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Clinical Significance of TGF Alpha, TGF Beta1 and VEGF in Sera of Egyptian Patients with Breast Cancer

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Abstract:

Background: breast cancer (BC) is the most prevalent cancer among women and affects approximately one million women worldwide each year and it is the most prevalent cancer among Egyptian women and constitutes 29% of National Cancer Institute cases. This study was designed to determine the crucial role of TGF-α, TGF-β1 and VEGF in patients with Breast carcinoma. Patients & method: serum level of TGF-α, TGF-β1 and VEGF were determined by ELISA in 51 patients with preoperative & postoperative primary (BC), as well as 30 healthy female persons. **Results**: this study showed that the TGF-α, TGF-β1 and VEGF levels were significantly high ($p = 0.001$) in patients with primary breast cancer compared to control healthy female group. Meanwhile the levels of these growth factors did show significant decrease after treatment. **Conclusion**: this study revealed that serum levels of TGF-α, TGF-β1 and VEGF in patients with breast cancer could be useful biomarkers for prognosis of such type of malignancy.

Key words: Primary BC, VEGF, TGF-α, TGF-ß1

INTRODUCTION:

As general consideration breast cancer is the second most common type of cancer after lung cancer (1), and the fifth most common cause of cancer death after lung cancer, stomach cancer, liver cancer, and colon cancer (2).

Breast cancer caused 502,000 deaths worldwide (2). The number of cases worldwide has significantly increased, a phenomenon partly blames on modern lifestyles in the western world (3).

In Egypt, breast cancer is the most common cancer among women, representing 18.9% of total cancer cases (35.1% in women and 2.2% in men) among the patients in National Cancer Institute (NCI) (3).

Metastasis is regulated not only by changes in tumor cells but also by reciprocal interactions with the surrounding microenvironment (4). Moreover, breast cancers metastasize to lungs, bone, liver, and brain (5).

Transforming growth factor alpha (TGF-α) is a 50-amino-acid polypeptide that are derived from a 160-residue precursor by proteolytic cleavage. TGF-α is physically constrained into three ring structures by disulfide bridges formed between six cysteine residues. It has been isolated from a retrovirus-transformed mouse cell line (6), it has subsequently been found in human tumor cells, in early rat embryo cells, in cell cultures from the bovine pituitary gland, and normal keratinocytes from human adults (7). The roles for TGF-α have been proposed in transformation, wound healing, bone resorption, angiogenesis, and cell migration (8). It is a 6 kDal polypeptide with 40% sequence homology to the epidermal growth factor (EGF), with which it shares a common receptor. Like many growth factor receptors. In breast cancer cell lines, TGF-α appears to stimulate cellular growth.

The transforming growth factor beta (TGF-β1) superfamily contains proteins that serve a wide variety of biologic functions, including growth control, cellular differentiation, embryologic morphology, and immunity. (TGF-β1) is the predominant

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form found in humans and is expressed widely in a variety of normal cells and organs. TGF-β1 is a multifunctional polypeptide, promoting angiogenesis, accumulation of extracellular matrix glycoproteins, and cell adhesion proteins, while inhibiting growth of both epithelial and immune cells (9). TGF-β1 has been found to be overexpressed locally in many tumors, and is believed to play a role in tumor transformation and progression, as well as in tumor regression (10, 11). Although TGF-β1 acts as a potent inhibitor of cell growth and tumor progression, loss of this negative regulation can contribute to tumor development (12). This was explained by defects in the activation of TGF-β1 or defects in the regulation of TGF-β1 receptor in the TGF-β1 signaling pathway (13, 14). Therefore it is now believed that the growth factor TGF-β1 is a growth inhibitor that becomes a stimulator of both growth and invasion to a more invasive state no matter what mechanisms are involved. In vivo studies also have shown elevated levels of TGF-β1 mRNA in tumor tissues and elevated levels of TGF-β1 in the serum or plasma of patients with various malignant tumors (15, 16).

VEGF induces angiogenesis and endothelial cell proliferation and it plays an important role in regulating vasculogenesis. VEGF is a heparin-binding glycoprotein that is secreted as a homodimer of 42 kDa. Three receptors tyrosine kinases have been described as putative VEGF receptors and have been shown to bind VEGF with high affinity. They are VEGFR-1 (Flt-1) (fms-like tyrosine kinase), VEGFR-2 (KDR/Flk-1) (kinase-insert-domain-containing receptor) and VEGFR-3 (Flt-4). Neuropilin has been implicated as a co-receptor for VEGF and may enhance VEGFR-2 activity (17).

SPECIMEN COLLECTION:

Blood samples were collected preoperatively and postoperatively then preserved on ice till reaching the lab, blood samples were collected with no additives to obtain serum. Samples were centrifuged for 15 minutes at 3000 rpm and the serum was separated and kept frozen at (-80˚c) until assayed.

MATERIALS AND METHODS:

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The study was carried out on 51 patients admitted to the medical oncology unit of the National Cancer Institute and 30 normal apparently healthy persons as a control, from December 2010 to June 2011. The work was carried out in the Biochemistry Unit, Cancer Biology Department, National Cancer Institute. All patients` available data were collected: history, medical examinations, pathology reports, laboratory investigations, ultrasound, CT scanning, operation details and the postoperative follow up. Patients included and normal persons were categorized into the following:

- **Group I**: includes 30 normal apparently healthy females as a control.
- **Group II**: includes 51 females with preoperative & postoperative primary non-metastatic Breast Carcinoma (BC).

Patients with malignant masses had their appropriate surgical treatment. The final diagnosis of the cases was based on histopathological examination of surgical specimens. Tumor staging and grading were determined according to TNM and World Health Organization Classification.

Human TGF-α ELISA (Enzyme-Linked Immunosorbent Assay) kit is obtained from Ray Bio®. The TGF-β1 and VEGF ELISA (Enzyme-Linked Immunosorbent Assay) kits are obtained from eBioscience. They are in vitro enzyme-linked immunosorbent assay for the quantitative measurement of human TGF-α, TGF-β1 and VEGF in serum.

The mean absorbance for each set of duplicate standards, controls and samples are calculated and the standard curve is plotted on log-log graph paper or using Sigma plot software, with standard concentration on the x-axis and absorbance on the y-axis. Draw the best-fit straight line through the standard points.

RESULTS:

The age of the control group ranged from 20 to 75 years. While the age of primary breast cancer females ranged from 27 to 83 years. The age of breast cancer metastasis females ranged from 48 to 61 years.

Transforming Growth Factor alpha (TGF-α):

The distribution of serum immunoreactive TGF-α level in preoperative, postoperative breast-cancer patients and in normal individuals is shown in (Fig. 1).

Analysis of variance showed that the difference between the preoperative serum TGF-α levels in breast cancer patients and normal individuals was statistically significant (p=0.001), where the difference between the postoperative serum TGF-α levels in cancer patients and normal individuals was statistically non-significant (p>0.05).

Transforming Growth Factor beta1 (TGF-β1)

As shown in (Fig. 3), the mean serum TGF-β1 level in the postoperative patients decreased significantly which represents no significant difference with healthy controls (p > 0.05).

Vascular Endothelial Growth Factor (VEGF)

The distribution of serum immunoreactive VEGF levels in preoperative, postoperative breast-cancer patients and in normal individuals is shown in (Fig. 4).

Analysis of variance showed that the difference in the preoperative serum VEGF levels in breast cancer patients and postoperative patients was statistically significant difference (p=0.001), where the difference in the postoperative serum VEGF levels in cancer patients and normal individuals was statistically non-significant (p>0.05).

DISCUSSION:

TGF-α is expressed and mitotically active in numerous breast cancer cell lines and has been directly implicated as a moulator of transformation in vivo. In fact, the discovery of TGF-α was based on its ability to transform retrovirally-infected cultured fibroblasts (18, 19).

Our experimental results revealed that the mean serum level of TGF-α in normal healthy group was found to be 103 pg/ml, which is consistent with the normal level obtained from the study done by Chakrabarry et al. (20), who reported that of the 74 normal sera used, 24 had TGF-α levels below the threshold of detectability of the assay (100 pg/ml) while the remaining 50 normal individuals had TGF-α level in the range of (120-207) pg/ml, with a mean of 147 pg/ml. Moreover, our results showed that the mean serum level of TGF-α in the preoperative breast cancer patients was found to be significantly higher than the control group (p = 0.001), While the mean serum level of TGF-α in the postoperative breast cancer patients revealed non-significant difference with the control group (P>0.05), these findings clearly showed the ability of TGF-α to be a useful marker in the prognosis of the breast cancer.

Both epidermal growth factor and TGF-α cause proliferation of breast carcinoma cell in vitro and several reports have indicated that levels of EGFR are related to prognosis in breast cancer (21-25). Other studies have confirmed the clinical importance of TGF-α in breast cancer as a good prognostic marker.

In our study we have obtained the mean serum level of TGF-β1 in control group and it was found to be 3.7 ng/ml, which is agreed with the normal level obtained from the study Kong et al.(26) who have reported that the normal range of serum TGF-β1 is (4.3 ± 0.3 ng/ml). Regarding to the normal mean serum level of obtained in our study, this work revealed that the mean serum level of TGF-β1 in the preoperative primary breast cancer patients was found to be significantly higher than the control group (p = 0.001), while the mean serum level of TGF-β1 in the postoperative primary breast cancer patients showed non-significant difference with the control group. These findings clearly revealed the ability of TGF-β1 to be a good prognostic marker in the management of breast cancer. Our results agreed with what

have been reported by Kong et al. (26) who have shown an elevated level of TGF-β1 in serum of patients with breast carcinoma as compared with those in normal adults. They also found that patients with more advanced tumors have higher serum levels of TGF-β1. Therefore, they suggested that serum TGF-β1 may reflect the severity of invasive cancer. This could indicate that, TGF-β1play a crucial role in metastasis.

Although TGF-β1 is a growth inhibitor for tumor progression in many cases, the expression of TGF-β1 in cancer cells was found to be strongly correlated with progression and metastasis of malignancy such as prostate and colorectal cancers (9, 27, & 28). TGF-β1 promotes proliferation of some mesenchymal cells, such as smooth muscle cells, as well as glioma and osteosarcoma tumor cells through the induction of platelet-derived growth factor (PDGF) A or B (29, 30, & 31).

Regarding VEGF findings, our experimental results revealed that its mean serum level control group was found to be 74.8 pg/ml, which is consistent with what have been shown by Ali et al. (32) who has reported that the normal range of serum VEGF is (45 -280 pg/ml). With respect to the normal mean value obtained in our work, the mean serum level of the preoperative VEGF in primary breast cancer was found to be significantly high ($p = 0.001$), while the mean serum level of the postoperative VEGF in primary breast cancer was significantly decreased compared to preoperative patients. These findings clearly showed a good possibility of VEGF to be a useful marker in the prognosis of primary breast cancer. Our results agreed with what have been noticed by Ali et al. (32) who found that serum preoperative VEGF is significantly high in BC patients than in normal adults ($p < 0.001$).

VEGF is a significant marker for overall survival (33), and the presence of VEGFR1 in breast cancer cells was shown to be significantly correlated with high metastasis risk and relapse and was considered a marker for breast tumor aggressiveness (34), but some authors did not detect the expression of VEGFR1 in breast tumors. Wulfing P, et al. showed that bone metastasis of breast cancer tends to be osteolytic, express VEGF receptors, therefore, a relation between VEGF and breast cancer-derived bone metastasis is suggested .This finding is out scope of our work since our cases were all non-metastatic (35).

CONCLUSION:

Our finding in this work suggests that serum TGF-α, TGF-β and VEGF could be useful as tumor markers for prognosis of breast carcinoma (BC), as their serum levels are markedly elevated in patients with primary malignancy, and decrease to normal healthy level after treatment.

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Table 1. Major characteristics of the breast cancer patients and controls:

	Breast cancer patients	Control	P-value
Number (%)	56	30	
Age (yrs)	49.78 ± 1.39	37.33 ± 2.36	0.001
Tumor size (cm) < 3 cm >3 cm	 21(37.5%) 35(62.5%)	-	
Tumor grade II III	 49 (87.5%) 7(12.5%)	-	
Stage I II III IV	 10 (17.86%) 35(62.5%) 6(10.71%) 5(8.93%)	-	
Type Ductal Medullary Lobular Mucinous tubular	 45(80.36%) 4(7.14%) 5(8.93%) 1(1.8%) 1(1.8%)	-	
LN status Positive Negative	 51 (91%) 5 (9%)	-	
Metastasis Yes No	 5 (9%) 51(91%)	-	

Table 2. Marker levels in the patients pre-and post-operatively compared with the controls.

	Patients		Control
Marker	Preoperative	postoperative	
TGF alpha	250 ± 19.73 *#	121.43 ± 8.23	103 ± 8.96
TGF beta	18.55 ± 0.89 *#	11.78 ± 0.52 #	3.68 ± 0.33
VEGF	791.38 ± 97.43 *#	298.67 ± 33.51 #	74.77 ± 5.78

#: Significant with control at $p < 0.001$
*: Significant with postoperative at $p < 0.001$

Table 3. Marker levels in the patients and the clinicopathological observations.

	TGF alpha	TGF beta	VEGF
Stage			
I	149.1 ± 12.4a	16.26 ± 1.78a	554.21 ± 176.16a
II	247.24 ± 22.6a	17.53 ± 1.03a	769.87 ± 127.13ab
III	285.5 ± 74.7a	19.41 ± 2.44a	832.85 ± 330ab
IV	427.5 ± 83.7b	29.28 ± 2.04b	1366.58 ± 291.6b
	F = 4.8 p= 0.004	F = 6.4 p= 0.001	F = 1.4 p= 0.242
Type			
ductal	271.73 ± 23.34	19.35 ± 1.03	839.4 ± 114.9
medullary	138.9 ±7.87	12.40 ± 1.80	484 ± 263.3
lobular	158 ± 7.68	18.57 ± 2.07	828.7 ± 236.4
	F = 2.65 p= 0.08	F = 2.04 p= 0.14	F = 0.42 p= 0.65
Tumor size			
< 3	207.12± 27.8	16.8 ± 1.39	739 ± 168.4
> 3	275.75 ± 26.13	19.61± 1.14	822.78 ±120.3
LN			
-ve	427.5 ± 83.7*	29.28 ± 2.04*	1366.6 ± 291.6
+ve	232.6 ± 18.64	17.5 ± 0.83	735.0 ± 97.4
Metastasis			
No	232.6 ± 18.64	17.5± 0.83	735± 100.4
Yes	427.5 ± 83.7*	29.28± 2.04*	1366.6± 291.4
Grade			
II	255.35 ± 21.86	17.82 ± 0.89	806.8 ± 106.7
III	212.64 ± 39	23.71 ± 3.06*	683.18 ± 237.3

The different letters indicate significantly different means according to Duncan's multiple range test.
*: significant at $p < 0.05$ (independent t-test).

Table 4. Sensitivity, specificity, diagnostic accuracy, positive predictive value, negative predictive value and differential positive rate of VEGF at the optimal cut-off values for the studied markers.

	Cut-off	Sensitivity	Specificity	Diagnostic accuracy	Positive predictive value	Negative predictive value	Differential positive rate
TGF alpha	123	92.86	86.67	90.70	92.86	86.67	79.52
TGF beta	8.7	98.21	100.00	98.84	100.00	96.77	98.21
VEGF	144	83.93	96.67	88.37	97.92	76.32	80.60

Table 5. Area under the ROC curve for the studied markers.

	Area	Standard error	95% confidence interval	
			Lower limit	Upper limit
TGF alpha	0.924	0.034	0.857	0.990
TGF beta	0.999	0.001	0.997	1.001
VEGF	0.938	0.025	0.889	0.986

Fig. 1.

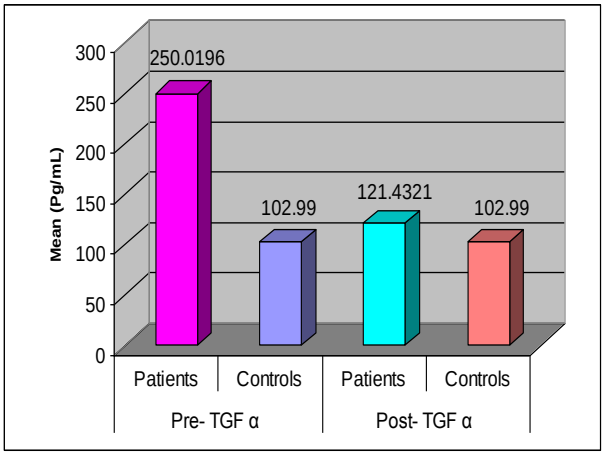

Fig. 1. Change of TGF-α in sera of patients before & after treatment

Fig. 2.

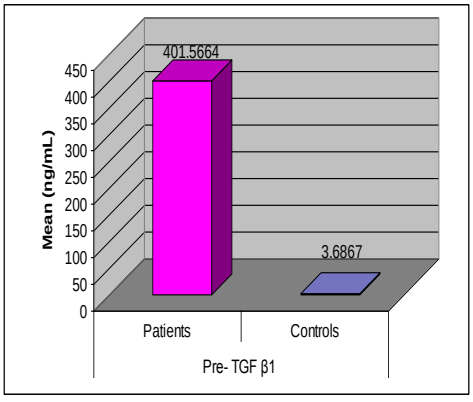

Fig. 3.

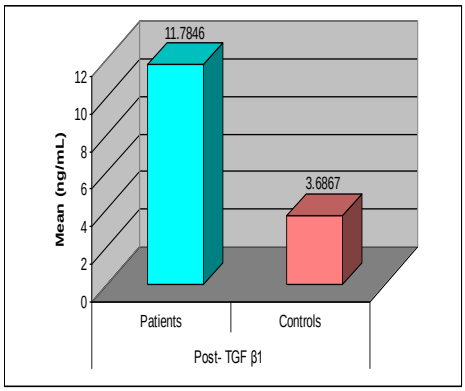

Fig. 2. Change of TGF-β1 in sera of patients before treatment
Fig. 3. Change of TGF-β1 in sera of patients after treatment

Fig. 4.

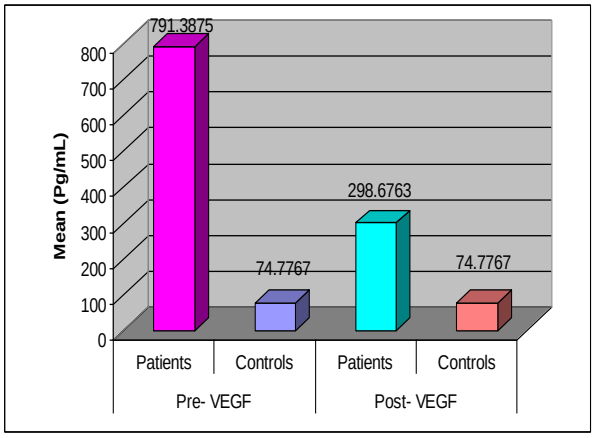

Fig. 4. Change of VEGF in sera of patients before & after treatment

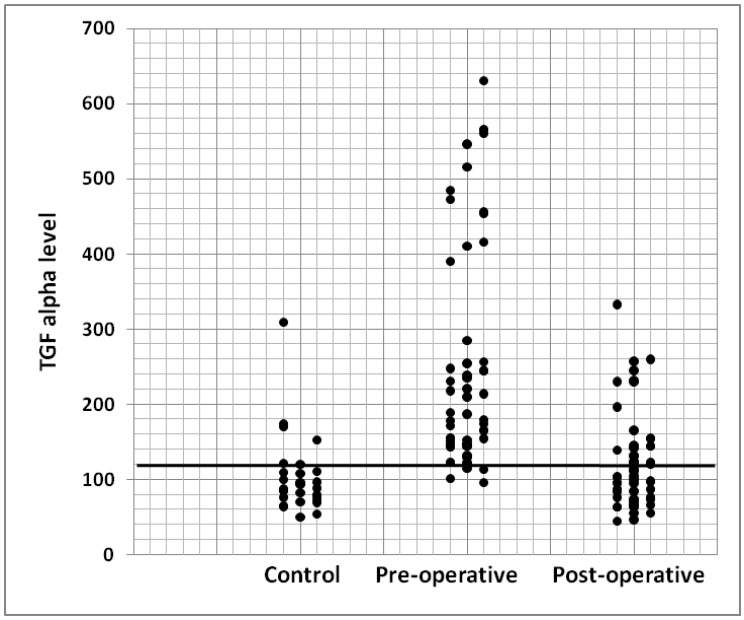

Fig. (5) Scatter diagram of TGF alpha level in the breast cancer patients (pre- and postoperatively) and the control. The horizontal line represents the optimal cut-off value (123 ng/ml) at which the sensitivity and specificity were 92.86 % and 86.67%, respectively.

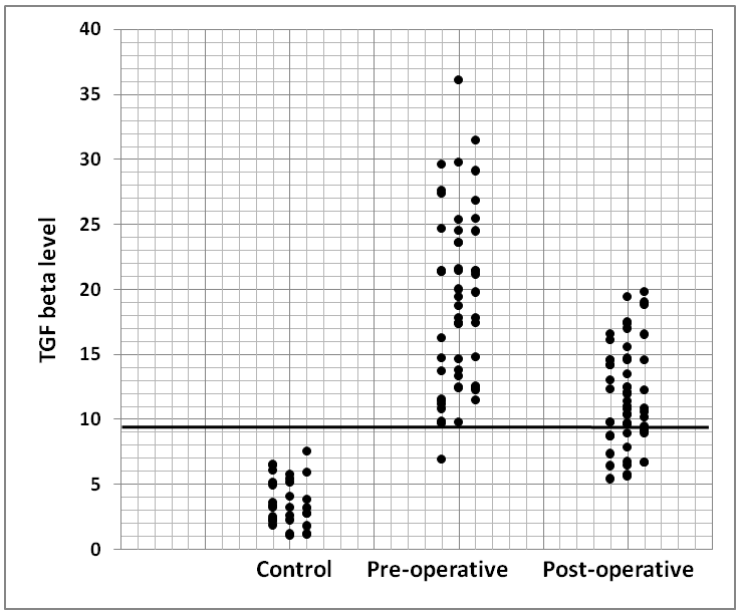

Fig. (6) Scatter diagram of TGF beta level in the breast cancer patients (pre- and postoperatively) and the control. The horizontal line represents the optimal cut-off value (8.7 ng/ml) at which the sensitivity and specificity were 98.21 % and 100%, respectively.

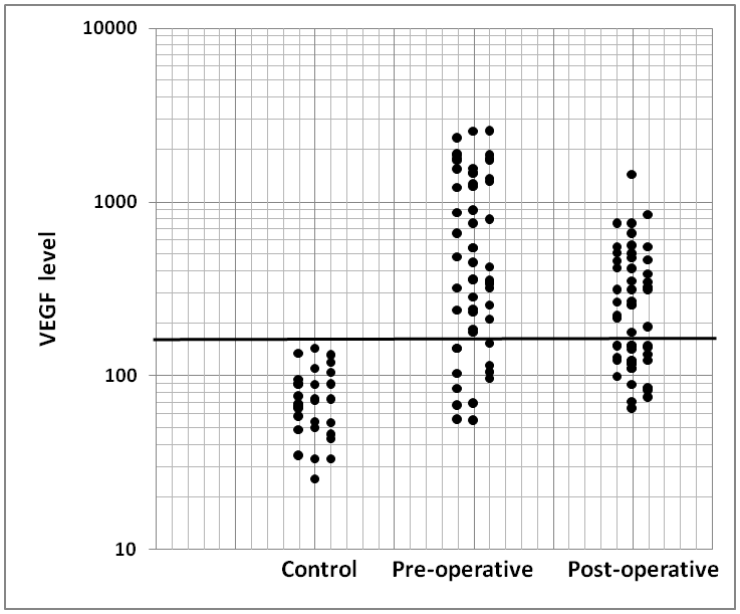

Fig. (7) Scatter diagram of VEGF level in the breast cancer patients (pre- and postoperatively) and the control. The horizontal line represents the optimal cut-off value (144 ng/ml) at which the sensitivity and specificity were 83.93 % and 97%, respectively. Logarithmic scale was used for good spread of points.

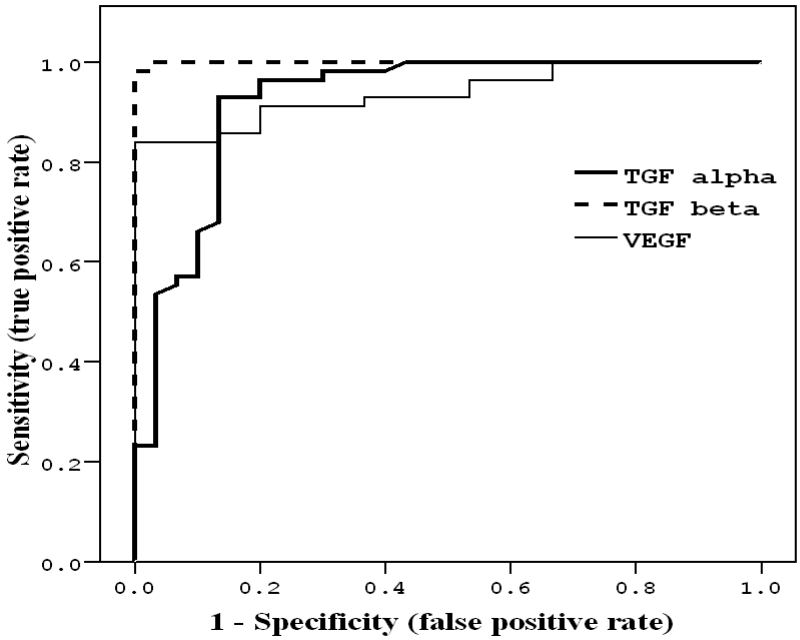

Fig. (8) ROC curves of TGF alpha, TGF beta and VEGF. The area under the curve is 0.924, 0.999 and 0.938, respectively indicating the great power of the studied markers in differentiating between breast cancer patients and controls. The area is excellent if exceeded 0.9 and accepted only if exceeded 0.7.