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Plasma Levels and Diagnostic Utility of VEGF and M-CSF in the Diagnosis of Iraqi Women with Breast Tumor: A Comparative Study with CA 15-3

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Abstract:
Background and objective: Breast cancer is the extreme widespread carcinoma in women and considered to be as the second major reason of demise in human being. Tumor markers are particularly helpful in controlling medicament also in identification of metastasis and early discovery of returning. The present research was designed to examine the plasma levels, diagnostic and differentiating utilities of vascular endothelial growth factor A (VEGF A) and macrophage-colony stimulating factor (M-CSF) in Iraqi women with breast cancer (BC), benign breast tumor women , well control women and to compare their results with that of routine used one, the CA 15-3. Subjects and Techniques:The present research was executed at the Department of Biochemistry, College of Medicine – University of Baghdad and at the Central Office for the Soon Disclosure of Breast cancer at Baghdad Teaching Hospital for Oncology/Medical City, over the interval of February 2017 until the end of November 2017. Eighty-eight Iraqi women were involved in the study; 38 women with primary breast cancer (BC), 25 women with benign breast tumor and 25 well control women. BC women were also classified in respect to their stage of cancer; stage I, II, and III. Seeking involved plasma measuring of VEGF A, M-CSF and CA 15-3 levels in whole enclosed women by using enzyme-linked immunosorbent assay (ELISA) technique. Results: The results indicate significant increase of VEGF A, M-CSF, and CA 15-3 in BC women in comparison to benign tumor and healthy women (p=0.001), with non-significant differences between benign tumor and healthy women. However, plasma VEGF A has the highest diagnostic utility (AUC=0.958) in differentiation between women with BC from those with benign tumor or healthy ones compared with M-CSF (AUC=0.662) and CA 15-3 (AUC=0.0.934). In addition, plasma VEGF A has the best ability (AUC=0.877) in differentiating early stage of BC from benign tumor or healthy women in comparison to CA 15-3 (AUC=0.807). Plasma M-CSF has the poor efficiency in such clinical application (AUC=0.68). Moreover, plasma VEGF A was suggested to be the accurate one in differentiating women with benign tumor from healthy control women.Conclusion:The present study found that plasma VEGF A has superior diagnostic utility of early BC and differentiating efficiency from benign tumor and healthy women of that of routine used one plasma CA-15-3. In addition, VEGF A was the best one in discriminating of benign tumor from control women. Plasma M-CSF was the poor one in such clinical uses. The replacement of plasma VEGF A instead of CA 15-3 in assessment of early BC and benign tumor is required and could be to reduce the biopsy.
Keywords: Tumour markers, VEGF, M-CSF, Breast cancer, Benign breast tumor.

1.Introduction

Breast cancer is a malignant tumor that starts in breast tissues and spread into remote areas encompassing many structures correlated with distinctive histological and biological characteristics. Cancer of breast is believed to be the utmost prevalent cancer in the female

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and considered to be the second in causing death in the universe [1]. It is related to the truth that tumor markers display low sensitivity (SE), and therefore, the tumor is not discovered previously. According to the raised numbering of patients, soon diagnosis is necessary, particularly in the primary periods of the cancer [2] . Tumor markers are particularly helpful in treating detection and monitoring, identification of metastasis and soon discovering of recurrence. CA 15-3 (recognized as MUC1) is the utmost excessively applied serum tumor marker in breast cancer follow-up [3, 4] and clearly glycosylated in cancer [5] . Nowadays, the major uses of CA 15-3 are in the pre-clinically detection of recurrence and treatment monitoring of patients with proceeding breast cancer [4, 5, 6]. Vascular endothelial growth factor (VEGF), primarily recognized as vascular permeability factor (VPF), which is a signal protein generated by cells that prompts blood vessels genesis. VEGF is secreted as a homodimeric disulfide-linked glycoprotein of 45 kDa [7]. VEGF is significant signaling proteins included in both vasculogenesis (the new formation of the embryonic circulatory system) and encouraging angiogenesis in several pathological situations, involving malignant conditions [8,9]. It enhances the progress of BC by stimulating angiogenesis and lymphangiogenesis [10, 11]. An elevated level of VEGF with its (mRNA) has been originated in breast tumor cells. In breast tumor, the VEGF (intratumoral) with the microvascular density significantly get together for reduced relapse-free survival [12]. Colony Stimulating Factor 1 (CSF1), as well recognized as macrophage colony-stimulating factor (M-CSF), Macrophage-colony stimulating factor (CSF1) is a growth factor manufactured by macrophages, endothelium, fibroblasts and CD3-activated T-cells [13]. M-CSF is a secreted cytokine that effects hematopoietic stem cells to distinguish into macrophages or other cell types [14]. It controls the growth and differentiation of hematopoietic progenitor cells and functionally stimulates the maturation of macrophages. M-CSF can as well activate the proliferation of non-hematopoietic cells in vitro, principally the cancer cells [15, 16]. Some studies showed that M-CSF mRNA is expressed in tumor cells [17]. Moreover, the stimulation of M-CSF receptors induces generation of new cancer cells [18].

2.Materials and Methods

The present work was carried out at the Department of Biochemical Studies, Medicine entirety of University of Baghdad and at the central office for the soon disclosure of breast cancer at the Oncology Teaching Hospital of the Medical City, through the interval from February 2017 until the end of November 2017. Eighty-eight Iraqi patients were included in this study; 38 patients with breast tumor, 25 patients with benign breast cancer who were diagnosed by Oncology group and 25 healthy control women. The diagnosis of breast tumor was based on the triplex estimation methods, i.e.; Mammography or Ultrasonography, fine needle aspiration cytology (FNAC), both with the clinical examination of breast. Women of this group were then subdivided into subgroups based on the stage of their cancer. Identification of the tumor class and stage was completed with respect to the standard from the International Union against Cancer Tumor-Node-Metastasis (IUAC-TNM) ranking with the American Committee on Cancer Staging. Histopathological study performed by histopathology consultants group established by taking biopsy from breast tumor or next to mastectomy. Determination of breast cancer stage was carried out using blood tests, ultrasonography, x- rays, physical screening tests, mammography and breast biopsy. In addition, radio isotopic bone scans; MRI and CT scanning of chest and brain were accomplished if essential to eliminate metastasis. Radio- and chemotherapy were excluded before blood sampling. Also, women of stage IV of breast cancer, ovarian, endometrial, cervical, colorectal-, head-, neck- and lung tumors, hepatic disease, chronic renal failure, diabetes mellitus, pancreatic-, were precluded. In addition, traumatic injuries especially of

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central nervous diseases, autoimmune diseases particularly rheumatoid arthritis, smoking and alcohol drinking were also excluded from this study. The diagnosis of benign tumor was based on clinical examination, ultrasonography or mammography with cytology using Fine Needle Aspiration Cytology (FNAC), and all included 25 women were found to have had fibroadenoma. Furthermore, 25 apparently healthy volunteer women aged (25-65) years who had no clinical features or history of malignant diseases, non-smokers and non-alcoholics were enclosed in the present work as control.

2.1. Biochemical analysis

Blood sample (5 mls) was withdrawn from each patient; 3 ml transported into heparin sodium tube, clotting took place for 15 minutes and then centrifugation at 2500 rpm was carried out to separate the plasma, which kept at - 40◦C till the timing of measurements of VEGF A and M-CSF. The reminder 2 ml of the aspirated blood sample was transferred into gel tube, then clotting was carried out for 15 minutes, then after centrifugation at 2500 rpm to obtain serum sample that used for determination of CA15-3. Plasma VEGF A, M-CSF and serum CA15-3 measurements were accomplished by applying the quantitative sandwich Enzyme Linked Immunoassay (ELISA) method in all patients. Plasma VEGF A quantitative sandwich enzyme immunoassay involved antibody specific for VEGF A has been pre-painted onto a microplate. Then, samples and standards are drawn into the wells then the immobilized antibody binds each VEGF A existing. Next, all the unbound materials are removed, after that a biotin- bound antibody particular for VEGF A is supplemented. Next to washing process, avidin conjugated Horseradish Peroxidase (HRP) is inserted into the wells. Then, the substrate solution is appended into the holes with the appearance of color proportionally to the bound VEGF in the first step. CUSABIO BIOTECH CO., LTD /CHINA provided this kit. The same quantitative method was used for measurement of plasma M-CSF. Kit for measurement of CA 15-3 was provided by HUMAN CO./GERMANY.

2.2. Statistical analysis

Statistical analysis was carried out using SPSS 20.0.0, p value of less than 0.05 was believed to be significant. Chi square test used to analyze the discrete variable (and if not proper, Fisher test was used) .Two samples t-test applied to analyze the variation in means between two groups, while one-way ANOVA test was applied when the difference was between more than two groups, next post HocTukey test will be applied to detect the significant pair . Linear regression test utilized to estimate the relation among various variables, Pearson regression used if one or both variables follow the normal distribution but if not, Spearman correlation shall be applied. Scatter plot was performed for the regression analysis. Receiver operator curve applied to recognize active cases from control cases.

3. Results

The demographic characteristics of patients and controls are illustrated in table 1. The mean (±SD) values of the age of BC women were (48.3 ± 9.2 years) comparable with the control women (42.4±9.6 year). However, women with benign tumor were found to be of age (32.3 ± 11.3 year) which is significantly lower than those of BC and controls (p=0.001, p= 0.002, respectively). The mean (±SD) values of BMI of women with BC (29.3 ± 4.1 Kg/m2) was significantly higher than that of control women (26.8 ± 4.1 Kg/m2, p=0.046) and benign tumor women (25.4 ± 3.9 Kg/m2, p=0.001). Table 1 also shows that number of patients with different stages of BC were comparable.Table 2 displays the mean (±SD) estimates of the studied biochemical markers. The mean (±SD) value of plasma VEGF A levels of BC group (217.6 ± 77.2 pg/ml) was significantly higher than that of benign tumor group (72.6 ± 9.2 pg/ml) and controls (42.5 ± 9.7 pg/ml, for both p <0.001). However, there was no significant

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difference between benign tumor group and controls. Similarly, the mean (±SD) value of plasma M-CSF levels of BC group (170.2 ± 36.3 pg/ml) was significantly increased in comparison with that of benign tumor women (88.0± 16.4 pg/ml, p= 0.001) and control women (82.7± 14.4 pg/ml, p= 0.001), with no significant variation between benign tumor with control women. Also, the mean (± SD) estimate of the serum CA15-3 of BC women (27.3 ± 11.0 u/ml) was higher significantly in comparison with that of benign tumor women (10.6 ± 3.3 u/ml, p=0.001) and controls (8.6 ± 3.1 u/ml, p=0.001), with comparable levels between women with benign tumor and control ones.

Table 1. Mean (±SD) estimates of Age, Body Mass Index, and percentage of Cancer Stages of studied groups.

Parameter	Controls (n=25)	Breast Cancer (n=38)	Benign Tumor (n=25)
Age (years)	42.4±9.6 ^{NS}	48.3 ± 9.2*	32.3 ± 11.3
BMI (kg/m^2)	26.8 ± 4.1	29.3 ± 4.1**	25.4 ± 3.9 ^{NS}
Stages of BC (Number of patients, %)		I (12, 31.58) II (14, 36.84) III (12, 31.58)	

t-test and ANOVA appeared, *significant rise in age of each of GI (p=0.001) and controls (p=0.002) compared with GII, ** significant increase in BMI in GI than in controls (p=0.046) and GII (p=0.001). NS: non- significant variation between GI and controls in age, between GII and controls in BMI.

However, the receiver operator curve (ROC) study revealed that area under curve (AUC) of VEGF A at cut-off value 88.67 pg/ml in discriminating women of BC from benign tumor was 0.958, sensitivity 79 % and specificity 100 % compared to that of M-CSF (AUC=0.662, sensitivity 34.2 % and specificity 100 % at cut-off value 122.5 pg/ml) and CA-15-3 (AUC=0.934, sensitivity 76.30 % and specificity 100 % at cut-off value 17.26 ng/ml), Figure 1. In addition, ROC study showed that AUC for VEGF A in discriminating women with BC from healthy control women was 1.0 with sensitivity 100 % and specificity 100 % at cut-off value 55.73 pg/ml, while that of M-CSF (AUC=0.70, sensitivity 42.1 % and specificity 100 % at cut-off 102.38 pg/ml) and CA-15-3 (AUC=0.996, sensitivity 97.4 %, specificity 100 %, at cut-off 10.82 ng/ml), Figure 2.

Table 2. Mean (±SD) Values of the measured Plasma Biomarkers (VEGF, M-CSF and CA 15-3) of the Studied Groups.

Parameter	Controls (n=25)	Breast Cancer (n=38)	Benign Tumor (n=25)
VEGF (pg/ml)	42.5 ± 9.7	217.6 ± 77.2 *	72.6 ± 9.2 ^{NS}
M-CSF (pg/ml)	82.7 ± 14.4	170.2 ± 36.3*	88.0 ± 16.4 ^{NS}
CA15-3 (ng/ml)	8.6 ± 3.1	27.3 ± 11.0 *	10.6 ± 3.3 ^{NS}

ANOVA & t-test revealed *significant increase of (VEGF, M-CSF, CA15-3) levels in BC compared to each of benign tumor and controls (for both, p<0.001). NS: Non-significant differences in (VEGF, M-CSF, CA15-3) levels between benign tumor and controls.

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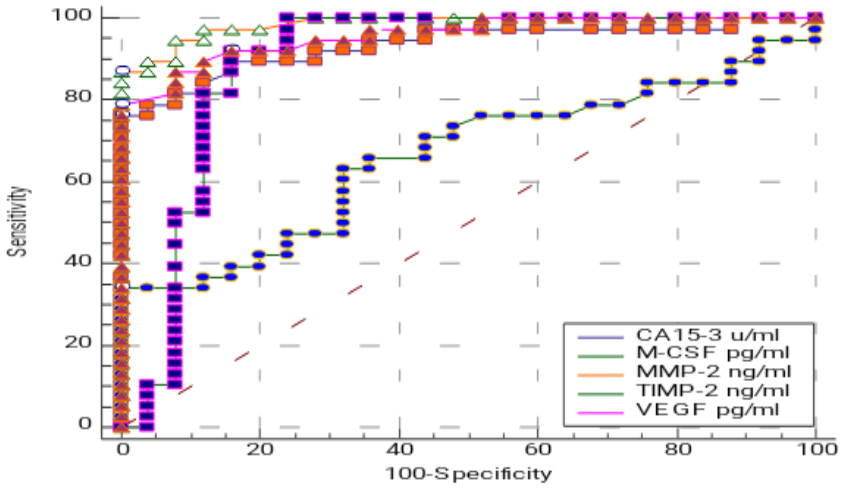

Figure 1. ROC curve of different markers as differentiator ofBC group from benign group.

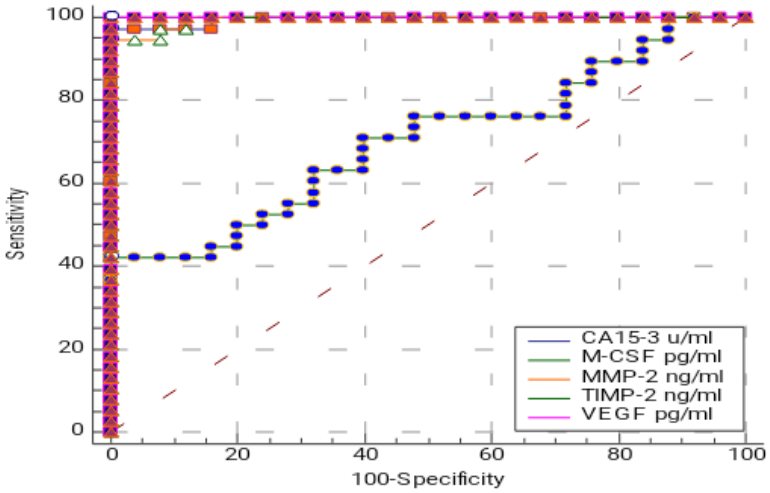

Figure 2. ROC curve of different markers as differentiator of BC group from healthy control group.

Table 3 shows the mean estimate of the measured biomarkers of BC women in accordance with their stage of cancer. ANOVA and t-test revealed that the mean (± SD) of serum VEGF A of BC women of stage III (454.3 ± 14.8 p/ml) was higher significantly in comparison with that of stage II and stage I (125.5 ± 43.0 pg/ml, 88.4 ± 10.2 pg/ml; respectively, for both p=0.005), with no significant difference among stage II and stage I. Similar results were found for each of M-CSF and CA15-3. However, ROC study found that AUC for plasma VEGF A in differentiating women with early stage of BC (stage I) from healthy women was 1.0 at cut-off value 55.73 pg/ml with sensitivity and specificity 100%, while that of M-CSF (AUC=0.70 at cut-off value 79.79 pg/ml) and CA-15-3 (AUC= 0.987 at cut-off 10.82 ng/ml), Figure 3. In addition, the AUC value of plasma VEGF A in discriminating women with stage I BC from benign tumor women was 0.877 at cut-off value 80.59 pg/ml, while that of M-CSF (AUC=0.68 at cut-off value 87.27 pg/ml) and CA 15-3 (AUC= 0.807 at cut-off value 14.26 ng/ml), Figure 4.

Table 3. Mean (±SD) values of the measured biomarkers of BC women in accordance with the stages of cancer.

Parameter	BC Patient Groups n=38

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	(Stage I; n=12)	(Stage II; n=14)	(Stage III; n=12)
CA15-3 (U/ml)	15.1 ± 3.9	29.2 ± 5.2 ^{NS}	37.2 ± 17.1 *
VEGF (pg/ml)	88.4 ± 10.2	125.5 ± 43.0 ^{NS}	454.3 ± 14.8 *
M-CSF (pg/ml)	74.0 ± 9.5	98.5 ± 12.5 ^{NS}	350.1 ± 10.3 *

* ANOVA & t-test showed a significant rise in (CA15-3, VEGF, M-CSF) levels of stage III in comparison with each of stage I and II ($p<0.005$). NS: Non- significant difference in (CA15-3, VEGF, M-CSF) levels between stage I & II.

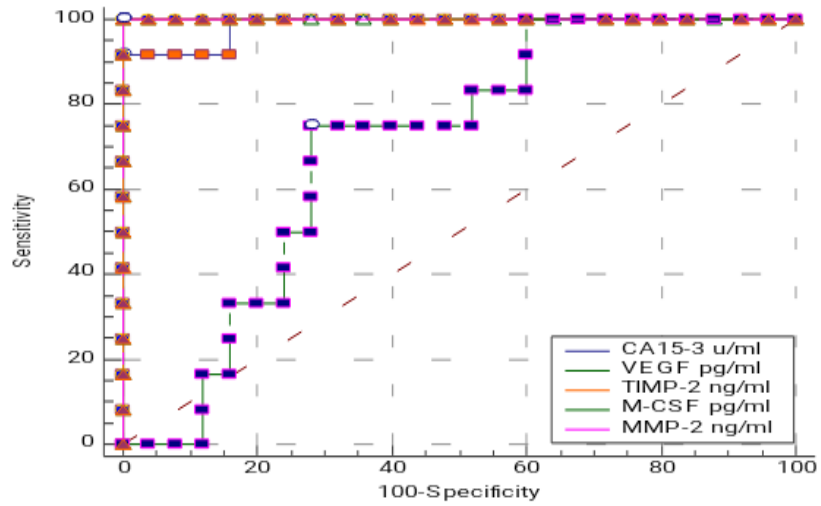

Figure 3. ROC curve of various marker that differentiate BC stage I from control.

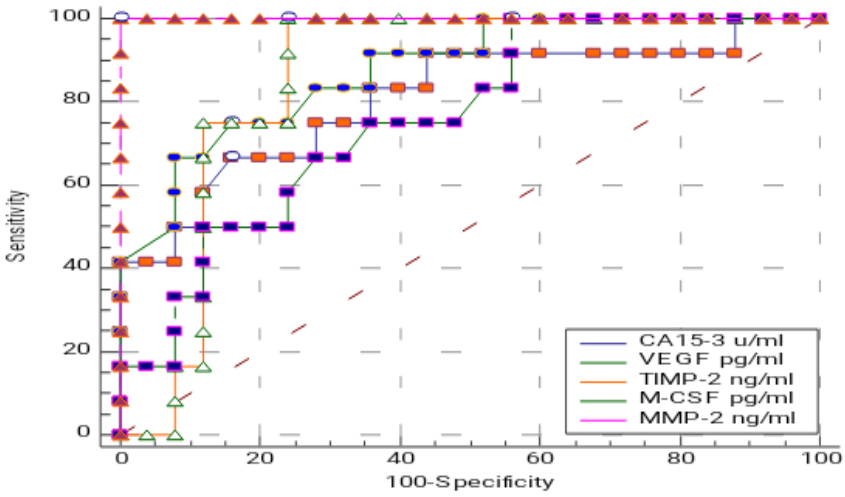

Figure 4.ROC curve of various marker that differentiate BC stage I from benign.

Figure 5 shows that AUC value of plasma VEGF A in differentiating benign tumor women from healthy ones was 0.998, sensitivity 100 % and specificity 96 % at cut-off value 54.93 pg/ml. The AUC values for M-CSF was 0.547 at cut-off value 102.38 pg/ml and for CA-15-3 was 0.945, sensitivity 100 % and specificity 84 % at cut-off value 6.05 ng/ml in performing such differentiation.

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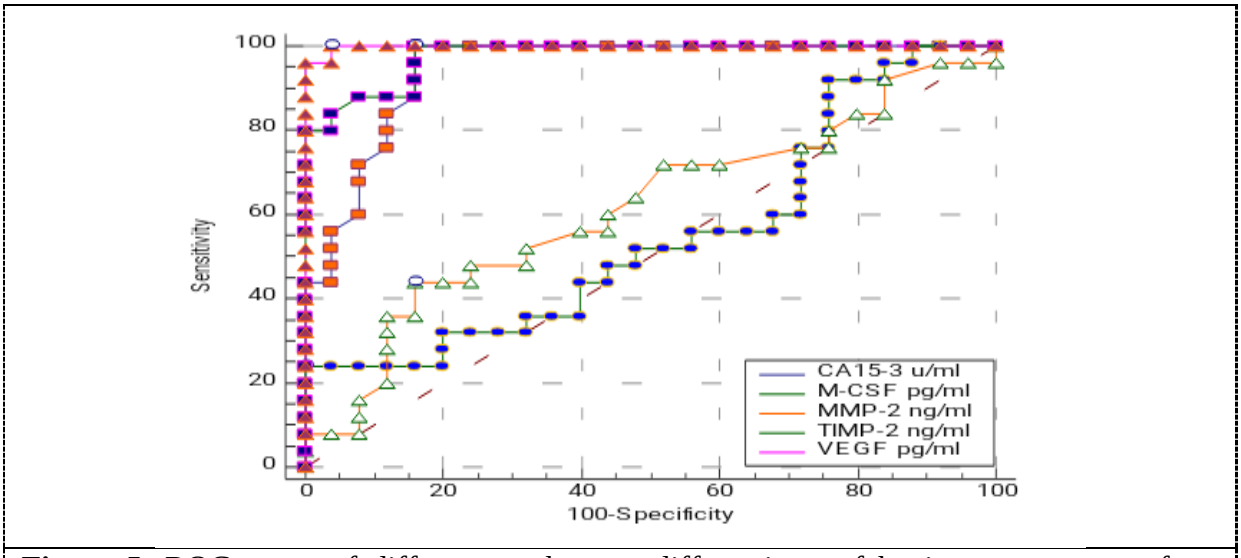

Figure 5. ROC curve of different markers as differentiator of benign tumor group from healthy control group.

The findings of the recent research also found that there were significant positive correlation among CA15-3 and VEGF ($r=0.382$, $p<0.05$) as well as between M-CSF and each of VEGF ($r=0.812$, $p<0.001$) in women with BC (Figures 6, 7).

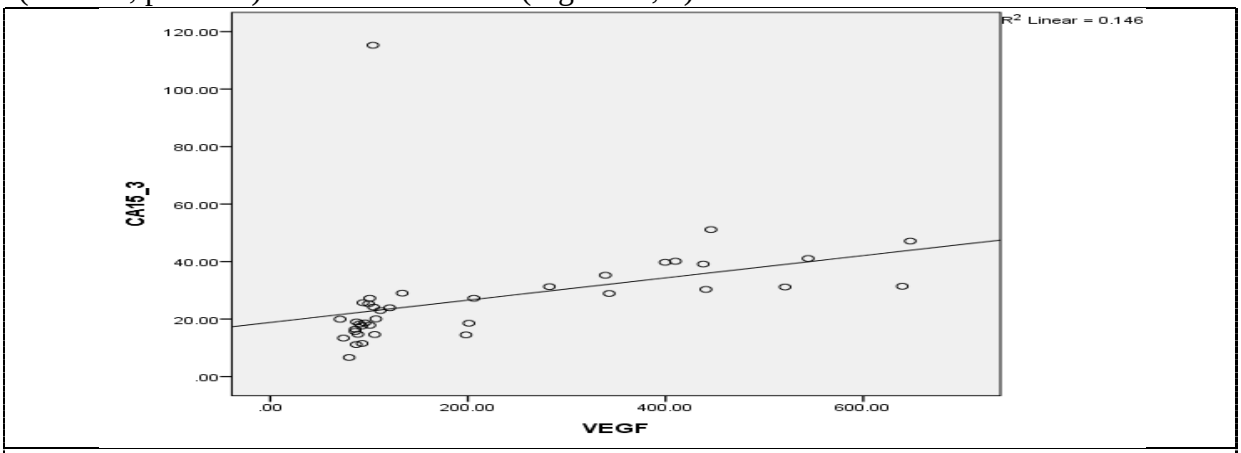

Figure 6. Direct correlation between VEGF and CA15-3 in BC women.

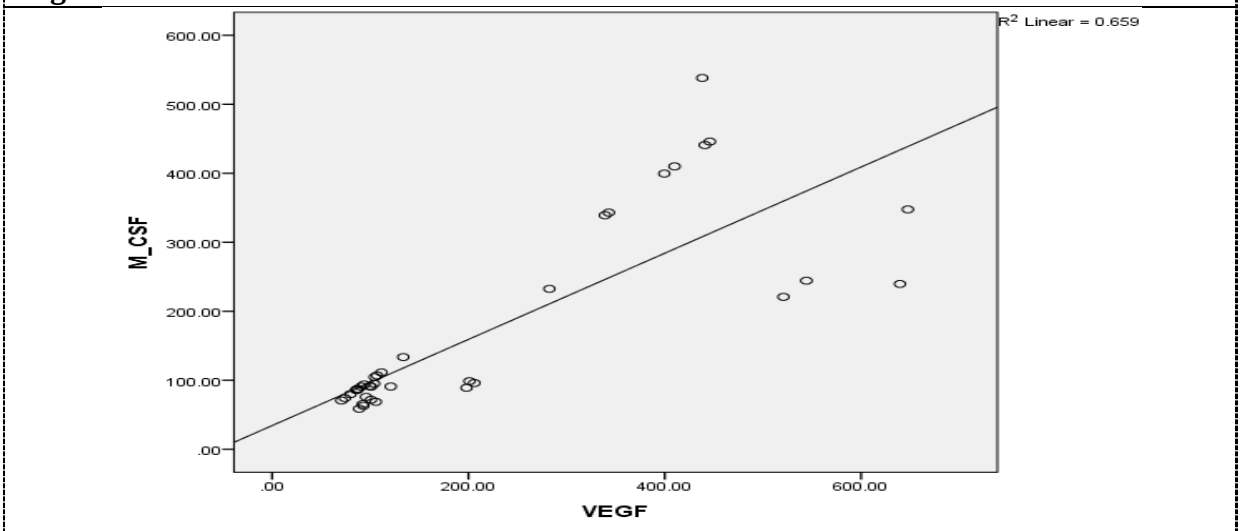

Figure 7. Direct correlation between M-CSF and VEGF in BC women.

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4. Discussion

The result of the current research showed that incidence of BC in Iraqi women is high at end of forty year of age, while that with benign tumor is near thirty year and these findings may be useful clinically in differentiating both tumor. In addition, women with BC are overweight and may be obese which confirmed the risky of obesity in occurrence of BC, which is not found by benign tumor. Also, the present results showed that the mean values of VEGF, M-CSF andCA15-3 levels of BC women were significantly elevated in comparison to that of benign tumor group and controls (Table 2), these results are consistent with that of prior studies [19, 20, 21, 22, 23, 24, 25]. The outcomes of the current study also revealed non-significant differences in mean values of VEGF, M-CSF and CA15-3 levels between women with benign tumor and controls (Table 2). The same observation on M-CSF and CA15-3 were reported previously [12, 19, 26], while previous studies observed significant increase in mean value of VEGF, M-CSF and CA15-3 levels in FA benign tumor compared to controls. The disagreement between the results of the current work and former researches may take part due to variations in sample size, population, age, types and progression of breast tumor.The present study found that measurement of plasma level of VEGF has the highest diagnostic and differentiating efficiency of early stage BC women (stage I) from benign tumor and healthy women (Figures 3, 4). [27] concluded that VEGF A is useful in breast cancer diagnosis and reported that VEGF A was the preferable parameter for diagnosis of the early stages of breast cancer (stage I) if added to CA 15-3 as novel panel for diagnosis. [25] found that serum levels of VEGF displayed greater diagnostic sensitivity and specificity than CA 15-3 in diagnosis of primary stages of breast cancer (BC). The current work noticed that serum levels of VEGF, M-CSF and CA 15-3 of BC women of stage III were higher in significance in comparison with that of stage II or stage I, however with no significant differences between stage I and stage II (Table 2). These results were in agreement with previous studies [12, 19, 23, 25, 27-30] and indicated that concentrations of these parameters were significantly increased with advancement of BC compared to benign tumor and control women. In addition, [23] reported the advantage and the great diagnostic potential of VEGF A in the discovery of BC. They revealed that VEGF A was better than CA15-3 in the early diagnosis of breast cancer as well as in discrimination among BC and benign breast tumor. While, M-CSF has shown to be the low one in comparison to CA 15-3 and VEGF A in BC diagnosis and differentiation. VEGF is one of the most potent angiogenic cytokines in normal tissues and tumors [8] stimulating endothelial cell proliferation in vitro and inducing angiogenesis *in vivo* [31]. VEGF is a selective cytokine (unlike other cytokines), acting specifically on vascular endothelial cells [32].Furthermore, it was noticed that else moderators of neovascularization involving growth factors, oncogenes and interleukins might yield actions via modifying VEGF expression, thus VEGF probably the final mutual path for whole pathological angiogenesis *in vivo* [33]. Cancer cells excrete VEGF to stimulate the VEGFR-2 path that activates cancer propagation, transmigration, infestation and angiogenesis. Angiogenesis is principally substantial for tumor nourishment, so it functions in all stages of cancer formation involving expansion, infestation, and metastasis [34]. Macrophage-colony stimulating factor (M-CSF) is one of the hematopoietic growth factors (HGFs) cytokine have a potent function in regulation of hematopoietic progenitor cell growth and differentiation, also M-CSF influences WBCs phagocytosis, chemotaxis and cellular cytotoxicity [35]. The present study found that plasma VEGF A was also the best one of the measured parameters in discriminating benign tumor women from healthy ones (Figure 5), with to our knowledge no previous report that deal with that.

5. Conclusion

The present study confirmed that plasma VEGF A has superior diagnostic utility of early BC and differentiating efficiency from benign tumor and healthy women of that of routine used one plasma CA 15-3. In addition, VEGF A was the best one in discriminating of benign tumor from control women. Plasma M-CSF was the poor one in such clinical uses. This study suggested the importance of replacement of plasma VEGF A instead of CA 15-3 in assessment of early BC and benign tumor in combination with imaging and other laboratory available parameters and may be to reduce for biopsy.

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