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Assessment of CA 15-3 and P53 biomarkers in diagnosis of breast cancer

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ABSTRACT— Breast cancer represents a heterogeneous categorization of diseases which is on the increase in Basra/Iraq. Cancer antigen 15-3 (CA15-3) is frequently used tumor biomarker (TM) in metastatic breast cancer but may not be elevated. Conventional, diagnosis of breast cancer is achieved by clinical examination and histopathological confirmation. Therefore this study aim to estimate the role of CA -15-3 in diagnosis and confirming the diagnosis breast cancer. Present study included (51) women diagnosed with breast cancer. In study population age ranged from (30-70 years) old. The control group has consisted of (32) healthy women. Enzyme Linked Immunosorbent Assay (ELISA) was once used to measure Serum CA 15-3, p53. The study referred a raised amount of (CA15-3) in breast cancer patients (82.14±66.22) in comparison to healthy control group (8.72±2.06). Conversely, the data of this study were illustrated opposite result that there is a significance decrease P53 level in serum (P<0.01) in BC patients in comparison to healthy group. The current study appeared that there is a high significant increase (P value < 0.001) in CA 15- 3 levels in BC patients at stage III in comparison to coming primary stages; while P53 has decreased in the progressive stages. Regarding BC of patients. While there was no significant difference in the levels of biomarkers despite the progression of the grads of the disease. That there is an increase in the biomarker (CA15.3) in women with breast cancer, while there is a decrease in P53 in breast cancer patients, may be helpful as prognosis in diagnosis of breast cancer patients.

KEYWORDS: Breast cancer, CA15-3, P53, Grads, stages

1. INTRODUCTION

Breast cancer is the most common malignancy in women and major causes of cancer-related death, It represent about 24.2 percent of newly diagnosed cancer cases and 15.0 percent of cancer-related deaths in women globally [7], it's a heterogeneous disease with many different clinical, histological, and molecular manifestations, Unfortunately, no diagnostic or screening test is now available for early diagnosis of breast cancer, other from conclusive diagnosis by getting biopsy and histopathology [19]. Breast cancer usually does not cause any symptoms, In early stages, so usually this disease detected at an advanced stage with poor treatment effect and poor prognosis, so determined of non invasive manner to enable early diagnosis of breast cancer are needed for bettering clinical outcomes and lowering death rate [33]. At the time of clinical diagnosis Breast cancer was developed to high mass, and become very difficult to treat, so biomarkers needed to predict, screen and identify treatment as early as possible, biomarker can be defined as a "biochemical, cellular, molecular alterations, biological characteristics or that can be objectively evaluated and measured as an signal of normal biological and pathogenic processes or pharmacological responses to a therapeutic intervention" [24]. On their surfaces, tumor cells have protein molecules, which called tumor-associated antigens, their rate in cancer cells, higher than normal tissues, serum, body fluids or urine of cancer patients, so, the increase in these biomarkers could be assist in early detection, prognosis determine,

after a course of therapy, expect the response or resistance to specific treatments and monitoring following primary surgery [1].

Blood biomarkers could be proteins in the plasma / serum, circulating MicroRNAs (miRNAs), DNA, circulating tumor cells, metabolites etc., CA15-3, carcinoembryonic and Antigen 27-29 antigen consider a classical soluble biomarkers in breast cancer, CA 15-3 is a one of the mucin-like membrane protein family, the members of MUC1 family are big glycosylated molecules and their physiological functions are indefinite but they have been participated in metastasis and cell adhesion [14].

CA 15-3, which is (MUC-1) protein soluble forms and often used as serum biomarker in breast cancer patients, its essential use for observing treatment in metastatic disease patients [13]. Its a great molecular mass mucin like glycoprotein, expressed on the luminal surface of majority secretary epithelia and participated with mammary cancers [24]. it is used for predicting prognosis after treatment in primary breast cancer, it with alkaline phosphatase predicts breast cancer metastasis and recurrence [23].

It is a glycoprotein produced by cells of breast cancer and can be detected by reactivity with monoclonal antibodies, the level of (CA15-3) is little increased in early stage of breast cancer at or localized cancer, the great of patients with metastatic breast cancer have displayed increase of (CA15-3) serum levels [10].

Decrease in CA 15-3 level to 50% refer to inhibition of tumor growth [14]. Level of (CA15.3) is relate to age, menarche age, menopause, and parity among healthy, benign and females with Breast cancer groups. Increased level of (CA 15.3) was showed in breast cancer and mainly dependent on high: stages and grades of tumor in females within breast cancer [3].

However, (p.53) was the initial tumor inhibitor gene to be identified. Its roles to inhibit and eliminate abnormal cells propagations, thereby suppressing neoplastic development. Inhibition of the negative growth regulatory functions of (p53) is happening in many, may be all, tumors [17]. Nevertheless, numerous investigations have revealed coding mutations in (p53) in breast cancer and this is now detected as a common, but by no means ubiquitous, somatic genetic alter in breast cancer [31].

Therefore our study carry out to reveal the impact of (CA 15-3) in early clinical diagnosis of breast cancer in comparative of histopathological biopsy.

2. Subject, material and methods

This study included (51) women diagnosed with breast cancer. In study population age ranged from (30-70 years) old. The study also included (32) healthy women as control. Who visited Al-Basrah Oncology Center in Al-Sadr Teaching Hospital in Basra. All patients in this work were confirmed diagnosed by histopathologist in Al-Basrah Oncology Center and emphasized by all clinical and laboratory seeking.

2.1 Sample collection

Four ml of human blood were obtained from each subject (patients and controls). 2 ml were transferred to sterilized test tubes and leave it to coagulate at room temperature for 30 minute, the sample was separated by centrifugation at 3000 rpm for 15 minutes and the serum was then isolated and deposited at-20 (0C) until analyzed. Enzyme-Linked Immunosorbent Assay (ELISA) was used to measure Serum CA 15-3 and p53. Number of patients have been exclude from the study who's affected by diabetes, kidney and heart diseases, were excluded from the study.

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Informed approval from the patients and ethical approval of the Teaching hospital had been obtained prior to assay.

2.2 Statistical Analysis

Data are stated as (means ± SD). Variations between groups were tested by Mann Whitney U Test (for means), chi-square test (for frequencies). All statistical analyses were performed using SPSS for Windows (version 23, USA). A value of $P < 0.05$ was considered statistically significant and $P > 0.05$ non-significant.

3. Results

As show in table (1) is about statistical distribution (frequency and percentage) of study groups according to their subgroups age were appeared that the highest percentage of the age subgroup is (40-49) years which constituted (54.90%) for patients group, and (25.49%) for control group.

Table1: shows the frequencies and percentage of breast cancer group and control according of age sub group.

Age subgroups	**patients(BC) (N=51)**		**Health(control) (N=32)**		**p- value**
	N	**%**	**N**	**%**	
30-39	12	23.52	10	19.60	1.62 (o.42) NS
40- 49	28	54.90	13	25.49	
50-59	9	17.64	8	15.68	
≥60	2	3.92	1	1.96	

NS; Not significance

Regarding the measurements of biomarkers in this study relates to subgroups age of breast cancer patients, as shown in table (2) that there is no significant difference ($P > 0.05$) in the levels of both biomarkers(CA15.3) and (P53) among age of all subgroups in breast cancer patients group

Table 2: shows differences in the measurement of biomarker among age subgroups of Breast cancer, value were expressed as mean ± SD.

Age sub group	**Biomarkers**	
	CA15-3((JU/ml) **Mean ±SD**	**P53(**U/ml**) Mean ±SD**
30-39(N=6)	293.74±142.81	244.62±231.73
40- 49(N=14)	301.82±291.72	124.18± 130.67
50-59(N=20	246.82±198.62	132.51±137.81
≥60(N=11)	126.91±47.69	236.16±132.58
Sig	0.41	0.54
p-value	NS	NS

NS; Not significance

However, the data for this study showed regarding differences in the measurement of serum biomarkers (CA15-3) and (P53) of BC patients and control group. Where shows that there is strong significance

increase in the serum level of (CA15-3), (P<0.001) in BC patients in comparison to healthy group.

Conversely, the results was illustrated that there is significance decrease in the serum level of (P53) (P<0.01) in BC patients in comparison to healthy group Table (3)

Table 3: show differences in the measurement s of serum biomarker between Breast cancer and control group, value were expressed Mean ± SD

Biomarker	**Health group (N=32)**	**patients(BC) group (N=18)**	**P-Value**
	Mean ±SD	**Mean ±SD**	
CA-15-3 (JU/ml)	8.72±2.06	82.14±66.22	0.001
P53(U/ml**)**	282.62±110.41	176.09±98.93	0.01

With regard to the grade and staging of breast tumor, the results were revealed that there is clearance difference in the measurement of biomarkers between BC patient groups categorized according to grade of breast cancer except it was appeared no significant difference in the levels of the both biomarkers (CA15.3) and (P53) as shown in table 4. On the other hand, as shown in same table (4), there is high significance in level of (CA15-3) biomarker among all degrees of stages of BC patients. Also the result of current study was revealed that there is a high significant deference (P < 0.01) in the level of P53 among all degrees of BC stages.

Therefore the data of this study proposes that higher levels of (CA 15.3) might be a dependable prediction biomarker as they were directly associated with progress stages and repetition.

Table 4: Variation in the measurement of biomarkers among patients subgroup according to grade and stage of Breast cancer, expressed values as mean ± SD

Biomarkers	**Grades subgroups**			**P-Value**	**Stage subgroups**				**P-Value**
	Grade 1	**Grade II**	**Grade III**		**StageI**	**Stage II**	**Stage III**	**Stage IV**	
CA15-3 (JU/ml)	82.62±90.71	112.42±1 1067	124.14± 121.16	0.42 NS	94.02± 101.16	66.42± 62.51	126.81 ±33.61	82.81 ±46.49	0.001
P35(U/ml)	234.62±182.21	297.81±2 56.93	189.74± 121.42	0.12 NS	421.32 ±276.2 6	291.81 ± 147.94	301.51 ±206.4 1	188.71± 122.09	0.01

4. Discussion

Breast cancer, the most frequent cancer in women, is still remains the secondary cause of cancer death among females worldwide [28], biomarkers are biomolecules that serve as indicators of disease processes or as a source of clinical data on pharmacological reactions to drug treatment [22]. Breast cancer can be detected early, which allows for less aggressive therapy and improved patient survival., In a variety of epithelial malignancies, there is overexpressed in the transmembrane glycoprotein mucin(CA15-3) which has great role in the disease progression [2]. The tumor suppressor (p53) has a well-established involvement in cancer., in particular, In response to DNA damage, it has been demonstrated to trigger apoptosis and cell-cycle arrest. It is the most frequently silenced or mutated genes in cancer, so targeting of p53 may therefore be a novel strategy for the estrogen-dependent breast cancer treatments [29].

Regarding the measurements of biomarkers in this study relates to subgroups age of breast cancer patients that there is no significant difference ($P > 0.05$) in the levels of both biomarkers(CA15.3) and (P53) in BC patients compared to all ages subgroups this result agree with [15] showed that (CA15.3) is a well known tumor marker a, assuming that the increase levels of this marker can be has great risk, [30] demonstrated that the expression of(p53) was not age- related, the present study showed that there is strong significance increase in the serum level of (CA15-3) ($P<0.001$) in BC patients in comparison to healthy group. Conversely, the results was illustrated that there is significance decrease in the serum level of (P53) ($P<0.01$) in BC patients in comparison to healthy group.

There was no difference in (CA15-3) serum level among patients in age group, it has been submit that (CA15.3) is a contributory parameter in evaluate metastasis occurrence, (CA15.3) might be a caution sign for metastases of bone in implicated patients through the therapy process [15].

Increase serum level of (CA15-3) was significantly noted among breast cancer patients compared to benign females and both healthy, but no significant association was detected between age of cancer and serum level (CA15-3) [4].

[5] refered to that the rate of (p53) antibodies in lower than 17% of breast cancer patients.

Moreover, in stage associated analyses prognosis of patients with increased serum(CA15-3) was worse than those with normal values, another studies reveals that CA 15-3 levels were serially monitored postoperatively through Follow-up care may be beneficial in the early diagnosis of recurring breast cancer [25], [26], [11].

Expression level of p53 in normal cells, is in minutes and has a very short half-life by proteasome degradation and virtue of ubiquitylation [8].

With regard to the grade and staging of breast tumor, the results were showed that there is clearance differences in the measurement of biomarkers between BC patient groups categorized according to grade of breast cancer except it was appeared no significant difference in the levels of the both biomarkers (CA15.3) and (P53). On the other hand, as shown in same table (4), there is high significance in level of (CA15.3) biomarker among all degrees of stages of BC patients. Also the result of current study was revealed that there is a high significant deference ($P < 0.01$) in the level of P53 among all degrees of BC stages. This result agree with [18] refer to there are relation of (CA 15.3) levels with clinicopathological parameters in breast cancer metastatic, [16] reported that significantly elevated of serum levels of CA15-3 in patients with malignant breast cancer, but there's not in those with benign tumors, In addition, found that CA15-3 level is greatly linked with tumor stages, and [20] indicate an increased level of CA15-3 in breast cancer patient associated to advanced stages.

However, once therapy failure happens the quality of life and Patients' survival rates are greatly significant., so, it is important to detect accurate prognostic factors to guide decision making through the breast cancer treatments of to improve prognosis, relation to the conventional pathological factors such as tumor grade, tumor size, lymph node status, molecular biomarkers such as human epidermal growth factor receptor 2 (HER2)and hormone receptor status expression [27], so serum tumor markers have an great roles in screening, ,treatment of many malignancies and early diagnosis of recurrence [21], [9].

The early diagnosis of breast cancer help in use of decrease aggressive treatment and raise patient survival.

The (CA15-3), is aberrantly glycosylated and high expressed in different epithelial of cancers, and act a vital role in the progression of the disease.

[12] demonstrated that (p53) positive was related to the progression of carcinoma according to a advance histological grade, so it might be a predictive sign for breast cancer, while [6] showed no significant difference have been showed between the expression of (p53) antibodies and the tumor grade.

High (p53) expression was related with high TNM stage, so (p53) was associated with tumor aggressiveness, However, [30] refer to that (p53) plays a great role in suppression of invasion, migration, and metastasis of cancer cells [32].

In conclusion; the current study clarifies that promote precise estimate of the clinical utility of two significant biomarkers, CA15-3 and P53, may be helpful in breast cancer surveillance. Therefore, our study will highlights on the warning of breast cancer patients.

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