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The role of VEGF levels in the differentiation between malignant and benign breast tumor

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ABSTRACT: Globally, more than 1,500,000 women are diagnosed with breast cancer annually which is considered as a big health challenge with the highest priority for investigation. Vascular endothelial growth factor (VEGF) is considered as promising tumor markers due to its role in the progression of cancer. To determine the role of VEGF in the differentiation between the females with breast cancer and those with benign tumor in a sample of Iraqi Females from Baghdad/Iraq. A comparison study was done on 60 female patients with breast cancer and 60 female patients with benign breast tumor who were recruited from Al Imamain Al-Kadhemain Medical City, Baghdad, Iraq between May 2022 and December 2022. Samples were collected from subjects and used to determine the levels of VEGF, carcinoembryonic antigen (CEA) and Cancer antigen 15-3 (CA 15-3) and compare their levels in both studied groups. There were highly significant increases in VEGF levels in patients with malignant breast tumor in comparison with patients with benign breast tumor. VEGF levels showed to be significantly correlated with CA 15-3 levels and provide better sensitivity and specificity when used in combination with CEA and CA 15-3. VEGF showed to be more significant biomarkers in differentiation between benign and malignant tumor with higher sensitivity and specificity when compared with classical breast tumor markers (CEA and CA15-3) and the combination between these markers showed to be a promised diagnostic panel for the differentiating of benign breast tumor from malignant one.

KEYWORDS: Benign breast tumor; breast cancer; cancer antigen 15-3; carcinoembryonic antigen; vascular endothelial growth factor.

1. INTRODUCTION

Breast cancer is a malignant tumor arising from epithelial cells of glandular lacteferons ducts or terminal ductilobular unit (TDLM) of the breast. Breast carcinoma is classified as either non-invasive (carcinoma *in situ*) or invasive, depending on whether or not the tumor has started to grow outside the basement membrane [1,2] It is considered as one of the most common women cancers in globally, accounting for about 570,000 deaths in 2015. More than 1,500,000 women (25% of all women with cancer) are diagnosed with breast cancer annually throughout the World [3,4]. Breast cancer is a metastatic cancer and can commonly transfer to distant organs such as the bone, liver, lung and brain, which mainly accounts for its incurability. Early diagnosis of the disease can lead to a good prognosis and a high survival rate [5].

The currently used serological breast cancer markers include (CA 15-3) and (CEA), their levels in serum are related to tumor size and nodal involvement and are recommended for monitoring patients with metastatic disease during active therapy. Recently, VEGF are considered as promising tumor markers, they may have roles implicated in the progression of cancer. Angiogenesis is a vital step in the development of cancer and is necessary for primary tumor growth, invasiveness, and metastases. Overexpression of VEGF was found in several tumor tissues [6,7]. Breast cancer is involving lymph angiogenesis, which is the recruitment of blood and lymphatic vessels, to a growing tumor [7,8]. Large number of evidence from in vitro and in vivo experiments has shown that increased VEGF expression is associated with tumor growth and metastasis [7,9].

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2. RESULTS

Age and BMI of the patients subjected to the study were summarized in table 1. Table 1 showed non-significant differences in age and body mass index (BMI) among all studied groups.

Table 1. Age and BMI of the patients with malignant in comparison with benign tumor.

	Benign breast tumor	Malignant breast tumor	P[b]
n	60	60	
Age (year)	37.53±5.46	36.75±5.88	0.971
BMI (Kg/cm^3)	26.4±3.71	27.07±3.62	0.389

Results illustrated in Table 2 revealed that there were non-significant differences in the levels of CA15-3 and CEA between patients with benign and malignant breast tumor (p=0.286, p=0.704; respectively) whereas VEGF levels showed significant increases in malignant breast tumor patients in comparison with benign tumor patients (p=0.018).

Table 2. CA15-3, CEA and VEGF levels in patients with benign and malignant breast tumor.

	Group	mean±SD	P[c]
CA 15-3 (U/ml)	Benign tumor (n=60)	25.9±6.49	0.286
	Malignant Tumor (n=60)	27.93±	
CEA (ng/ml)	Benign tumor (n=60)	1.56±0.42	0.704
	Malignant Tumor (n=60)	1.62±0.4	
VEGF	Benign tumor (n=60)	219.91±145.37	0.018
	Malignant Tumor (n=60)	271.83±97.38	

Results presented in Table 3 clarified that the correlations between the studied biochemical parameters in patients with benign tumor were non-significant with p>0.05.

Table 3. Correlations between the levels of all studied biochemical parameters among benign patient.

		CA153	S. VEGF	Age	BMI
CEA	**r**	0.058	0.114	-0.033	-0.227
	p	0.659	0.385	0.801	0.082
CA153	**r**		0.223	0.029	-0.186
	p		0.086	0.827	0.154
S. VEGF	**r**			-0.215	0.178
	p			0.100	0.173
Age	**r**				-0.077
	p				0.559

The correlations among patients with malignant tumor that demonstrated in Table 4 showed that there were significant positive correlations between CA 15-3 with both of CEA (r=0.305; p= 0.018) and VEGF (r=0.28; 0.03) and also a positive significant correlation between VEGF and the BMI of the patients. On the other hand, VEGF levels were negatively and significantly correlated with the age of patients subjected to the current study.

Table 4. Correlations between the levels of all studied biochemical parameters among malignant patient

		CA153	S. VEGF	Age	BMI
CEA	**r**	0.305*	0.228	-0.047	-0.138
	p	0.018	0.080	0.723	0.294
CA153	**r**		0.280*	-0.246	0.072
	p		0.030	0.058	0.583
S. VEGF	**r**			-0.291*	0.284*
	p			0.024	0.028
Age	**r**				-0.115
	p				0.381

Table 5. ROC curve results of all studied markers between malignant and benign group

Parameters	AUC	Sensitivity (%)	Specificity (%)	Cut-Off value
CEA	0.553	46.7	60	1.63
CA 15-3	0.566	56.7	63.3	26.79
VEGF	0.712	71.7	65	214.21
Combined CEA, CA 15-3 and VEGF	0.705	70.8	60	-

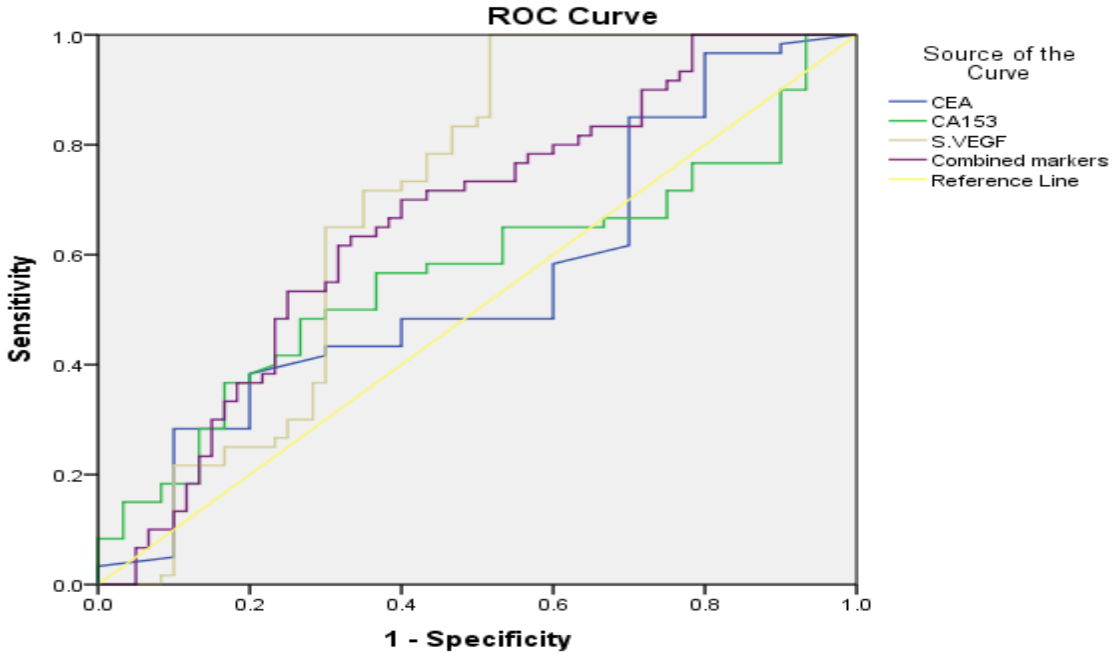

Figure 1. ROC curve of all studied markers between malignant and benign group

3. DISCUSSION

Current work was aimed to assess the levels of a new marker that can be used in combination with other classical markers for the differentiation between the benign and malignant tumor. For that reason, the patients with benign tumor included in this study were chosen to be non-significantly differ from patients who suffered from a breast cancer in their age and body mass index (BMI) to exclude any effect of these variables on the levels of vascular endothelial growth factor (VEGF) that studied profoundly in this research for its possible role in progression of cancer and its promising role as a markere for the differentiation of cancer from non-cancerous tumor.

The basis of choosing age and BMI matched groups originated from the previous study which reported that the expression of vascular growth factors is attenuated in elderly persons. Production of vascular

endothelial growth factor (VEGF), which is one of the key regulators of physiological and pathological angiogenesis is decreased in the elderly at both basal levels and in response to tissue injury [10]. Additionally, Olsen et al., also reported that the amount of VEGF protein in skeletal muscle cells and in media surrounding muscle cells tended to be lower in cultures derived from the aged compared to the young women which provide evidence that aged women have a reduced angiogenic potential due to impaired proliferative capacity of endothelial cells and a lower availability and release of VEGF protein, with potential implications for the training induced angiogenic response [11].

On the other hand, BMI showed to affect the levels of VEGF significantly as reported by Zaki and his colleagues who demonstrated that obese women showed significantly higher levels of serum VEGF as compared with the non-obese group [12]. So, collectively, patients with benign tumor included in this study were chosen to be comparable to patients with breast cancer in age and BMI to avoid an effect of these variables on VEGF levels.

The results from table 2 showed that there were non-significant differences in CA 15-3 levels ($p>0.005$) between patients undoubtedly have breast cancer and those proven to have benign breast disease. This finding was typically supported by Chukwurah et al., 2018, which say there is about (30%-60%) of patients with benign breast tumor presented with increased concentration of pre-treatment CA 15-3 that give mixed feelings on the diagnostic utility of this marker [13].

Furthermore, the data presented in this study support the fact that CA 15-3 cannot be used in a differentiation between patients with malignant and benign tumors as it showed a non-significant elevation in early diagnosed cancerous patients in comparison with benign tumor patients which is in agreement with results obtained in a meta-analysis study conducted by Fu and li who reported that there was no difference in CA15-3 expression between benign tumors and patients with stage I and II malignant breast tumor and the only significant differences were obtained at stage III and IV in comparison with healthy subjects and patients with benign breast tumor [14]. So, in an agreement with other studies, CA 15-3 is a tumor marker for many types of cancer, most notably breast cancer that used widely for several years. It do not play great role in screening for primary breast cancer, but it more useful in follow-up care as reported previously in several studies [15,16].

Zaleski et al., 2018 also found that tumor marker CA 15-3 was significantly higher in serum of breast cancer patients as compared with healthy women, however, not as compared to patients with benign breast diseases. This study also reviewed that cancer antigen 15-3 (CA15-3) is only valuable in late stages of breast cancer and support therapy response assessment and early detection of recurrent disease. In early stages, their sensitivity is limited [17].

Carcinoembryonic antigen (CEA) levels showed that their levels showed a non-significant difference between benign and malignant group as showed in table2 which revealed that this marker cannot be used in the differentiation between malignant and benign tumors. As reported previously, the serum tumor marker carcinoembryonic antigen (CEA) plays a significant role in the diagnosis and follow-up of colorectal cancer [18]. The previous literatures stated that CA15-3 and CEA are two of the most widely utilized serum tumor indicators for breast cancer. Elevated levels of serum CEA are frequently observed in patients with metastatic and recurrent breast cancer [19] while the results obtained in the current work differ from previously reported articles in that the patients subjected to the study were early diagnosed and literatures regarding this stage of cancer reveled that both markers cannot be used in the deferential diagnosis due to their low sensitivity and specificity [20] which is also agreed with results obtained by Yang et al. who found that elevated serum CEA levels are particularly noted in metastatic and recurrent disease [21] and Wang et al. who demonstrated that serum CEA is less widely investigated as a prognostic factor than CA15-3 because of its poor sensitivity and specificity [22].

Results obtained in this study (table 4) revealed that CEA and CA15-3 were significantly correlated with each other in patients with malignant tumor which is consistent with previous research which demonstrated that the levels of these two markers elevated in parallel with each other and also in parallel with the stage of tumor and clinicopathological parameters [23]. These findings caused by the role of these two markers in the progression and development of cancer given that the possible explanation of the high levels of CA15-3 in patients with breast cancer owned to the site of expression and function. CA 15-3 is a high molecular weight glycoprotein (300-450 kDa) that synthesized by apical surface of epithelial ducts and acinic breast cells and is then secreted in milk normally. In cancerous statue, CA15-3 drains into the blood perfusion because of disrupted breast morphology [15,24] while the possible link between the levels of CEA and the late stages of cancer emerged from its close association with various functions of endothelial cells, including

adhesion, proliferation, and migration of cells both in vivo and in vitro [25] which may explain why these markers lack of sensitivity as it increased widely and appeared obviously in the circulation in late stages when the cancer metastasized, which need CEA in cell migration and disrupt breast morphology that causing the release of CA 15-3 in the circulation.

Ultimately, CEA and CA 15-3 showed to have a nearly similar pattern of increase in malignant and benign breast tumor with a superiority for CA 15-3 which showed a higher increase in breast cancer patients than that of CEA but both of them considered as a non-significant markers and cannot be used either alone or in combination in the early diagnosis of breast cancer and their levels need to be combined with more sensitive and specific markers to establish a new diagnostic panel which improve the sensitivity and specificity to provide a powerful tool for the diagnosis of breast tumor and also for the deferential diagnosis between benign and malignant breast tumor.

The Receiver Operating Characteristic (ROC) analysis results in tables 5 showed that CA 15-3 had low sensitivity and specificity in differentiate breast cancer from other benign or non-breast diseases. Furthermore, the low sensitivity and specificity obtained by ROC curve results in this study were in agreement with many previous studies such as Kabel, 2017 review who stated that "Because CEA lacks disease sensitivity and specificity, it cannot be used for screening the general asymptomatic population" he also stated that CA15-3 has low sensitivity [26] CEA and CA15-3 levels showed to be non-useful parameters for differentiation between malignant and benign breast tumor patients as they showed non-significant differences (table 2) beside the low sensitivity and specificity of these two parameters between these two subgroups (table 5). The possible explanation of poor deferential ability of CA15-3 may be due to the fact that it can be raised in benign breast tumor as well as malignant which make it difficult to be used as a deferential tool as mentioned previously in many studies (14, 26-28).

Ultimately, the results obtained by ROC analysis can be considered as a confirmation for the results obtained by t-test and the possible cause of the low sensitivity owned to the overexpression of these markers in late stages whereas the possible reason for the lack of specificity is that the expression of these markers in several body organs and tissues as discussed above.

Results reported in this study showed that the levels of VEGF showed a significant elevation in cancerous patient which is nearly comparable to that of CA15-3 with a superiority in that it showed a significant difference between malignant and benign patients which may provide a promising result that may allow a differentiation between benign and cancerous tumor given that the metastasis and cancer progression depend on the formation of new vessels which is not occur in benign tumor that may explain the non-significant increase in VEGF levels in female with benign mass in which the pathogenesis of this tumor doesn't depend on the angiogenesis primarily. These findings are in a consistency with previous studies which reported that the levels of VEGF were increased in cancerous patients due to its role in angiogenesis [29] which is considered as a one of the hallmark features of cancer [30]. It is a complex and coordinated process regulated by different growth factors like platelet derived growth factor, transforming growth factor and angiopoietins among which vascular endothelial growth factors (VEGF) play a crucial role [31]. VEGF is one of the most powerful endothelial cell mitogen and has a very critical role in normal physiological and tumor angiogenesis. It enhances tumor vessel permeability and endothelial cell proliferation, migration, differentiation, capillary formation and also has pro-inflammatory actions [32].

The results of the current work are in agreement with recently published research which demonstrated that the VEGF levels showed a significant increase in patient with malignant tumor in comparison with patients with benign breast tumor which may provide a powerful tool for deferential diagnosis of breast cancer beside the other classical tumor markers such as CA 15-3 [33]. Recently, several studies focused on the levels of VEGF and its expression in cancer in an attempt to discover a powerful treatment for cancers in that it halts the expression of VEGF which may cause prevention and hindered cancer progression [34,36].

All the discussed literatures prove the results of the present study which demonstrated that the levels of VEGF can be considered as more reliable tumor marker than the classical tumor markers (CEA and Ca 15-3) and confirm the possibility of using it in a combination with them to improve the diagnostic and also the prognostic value as it increased earlier to the classical markers with a more obvious increment that can be noticed clearly in the highly significant difference between patients with malignant and benign breast tumor.

Levels of VEGF showed to be affected by the age and body mass index as it appears clearly in the significant directly proportional correlation (positive correlation) with BMI ($r= 0.284$, $p= 0.028$) and the significant inversely proportional correlation (negative correlation) with age ($r=-0.291$, $p= 0.024$) in patients

with breast cancer (table 4) and for that reason, the subjects of both studied groups were selected to be age, gender and BMI matched. These findings agreed with previous studies which stated that VEGF mRNA and protein levels are lower in aged individuals at baseline, after exercise, and after ischemia in comparison to younger control subjects [37] and also recent study which found that VEGF released from myocytes were lower in elderly women in comparison with young ones [11]. Furthermore, high levels of VEGF showed to be associated with the high visceral fat index in obese women [12].

We can conclude from these results that women with high BMI may experience an increase in the levels of VEGF which in turn may affect the risk of cancer progression and metastasis. In addition to that, the obesity may be considered as risk factor for the breast cancer as it causes an elevation in the levels of VEGF and these conclusions need more profound investigations to elucidate the exact role of obesity in the tumorigenesis.

It was demonstrated that the levels of VEGF in cancerous patient correlated directly with the levels of CA 15-3 in that they increased in parallel to each other which prove the concept of using these two markers in a combination as new diagnostic panel for differentiating benign breast tumor from breast cancer [33,38].

ROC analysis results showed that VEGF alone can be used in differentiating between benign and malignant tumor as it provides values of AUC, sensitivity and specificity of 0.712, 71.2% and 65%; respectively that need more profound studies with larger sample size to elucidate the exact role of VEGF in the deferential diagnosis of breast tumor.

4. CONCLUSIONS

The levels of CEA showed to be of a lowest sensitivity and specificity among studied markers in differentiating cancer from benign tumors whereas CA15-3 showed to have slightly higher sensitivity and specificity than those of CEA whereas VEGF showed to be more significant biomarkers in diagnosis and differentiation between benign and malignant tumor with higher sensitivity and specificity when compared with classical breast tumor markers (CEA and CA15-3).

5.MATERIAL AND METHODS

5.1. Study Protocol

The study was done on 60 female patients with breast cancer and 60 female patients with benign breast tumor who were recruited from Al Imamain Al-Kadhemain Medical City, Baghdad, Iraq between May 2022 and December 2022. Ages of the malignant group ranged between 29 and 48 years (mean± SD 36.75±5.88 years) and benign group's ages were ranged between 29 and 46 years (mean± SD 37.53±5.46 years). The practical part of the study was conducted at Department of Chemistry and Biochemistry, College of Medicine, Al-Nahrain University, Baghdad, Iraq.

In the current research women were eligible for this study if they had a suspicious breast lesion (early diagnosed) which was recorded by clinical breast examination and/or imaging technology. Patients were subjected to physical breast examination (PBE), mammography and approved by histopathologist.

5.2. Exclusion criteria:

1. Subjects that had a history of any acute or chronic diseases (eg. Acute and chronic hepatitis.
2. Subjects that had a history of any type of cancer (eg. Colorectal and endometrium cancer).
3. Patients received hormonal treatment or chemotherapy.
4. Pregnant women.

The study has approved by the Institutional Review Board (IRB) of the College of Medicine, University of Al-Nahrain, Baghdad, Iraq. In addition, an informed written consent for participation in the study was signed by investigated subjects according to the Helsinki principles.

All eligible studied patients were subjected to baseline evaluation of the following:

- Thorough clinical examinations in addition to full medical history
- CEA, CA15-3 and VEGF.

5.3. Sample collection and preparation

About 5 ml of blood samples were collected from patients and put into serum separating tube (SST) and left to clot for 30 min at room temperature then were centrifuged at 4000rpm (1252 x) g for 10 min, the separated sera were divided into small aliquots and stored at (-20^{o}C) until assayed for the evaluation of CEA,

CA15-3 and VEGF by ELISA technology according to manufacturer instructions. Kits were supplied by Cell Biolabs/ USA (Catalog Number. PRB- 5069) and Elabscience/China for CEA (Catalog Number. E-EL-H6047) and VEGF (Catalog No: E-EL-H0111).

5.4. Statistical analysis

The data of the study were analyzed using the SPSS software 20. Numeric variables were expressed as mean ± SE and all statistical comparisons were made by t-test with P ≤0.05 was considered statistically significant. The correlation was done between all parameters using Pearson correlation test with P ≤0.05 was considered statistically significant [39], Receiver Operating Characteristic (ROC) analysis was performed as a comprehensive way to assess the accuracy of the studied markers. The area under the curve (AUC) provides a useful tool to compare different biomarkers. Whereas an AUC value close to 1 indicates an excellent diagnostic and predictive marker, a curve that lies close to the diagonal (AUC = 0.5) has no diagnostic significance. AUC close to 1 is always accompanied by satisfactory values of specificity and sensitivity of the biomarker [40].

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