CA 27-29: A Valuable Marker for Breast Cancer Management in Correlation with CA 15-3

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Abstract

Background: Breast cancer is the most common invasive cancer in women, and second main cause of cancer death in women after lung cancer. Tumor markers that showed evidence of clinical utility and were recommended for use in practice include CA15-3, CA27-29 and Carcinoembryonic.

Aim of the study: This study was designated to measure the levels of CA15-3, CA27-29 and CEA and evaluate their clinical significance in metastasis and non- metastasis patients with breast carcinoma, comparing their sensitivity and specificity.

Patients, Materials and Methods: Serum levels of (CA27-29) and (CA15-3) were measured in 62 patients (40 in non-metastasis and 22 in metastasis patients). The age of patients was from (30 to 90) years old. Samples were collected from the Oncology Teaching Hospital in Baghdad Medical City.

Results: The levels of CA15-3, CEA were significantly higher in metastasis group compare to non-metastasis group. while the levels of CA27-29 were no statically significant difference between the two groups.

Conclusion: In this study, measurement of CA15-3 and CEA correlated with disease activity especially in the metastatic setting. on the contrary, CA27-29 did not show such clear correlation. CA15-3 was more sensitive in follow up of breast cancer patients followed by CEA and then CA27-29.

Key Words: *Breast cancer, Tumor marker, Cancer antigen27-29, Cancer antigen15-3*

Introduction

According to the WHO, cancer is the second-largest cause of death worldwide. Cancer in 2015 was responsible for 8.8 million deaths, and it used to be the reason of about 1 in 6 deaths worldwide. Cancer is the fourth-ranked cause of death within the Eastern Mediterranean region following infectious diseases, cardiovascular diseases, and injuries [(1)].

Tumor markers are quantifiable biochemical that are related with a malignancy. These markers are created either by tumor cell (tumor-derived) or with the body in response to cancer cell. They are ordinarily substances that are discharged into the circulation and hence measured in the blood [(2)]. Tumor markers are not the primary methods for diagnosing cancer, but can be used as a laboratory test to assist the diagnosis [(3)].

The tumor markers that demonstrated evidence of clinical efficacy and have been advised for use in practice for breast cancer include Cancer Antigen (CA15.3), (CA27.29) and Carcinoembryonic Antigen (CEA).

(CA15-3) is an epitope of a large transmembrane glycoprotein derived from the gene called MUC1. The protein MUC1, also identified as polymorphic epithelial mucin or epithelial membrane antigen, has a big extracellular region, transmembrane sequence, and

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a cytosolic domain. In breast cancer this protein can often be over-expressed and in its extracellular region is aberrantly glycosylated [4].

(CA27.29) is consider a carbohydrate contains a protein antigen which performs as a tumor marker for breast cancer. It is also named breast carcinoma associated antigen [5]. Cancer Antigen 27.29 has similar clinical performance to that of CA15.3 in breast cancer patients.

Carcinoembryonic antigen (CEA) is a cell surface glycoprotein with a high molecular weight (200 KDa) [6]. CEA is a family of associated cell surface glycoproteins; it may be the most commonly utilized tumor marker for lung, colorectal, gastrointestinal and breast cancer [7].

Estrogen is the most common sex hormone in menstruating women. It is made from cholesterol in the body. A significant proportion of breast cancers are positive estrogen receptors. This means that they have a larger number of estrogen receptors (ER), which indicate that estrogen helps feed their growth. Receptors are proteins in or on certain cells, which can attach to certain substances, such as hormones, that circulate in the blood. Normal breast cells and some cells of the breast cancer contain receptors that attach to estrogen (ER) and progesterone (PR). These two hormones often enhance breast cancer cell growth [8].

Patients, Materials and Methods

This study (cross section) was conducted at the Oncology Teaching Hospital/Medical City/ Baghdad from February 2020 to the end of April 2020, where 62 patients with breast carcinoma (40 in non-metastasis and 22 in metastasis patients). All of them female patients and were diagnosed by specialists, their age range between (32-90) years have been submitted to a cross-sectional study. Whole blood samples were taken and allowed to clot for two hours at room temperature using a serum separator tube. Serum was separated by centrifuge for 15 minutes at 1000 rpm.

Non hemolysed and clear sera were taken and divided into two aliquots in Eppendorf tubes. Hemolysed samples discarded. Samples were stored at (-80 C^{o}) for one month. Repeated freeze-thaw cycles were avoided.

Serum level of CA15-3was measured by the Access BR Monitor Assay technique. This assay is a paramagnetic particle, chemiluminescent immunoassay for the quantitative determination of CA15-3 antigen levels in human serum and plasma (heparin) using the Access Immunoassay System.

The serum concentration of CA27-29 was tested by using Enzyme Linked Immunosorbent Assay (ELISA) technique. This technique uses a quantitative immunoassay enzyme sandwich technique.

Serum level of CEA and E2 was measured by Minividas technique. The test principle contains a method of "2-step immunoassay sandwich enzyme" with an ultimate fluorescent detection (ELFA).

Statistical Analysis

Data were translated into computerized database, and used spss version 25 program to do the analysis. In order to test the statistical significance and the strength of association between the qualitative variable and quantitative variable ETA is calculating. P value less than 0.05 is consider to be statically significant result for both tests.

Results

Study conducted on 62 female patients in who are known to have a breast cancer, mean of age was (58.81±11.57) years, the youngest patient in the study was 30 years old and the oldest patient was 90 years old. Patient splitter into two group according to M staging system: metastatic group (21, 33.9%) mean of age in this group was (56.81±12.92) years and the non-metastatic group (41, 66.1%) mean of age in this group was (52.27±10.651) years as in table 1.

Table 1: subgroups of each variable, their frequency and percentage.

variable	Subtype	n. of patient (percentage)
Grade	I	3 (4.8 %)
	II	48 (77.4%)
	III	11 (17.7%)
Hormonal receptor	+ve	20 (32.3 %)
	-ve	42 (67.7%)
Her2 receptor gene changes	Negative	58 (93.5 %)
	Equivocal	4 (6.5%)
	Over expressed	0 (0.0%)
M stage	no metastasis	41 (66.1%)
	metastasis to bone	8 (12.9 %)
	to liver	4 (6.5 %)
	to brain	1 (1.6 %)
	Metastasis to other part	8(12.9%)
	To lung	0(0.0%)
T. stage	T0	1 (1.6 %)
	T1	8 (12.9 %)
	T2	43 (69.4 %)
	T3	3 (4.8 %)
	T4	7 (11.3 %)
N stage	N0	15 (24.2 %)
	N1	18 (29.0 %)
	N2	25 (40.3%)
	N3	4 (6.5%)
Age group	premenopausal	28 (45.2%)
	Post-menopausal	34 (54.8%)
Total number		62 (100 %)

M stage: Describe the metastatic state. T stage: Describe the size of the tumor.

N stage: Describe the spread to lymph node.

In this study four biomarker was measured CA15-3, CA27-29, CEA, and E2 in metastatic and non-metastasis group are shown in table 2.

The CA15-3 result shows a difference in the mean level of CA15-3 between the two groups which is significantly higher in the metastatic group compare to the non-metastatic group, which is statistically approved as p-value, is≤ 0.003.

The same for CEA mean level, which is higher in metastatic group compare to non-metastatic and it is statically approved, as p-value is ≤0.02.

The result of CA27-29 was different from other biomarker as there is no statically significant difference in mean level of CA27-29 between the two groups as p-value is ≥0.62.

To determine which one of the 3 test is more sensitive and more specific than the others in the metastatic and non-metastatic group, the result of each test for each patient split into positive or negative according to their cut off value of normal range CA15-3 NR < 31.3 U/ml , CA27-29 NR < 15U/ ml, CEA NR< 4.1ng/ml.

Table 2: level of each biomarker in both metastatic and non-metastatic group

		N	Mean ±S. D	Std. E	p-value
CA15-3	Non-met.	41	13.88±14.37	2.24	0.003
	Met.	21	93.74±164.96	35.99	
CA27.29	Non-met.	41	78.30±466.91	72.92	0.63
	Met.	21	28.20±48.42	10.57	
CEA	Non-met.	41	2.00±4.69	0.73	0.02
	Met.	21	18.08±43.89	9.58	

In table 3 the sensitivity, specificity, positive predictive value, negative predictive value and p-value for each marker is shown, CA15-3 is more sensitive and specific followed by CEA. But CA27-29 is less sensitive and specific.

Table 3: The Sensitivity, Specificity, positive predictive value, negative predictive value and p-value for each marker.

	SN	SP	PPV	NPV	p-value
CA15-3	92.9%	83.3%	61.9%	97.6%	0.000
CA27.29	75.0%	76%	42.9%	92.7%	0.002
CEA	91.7%	80.0%	52.4%	97.6%	0.000

To determine the relation between each biomarker and the grade, M stage, N stage and T stage Eta, Eta^2 was calculated in table 4

Table 4: Effect of M stage on the biomarkers.

Biomarker	Eta	Eta2	p-value
CA15-3	0.37	0.14	0.003
CA27-29	0.06	0.004	0.63
CEA	0.28	0.08	0.03

Relation between biomarker and N stage in metastatic and non-metastatic group shown in table 5. The Eta indicates a very weak relation between the biomarkers and N stage in metastatic and non-metastatic group.

Table 5: Effect of N stage on biomarker level in non-metastatic and metastatic group.

	Biomarker	Eta	Eta2	p-value
non metastatic	CA15-3	0.25	0.06	0.50
	CA27-29	0.21	0.04	0.65
	CEA	0.23	0.05	0.56
metastatic	CA15-3	0.16	0.03	0.92
	CA27-29	0.15	0.02	0.94
	CEA	0.19	0.04	0.88

Relation between biomarker and T stage in metastatic and non-metastatic group shown in table 6. The Eta indicates a very weak relation between the biomarkers and T stage in the two group.

Table 6: Effect of T stage on biomarker level in non-metastatic and metastatic group.

	Biomarker	Eta	Eta2	p-value
non metastatic	CA15-3	0.17	0.03	0.89
	CA27-29	0.10	0.01	0.98
	CEA	0.09	0.009	0.99
Metastatic	CA15-3	0.11	0.01	0.97
	CA27-29	0.17	0.03	0.92
	CEA	0.22	0.05	0.84

Discussion

Study (cross section) conducted on 62 patient who are known case of breast cancer, in table 1 patient data divided into groups.

Age range 30-90 years old, 45.2% of them are younger than 50 years and 54.8% are older than 50 this result agree with (Mohammed TM, *etal.*, 2018)[1], carried on Iraqi patient who found also that 45% were younger than 50, the other result will also be compare to

this study as it done on Iraqi patient in recent time, and TMN staging was taking in account. M stage: metastasis found in 33.9%, and no metastasis found in 66.1, which slightly similar to (Mohammed Tm, *etal*, 2018) [1] study has found that 24.1% has metastasis, this difference may be due to difference in sample size as the study conduct on 171 patients.

In this study three biomarker was measured, it found that CA15-3 has a higher mean level in metastatic group compare to non-metastatic group in table 2.

This result is agreed with (Farzaneh Agha-Hosseini, *etal*.,2009) [9] study in which was found that serum level of CA15-3 was higher in patient with breast cancer in the 2nd stage of disease.

And agree with (Kifah H. Alani, *etal*., 2018) [10] study in which found that serum level of CA15-3 was higher in metastatic breast cancer compare to non-metastatic group.

The CEA mean serum level is higher in metastatic group compare to non-metastatic group in table 2. This result agrees with the result found by (KA Pathak, *etal*,.1996) [11], in which CEA level was higher in patient with breast cancer and patient with benign breast disease.

The CA27-29 serum level was not statically significant difference between the two groups in table 2. Result could be related to small sample size or due to other causes as CA27-29 level increase or decrease in many different ways as its level may be not elevated in early-stage or absent or high levels of CA27.29 can indicate metastatic disease in many studies. Also, malignant and benign conditions beside breast cancer can cause increase in serum levels, as found in (Donepudi MS, *etal*., 2014) [12], and (Van Poznak C, *etal*., 2015) [13].

In the current study, found that CA15-3 as biomarker is most sensitive biomarker in metastatic group followed by CEA, then CA27-29, as shown in table 3. Positive predictive value of CA15-3 is (61.9%). The test is useful to detect the metastasis in breast cancer patient more than the two other biomarkers making them less useful than CA15-3.

In table 4, the relation of CA15-3 to M stage, and if the level of CA15-3 increases with M stage Eta which is measure effect of size indicate increase in CA15-3 level with increase M stage even that increase not strong, this agree with (Yijie Fu, *etal* ,2016) [15] who found increase in serum level as tumor progress and it's more useful to monitor advance tumor.

Relation between biomarker and T and N stage in table 5, and 6. Eta of CA15-3, CA27-29 and CEA indicate no relation between the biomarkers and the staging to T or N state. The result agrees with study done by (Franco Lumachi *etal*, 2004) [14], who found no relation between CEA and T and N stage, and no relation between CA15-3 with N stage but there is a relation with T stage.

A high proportion of breast cancers tent to express a greater number of estrogen receptor, as increase time exposure to estrogen is a risk factor to develop breast cancer.

Conclusion

Measurement of cancer antigen15-3 (CA15-3) and Carcinoembryonic (CEA) (to a lesser extent) correlated with disease activity especially in the metastatic setting. While (CA27-29) did not show such clear correlation. Also, the tumor marker (CA27-29) failed to show concordance results with Cancer antigen 15-3 (CA15-3) and Carcinoembryonic (CEA) in breast cancer patients.

The tumor marker Cancer antigen 15-3 (CA15-3) was more sensitive in follow up of breast cancer patients followed by Carcinoembryonic (CEA) and then Cancer antigen 27-29 (CA27-29).

Also, there was no correlation between (CA15-3, CA27-29, and CEA) and the staging system (TNM) and there was no correlation between the tumor markers and the levels of estrogen.

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