequate bone marrow reserve were also included.

We excluded the patients with renal impairment, pregnant or lactating patients, those with cardiac problems (ejection fraction <50%), and patients who had a life expectancy below 6 months.

Pathological study

We have retrieved the paraffin blocks of the treated patients; all had invasive triple negative breast cancers and were treated at the oncology department, Ain Shams University hospitals. We evaluated the tumor histological type and grade (1-3) using the modified Scarf-Bloom Richardson grading system comprising an architectural grade, nuclear grade and mitotic grade **[25].**

For immunohistochemical studies, sections of 4 µm thickness from formalin-fixed and paraffin-embedded tumors were cut and mounted. Following deparaffination in xylene, slides were rehydrated through graded ethanol series, and then placed in running water; endogenous peroxidase activity was blocked with 6% hydrogen peroxide and methanol. Samples were steamed for antigen retrieval with 10mM citrate buffer (PH6.0) for 30 minutes. Following protein block, slides were stained with antibodies against BRCA1-protein (clone GLK-2, 150, DAKO) and EGFR (prediluted, DAKO) and incubated with streptavidin-conjugated horseradish peroxidase using DAKO En vision kit protocol.

For the visualization of the antibody-enzyme complex, we used 3, 3-diamino benzidine tetrahydrochloride (DAB) counter stained with hematoxylin and examined by light microscopy.

For BRCA1 protein, we consider that cytoplasmic immunostaining within the tumoral cells was positive for gene BRCA1-mutations (abnormal phenotype), and nuclear or nuclear and cytoplasmic immunostaining within tumoral cells as negative for gene BRCA1-mutation (normal phenotype) **[26].** Immunostaining for EGFR was interpreted as positive or overexpressed when at least 10% of tumor cells showed membranous staining **[27, 17].**

Pre treatment assessment

Before being included in the trial, all the patients had to be assessed radiologically as part of the metastatic work up with bone scan, ultrasound (U/S) or computed tomography (CT), for the chest, abdomen and pelvis, we also had complete history of the previous chemotherapy, radiotherapy given to the patient in the adjuvant setting. Clinical examination, complete blood picture, kidney and liver function tests were done before each cycle of chemotherapy.

The treatment protocol

The chemotherapy schedule consisted of cisplatin 75 mg/m^2 and Docetaxel 75mg/m^2 both given every 3 weeks, proper hydration was given before and after treatment with adding granisterone 3 mg for nausea prevention and Dexamethosone 8mg was to be given a day before and 3 days after the chemotherapy. The plan was to administer the doses until progression or until encountering unacceptable toxicities.

Response evaluation and toxicity assessment

The patients were subjected to clinical and radiological examinations after 2, 4 and six courses or at withdrawal if happened earlier. We used the standard **RECIST** guidelines for evaluating response of the treated patients into complete response **CR** (complete disappearance of disease), partial response **PR** (≥30% reduction in 2 largest diameters of the mass), stable disease **SD** (no change in size), and **PD** progressive disease **[28].**

Study end points

This phase II study had the progressive free survival (PFS) and over all survival (OS) as the primary end points depending mainly on clinical and radiological responses as no pathological biopsy specimens were done for proving pathological complete response. The secondary endpoints were the correlation of the BRCA1, and EGFR overexpression with the clinical outcome of the studied protocol.

Statistical analysis

We calculated PFS from the first day of treatment till the time of disease progression, or to the moment of the patient being withdrawn from the study due to any reason. On the other hand, OS was calculated from the first day of treatment until death or when the patient was lost to the follow up visits. We used the Kaplan-Meir's method for statistical analysis of PFS and OS. The univariate analysis of the patient's characteristics was done using the chi-square test, while the multivariate analysis (Cox proportional hazard model) was done on these variable having P-value of <0.05 at the univariate analysis.

Results

Patient characteristics and Immunohistochemical results

Between January 2006 and March 2009, 40 eligible patients with TN MBC were included in the study, all had unilateral invasive ductal carcinoma and the specimens were obtained from mastectomy blocks.

The clinicopathological characteristics and the correlation of them to the BRCA1 and EGFR protein expression are listed in table 1.

Most of the patients were young (median age 43.5 years), had GIII tumors

(65%), and mostly had visceral metastasis in the liver (n=18), bone (n=18), lung (n=20), soft tissue (n=6), and cervical lymph nodes (n=2), One metastatic site was found in 14 patients (35%). Half of the patients had a performance status of 0 or I according to the ECOG scale.

The immunostaining for BRCA1 was diffuse, intense, and cytoplasmic within the tumor cells with loss of nuclear staining in 22 cases (55%) as shown in ***(figure 1)***, while 18 cases (45%) were negative. BRCA1 protein is localized in the nuclei of the adjacent normal mammary glands.

The EGFR showed weak cytoplasmic staining in the myoepithelial cells in the adjacent normal mammary glands. The overexpression of EGFR was detected in 26 cases (65%) as shown in ***(figure 2)***.

Breast carcinomas positive for BRCA1 had a significant correlation to poor tubular differentiation (higher tumor grade, grade III), toward an overexpression of EGFR protein, and number of metastasis ***(p< 0.05)***.

BRCA1 was significantly positive with younger age, in patients with worse performance, with more than one metastatic site ***(p< 0.05)***. EGFR was highly significantly overexpressed in patients with worse performance, higher tumor grade (grade III), nodal positivity at presentation, with more than one metastatic site that are mostly visceral ***(p< 0.005)***.

Response rates and survival

The overall disease control rate (DCR), i.e. complete response (CR), partial response (PR), together with the stable disease (SD) to all the studied cases was 60%, and 16 patients (40%) had a progressive disease. The response was significantly lower in the patients with the EGFR positive tumors, while those with positive BRCA1 showed highly significant improved response than the BRCA1 negative cases ***(p<0.0001)***.

The median progressive free survival (PFS) was 8 months (range 2-17 months), while the overall survival (OS) was 17.5 months (range 7-32 months), while the median OS among responders was significantly higher reaching 23 months (95% CI 21.35 to 25.32). The patient with positive EGFR showed highly significant decreased PFS below 8 months (95% CI 2.34 to 6.43), and OS below 17.5 months (95% CI 11.09 to 15.83) than the negative patients ***(p<0.0001),*** while BRCA1 positivity had no significant correlation to PFS or OS.

Those correlations of BRCA1 and EGFR expression to the PFS and OS are shown in the Kaplan Mayer's survival curves as in ***(figures 3- 6)***. The multivariate regression analysis found no significant correlations.

Treatment compliance

The regimen was well tolerated, the 40 patients received 197 doses of chemotherapy with a mean of 5 doses per patient (range 4-8 doses), no toxic death was recorded, G III vomiting and neurological side effects were observed in 8 patients (20%), other toxicities as G I and II mucositis was recorded in 12 patients (30%), neutropenia of G III was recorded in 9 patients (22.5%), all were given colony stimulating factor support to decrease dose delay. Dose reduction was done for 4 patients (10%) due to side effects. Overall the toxicities were generally mild and medically manageable **[29].**

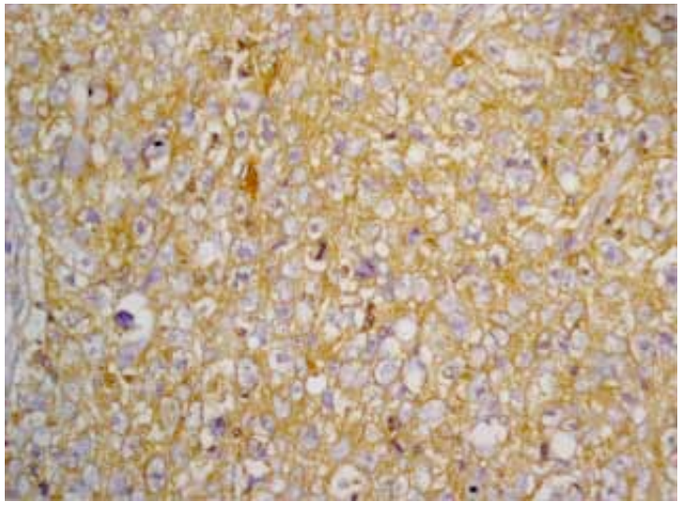

Fig 1: BRCA1 expression in TN breast carcinoma: showed positive cytoplasmic immunostaining and with total loss of nuclear staining (abnormal phenotype) (x400).

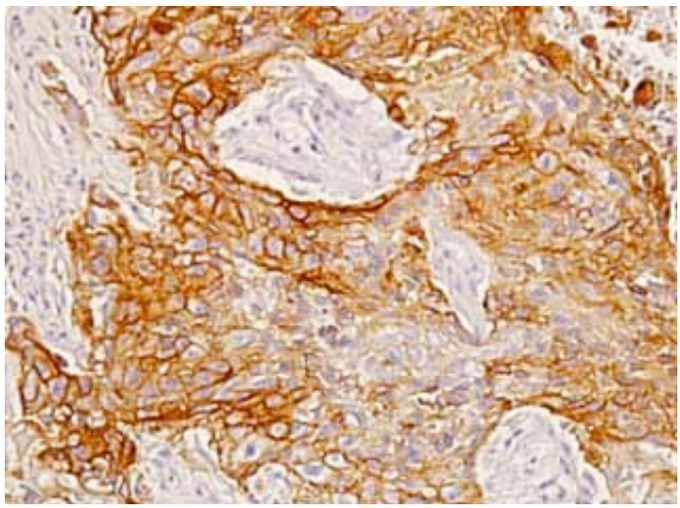

Fig 2: EGFR overexpression in TN breast carcinoma: showed strong positive membranous immunostaining (x200).

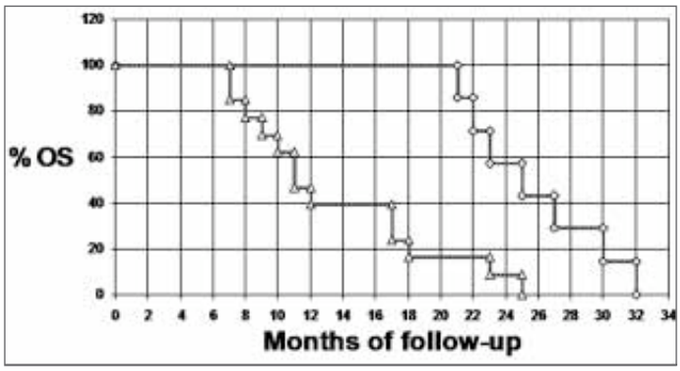

Fig 3: Correlation of OS to EGFR, Neg. EGFR (◊), Pos. EGFR (Δ) (p=0.001)

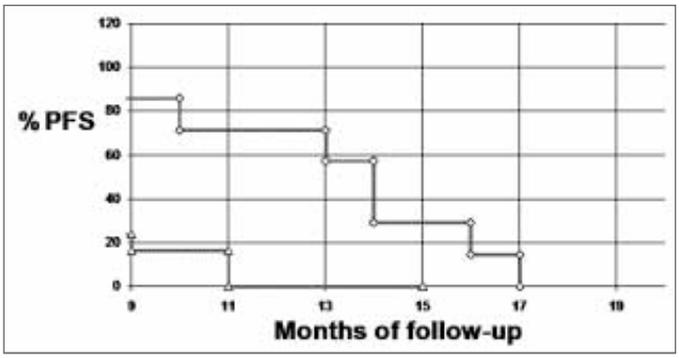

Fig 4: Correlation of PFS to EGFR, Neg. EGFR (◊), Pos. EGFR (Δ) (p=0.001)

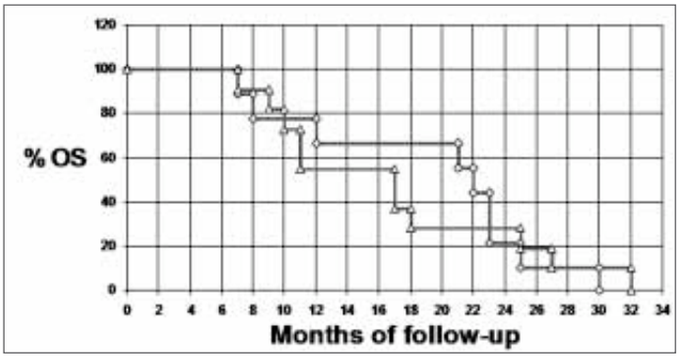

Fig 5: Correlation of OS to BRCA1, Neg. BRCA1 (◊), Pos. BRCA1 (Δ) (p=0.057)

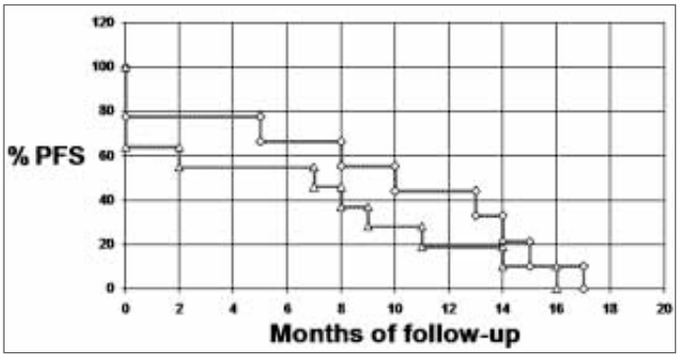

Fig 6: Correlation of PFS to BRCA1, Neg. BRCA1 (◊), Pos. BRCA1 (Δ) (p=0.225)

Discussion

DNA-damaging drugs cause DNA DSBs either directly or indirectly, and it is widely accepted that the absence of BRCA1 expression leads to hypersensitivity of cells to DNA damage-based chemotherapy. It was initially reported that overexpression of BRCA1 in human breast cancer cell lines resulted in increased resistance to cisplatin. Furthermore, antisense inhibition of endogenous BRCA1 expression promoted increased sensitivity to cisplatin that was associated with decreased DNA repair and increased apoptosis **[30]**.

In a retrospective study of 278 women, it was demonstrated that patients with BRCA1 mutation faired worse if they did not receive chemotherapy when compared with similar non carrier patients who also did not receive adjuvant chemotherapy (relative ratio of 3.3). Therefore, it seems that patients with BRCA1 mutation gained more benefit from chemotherapy **[31]**. Another study confirmed that tumors arising in BRCA1- mutation carriers are more chemosensitive **[32]**.

Clinical evidence has suggested an increased benefit from DNA damage-based chemotherapy for patients with BRCA1 germ line mutations. In sporadic breast cancer cases, there is conflicting evidence as to whether tumors with epigenetic inactivation of BRCA1 will derive a similar benefit to DNA damage-based chemotherapy **[33]**.

Immunohistochemical assessment of BRCA1-mutation is less expensive, less time consuming than genetic testing, and could be of clinical and therapeutic benefit **[34, 26],** demonstrated that BRCA1 protein is exclusively localized in the nuclei of normal mammary epithelial cells, suggesting that nuclear localization could be the normal phenotype, while loss of nuclear BRCA1 expression represents an altered BRCA1 phenotype.

We detected loss of BRCA1 nuclear expression in 22 out of 40 TNBC patients (55%). ***Lee W.Y. et al., in 1999,*** demonstrated such loss in 22 out of 108 sporadic cases of invasive ductal carcinoma (20.4%), this correlated well with the higher histologic grade and ER-negativity, they suggested that BRCA1 expression may play an important role in the pathogenesis and prognosis of breast carcinoma **[26].**

Interestingly, we observed loss of the BRCA1 protein expression in breast cancer cells in comparison to that in normal mammary epithelial cells, and an association between the loss of BRCA1 protein expression and poor tubular differentiation, high histologic grade and an overexpression of EGFR protein, these results were in accordance to ***Comanescu M and Popescu C.F, in 2009*** **[35].**

Positive correlation between BRCA1 and ER was reported by **[36, 26]**, their data suggested that BRCA1 expression is regulated by the steroid hormones in human breast cancer cells. Recently, BRCA1 has been demonstrated to play a critical role in the differentiation of ER-negative stem progenitor cells to ER-positive luminal cells, and it was reported that loss of BRCA1 function resulted in accumulation of ER negative stem/progenitor cells, candidates for a cancer stem cell in the basal-like subtype **[37].**

Altogether those findings suggest that reduced BRCA1 expression in breast cancers may somehow be related to their malignant phenotype and may also indicate a crucial role of the BRCA1 protein in the development of sporadic breast cancers. Analysis of the association between BRCA1 protein expression and the disease-free or overall survival rates of patients with breast cancer might clarify the significance of reduced expression of BRCA1 protein in sporadic breast cancers **[38].**

Sixty five percent of our specimens stained positive for EGFR protein by immunohsitochemistry, that was in agreement with the results of ***Nielsin TO et al. in 2004***, but disagreed with ***Toyama T et al., in 2008*** who found that only 31% of TNBCs were positive for EGFR protein **[39- 40].**

Our results agree with ***Rhee et al., 2008,*** that TNBC was linked to younger age (median 43 years), higher histologic grade (GIII=65%), visceral metastasis (80%), and overexpression of EGFR, all indicated poor prognosis **[41].**

The study demonstrated that loss of nuclear expression of BRCA1 and overexpression of EGFR protein could be associated with poor prognosis in TNBC. Likewise ***Toyama T. et al., in 2008*** found that, frequently increased EGFR copy number and EGFR protein expression, and decreased BRCA1-mRNA expression were observed in Japanese triple-negative breast cancers, and concluded that EGFR and BRCA1 might be candidates for targeted therapies **[40].**

A recent study ***by Corkey and his colleagues***, discussed the effect of gifitinib (targeted therapy to EGFR) on the cell lines given with docetaxel and carboplatin, they suggested that this was a rational combination that might provide an additional benefit in TNBC patients and warrants further studies **[42].**

The known aggressiveness of TNBC could be correlated to the increased deaths in those patients in the first 5 years of diagnosis **[43, 44],** and those patients with metastatic disease have a significantly shorter survival **[45- 46].**

The only systemic therapy currently available for TNBC patients is chemotherapy with high rates of response to neoadjuvant anthracycline plus toxane **[18, 23].** There may be some clinical gain with platinum chemotherapy for TNBC patients which are mostly modest **[47].**

Sirohi et al., in 2008 presented 34 TNBC patients who received platinum-based chemotherapy regimen and showed response rates and survival data equivalent to ours where they had CR in 3% PR in 38%, SD in 35% and PD in 35% of the patients compared to 5%, 40%, 15%, and 40% in our study respectively **[47].**
Byrski and his associates in 2009 found that pathologic complete response to cisplatinum in BRCA1 positive breast cancer patients were observed in nine out of ten patients (90%). Considering the small number of patients, they suggested that clinical studies of cisplatinum might also be extended to BRCA1 carriers with other solid tumors **[48].** We reported PFS of 8 months compared to 6 months in the previously mentioned study, and OS of 17.5 months compared to 11 months, this could be due to the added effect of docetaxel in study rather than vinblastine & mitomycin used with cisplatin in such study.

Conclusion

Our study confirms the potential benefit of cisplatin plus docetaxel in the subgroup of BRCA1 positive TN breast cancer patients. Also, we concluded that BRCA1 protein positivity and EGFR overexpression are poor prognostic markers in TNBC, such group of patients could be the subject of larger randomized trials to evaluate the benefit of adding anti EGFR to this protocol.

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Tables

Table 1: The correlation of the clinicopathological characteristics of the studied cases with BRCA1 and EGFR expression

Clinicopathological characteristics		N=40	%	BRCA1		EGFR	
				Positive 22 (55%)	Negative 18 (45%)	Positive 26 (65%)	Negative 14 (35%)
1- Age in years Median 43 years Range 36-59	<45years ≥45 years	20 20	50 50	12 10	8 10	14 12	6 8
				0.005 S		0.507 NS	
2- Menopausal status	Pre post	26 14	65 35	14 8	12 6	18 8	8 6
				0.842 NS		0.445 NS	
3- Performance status	0 I II	10 10 20	25 25 50	8 2 12	2 8 8	4 4 18	6 6 2
				0.022 S		0.004 HS	
4- Grade	II III	13 27	32.5 67.5	5 17	8 10	4 22	9 5
				0.04 S		0.0001 HS	
5- Nodal state at diagnosis	Positive Negative	24 16	60 40	14 8	10 8	20 6	4 10
				0.604 NS		0.003 HS	
6- Number of metastasis	1 2 ≥ 3	14 14 12	35 35 30	4 12 6	10 2 6	6 8 12	8 6 0
				0.009 S		0.007 S	
7- Metastatic sites	NV V	8 32	20 80	4 18	4 14	4 22	4 10
				0.912 NS		0.027 S	
8- Response	CR PR SD PD	2 16 6 16	5 40 15 40	2 11 3 6	0 5 3 10	0 9 2 15	2 7 4 1
				0.004 HS		0.005 S	
9- PFS	<8 months ≥8 months	22 18	55 45	14 8	8 10	20 6	2 12
				0.225 NS		0.0001 HS	
10- OS	<17.5 months ≥17.5 months	20 20	50 50	14 8	6 12	20 6	0 14
				0.057 NS		0.0001 HS	

V= visceral, NV= non visceral.