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Serum CYFRA 21-1 in Egyptian women with breast cancer

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KEYWORDS

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Abstract *Introduction:* Cytokeratin fragment 21.1 (CYFRA 21.1) assay detects a serum fragment of cytokeratin 19 (CK19) and is employed in the diagnosis and management of lung cancer, particularly of squamous cell histotype. Breast carcinoma has been demonstrated to express CK19 fragments in the primary and metastatic lesions and CK19 mRNA is detectable in peripheral blood from patients affected by breast cancer. *Aim of the work:* The aim of the present study was to evaluate the clinical significance of serum CYFRA21-1 in patients with breast cancer by analyzing the correlation between serum CYFRA21-1 titers, clinicopathological factors and prognosis in comparison with serum CA15.3 and CEA tested in the same samples. *Subjects and methods:* This study included 60 breast cancer patients and 25 healthy females as control group. Three blood samples were drawn from each patient, before surgery, two weeks after surgery and after 6 cycles of chemotherapy. One blood sample was drawn from each subject of control group. Serum was separated and kept frozen till used for estimation of CYFRA21-1 by enzyme linked immunosorbent assay (ELISA) and serum CA15.3 and CEA by immunoradiometric assay (IRMA). *Results:* Serum CYFRA21-1 was highly elevated in breast cancer patients than in controls and was significantly associated with tumor size, clinical stage and axillary lymph node involvement. Serum CYFRA21-1 was superior to CA15.3 and CEA as a diagnostic marker for breast cancer using ROC curve analysis. Higher levels of serum CYFRA21-1 and CA15.3 were significantly associated with poor prognosis in primary breast cancer patients. *Conclusions:* The measurement of serum CYFRA 21-1 in breast cancer patients may be useful for detecting disease relapse and for assessing surgical and chemotherapeutic efficacy. Further prospective studies using greater number of patients are required to confirm our findings.

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1. Introduction

Breast cancer is the most common cancer and second cause of cancer-death among females in most Western countries where there is an overall lifetime risk of >10% of developing breast cancer.[1] Although diagnosis by screening mammography is believed to be responsible for the significant decline in breast cancer mortality, the limitations of mammography are well recognized, especially for women with premenopausal breast cancer.[2] Thus, alternative approaches to breast cancer detection are clearly needed for improving diagnosis/prognosis.[3]

The clinical usefulness of serum tumor markers for breast cancer surveillance has not yet been established. Indeed, until sufficient data on this subject are obtained, the American Society of Clinical Oncology (ASCO) recommends against the routine use of even well-known antigens such as carcinoembryonic antigen (CEA) and carbohydrate antigen 15-3 (CA 15-3) after primary treatment or in monitoring responses to treatment.[4] Therefore, it is clearly a worthwhile goal to find a reliable serum tumor marker with high sensitivity and high specificity for breast cancer.[5]

It has been demonstrated that breast cancer cells express fragments of cytokeratin-19, which are one of the various kinds of cytokeratins comprising the intermediate filaments of the cytoskeleton. Serum fragments of cytokeratin-19 can be detected using anti-CYFRA21-1 antibody. Most of the epitopes that are detectable by clinically useful tumor markers such as CEA, CA 15-3, CA 19-9, and alpha-fetoprotein are glycoproteins shed from the cell surface. CYFRA 21-1 is unique in that its epitope is a polypeptide, which is most likely released following cell death.[6] Elevated levels of serum CYRFA 21-1 titers have been observed in various malignancies, especially in lung cancer. Healthy individuals with an abnormal level of serum CYFRA 21-1 are quite rare.[7] Patients with nonmalignant disease also showed almost no elevation of serum CYFRA 21-1, except in cases of cirrhosis, renal failure, or infectious lung disease. In previous studies, 20–30% of patients with one of these three benign diseases showed elevated levels of serum CYRFA 21-1. However, little is known about this tumor marker for breast cancer.[7–9] Serum CYFRA 21-1, the most sensitive tumor marker used in monitoring non-small cell lung cancer[10,11] has been reported to be detected in the peripheral blood from patients with metastatic breast cancer and proposed to be used in monitoring disease relapse and treatment response in breast cancer patients.[9]

The aim of the present study was to evaluate the clinical significance of serum CYFRA21-1 in patients with primary breast cancer by analyzing the correlation between serum CYFRA21-1 titers, clinicopathological factors and prognosis in comparison with serum CA15.3 and CEA tested in the same samples.

2. Subjects and methods

This study included 60 primary breast cancer patients and 25 healthy females. Patients were recruited from Menofiya Clinical Oncology Institute, Menofiya University. A written consent for participating in the study was taken from all contributors according to the declaration of Helsinki. Patients were subjected to preoperative evaluation including history taking, and clinical examination to detect the site of the tumor. Radiological investigations included mammogram, abdominal ultrasound and chest X-ray. Preoperative investigations also included fine needle aspiration cytology (FNAC) to diagnose the presence of malignancy. Patients were subjected to surgery and postoperative pathological evaluation of the tumor. Patients received 6 cycles of chemotherapy and followed up for 15 months to assure the prognostic value of the markers. Patients who lost follow-up were excluded from this study.

2.1. Blood samples collection

Three blood samples were drawn from each patient, before surgery, two weeks after surgery and after 6 cycles of chemotherapy. One blood sample was drawn from each subject of control group. Blood samples were allowed to clot for 30 min before centrifugation and centrifuged at 3000 rpm for 10 min to isolate sera. The serum was stored at −20 °C until used. Circulating serum CYFRA 21.1 was measured by enzyme linked immunosorbent assay (ELISA) (DRG, USA) and serum CA15.3 and CEA by immunoradiometric assay (IRMA) (IBL, Germany) according to the manufacturer's instructions.

2.2. Statistical analyses

Statistical analyses were conducted using the statistical software package of SPSS version 17 (SPSS Inc, Chicago, USA). Quantitative data were described using minimum and maximum as well as mean and standard error. Differences between groups were assessed by the Mann–Whitney U test for non-parametric variables. Diagnostic performance for studied parameters was evaluated using ROC curve analysis. Disease-free survival (DFS) curves were plotted using the Kaplan–Meier method. We defined DFS as the time between the date of diagnosis and the date of unfavorable outcome including local recurrence, distant metastasis or contralateral breast cancer. Correlations between quantitative variables were assessed using spearman correlation coefficient. Statistical significance was set at $p \leqslant 0.05$.

3. Results

3.1. Clinicopathological characteristics of breast cancer patients

Clinicopathological characteristics of breast cancer patients are represented in Table 1.

3.2. Serum CYFRA 21.1 in breast cancer patients

As shown in Table 2, serum CYFRA21-1 was highly elevated in breast cancer patients before surgery than in control group, and the difference was statistically significant ($P1 = 0.003$). It was also noticed that the levels of this parameter after 2 weeks of surgery and after 6 cycles of chemotherapy were significantly decreased than before surgery ($P2 = 0.002$ and 0.001 respectively) and became within the normal control value ($P1 = 0.421$ and 0.089 respectively). On comparing serum CYFRA21-1 levels after 2 weeks of surgery with those after 6 cycles of chemotherapy, the difference was not significant ($P3 = 0.710$).

Table 1 Clinicopathological characteristics of breast cancer patients.

	Breast cancer patients (n = 60)
Age	
Mean ± SE	47.72 ± 8.92
Range	31–65
Menopausal status	
Pre	25 (41.6%)
Post	35 (58.4%)
Histological grade	
II	41 (68.3%)
III	19 (31.7%)
Clinical stage	
II	40 (66.7%)
III	20 (33.4%)
ER status	
Positive	43 (71.7%)
Negative	17 (28.3%)
PR status	
Positive	39 (65%)
Negative	21 (35%)
Her-2/neu expression	
Positive	17 (28.3%)
Negative	43 (71.7%)
Tumor size	
⩽5	52 (86.7%)
>5	8 (13.3%)
Axillary lymph node involvement (%)	
Positive	41 (68.3%)
Negative	19 (31.7%)

Abbreviations: ER: estrogen receptor, PR: progesterone receptor, Her-2/neu: human epidermal growth factor receptor 2.

3.3. Serum CA 15.3 in breast cancer patients

Table 2 reveals that serum levels of CA15.3 in breast cancer patients before surgery were significantly higher than in control group ($P1 = 0.015$). It was also noticed that the levels of this parameter after 2 weeks of surgery and after 6 cycles of chemotherapy were significantly decreased than before surgery ($P2 = 0.004$ and 0.002 respectively) and became within the normal control value ($P1 = 0.511$ and 0.491 respectively). On comparing serum CA15.3 levels after 2 weeks of surgery with those after 6 cycles of chemotherapy, the difference was not significant ($P3 = 0.735$).

3.4. Serum CEA in breast cancer patients

Table 2 shows that serum CEA levels in breast cancer patients before surgery were significantly higher than in control group ($P1 = 0.013$). We also found that CEA levels after 2 weeks of surgery and after 6 cycles of chemotherapy were nearly within the same range ($P3 = 0.853$), significantly decreased than before surgery ($P2 = 0.022$ and 0.015 respectively) and became within the normal control value ($P1 = 0.539$ and 0.435 respectively).

3.5. Diagnostic performance of serum CYFRA 21-1, CA 15.3 and CEA in breast cancer patients

The receiver operating characteristic curve (ROC) analysis was used to compare the diagnostic value of serum CYFRA21-1, CA15.3 and CEA depending on the area under the curve (AUC). The higher AUC corresponds to a better diagnostic test. Serum CYFRA21-1 showed significant AUC (0.921, $P < 0.001$) with sensitivity (88%) and specificity (85%) at a cutoff value 2 (ng/ml). Serum CA15.3 showed significant

Table 2 The statistical analysis of serum CYFRA21-1, CA15.3 and CEA levels in normal control subjects and breast cancer patients.

	Control group (n = 25)	Breast cancer patients (n = 60)		
		Before surgery	After 2 weeks of surgery	After 6 cycles of chemotherapy
CYFRA 21-1 (ng/ml)				
Range	0.3–1.65	0.3–32	0.4–1.8	0.3–1.6
Mean ± SE	1.1 ± 0.092	5.72 ± 1.71	1.0 ± 0.081	0.78 ± 0.065
$P1$		0.003*	0.421	0.089
$P2$			0.002*	0.001*
$P3$				0.710
CA-15-3 (U/ml)				
Range	5–32	7–180	8–29	6.9–26
Mean ± SE	16.13 ± 1.57	43.2 ± 1.96	17.01 ± 0.86	14.76 ± 0.17
$P1$		0.015*	0.511	0.491
$P2$			0.004*	0.002*
$P3$				0.735
CEA (ng/ml)				
Range	0.4–4.2	0.8–60.5	0.6–18	0.8–19
Mean ± SE	1.86 ± 0.23	7.21 ± 2.29	1.98 ± 0.13	2.03 ± 0.59
$P1$		0.013*	0.539	0.435
$P2$			0.022*	0.015*
$P3$				0.853

$P1$: p value compared to normal control subjects.
$P2$: p value compared to before surgery.
$P3$: p value compared to after 2 weeks of surgery.
* Statistically significant at $p \leqslant 0.05$.

AUC (0.818, $P = 0.002$) with sensitivity (70%) and specificity (80%) at a cutoff value 30 (U/ml). Serum CEA showed significant AUC (0.738, $P = 0.018$) with sensitivity (60%) and specificity (66%) at a cutoff value 4 (ng/ml). Based on these results, serum CYFRA21-1 had higher diagnostic performance than serum CA15.3 and serum CEA.

3.6. Prognostic value of serum CYFRA 21-1, CA 15.3 and CEA in breast cancer patients

To study the prognostic value of these parameters, the Kaplan–Meier survival curves were constructed. As shown in Figs. 1–3 Kaplan–Meier analysis revealed that, patients with elevated levels of serum CYFRA 21-1(>2 ng/ml) and CA15.3 (>30 U/ml) than their corresponding cutoff points had shorter disease free survival time than patients with lower levels ($P < 0.000$ and $P = 0.001$ respectively). On the other hand, insignificant difference between high and low levels of serum CEA was observed ($P = 0.317$).

3.7. Correlation between studied parameters and patients clinicopathological characteristics

According to Table 3, it was observed that, there was a significant positive correlation between serum CYFRA21-1 and axillary lymph node involvement ($P = 0.024$), tumor size ($P = 0.031$) and clinical stage ($P = 0.042$) while insignificant correlation was observed with age ($P = 0.532$), histological grade ($P = 0.326$), ER and PR status ($P = 0.954$ and 0.450 respectively) and Her/2neu expression ($P = 0.076$). On the other hand, serum CA15.3 and serum CEA showed insignificant correlation with all studied clinic-pathological parameters.

4. Discussion

Breast cancer is the most common neoplasm affecting women in the Western world. Many studies are still conducted with the purpose of finding markers that could be used for early diagnosis and/or serve as possible reliable prognostic or predictive parameters, but with conflicting results. At present, no markers are available for an early diagnosis of breast cancer. For surveillance of patients with diagnosed breast cancer, the most widely used serum markers are CA 15-3 and CEA which, in combination with other clinical parameters, could have clinical significance.[12] Serum CYFRA 21-1, the most sensitive tumor marker used in monitoring non-small cell lung cancer[10,11] has been reported to be detected in the peripheral blood from patients with metastatic breast cancer.[9]

The present study showed that, serum CYFRA21-1 in breast cancer patients before surgery was highly elevated than that in control group. In agreement with our result Giovanella et al.[7] reported that serum CYFRA21-1 increased in patients affected by breast cancer in comparison with controls. Bidard et al.[13] investigated that CYFRA 21-1 is the most commonly elevated serum tumor marker (65% of patients) in metastatic breast cancer patients, supporting our result. CYFRA21-1 determines only fragments of cytokeratin 19 (CK19). Cytokeratin 19 is a

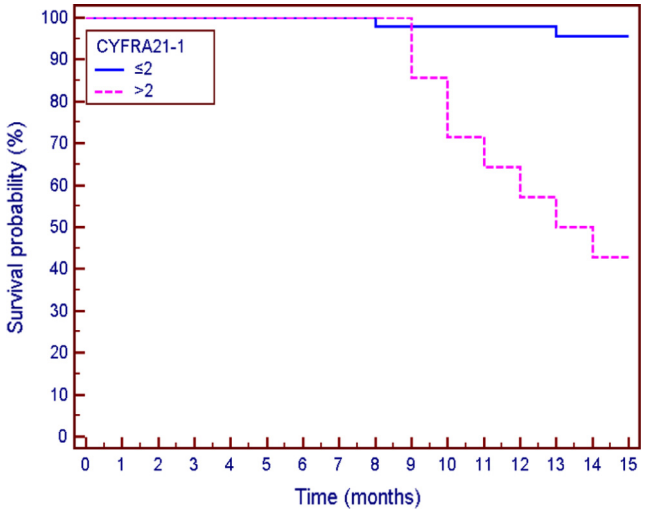

Figure 1 Kaplan–Meier disease free survival of serum CYFRA21-1 for breast cancer patients.

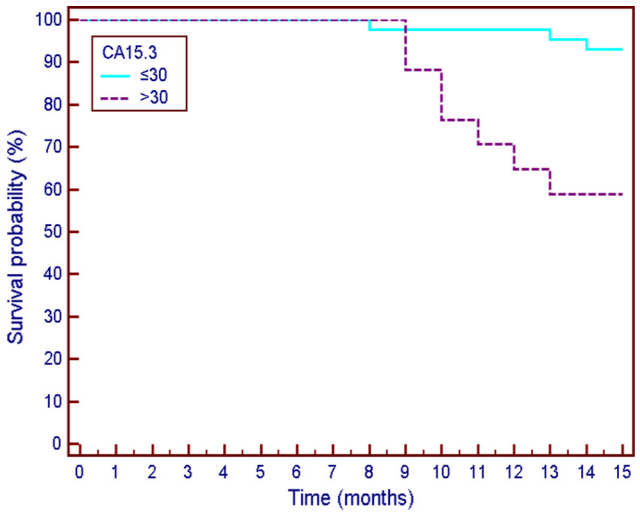

Figure 2 Kaplan–Meier disease free survival of serum CA15.3 for breast cancer patients.

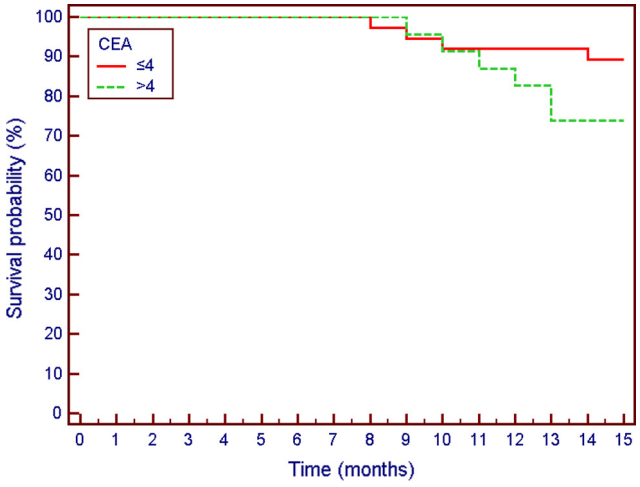

Figure 3 Kaplan–Meier disease free survival for serum CEA for breast cancer patients.

Table 3 Correlation between studied parameters and patients clinicopathological characteristics.

	CYFRA 21-1		CA 15.3		CEA	
	rs	*P*	rs	*P*	rs	*P*
Age	0.086	0.532	0.047	0.724	0.001	0.992
Axillary lymph node involvement (%)	0.291	0.024*	0.026	0.846	0.028	0.833
Histological grade	0.129	0.326	0.049	0.721	0.005	0.968
Tumor size	0.279	0.031*	0.082	0.534	0.057	0.663
Clinical stage	0.265	0.042*	0.209	0.110	0.127	0.335
ER status	0.008	0.954	0.036	0.772	0.019	0.886
PR status	0.099	0.450	0.065	0.621	0.131	0.317
Her-2/neu expression	0.213	0.076	0.003	0.980	0.134	0.307

rs: Spearman coefficient.
* Statistically significant at $p \leqslant 0.05$.

protein component of the intermediate filaments protein in epithelial cells. The mechanism of the release of CK19 fragments (CYFRA21-1) is a proteolytic cleavage. During apoptosis CK19 protein is cleaved by caspase 3 and produces soluble CYFRA21-1 fragments. When epithelial cells transformed into malignant cells, the keratin content increased. Due to necrosis of tumor cells, the soluble fragment CYFRA21-1 of CK19 is released into the blood. CYFRA21-1 provides a useful marker for epithelial malignancies, distinctly reflecting ongoing cell activity.[14]

In our study, we also noticed that serum CYFRA21-1 after 2 weeks of surgery significantly decreased than before surgery and became within the normal control value. This result is similar to that reported by Nakata et al.[5] who found that the elevated preoperative levels of serum CYFRA 21-1 decreased to normal levels after curative operation. Suggesting that serum CYFRA21-1 may be a good marker for surgical curability. Furthermore, our results demonstrated that serum CYFRA21-1 after 6 cycles of chemotherapy was significantly decreased than corresponding values before surgery and approaching normal control values. This finding is in line with study done by Nakata et al.[5] who reported the changes of serum CYFRA 21-1 levels after chemotherapy and found that serum CYFRA21-1 in five of seven patients with postoperative recurrence decreased to the normal range after chemotherapy. He also stated that, in patients with stable disease, chemotherapy reduced the serum CYFRA 21-1 levels to approximately 20–90% of the prechemotherapy levels. These results lead us to suggest that serum CYFRA21-1 may be a reliable marker of chemotherapy efficacy in patient with breast cancer.

The relationship between serum CYFRA21-1 and clinicopathological characteristic of the disease has been investigated in various malignancies. Holdenrieder et al.[15] demonstrated that CYFRA21-1 levels were significantly associated with clinical stage in patients with lung cancer. In non-small cell lung cancer Lee et al.[16] reported that tumor size and histological grade were correlated with CYFRA21-1 levels. In colorectal cancer Lee [17] found that serum CYFRA21-1 was not correlated with lymph node involvement, histological grade, clinical stage or tumor size. In the present study, we found that high levels of CYFRA21-1 in serum of breast cancer patients were significantly correlated with tumor size. This may point to the growth advantage for cells possessing high levels of CYFRA21-1. This hypothesis is coincided with that reported by Nakata et al.[9] who suggested that elevated levels of CYFRA21-1 function to promote cell growth.

Similarly, the concentration of this parameter in serum was positively correlated with clinical stage of the disease. This finding indicated that serum CYFRA21-1 may be considered as a biological marker for estimating the occurrence and progression of breast cancer and reflecting an unfavorable prognosis for breast cancer patients regardless of the treatment. This correlation was proven in a previous study carried out by Nakata et al.[9] who reported that increasing serum CYFRA21-1 was related to higher clinical stage.

Whereas axillary lymph nodes involvement is known as an effective and independent factor in breast cancer prognosis, its correlation with serum CYFRA21-1 can help to determine prognosis of breast cancer. In our study, a positive correlation was found between serum CYFRA21-1 and axillary lymph node involvement. Since serum CYFRA21-1 was positively correlated with clinical stage, tumor size and axillary lymph node involvement already tested as prognostic markers may throw light on the possibility of using this parameter as an effective marker for the determination of breast cancer prognosis. Regarding the correlation between serum CYFRA21-1 and ER, PR and Her-2/neu expression, our study showed insignificant correlation between serum CYFRA21-1 and ER, PR status and Her-2/neu expression. Bidard et al.[13] confirmed our results as they found that serum CYFRA21-1 not correlated with hormone receptors status or Her-2/neu positivity.

CA15.3 is the mucin-1 marker that is the most widely used serum marker in breast cancer. Currently, its main uses are in surveillance of patients with diagnosed disease and monitoring the treatment of patients with advanced disease.[18] CA15.3 has been implicated in cell adhesion, immunity and metastasis compared with healthy breast tissue.[19] In the present study serum CA15.3 before surgery was highly elevated in patients group than normal control group. These elevated levels of CA15.3 in breast cancer patients may be attributed to the presence of malignant disease. This result is similar to that reported by Ali et al.[20] who reported that serum CA15.3 was significantly elevated in breast cancer patients than in controls. Moreover, our result showed that serum CA15.3 levels after 2 weeks of surgery were significantly decreased than before surgery and became within the normal control value. This result may be due to the removal of the bulk mass of the tumor.[20] After 6 cycles of chemotherapy CA15.3 significantly decreased than corresponding values before surgery. These results mean that serum CA15.3 plays a role in monitoring the response of breast cancer patients to surgery and chemotherapy. These results are supported by many previous studies.[21,22]

Carcinoembryonic antigen was detected in cancer and embryonic tissue. Since CEA is rarely detected in the blood of healthy individuals, so its elevated levels in breast cancer patients than in normal control subjects may indicate the presence of breast carcinoma. This result is similar to that reported by Lee et al.[21] Our result is supported by Duffy et al.[23] who found that serum CEA from individuals with colorectal, gastric, pancreatic and lung cancer had higher levels of CEA than healthy individuals.

After 2 weeks of surgery and after 6 cycles of chemotherapy, serum CEA levels were nearly within the same range, significantly decreased than before surgery and became within the normal control value. The significant decrease in CEA levels post

2 weeks of surgery or after chemotherapy may be attributed to the removal of the malignant mass and the effectiveness of chemotherapy. These results indicated that serum CEA levels may be a useful tumor marker in that it may reflect cancer status and could be considered a reliable marker for chemotherapy response in breast cancer patients.

The significant preoperative elevations of CYFRA21-1, CA15.3 and CEA in serum of breast cancer patients than in control subjects suggest the possibility of using any one of these parameters for diagnosis of breast cancer. This directs us to compare the diagnostic performance of these parameters. Using ROC curve analysis, serum CYFRA21-1 showed higher AUC, sensitivity and specificity followed by CA15.3 then CEA. So serum CYFRA21-1 was sensitive and specific in breast cancer diagnosis than CA15.3 and superior to CEA. Rodriguez et al.[8] examined the serum CYFRA 21-1, CEA, and CA 15-3 titers in 40 patients with metastatic breast cancers and found that CYFRA 21-1 was a sensitive tumor marker for breast cancer when compared with CEA or CA 15-3, supporting our results. However, contrary to our results, Giovanella et al.[7] reported that serum CYFRA 21-1 was less accurate for the evaluation of primary and recurrent breast cancer than serum CA 15-3. The low diagnostic value of CYFRA 21-1 in breast cancer reported by Giovanella et al.[7] might be due to some issues such as time of measurement and their higher serum CYFRA 21-1 cutoff value (3.3 ng/ml) compared to our cutoff value (2.0 ng/ml).

Moreover, follow-up study was made to answer the question of whether preoperative serum CYFRA21-1, CA15.3 and CEA levels are of prognostic significance. Notably after 15 months, our results showed that breast cancer patients with preoperative higher levels of serum CYFRA21-1 had shorter disease free survival time and poor prognosis than patients with lower levels. This study supports the finding that serum CYFRA21-1 may add prognostic information to that obtained from classical prognostic factors and be useful in detecting early recurrence in breast cancer patients. This suggestion is coincided with that reported by Nakata et al.[5] who reported that elevated levels of CYFRA21-1 function to promote invasion and metastasis. Furthermore, we find relation between CA15.3 and prognosis by evaluating disease free survival (DFS) in patients group. The analysis of DFS showed that higher levels of serum CA15.3 are significantly associated with poor prognosis in breast cancer patients. This result is in agreement with that of Di Gioia et al.[22] who showed independent prognostic value of CA15.3. Moreover, Ali et al.[20] found that CA15-3 is an important prognostic indicator and good predictor for relapse. In contrast to our result Velaiutham et al.[24] found that preoperative CA15.3 had no prognostic value. Regarding serum CEA and DFS, our study demonstrated that preoperative serum CEA failed to predict disease free survival in primary breast cancer patients. In fact, our result is in line with Bartsch et al.[25] who reported that preoperative CEA had no prognostic value and CEA is not a useful prognostic marker in a subset of patients in which it was initially increased above the normal levels. In contrast to our result Lee et al.[21] reported that elevated preoperative CEA levels are associated with tumor burden and showed independent prognostic significance.

In conclusion: Serum CYFRA21-1 is highly elevated in Egyptian breast cancer patients in comparison with controls and adds significant improvement in breast cancer diagnosis superior to CA15.3 and CEA. Moreover, the measurement of serum CYFRA 21-1 may be useful for detecting disease relapse and for assessing surgical and chemotherapeutic efficacy. Further prospective studies using greater numbers of patients are required to confirm our findings.

Conflict of interest

No conflict of interest is declared.

Acknowledgment

All authors have contributed significantly to this work.

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