RESEARCH ARTICLE

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Serum Cytokeratin 18 as a Potential Early Marker for Chemotherapy Response in Breast Cancer Patients: A Prospective Study

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Abstract

Background: Currently, it is well recognized that response to neoadjuvant chemotherapy is an important predictive factor for survival in breast cancer patients. However, it is still an area of research about which patient would respond to the neoadjuvant chemotherapy. **Methods:** Serum CK18 levels were measured using ELISA from 52 newly diagnosed breast cancer patients, at presentation and after first cycle of neo-adjuvant chemotherapy. Pre- and post-treatment CK-18 levels were correlated with several clinical and pathological parameters. At the end of neoadjuvant treatment, changes in serum CK18 levels were correlated with tumors' response to therapy. **Results:** Significant elevation of pre-chemotherapy CK18 level was observed in patients who had progressive disease compared to those who had complete or partial response to therapy (P=0.006 and P<0.001, respectively). Significantly higher CK18 levels were observed post-chemotherapy in complete and partial responders, in contrast to patients with stable or progressive disease (P=0.012% and P=0.001%, respectively). The percent of change was significantly higher in complete responders compared to patients who had stable or progressive disease (P=0.043% and P=0.045%, respectively). **Conclusion:** Our results suggest that patients with increasing CK18 level following chemotherapy are potential responders to their neoadjuvant protocol. Thus, the measurement of serum CK18 early in the treatment course could be a simple, noninvasive way to predict tumor response to neoadjuvant chemotherapy.

Keywords: Breast Cancer- Cytokeratin 18- Chemotherapy

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Introduction

Breast cancer is a heterogeneous disease with diverse clinical, and molecular features (Li et al., 2005). In recent decades, significant advances have been achieved in systemic therapies utilized for breast cancer patients, both in the early and advanced settings (Tong et al., 2018). Currently, it is well recognized that response to neoadjuvant chemotherapy is an important predictive factor for survival in breast cancer patients. Therefore, neoadjuvant chemotherapy is being utilized more and more in the management of patients with early-stage breast cancer (Thompson and Moulder-Thompson, 2012; Asaoka et al., 2020). However, it is still an area of research about which patient would respond to the neoadjuvant chemotherapy. Therefore, in order to gain the maximal benefits of therapy, a more personalized approach which utilize predictive markers is encouraged.

Cytokeratins are recognized as major structural proteins within the epithelial cells. The expression of cytokeratins is dependent mainly on the type and degree of differentiation of epithelial cells; thus, cytokeratin expression is useful for distinguishing carcinomas from other types of tissues (Chu and Weiss, 2002). Several subclasses of cytokeratin have been studied regarding their diagnostic and prognostic utility in various malignancies (Caviglia et al., 2020; Kiani et al., 2020; Vasilevska et al., 2022). Cytokeratin 18 (CK18) is expressed by most carcinomas, such as breast, prostate, lung, and colon (Linder et al., 2004). The dysfunctional regulation of CK18 was shown to have a role in the pathogenesis and progression of different cancers. The increased expression of CK18 has been associated with poor patient prognosis in several cancers (Fillies et al., 2006; Makino et al., 2009). In contrast, low CK18 expression was associated with increased tumor progression in breast and colorectal adenocarcinomas (Woelfle et al., 2004; Knosel et al., 2006).

Most chemotherapies induce cell death by activation of the apoptotic pathway (Hickman et al., 1992). This

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process is mediated by a number of proteases which cleave intracellular substrates such as CK18. CK18 is cleaved during apoptosis, and its fragments are released into the serum (Caulin et al., 1997). Thus, it has been proposed that detecting circulating forms of CK18 in the serum can serve as a surrogate biomarker of cell death (Kramer et al., 2004). Previous studies have investigated the usefulness of serum CK18 as a potentially predictive marker of chemotherapy response in different types of tumors (Demiray et al., 2006; Scott et al., 2009; Fazilat-Panah et al., 2020). However, the clinical utility of CK18 as a marker of response remains unclear.

Therefore, in this study we aimed to evaluate changes in serum CK18 level during neoadjuvant chemotherapy and its relation to tumor response in breast cancer patients.

Materials and Methods

This was a single-center, prospective, non-randomized study that was undertaken on 52 patients with previously untreated breast carcinoma who attended the Oncology center of Mansoura University between August 2020 and April 2021. Eligible patients were those with pathologically confirmed breast cancer who were due to start neoadjuvant chemotherapy. Baseline pretreatment serum samples were collected from all patients. The estimation of CK18 was done using ELISA kit at presentation and after first cycle of neo-adjuvant chemotherapy (combination of adriamycin and cyclophosphamide).

The initial values of CK-18 were correlated with several parameters such as stage of breast cancer, menopausal status, tumor grade and hormonal receptor status. After the first cycle of chemotherapy, changes in the level of serum CK18 were also correlated with these parameters, and with tumors' response to neoadjuvant treatment at the end of therapy. This study received approval of the local Ethics Committee of Mansoura University, Faculty of Medicine. Written informed consent was obtained from all patients.

Statistical analysis of data was done using IBM-SPSS software (IBM Corp. Released 2017. IBM SPSS Statistics for Windows, Version 25.0. Armonk, NY: IBM Corp.). Qualitative data were expressed as number and percentage (%). Quantitative data were initially tested for normality using Shapiro-Wilk test. Quantitative data were expressed as mean ± standard deviation (SD) if normally distributed, or median and range if not. Mann-Whitney U test was used for comparison of non-parametric quantitative data between two groups, and Kruskal-Wallis test was used for more than two groups. For non-parametric data in one group (serial measurement), Wilcoxon test was used. The ROC Curve (receiver operating characteristic) evaluated the sensitivity and specificity for quantitative diagnostic measures. A p value was considered significant if <0.05 at confidence interval 95%.

Results

A total of 52 breast cancer patients were enrolled in this study. The mean age of participants was 47 ± 10.7 years. Postmenopausal women represented majority of the cases (69.2%). Most of the cases were diagnosed with invasive ductal carcinoma (92%). Around 72% of the cases enrolled in this study where either > T3 or > N2 stage at diagnosis (before receiving neoadjuvant chemotherapy). Other pre- and post-operative characteristic of the breast cancer patients are summarized in Table 1.

In our study, 41 out of 52 (78.9%) breast cancer patients demonstrated either complete (CR) or partial response (PR) to chemotherapy, with 15% of patients achieving CR. The complete pathological response rate, and breast conservation rate are shown in Table 2.

CK18 level compared pre- and post-chemotherapy as regard to different patients' and tumor characteristics, revealed that the median CK18 level post-chemotherapy showed some degree of increase across all patients' subgroups. The percent of change in CK18 level was calculated as =(post chemotherapy value-prechemotherapy

Table 1. Clinical and Pathological Characteristics of Breast Cancer Cases

Parameter		N (%)
Mean age (years ± SD)		47 ± 10.7
Menopausal status	Premenopausal	16 (30.8%)
	Postmenopausal	36 (69.2%)
Family history of breast cancer		8 (15.4%)
Hypertensive cases		7 (13.5%)
Diabetic cases		7 (13.5%)
Hepatitis C positive cases		8 (15.4%)
T stage pre-operative	T1	0 (0%)
	T2	14 (26.9%)
	T3	23 (44.2%)
	T4	15 (28.9%)
N stage pre-operative	N0	5 (9.6%)
	N1	10 (19.2%)
	N2/N3	37 (71.2%)
M stage pre-operative	M0	52 (100.0%)
Tumor grade	Grade 1	11 (21.2%)
	Grade 2	31 (59.6%)
	Grade 3	10 (19.2%)
Histological type	Invasive Duct Carcinoma	48 (92.3%)
	Invasive Lobular Carcinoma	4 (76.9%)
ER	Negative	12 (23.1%)
	Positive	40 (76.9%)
PR	Negative	14 (26.9%)
	Positive	38 (73.1%)
HER2	Negative	33 (63.5%)
	Positive	19 (36.5%)
KI67 Median (Min-Max)		20.0% (0-80)
T stage post-operative	T0	10 (19.2%)
	T1	12 (23.2%)
	T2	22 (42.3%)
	T3	6 (11.5%)
	T4	2 (3.8%)
N stage post-operative	N0	24 (46.1%)
	N1	13 (25.0%)
	N2	12 (23.1%)
	N3	3 (5.8%)

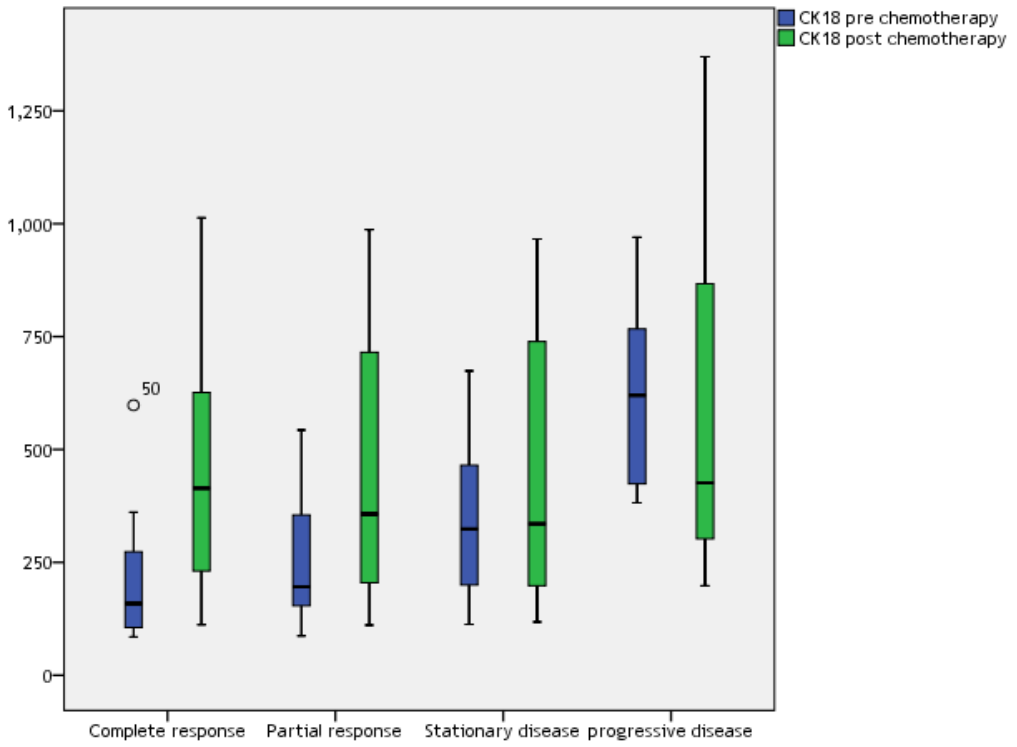

Figure 1. Comparison of CK18 Level Pre- and Post-Chemotherapy in Relation to Clinical Response to Neoadjuvant Treatment.

Table 2. Clinical and Pathological Response to Neoadjuvant Therapy

Parameter	N (%)
Clinical response	
Complete response	8 (15.4%)
Partial response	33 (63.5%)
Stable disease	6 (11.5%)
Progressive disease	5 (9.6%)
Complete pathological response	
No	44 (84.6%)
Yes	8 (15.4%)
Breast conservation	
No	30 (57.7%)
Yes	22 (42.3%)

value)/(prechemotherapy value)*100. Higher rates of increase in CK18 level were observed in T4, LN positive, Ki67 high, and HER2 positive tumors. However, the percent of change in CK18 level didn't show statistically significant difference among different patients' subgroups (Table 3).

In the current study, we noticed significant elevation of pre-chemotherapy CK18 level in patients who had progressive disease compared to those who had complete or partial response (P=0.006 and P<0.001, respectively). CK18 level compared pre- and post-chemotherapy in relation to the tumors' response to neoadjuvant treatment, revealed that significantly higher CK18 levels were observed post-chemotherapy in complete and partial responders, in contrast to patients with stable or progressive disease. The significantly higher CK18 level did not discriminate between patients who did or did not achieve complete pathological response post-chemotherapy (Table 4 & Figure 1). The percent of change was significantly higher in complete responders compared to patients who had stable or progressive disease (Table 5 & Figure 2).

To analyze the utility of CK18 level changes during treatment in predicting patients' response to neoadjuvant chemotherapy, we classified our patients into responders (patients who achieved complete or partial response) and non-responders (patients with stable or progressive disease). Receiver-Operating Characteristic (ROC) analysis was conducted to identify the optimal percent of change in CK18 level for discrimination between responders and non-responders. CK18 percent of change best cut-off value for identifying responders was 45.34%. The area under the curve (AUC) was 0.734 (p=0.018). Above this cut of value (45.34%), this study can predict responders to neoadjuvant chemotherapy by a sensitivity of 72.7% and specificity of 63.4% (Figure 3).

Discussion

Breast cancer is the most commonly occurring neoplasm and the leading cause of cancer death among women (Sung et al., 2021). Utilizing different clinical and biological markers has been an essential factor in choosing between different therapeutic options for breast cancer patients. In the neoadjuvant setting, commonly used regimens include an anthracycline followed by a taxane combination. Currently, monitoring of the response to neoadjuvant therapy is based mainly on clinical and

Table 3. CK18 Level as Regard Tumor Characteristics

Parameter		CK18 level Median (Min-Max)			
		Pre chemotherapy	Post chemotherapy	Percent of change	P value
Menopausal status	Premenopausal	193.5 (85-767)	357.0 (111-987)	76.75 (-74.1/524.6)	0.488
	Postmenopausal	265.0 (87-970)	392.5 (112-1370)	52.18 (-74.0/631.3)	
T staging	T2	185.5 (87-543)	247.0 (112-975)	67.2 (-74.03/631.3)	* P=0.567
					* P^1=0.486
	T3	206.0 (85-767)	357.0 (111-1013)	43.3 (-74.1/370.5)	* P^2=0.847
	T4	240.0 (113-970)	496.0 (118-1370)	58.4 (-45.9/524.6)	* P^3=0.329
N staging	N0	99.0 (87-103)	205.0 (132-357)	132.9 (30.1-250)	¶ P=0.150
					¶ P^1=0.953
	N1	233.0 (85-465)	427.0 (148-975)	133.8 (2.0-370.5)	¶ P^2=0.253
	N2/N3	276.0 (94-970)	439.0 (111-1370)	47.3 (-74.1/631.3)	¶ P^3=0.089
Tumor grade	Grade 1	185.0 (87-767)	357.0 (148-1013)	69.3 (-74.1/524.6)	§ P=0.820
					§ P^1=0.844
	Grade 2	196.0 (85-970)	357.0 (111-1370)	57.0 (-74.0/631.3)	§ P^2=0.654
	Grade 3	326.5 (141-674)	564.0 (117-966)	50.9 (-54.8/327.9)	§ P^3=0.580
ER	Negative	287.5 (85-970)	378.5 (198-1370)	49.8 (-54.8/370.5)	0.879
	Positive	203.0 (87-767)	371.0 (111-1013)	57.9 (-74.1/631.3)	
PR	Negative	324.0 (88-970)	391.5 (117-1370)	34.3 (-54.8/180.5)	0.076
	Positive	197.5 (85-767)	378.5 (111-1013)	74.0 (-74.1/631.3)	
HER2	Negative	276.0 (87-970)	426.0 (111-1370)	43.3 9-74.1/631.3)	0.073
	Positive	161.0 (85-620)	357.0 (118-1013)	95.0 (-51.2/524.6)	
KI67	<14% group	206.0 (88-543)	240.0 (112-987)	72.5 (-74.0/631.3)	0.983
	>14% group	220.0 (85-970)	427.0 (111-1370)	55.5 (-74.1/370.5)	

Kruskal-wallis, Mann whitney test. *P, significance between all T stages; *P^1, significance between T2 and T3; *P^2, significance between T2 and T4; *P^3, significance between T3 and T4; ¶P, significance between all N stages, ¶P^1, significance between N0 and N1; ¶P^2, significance between N0 and N2/N3, ¶P^3, significance between N1 and N2/N3. §P, significance between all grades, §P^1, significance between grade I and grade II, §P^2, significance between grade I and grade III, §P^3, significance between grade II and grade III.

radiological examination. In this context, early detection of chemo-sensitive tumors may facilitate individualized therapy, preventing undue toxicity in patients who would not benefit from certain chemotherapies. When chemotherapy is being used, the efficacy of the drug is based mainly on inhibition of tumor cell proliferation or induction of tumor cell death. Biomarkers that are released after cell death into the blood like cytokeratin fragments have been investigated regarding their relevance for therapy prediction and therapy monitoring in various cancers (Barak et al., 2004). This study investigated the utility of serum CK18 level as a potential early marker of response to neoadjuvant chemotherapy in breast cancer patients.

In our study, the median CK18 level following chemotherapy administration was higher compared to pre-chemotherapy levels across all patients' subgroups. Previous studies have reported significant increases in CK18 level after administration of chemotherapy (Kramer et al., 2006; Demiray et al., 2006; Olofsson et al., 2007). A study of patients with hormone refractory prostate cancer receiving palliative chemotherapy showed significant increases in CK18 level usually one week following each treatment cycle (Kramer et al., 2006). Another study by Demiray and colleagues found significantly higher levels of CK18 in the serum of breast cancer patients

Table 4. CK18 Level as Regard Response to Treatment

Parameter		CK18 level Median (Min-Max)		
		Pre chemotherapy	Post chemotherapy	P value
Clinical response	Complete response	159.0 (85-598)	414.0 (112-1013)	0.012*
	Partial response	196.0 (87-543)	357.0 (111-987)	0.001*
	Stable disease	324.0 (113-674)	335.5 (118-966)	0.116
	Progressive disease	620.0 (382-970)	426.0 (198-1370)	0.893
Pathological response	Negative	233.0 (87-970)	335.5 (111-1370)	0.001*
	Positive	193.0 (85-598)	427.0 (112-1013)	0.012*

Wilcoxon test; *Significant (P value < 0.05)

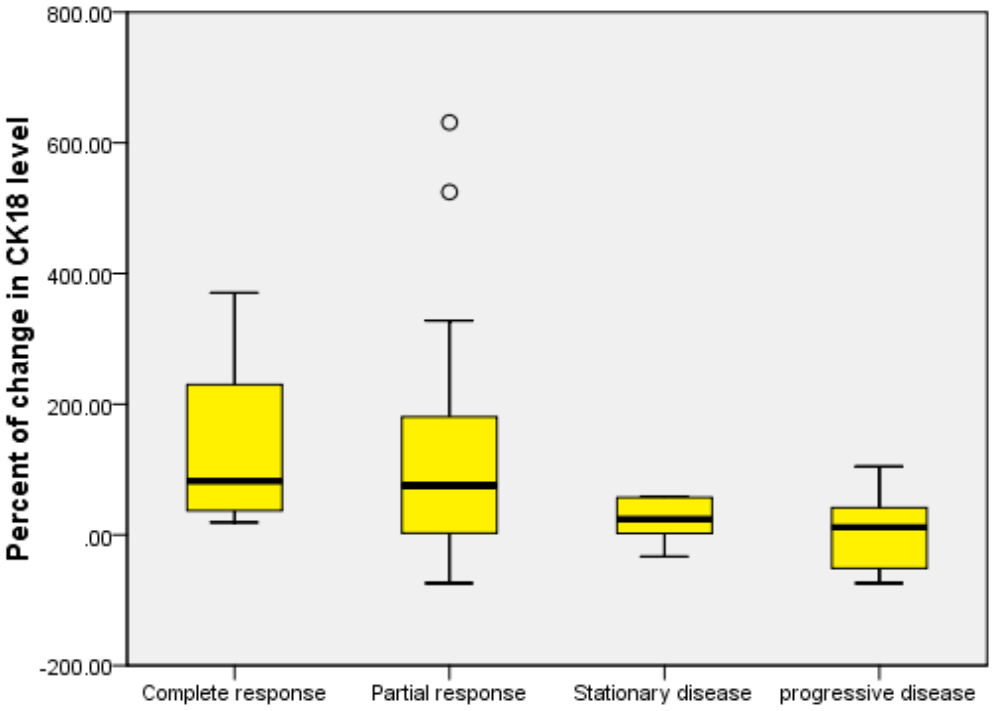

Figure 2. Median Percent of Change in CK18 Level as Regard to Clinical Response to Neoadjuvant Chemotherapy.

following chemotherapy administration, compared to pre-chemotherapy levels (Demiray et al., 2006). Similarly, Olofsson and colleagues reported elevations in serum CK18 levels in breast cancer patients receiving either anthracycline-based therapy or docetaxel (Olofsson et al., 2007). Thus, our observation comes in line with previous data indicating that CK18 could serve as a marker of cell necrosis as it is released to the circulation following cell death (Linder et al., 2004; Shah et al., 2016).

In contrast, a study by Fazilat-Panah et al. showed statistically insignificant increase in post-chemotherapy serum levels of CK18 fragments following neoadjuvant chemotherapy in breast cancer patients. In their study, there was no correlation between CK18 fragments level and pathologic response to neoadjuvant chemotherapy (Fazilat-Panah et al., 2020).

In the present study we noticed that pre-chemotherapy CK18 level was significantly elevated in patients with progressive disease compared to complete and partial responders. A study by Scott and colleagues who investigated the relation between plasma CK18 level and tumor response in patients with advanced gastrointestinal malignancy found that more patients with higher baseline level of CK18 tend to experience disease progression with chemotherapy compared to patients with lower baseline level (Scott et al., 2009). Another study conducted on breast cancer patients reported that patients with recurrent cancer had higher serum CK18 level than both primary breast cancer patients and normal controls. Furthermore, the serum CK18 level correlated with the disease burden in patients with recurrent cancer (Ueno et al., 2003). This finding may be an indicator that the higher CK18 level reflects the amount of cell death occurring in the tumor, whether as a part of an ongoing disease process or the

Table 5. CK18 Level as Regard Response to Therapy

Parameter		CK18 level Median (Min-Max)					
		Pre chemotherapy	P value	Post chemotherapy	P value	Percent of change	P value
Clinical response	Complete response	159.0 (85-598)	P=0.006*	414.0 (112-1013)	P=0.868	82.2 (19.1-370.5)	P=0.106
			P^1=0.322		P^1=0.961		P^1=0.594
			P^2=0.181		P^2=0.852		P^2=0.043*
			P^3=0.006*		P^3=0.724		P^3=0.045*
	Partial response	196.0 (87-543)	P^4=0.212	357.0 (111-987)	P^4=0.955	75.4 (-74.0/631.3)	P^4=0.159
	Stable disease	324.0 (113-674)	P^5=<0.001*	335.5 (118-966)	P^5=0.399	23.8 (-33.5/58.9)	P^5=0.093
	Progressive disease	620.0 (382-970)	P^6=0.082	426.0 (198-1370)	P^6=0.537	11.5 (-74.1/104.4)	P^6=0.662
Pathological response	No	233.0 (87-970)	0.575	335.5 (111-1370)	0.61	56.3 (-74.1/631.3)	0.361
	Yes	193.0 (85-598)		427.0 (112-1013)		63.1 (11.5/370.5)	

Kruskal-wallis, Mann whitney test. As regard clinical response: P, significance between all type of response, P^1, significance between complete response and partial response; P^2, significance between complete response and stable disease; P^3, significance between complete response and progressive disease; P^4, significance between partial response and stable disease; P^5, significance between partial response and progressive disease; P^6, significance between stable disease and progressive disease; *Significant (P value < 0.05)

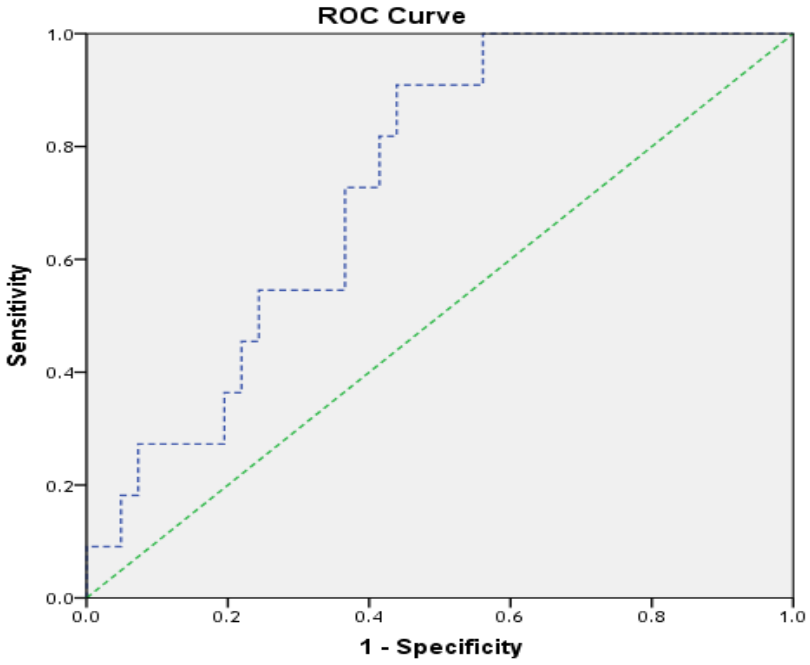

Figure 3. Performance Characteristics of CK18 Percent of Change for Discrimination between Responders and Non-Responders.

result of chemotherapy.

In our study, we documented the response of breast cancer patients at the end of the neoadjuvant therapy. As per RECIST criteria, 78.9% of our patients showed regression in the sum of diameters of target lesions by at least 30%, demonstrating partial response in 63.5% of cases and complete response (disappearance of all target lesions) in 15.4% of cases, whereas stable disease (neither partial response nor progressive disease) and progressive disease was seen in 21.1% of cases. We compared the clinical response to chemotherapy with changes in serum CK18 level. We noted that the percent of change in CK18 level was significantly higher in complete responders compared to those with stable or progressive disease (82.2% vs. 23.8% and 82.2% vs. 11.5%, respectively). Patients with progressive disease demonstrated a decline of CK18 level after chemotherapy. This result is in agreement with data from previously mentioned studies (Demiray et al., 2006; Olofsson et al., 2007), in which the increase in CK18 level post-chemotherapy was primarily observed in patients who responded to therapy.

ROC analysis in the current study proved that about 45% increase in CK18 level post-chemotherapy as compared to pretreatment level may predict clinical response to chemotherapy, with moderate sensitivity and specificity. Previously published data on breast cancer patients identified 34% increase in CK18 following chemotherapy to be the optimum cut-off value, with 70.4% sensitivity and 66.7% specificity (Demiray et al., 2006).

On the contrary to the above-mentioned data, a study evaluating CK18 in colorectal cancer found that the increases in CK18 observed during chemotherapy did not correlate with tumor response (Ausch et al., 2009). Similarly, another study conducted on patients with testicular cancer found that patients who eventually did not respond to BEP chemotherapy after an initial decline in tumor markers showed patterns of changes in serum CK18 comparable to responding patients (de Haas et al., 2008). Interestingly, another study by Fanipakdel and colleagues found that plasma CK18 levels decreased significantly after chemotherapy/chemoradiation in patients with esophago-gastric cancer (Fanipakdel et al., 2019). Thus, it seems that more questions still need to be answered regarding whether the utility of CK18 might be tumor and/or drug specific, and what would be the right timing for measurement of CK18 levels.

Conclusion, Our results suggest that patients with increasing CK18 level following chemotherapy are potential responders to their neoadjuvant protocol. On the other hand, patients with stable or decreasing CK18 level are probably not responding to their chemotherapy and an alternative protocol might need to be considered. Thus, the measurement of serum CK18 early in the treatment course could be a simple, noninvasive way to predict tumor response to neoadjuvant chemotherapy. However, although serum CK18 is a promising therapy response marker, larger studies with randomization of "potentially" non-responders between continuing or switching to an alternative therapy is needed to validate the utility of this marker.

Author Contribution Statement

Conception and design: Shimaa R. Hendawy. Collection and assembly of data: Manar Hamed and Amr Hossam. Data analysis and interpretation: Mostafa Mansour. Manuscript writing: Ahmed M. Ramez. Final approval of manuscript: All authors..

Acknowledgements

Ethical approval

This study was approved by the Institutional Research Board of Mansoura University, Mansoura, Egypt (IRB code number).

Availability of data

Data are available upon reasonable request to the corresponding author

Conflict of interest

None.

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