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Biomarkers in Women with Breast Cancer: I: CEA, CA 15.3, CA 27-29, BRCA1, and BRCA2 Predictive Value

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ABSTRACT

Background: Breast cancer is the most cancer affecting women worldwide and to date most of the investigated biomarkers are with low sensitivity and specificity in early diagnosis of primary cancer.
Aim: In the present study the relationship of tumor marker panel and breast cancer in an Iraqi population was investigated.
Patients and Methods: 100 women with breast cancer and 100 healthy controls were included in the study. All patients and control groups serum samples were subjected for determination of CEA, CA15.3, CA27.29, BRCA1, and BRCA2.
Results: Serum mean values of CA 15-3, CA 27-29, CEA, BRCA1 and BRCA2 were significantly higher in women with breast cancer than in controls. OR and relative risk confirm the association between serum increase of the five markers to breast cancer. AUC of ROC indicated the high sensitivity of their determination in breast cancer.
Conclusion:the present study show evidence that serum CA15-3, CA27-29 and CEA simultaneous determination arepotintial markers for early diagnosis of breast cancer metastasis and treatment minitoring.
Keywords: Breast, Cancer, CEA, CA 15-3, CA 27-29, BRCA1, BRRCA2, ROC, AUC

I. INTRODUCTION

Breast cancer is the most common cancer affecting women worldwide [1]. The disease can be diagnosed by clinical and physical examination, imaging and ultrasound, and histopathology [2]. Serum biomarker has not played a major role in diagnosis and prognostic monitoring of breast cancer [3]. However, effective biomarker panel may be established and developed which is used in conjunction with clinical and pathological approaches [4].

Breast cancer biomarkers are extremly various in number and type but their prediction value in diagnosis and detection of recurrence is controversial [5-7]. But with the development of new methods [2, 3], the goal for development of diagnostic, prognostic and monitoring panels may be achieved. For early diagnosis, prognosis and prediction in breast cancer, there are established biomarkers [8] and the newly emerging biomarkers [6, 9]. Although many research works worlwide reported the role and effectivenes of many markers in prognosis and prediction in breast cancer, a restricted studied were reported for Iraq.In the present study the relationship of tumor marker panel and breast cancer in an Iraqi population was investigated.

Tumor biomarkers are substances which show up or are elevated in blood, urine or tumor. These substances can be hormone, proteins, peptides etc. Tumor markers can be specific or non-specific, making it useful in detection, diagnosis and prognosis of cancer [10]. CA 15-3 is one of the first circulating prognostic factors for breast cancer. Preoperative concentrations thus might be combined with existing prognostic factors for predicting outcome in patients with newly diagnosed breast cancer. At present, the most important clinical application of CA 15-3 is in monitoring therapy in patients with advanced breast cancer that is not assessable by existing clinical or radiologic procedures [11].

The CA27.29 antigen is detected by the monoclonal antibody B27.29, specific for the protein core of the MUC1product [12]. Preliminary studies evaluated CA27.29 in comparison with either CA15.3 or other tumor markers .Using a manual RIA method, Chan et al [13].showed in a double-masked, prospective clinical trial that CA27.29 was effective for the early detection of recurrence in patients in follow-up after treatment of primary breast cancer. The diagnostic performance of CA27.29 found in the latter study seemed superior to those reported to date for CA15.3 in stage II patients [14].

II. METHODS AND MATERIAL

Study population

Hundred patients diagnosed as with breast cancer patients were included in the study. They were recruted from women attending Breast Clinic in Azadi Hospital in Kirkuk during the period from December 2012 till the end of May 2013. All patients were female, their ages ranged from 35-74 years. Aapparently healthy 100 women were selected as control group. All patients and control groups serum samples were subjected for determination of CEA, CA15.3, CA27.29, BRCA1, and BRCA2. The study protocol was approved by the ethical committee of Tikrit University College of Medicine and informed consent taken from each women agreed to participate in the study.

Methods

Serum CEA, CA15.3, CA27.29, BRCA1, and BRCA2 were determined by commercial ELISA kits according to manufacturer instruction

Statistical Analysis

The results presented as mean ± SD and comparsion between patients and control groups performed using SPSS (version 16) statistical package. P value of <0.05 considered significant. Odd ratio calculated using international standards and the present study control figures of mean values of the determined markers.

III. RESULTS AND DISCUSSION

The mean serum CEA was significantly (P<0.0001) higher in patients with breast cancer (8.01 ± 3.22 ng/ml) compared to control (1.89± 1.16 ng/ml) .Thus the mean value was about Four times higher in patients than in controls, Table (1) . Odd ratio confirmed the significant (p<0.0001) association between the increased serum level of CEA and Breast cancer whether calculated on international standard (OR = 81.6 , p< 0.0001) our present study control (OR = 76.5 , p< 0.0001). Furthermore, relative risk was significant for both cut-off, Table 1.

The mean serum CA 15.3 was significantly (P<0.0001) higher in patients with breast cancer (41.84 ± 7.02 U/ml) compared to control (10.31± 5.34 U/ml) .Thus the mean value was about Four times higher in patients than in controls, table (2). Odd ratio confirmed the significant (p<0.0001) association between of CA 15.3 antigen serum level and presence of Breast cancer for both international standard (OR = 72.81 , p< 0.0001) and present study control (OR = 92.76 , p< 0.0001). Relative risk was significant for both cut-off values, Table2.

The mean serum CA 27.29 was significantly (P<0.0001) higher in patients with breast cancer (55.03 ± 11.36 U/ml) compared to control (17.29± 7.62 U/ml) .Thus the mean value was about three times higher in patients than in controls, table (3). Serum CA 27.29 level increase was significantly associated with presence of Breast cancer as OR confirmed such association for both international standard (OR = 33.41 , p< 0.0001) and present study control (OR = 96.24 , p< 0.0001). Relative risk was significant for both cut-off values, Table 3.

The mean serum BRCA 1 was significantly (P<0.0001) higher in patients with breast cancer (21.24± 9.42 ng/ml) compared to control (4.48± 3.05 ng/ml) .Thus the mean value was about five times higher in patients than in controls as in table (4) . BRCA 1 increase in serum level was significantly (P<0.0001) associated with breast cancer for international (OR = 54.15) and present study control (OR = 44) . Relative risk was significant for both cut-off values, table (4).

The mean serum BRCA 2 was significantly (P<0.0001) higher in patients with breast cancer (28.51± 7.80 ng/ml) compared to control (9.41± 5.63 ng/ml) .Thus the mean value was about three times higher in patients than in controls, table (5). Odd ratio confirmed the associationbetween breast cancer and the increased serum level of BRCA 2 for international standard (OR =

66 , p< 0.0001) and this study control (OR = 85 , p< 0.0001) . Relative risk was significant for both cut-off values, table (5).

ROC curve analysis (Table 6) was used to quantify the diagnostic value of the CEA, CA 15-3, CA 27-29, BRCA1, and BRCA2 markers. All markers have AUC significantly better than 0.5, with CA 15-3 and CA 27-29 having the best performance (AUC=0.999, 95% CI [0.997, 1.001]). The superiority of CA 15.3 and CA 27-29 over the other three markers was also evident when we determined OR. The incremental values of AUC for CA 15-3 and CA 27-29 over that for CEA are statistically significant (Delong test, p <0.05).

Table (1) Mean CEA and odd ratio in patients with breast cancer compared to control

Variable		Mean ± SD
CEA ng/ml	Patient	8.012 ± 3.227
	Control	1.895 ± 1.166
	t test	17.27
	P value	<0.0001
CEA cut-off value	International standard	Present study control
	5 ng/ml	3.1 ng/ml
Odd ratio	81.6	76.5
Z statistic	9.05	8.70
P value	<0.0001	<0.0001
Relative risk	12.28	5.53

Table (2) Mean CA 15-3 and odd ratio in patients with breast cancer compared to control

Variable		Mean ± SD
CA 15-3 ng/ml	Patient	41.848 ± 7.027
	Control	10.310 ± 5.349
	t test	35.71
	P value	<0.0001
CA 15-3 cut-off value	International standard	Present study control
	30 ng/ml	15.66 ng/ml
Odd ratio	72.81	92.76
Z statistic	9.28	8.54
P value	<0.0001	<0.0001
Relative risk	8.9	5.59

Table (3) Mean CA 27-29 and odd ratio in patients with breast cancer compared to control

Variable		Mean ± SD
CA 27-29 ng/ml	Patient	55.039 ± 11.360
	Control	17.293 ± 7.62
	t test	27.59
	P value	<0.0001
CA 27-29 cut-off value	International standard	Present study control
	38 ng/ml	24.91 ng/ml
Odd ratio	33.41	96.24
Z statistic	8.71	8.95
P value	<0.0001	<0.0001
Relative risk	6.71	4.9

Table (4) Mean BRCA1 and odd ratio in patients with breast cancer compared to control

Variable		Mean ± SD
BRCA1 ng/ml	Patient	21.240 ± 9.420
	Control	4.480 ± 3.050
	t test	16.92
	P value	<0.0001
BRCA1 cut-off value	International standard	Present study control
	10	7.53 ng/ml
Odd ratio	54.15	44.33
Z statistic	9.144	7.46
P value	<0.0001	<0.0001
Relative risk	6.85	3.17

Table (5) Mean BRCA 2 and odd ratio in patients with breast cancer compared to control

Variable		Mean ± SD
BRCA2 ng/ml	Patient	28.510 ± 7.800
	Control	9.410 ± 5.630
	t test	19.83
	P value	<0.0001
BRCA2 cut-off value	International standard	Present study control
	20 ng/ml	15.05 ng/ml
Odd ratio	66	85.09
Z statistic	9.235	7.87
P value	<0.0001	<0.0001
Relative risk	8.8	4.36

Table 6. Area Under Curve (AUC) as a Predictive of Biomarkers in Patients with Breast Cancer.

Biomarker	AUC [95% CI]	Standard Error
CEA	0.980 [0.966-0.995]	0.008
CA 15.3	0.999 [0.997-1.001]	0.001
CA 27-29	0.999 [0.997-1.001]	0.001
BRCA1	0.969 [0.943-0.995]	0.013
BRCA2	0.971 [0.946-0.996]	0.013

Discussion

CEA as a member of the immunoglobulin supergene family and is expressed in a large variety of secretory tissues [15, 16].This biomarkers form with CA 15-3 the most extensively studed in breast cancer, while CA 27-29 less widely used serum marker in breast cancer [17].

The present study shows a significantly higher mean serum values in women with breast cancer for CEA, CA 15-3 and CA 27-29. The association between these three serum biomarkers was confirmed by a significant OR values .determination of area under curve indicated that CA 15-3 and CA 27-29 biomarkers may displays higher diagnostic sensitivity for breast cancer than the currently used tumor markers CEA. Moreover, 14% and 11% of women with breast cancer were with normal levels of CEA who have elevated CA 15-3 and CA 27-29 respectively. A finding that goes with ROC results as both CA 15-3 and CA 27-29 were with the higher area under curve. For this reason, CEA measurements will benefit from combining CA 15-3 and CA 27-29 measurements, to increase the diagnostic sensitivity of each of the markers alone. The relative risk was significant for CEA, CA 15-3 and CA 27-29 using international or the present study control.

High preoperative level of CA 15-3 associated with adverse patients outcome [17]. Serum increase in CA 15-3 was correlated to the stage of breast tumor, this biomarker increased in 10% of women in stage I, 20% in stage II, 40% in stage III, and 75% in stage IV and may be a predictor of metastasis when increased in 5-10 fold [18,19].
CA 15-3 is of value as prognostic factor of breast cancer and therapy monitoring [20,21], survellance after primary treatment [22-25], and monitoring response to therapy in advanced disease [26,27]. Thus, CA 15-3 increase of 5-10 times above normal upper limit can predicts breast cancer [28], however, a low value cannot exclude metastasis [**17,19**], making CA 15-3 more of prognostic rather than diagnostic marker [28-30] . .

The interpretation of tumor marker in women with b reastcance is influenced by tumor stage [18,29]. In the literature, CA 15-3 sensitivity range was 3% to 95.6% for women with breast cancer [18,30],however, the present study shows high sensitivity whether the results calculated using international standards [<30 U/ml, 100% sensitivity] or this study control [<16 U/ml, 83% sensitivity] cut off values. Although, ther337e was variation in percent of CA 15-3 positivity range in relation to breast cancer, elevated CA 15-3 values were decomented in all breast cancer subtypes [18,30,31]. Previous studies [18, 30, 32] suggest that CA 15-3 is the most sensitive tumor marker in breast cancer. Verring et al [30] study confirms that CA 15-3 serum levels was the sensitive tumor marker for all breast cancer subtupes, regardless of tumor site metastasis. The advances in breast cancer control will be aided greatly by early detection so as to diagnose and treat breast cance prior to metastasis [9]. In addition, the development of new sensitive and specific methods [4, 33, 34] suggest the advantages of determing other biomarkers for diagnostic and prognostic purposes.

American Society of Clinical Oncology (ASCO) in 1997 [35] considered CA 27-29, which had been evaluated in a well designed study [13] as an additional breast tumor marker in addition to CA15.3. Gion et al [36] suggest that CA 27-29 discriminated primary breast cancer from healthy individual better than CA 15-3, especially in patients with limited disease. The present study indicated that CA 27-29 was 3 times higher in women with breast cancer than in controls and the association of such marker with breast cancer was confirmed by high odd ratio for both international and present study cut off values. In addition the mean serum values in both patients and control groupd were high that the values of CA 15-3. This finding agreed with that reported for patients but not for healthy controls [36,37]. Furthrmore, the area under the ROC curve is similar for both CA 27-29 and CA 15-3, while Gion et al [36] reported that area under curve was greater for CA 27-29 than for CA 15-3. Reported studies [38-40] suggest an excellent correlation between CA15-3 and CA 27-29 and comparable sensitivity and specificity. Chan et al [13] found that CA 27-29 had a relatively higher sensivity to that of CA 15-3 reported in literature [12,38-46]. However, Bon et al

[45] in a large case series found that both CA27-29 and CA15-3 are with good correlation. Our study confirms that both markers are with high sensitivity and with same AUC. The finding of Chan et al [13] different from ours and that of Bon et al may contributed to that Chan et al did not compare CA27-29 and CA 15-3 in the same patient series, but to that in literature. In addition, published studies [46-54] suggest a higher sensitivity of CA15-3 for the detection of recurrence than that cited by them [13].

CA27-29 is directly associated with extent of disease [36], however, Bon et al [45] not find difference in CA27-29 and CA 15-3 serum valus in relation to breast cancer stage. CA27-29 serum concentration and positivity rates are higher than CA15-3 in early stage of breast cancer, but they are comparable in stage III [36].

Carcinoembryonicantige (CEA) is used as a marker of a wide range of maligancies, however, its monitoring should be considered inefficient and expensive follow up method in women with breast cancer [32]. However, the present study indicated a 4 times mean serum concentration in women with breast cancer than incontrol and with highly significant association with breast cancer whether the OR determined using international standard or this study standard as cut off. Although CEA less widely investigated as prognostic factor than CA15-3, high preoperative concentrations associated with poor prognosis in women with breast cancer [55-58]. In addition, CEA serum concentration elevation documented in the majority of women with metastatic breast cancer and in all stage types [18]. Molina et al [58] found that positivity rate of 13% for CEA in women with primary breast cancer. While Samy et al [59] found that CEA and CA 15-3 serum concentration significantly higher in women with recurrent breast cancer. Other study reported that CEA and CA 15-3 were predictable markers for recurrence of breast cancer in women with <64 years age [60]. However, Mariani et al [61] reported that CEA and CA 15-3 markers are weakly predictive in distant recurrence. Furthermore, Lumachi et al [62] suggest that CEA and CA 15-3 preoperative measurment are of little value in ealy stage breast cancer and not predictive for therapeutic decision making. The AUC of ROC suggests that CEA in our present study cohort was of predictable value in women with breast cancer. The variation in predictibility between different studies may be due to influence of study design, study population and method of detection of markers.

Serum based breast cancer markers, CA15-3, CA27-29 and CEA represent the most mature markers panel for monitoring women with metastatic breast cancer [44, 63-66]. These 3 biomarkers are overexpressed in stage IV breast cancer [63,67]. Although these 3 markers being endorsed by ASCO, their utility has been limited by specificity and sensitivity of the individual marker [68]. This problem could be overcome by multiple markers measurment andusing newly sophisticated procedure [69-71]. LI et al [4] developed a multiplexed detection of these 3 markers with plasmon-enhanced Raman spectro-immunoassay that provide higher sensitivity than conventional methods.

Breast cancer susceptibility antige 1 (BRCA1) mean serum value was 5 times higher in women with breast cancer than in control, while BRCA2 was 3 times higher than in controls. OR calculation using international standard and this study control confirmed the association between breast cancer and increased serum levels for both BRCA1 and BRCA2. In addition, AUC of ROC curve was significantly high for BRCA1 (0.969) and BRCA2 (0.971). Furthermore, the frequency of serum positivity was 89% for BRCA1 and 88% for BRCA2 in women with breast cancer. Both antigen high serum levels in breast cancer than in controls indicating the possibility that these two antigens may be potential markers for diagnosis and monitoring of breast cancer. Unfortunately, in literature no report on detection of BRCA1 and BRCA2 antigen in serum in order to compare this study finding with them. Relative risk was significant for both BRCA1 and BRCA2 using international or this study control.

IV. CONCLUSION

In conclusion, the present study show evidence that serum CA15-3, CA27-29 and CEA simultaneous determination arepotintial markers for early diagnosis of breast cancer metastasis and treatment minitoring.

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