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ORIGINAL PAPER

Taurine: a novel tumor marker for enhanced detection of breast cancer among female patients

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Abstract
Introduction The antioxidant Taurine found to display antineoplastic effect through down regulation of angiogenesis and enhancement of tumor cell apoptosis. It has been found that progressive inhibition of apoptosis and induction of angiogenesis may contribute to tumor initiation, growth and metastasis in the pathogenesis of breast cancer.
Aim of the study To correlate taurine level with the levels of some bioomolecules operating in both angiogenesis (VEGF, CD31) and apoptosis (TNF-α and Caspas-3) which could help for breast cancer pronostication and to evaluate a possible role of serum taurine level as an early marker for breast cancer in Egyptian patients.
Patients and methods Four groups of a total 85 female candidates were studied in this work. The first group consists of 50 female patients at National Cancer Institute (NCI), Cairo University were diagnosed and undergoing surgery for breast carcinoma. In the second group 10 having benign breast lesions, were included. The third group consists of five cases, with positive family history. Twenty healthy females were also recruited as control. A preoperative blood sample were taken from each patient to measure serum level of VEGF; Taurine; CA15.3 and TNF-α. Sample of fresh tumor and their corresponding safety margins were obtained from the first and second groups, for determination of caspase-3; histopathological examination and immunohistochemical assay of VEGF and CD31.
Result No significant differences in the serum level of CA15.3 between the breast cancer patients, the high risk and the control group. TNF-α (apoptotic biomolecule) level showed a significant difference only between breast cancer group and control group. The VEGF (angiogenic biomarker) showed a highly significant difference between breast cancer patients, the high risk and the control group. Regarding the antioxidant taurine (antiangiogenic biomolecule) serum level in breast cancer group exhibited a value strongly lower than the high risk and control group. Also the correlative ratio between the angiogenic/apoptotic biomarker (VEGF/TNF-α) showed a highly significant difference between the main previous three groups. Same observation were also noticed in the correlation between angiogenic/antiangiogenic (VEGF/taurine) ratio in the same groups. Moreover the enzymatic activities of Casp-3 in the tissue homogenate were statistically higher in adjacent normal tissues than in malignant tissues. The result of immunohistochemical investigation showed a significant increase in the density of intracellular VEGF and microvessel density expressed as CD31 in cancer cases compared to normal adjacent tissue.

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Conclusion It is suggested that assessment of taurine level in sera of patients with high risk for breast cancer are of great value in the early diagnosis of malignant changes in the breast.

Keywords Antioxidant taurine · Angiogenesis (VEGF, CD31) · Apoptosis (TNF-α and Caspas-3)

Introduction

Carcinoma of the breast is the most prevalent cancer among Egyptian women, it constitutes 29% of Cairo National Cancer Institute cases, and it is usually diagnosed at an advanced stage [1, 2]. There are several hypotheses regarding breast cancer etiology, including carcinogenesis by steroid hormones, chemical carcinogens and oxidative stress. Epidemiological studies suggest that a diet that is rich in antioxidants may help to prevent the development of breast carcinoma [3, 4].The lifetime risk for women of being diagnosed with breast cancer is currently between 1 in 7 and 1 in 8, the risk is even higher for women with certain risk factors, such as a strong family history or known BRCA1 or BRCA2 mutations [5].

Experimental studies suggest that angiogenic activity of tumor may result from down-regulation of inhibitors of angiogenesis or upregulation of endothelial growth factors [6, 7]. Several researches have emphasized that VEGF is now believed to be a key mediator of angiogenesis in numerous solid tumors, including breast cancer [7]. Also it has been found that serum VEGF levels was significantly elevated in the breast cancer patients compared with those of the controls and it was more sensitive than CA15.3 as a marker [8].

In breast cancer, immunohistochemical study of VEGF signal in pre-tumoral blood vessels correlated with image cytometric CD31 and D2-40 microvessel density, consistent with the role of VEGF in blood and lymphatic vascular growth. CD31 and D2-40 microvessel density were found to correlate significantly with several prognostic factors, including lymph node metastasis [9].

Caspase-3 is one of the biomolecules that operate in apoptosis [10]. The appearance of the active form of caspase-3 in the cytoplasm of the cells undergoing apoptosis is an early event and precedes the development of classical morphological features of apoptosis [11]. Moreover Caspase-3 deficiency or down regulation has been reported in breast cancer and other kinds of cancer [12].

Many antioxidants are being identified as anticarcinogens that characterizing and optimizing such defense systems may be an important part of a strategy of minimizing cancer and other age-related diseases [4, 13]. Taurine and its derivatives such as taurolidine and taurochloramin were found to display antineoplastic effect both in vitro and in vivo; through suppressing cell proliferation, enhancement of tumor cell apoptosis [14–21], and through an antiangiogenic effects [15, 22] while enhancing the therapeutic index of some antitumer agent[16]. Recently taurine level used as early marker in hepatocellular carcinoma (HCC) [17] and in cancer uterus [18].

The aim of this study is to correlate taurine level with the levels of some bioomolecules operating in both angiogenesis (VEGF, CD31) and apoptosis (TNF-α and Caspas-3) which could help for tumor pronostication and to evaluate a possible role of serum taurine level as an early marker for breast cancer in Egyptian patients.

Materials and methods

This prospective study involves four groups of a total eighty-five female candidates. The first group consists of fifty female patients at National Cancer Institute (NCI), Cairo University. Patients were diagnosed according to mamographic imaging, laboratory tests, and clinical investigation following the institutional protocol. They were undergoing surgery for breast cancer. After obtaining informed verbal consent, a preoperative blood sample was taken from each patient. Patients who received new adjuvant therapy were excluded. In the second ten having benign breast lesion, were included. The third group consists of five cases, with positive family history. Finally, twenty healthy females were also recruited as control. A preoperation blood sample were taken from each patient to measure serum level of VEGF; Taurine; CA15.3 and TNF-α. Fresh tumor and their corresponding safety margin were obtained from the first and second groups, for determination of caspase-3; histopathological examination and immunohistochemical assay of VEGF and CD31. This work approved by ethical committee of National Cancer Institute - Cairo University (approved by IRB).

Blood sample collection: for the whole four groups, after an over-night fasting, a 7 ml of venous blood was collected, in a plain tube and allowed to clot for half an hour, after which it was centrifuged at 3,000 rpm for 10 min. The serum was separated and stored at −80°C to avoid loss of biological activity until a batch analysis for serum VEGF, taurine TNF-α and CA15.3 analysis.

- *Serum VEGF Analysis*: Analysis for VEGF were performed using Oncogen Research Products VEGF ELISA, (cat#QIA51)
- *Serum taurine determination*: Serum taurine was determined by High Performance Liquid Chromatography (HPLC) according to the Pre-column Extraction and derivatization methodology [19]. In the present

work we use the shimadzu, japan, HPlC model Lc-10AT.

- *Serum TNF-α determination*: Human TNF-α ELISA were used for quantitative determination of tumor necrosis factor alpha, a monoclonal antibody specific for TNF-α is used to capture the TNF-α, and an enzyme-linked polyclonal antibody specific for TNF-α is used for quantification.
- *CA15.3 Analysis*: Microparticle Enzyme Immunoassays from Abbott Laboratories, Diagnostics Division (CEA, Dainabot, Tokyo Japan; CA15.3, Abbott Park, IL). A cutoff level of 30 units/ml is used for CA15.3.

Tissue samples: Fresh tumor and their corresponding safety margin normal tissues were obtained at the same time of surgical resection from breast cancer females who underwent radical mastectomy and the second group of the ten candidates having benign breast cancer lesion.

Tissue samples were subjected for determination of Caspase- three activity, histopathology examination and immunohistochemical assay of VEGF& CD31.

- *Caspase-3 determination*: Caspase-3 is determined using Caspase-3/Cpp32 Colorimetric Protease Assay (ApoTarget).
- *Monocolonal antibody to CD31*: CD31 was determined immunohistochemically using commercial kits purchased from DAKO Corporation, Carpinteria, CA, USA).
- *Monoclonal to VEGF*: VEGF was localized and determined on formalin fixed, paraffin wax embedded tissue sections of tumor samples. Monoclonal Mouse Anti-Human Vascular Endothelial Growth Factor, clone VG1 (Dakocytomation) from santa Cruse Biotechnology, Inc. is used. The antibody labels the VEGF-121, VEGF-165 and VEGF-189 isoforms of vascular endothelial growth factor. The antibody labels the characteristics doublet bands observed when VEGF is run under reducing condition.
- *Immunohistochemistry staining of VEGF and scoring of CD31 microvessel density*: All tissues were fixed in 10% neutral buffered formalin and embedded in paraffin using standard surgical pathology protocols. Diagnoses were established from H&E-stained slides using standard histopathological criteria. Immunohistochemistry was performed on a single representative block from each case. Using SLAB kit (DAKO Corporation, Carpinteria, CA, USA) and VEGF (1:160 dilution), Santa Cruz Biotechnology, Santa Cruz, CA, USA). For VEGF expression, immunohistochemical reactions were assessed in areas of invasive carcinoma and in non-neoplastic breast tissue. Cytoplasmic staining intensity was graded from 0 (no staining) to 3 (most intensely stained), and the percentage of positive cells was noted.

Statistical analysis

Data were analyzed using the Statistical Package for the Social Sciences (SPSS) version 10. Data were represented as mean ± SE. One way analysis of variance (ANOVA) followed by Duncan's post hoc test was used to clarify the statistical significance between means of the different patient groups. Unpaired t-test was used to compare the means of two groups. Pearson's correlation coefficient was calculated to study the correlation between the different parameters. *P*-value less than 0.05 were considered significant.

- The sensitivity, the specificity, the diagnostic accuracy, positive predictive value and negative predictive value, the differential positive rate (DPR) were calculated according to the following formulas [20]:

  1. Sensitivity = a/(a + c)
  2. Specificity = d/(b + d)
  3. Diagnostic accuracy = a + d/(a + b + c + d)
  4. Positive predictive value = a/a + b
  5. Negative predictive value = d/c + d
  6. DPR = sensitivity + specificity − 1

  where: a = true positive cases, c = false negative cases, d = true negative cases, b = false positive cases.

- The Mann–Whitney U-test was used to compare the medians of the non- parametric data; (tissue VEGF and CD31).

Results

Serum taurine level was estimated in different group illustrated in (Table 1). Its level showed highly significant difference in between the control groups, the high risk and breast cancer patients ($F = 380.7$, $P < 0.001$). in breast cancer patients taurine level was strongly lower than the high risk and control group (13.79 ± 0.65 μmol/l); recording a −0.72-fold decrease than the control (Table 2). In lymph node-positive breast cancer patients serum taurine was insignificantly changed from the lymph node-negative patients recording serum level of (14.33 ± 0.68 μmol/l) (13.37 ± 1.08 μmol/l), respectively, $P > 0.05$), this is shown in (Table 3). While in high risk group taurine level was lower than control group as it recorded (30.7 ± 1.38 μmol/l, recording a −0.59-fold decrease than control (Table 2). Among the high risk group; high risk patients with benign breast lesion recorded (22.3 ± 1.42 μmol/l) which is significantly lower than taurine level in those with positive family history have no breast lesion (47.4 ± 2.75 μmol/l) $P < 0.001$, this shown in (Table 4). The

Table 1 Characteristics of the investigated groups

Groups	Number	Percent (%)	Age (years)
Control (normal)	20	100	44.75 ± 1.54
High risk	15	100	46.40 ± 1.71
Family history with no breast lesion	5	33.33	
Cases with benign breast lesion	10	66.66	
Breast cancer patients Lymphnode status	50	100	47.25 ± 1.08
LN +ve	22	44	
LN −ve	28	56	

sensitivity, specificity, diagnostic accuracy, differential positive rate (DPR), positive and negative predictive values of taurine at different cut-off values are calculated whereas sensitivity and specificity of taurine at the optimal cut-off values was (18 μmol/l) was 100% (Table 5).

The results showed no significant difference in the level of CA 15.3 between the breast cancer patients, the high risk and the control groups ($F = 0.17$, $P > 0.05$). (Table 2). Also there was No statistical significant difference could be detected in the level of CA 15-3 between the high risk patients with positive family history (21.92 ± 1.61) and those with negative family history (19.63 ± 0.72, $P > 0.05$) (Table 4). The optimal cut-off value of CA 15-3 was 21.7 (Table 5). At this level, the DPR was maximal

Table 2 Serum level of VEGF, TNF-α, taurine and CA 15-3 among different studied group

Groups	N	VEGF (Mean ± SE)	TNF-α (Mean ± SE)	Taurine (Mean ± SE)	CA 15-3 (Mean ± SE)
Control	20	8.80 ± 1.12 b	16.55 ± 2.42 ab	53.10 ± 1.49 a	21.05±0.93
High risk group	15	17.13 ± 2.08 b (0.94)	14.00 ± 2.54 b (−0.154)	30.7 ± 1.38 b (−0.59)	20.93 ± 0.74 a (−0.006)
Cancer patients	50	146.18 ± 14.85 a (15.61)	26.46 ± 3.29 a (0.59)	13.79 ± 0.65 c (−0.72)	21.11 ± 0.65 b (0.003)
F-ratio		27.91	3.45	380.7	14.45
P-value		<0.001	<0.05	<0.001	NS

$P < 0.05$: significant, $P < 0.001$ highly significant

Table 3 Serum level of VEGF (pg/ml), TNF-α (pg/ml), taurine (μmol/l) and CA 15-5 levels in LN +ve and LN −ve among breast cancer patient

Patient groups	N	VEGF (Mean ± SE)	TNF-α (Mean ± SE)	Taurine (Mean ± SE)	CA 15-3 (Mean ± SE)
LN-Positive	22	199.13 ± 17.54	22.47 ± 4.33	14.33 ± 0.68	22.48 ± 1.05
LN-Negative	28	87.75 ± 8.46	20.53 ± 3.72	13.37 ± 1.08	19.97 ± 0.77
t-test (*P*-value)		<0.001	NS	NS	NS

Table 4 Serum level of VEGF (pg/ml), TNF-α (pg/ml), taurine (μmol/l) and CA 15-3 among high risk groups

Groups	N	VEGF (Mean ± SE)	TNF-α (Mean ± SE)	Taurine (Mean ± SE)	CA 15-5 (Mean ± SE)
H.R. have benign breast lesion	5	14.4 ± 1.63	17.5 ± 6.00	22.3 ± 1.42	21.92 ± 1.6
H.R with family history	10	16.7 ± 2.08	14.4 ± 4.61	47.4 ± 2.75	19.63 ± 0.72
t-test (*P*-value)		NS	NS	<0.001	NS

Table 5 Sensitivity, specificity, differential positive rate, positive predictive value and negative predictive value of CA 15-3, VEGF, Taurine and TNF-α under the cut-off values

Serum marker	Cut-off value	Sensitivity	Specificity	Diagnostic accuracy	Differential positive rate (DPR)	Positive predictive value	Negative predictive value
CA-15	21.7	40	73.33	47.69	13.33	83.33	73.17
TNF-α	15	60	66.66	61.53	26.66	85.71	66.66
VEGF	25	92	86.66	90.76	78.66	95.83	23.52
Taurin	18	100	100	100	100	100	100

(13.33%) while the sensitivity, specificity and the diagnostic accuracy were 40, 73.33 and 47.69%, respectively. The positive and negative predictive values were 83.33 and 73.17%, respectively.

Results for angiogenic biomolecules

Our results for serum VEGF showed a highly significant difference between different studied groups ($F = 27.91$, $P < 0.001$). The breast cancer patient group showed the highest VEGF level (146.18 ± 14.85 pg/ml) among all groups; recording 15.61-fold increase, than control, whereas the high risk group recorded (17.13 ± 2.08 pg/ml;) giving only a 0.94-fold increase in relation to control healthy females, in which VEGF was 8.80 ± 1.12 pg/ml (Table 2). No statistical significant difference could be detected in the level of VEGF between the high risk patients with positive family history without breast lesion (17.5 ± 6.00 pg/ml) and those with benign breast lesion (14.4 ± 4.61 pg/ml, $P > 0.05$) (Table 4). In lymph node-positive cases the level of VEGF recorded (199.13 ± 17.54 pg/ml) was highly significantly increased than the lymph node-negative cases (87.75 ± 8.46 pg/ml, $P < 0.001$) (Table 3). The optimal cut-off value of VEGF was 25 pg/ml (Table 5) At this level, the DPR was maximal 78.66, while the sensitivity, specificity and the diagnostic accuracy were 92, 84 and 90.76%, respectively. The positive and predictive values were 95.83 and 23.52%, respectively.

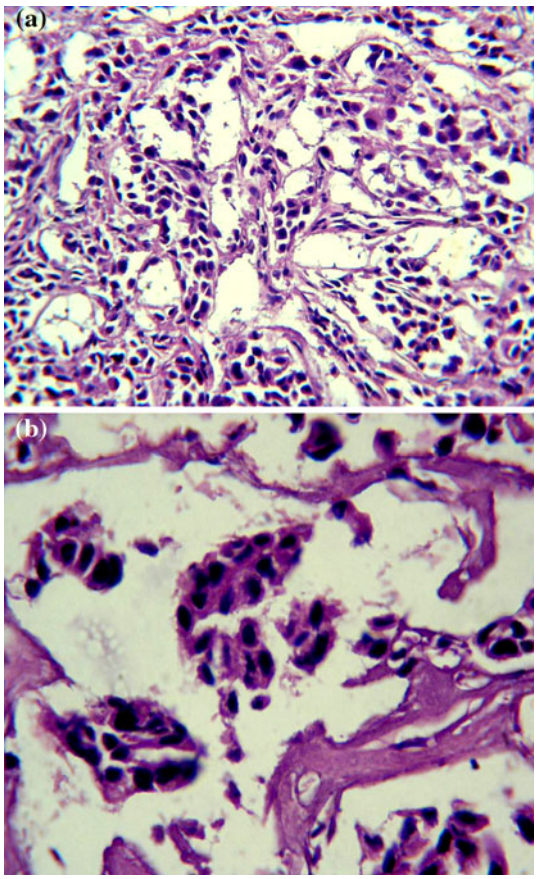

Plate 1 Hematoxline film for invasive duct carcinoma. **a** Invasive duct carcinoma grade II (H&E). **b** Invasive duct carcinoma grade III (H&E); DAB X400

Results of immunohistochemical investigation

Angiogenesis assessed by immunohistochemical staining of intracellular VEGF and microvessel density mark CD31 expression, in tissue sample, the results showed a highly significant increase in the density of VEGF in breast cancer cases, and this increase was more in lymph nod positive cases of higher grade (Plate 3a–c). It was also noticed that VEGF expression was at low levels in adjacent normal tissue and benign tissues in the high risk group. Also the microvessel density expressed by the CD31 staining showed significant increase among breast cancer cases as presented in and shown in Plates 1, 2 and 3 (Table 6).

Results of apoptotic biomolecules

- TNF-α level results showed a significant difference between the breast cancer patients and control group ($F = 3.45$, $P < 0.05$) The breast cancer patient group showed the highest TNF-α level (26.46 ± 3.29 pg/ml) in all groups, *recording only 0.59*-fold increase. Whereas the high risk group and the control group recorded comparable values not significant with each other (14.00 ± 2.54 pg/ml and 16.55 ± 2.42 pg/ml, respectively) (Table 2). The optimal cut-off value of TNF-α was 15 pg/ml, at this threshold, the DPR was maximal (26.66%), while the sensitivity, specificity, diagnostic accuracy were 60, 66.66 and 61.53%, respectively. The positive and predictive values were 85.71 and 66.66, respectively (Table 5).
- Caspase-3 enzyme activity: caspase-3 measured in tissue samples of the surgically resected malignant tissue and normal adjacent tissues. The enzyme activity in the tissue homogenates were statistically higher in adjacent normal tissues (105.33 ± 0.88 U/l) than the malignant tissues (64.75 ± 5.62 U/l, $P < 0.001$), Table 7, clarify this important finding.

The value of serum VEGF, taurine and CA15.3 in the early diagnosis of breast cancer in a group of high risk patients are evaluated by ROC curve analysis (Fig. 1a–c) respectively. The minimal area of acceptance of diagnostic markers = 0.7 (Sox et al. [20]). For serum taurine, the area

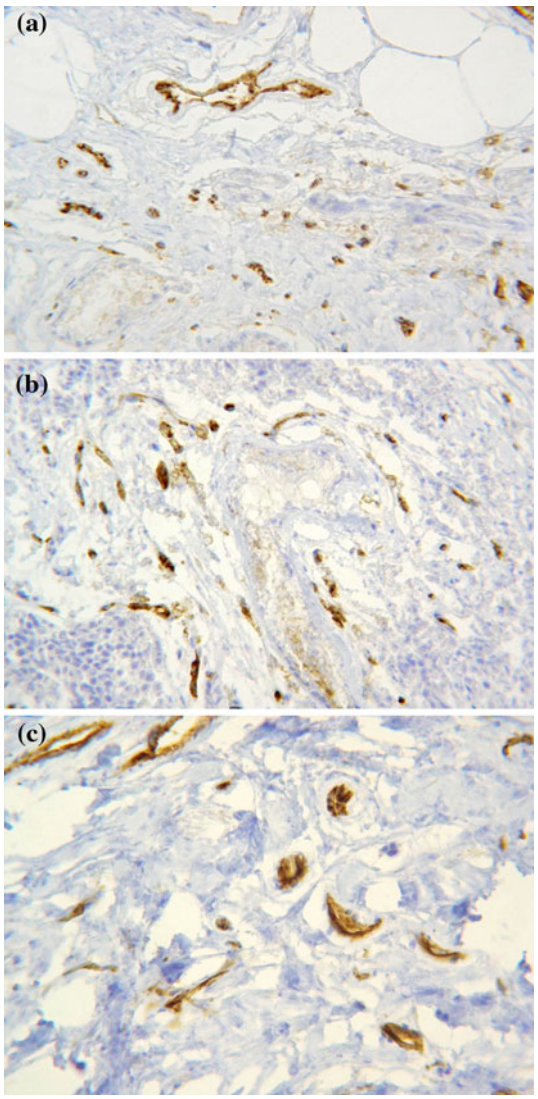

Plate 2 **a** CD 31 in invasive duct carcinoma with microvessel density score 1. **b** CD 31 highlight of micrvessel density score 2*. **c** CD 31 highlight of microvessel density score 3

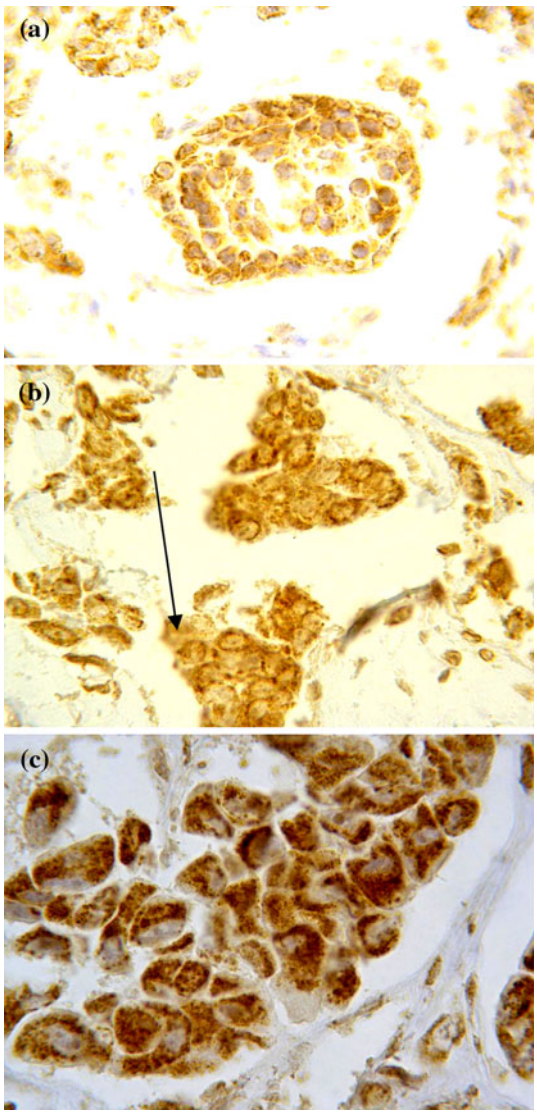

Plate 3 **a** VEGF score 1; DAB X400. **b** VEGF score; 2 DAB X400. **c** VEGF score 3; DAB X400

under the curve (AUC) = 1 indicating the validity of using taurine in diagnosis of breast cancer in patients with high risk of breast cancer, and for VEGF, AUC = 0.95 indicating also the validity of using VEGF in diagnosis. While for CA1503, AUC = 0.523 indicating the rejection of using CA 15-3 in diagnosis of breast cancer in patients with high risk.

The angiogenic/apoptotic ratio was calculated as VEGF/TNF ratio and statistically studied among the different group. There was a highly significant difference in the angiogenic/apoptotic ratio between the breast cancer patients, the high risk and the control groups ($F = 11.27$, $P < 0.001$). The breast cancer patient group showed the highest ratio (13.55 ± 2.03), among all groups whereas the high risk group and the control group showed comparable values insignificant from each other (2.21 ± 0.58 and 1.23 ± 0.36, respectively). The breast cancer group showed a *10.01-fold* increase than the control while the high risk group showed a *0.79-fold* increase only (Table 8).

The angiogenic/antiangiogenic ratio was also calculated as VEGF/Taurine ratio recording a highly significant difference in the angiogenic/antiangiogenic ratio between the breast cancer patients, the high risk and the control groups. The breast cancer patient group showed the highest ratio (11.21 ± 1.09), *giving* $F = 33.64$, $P < 0.00$; whereas the high risk group and the control group showed comparable values insignificant from each other (0.82 ± 0.08 and 0.16 ± 0.02, respectively). The breast cancer group showed a *69.06-fold* increase than the control while the high risk group showed *4.12-fold* increase only (Table 8).

Table 6 The result of immunohistochemical stain of VEGF and CD31

Groups	Number		CD31	VEGF
	Total N	Number of estimated cases	Median range	Median range
a Breast cancer patients and high risk group				
High risk group	15	4	2 (0–1)	1 (0–1)
Breast cancer patients	50	23	2 (1–3)	2 (0–3)
P-value (U-test)			<{0.05}	<(0.05)
b Lymph nod positive and lymph nod negative cases of breast cancer patients				
LN positive	28	15	2 (1–3)	1 (1–3)
LN negative	22	8	2 (1–1)	2 (0–1)
P-value (U-test)			<{0.001}	<(0.001)

Table 7 Caspase-3 enzyme activity (U/l) in some malignant and normal breast tissues

	Number of samples	Caspase-3 enzyme activity (U/l)
Malignant tissues	8	64.75 ± 5.62
Normal tissue	3	105.33 ± 0.88
t-test (*P*-value)		<0.001

Discussion

Circulating proteinic biomarkers are secreted by tumor cells or by their environmental cells and they have a variable specificity. CA15.3 is the most widely used serum marker in breast cancer, currently its main uses are in the surveillance of patients with diagnosed disease and monitoring the treatment of patients with advanced disease [21, 23].

The results of serum CA15.3 revealed no significant change compared to normal in all the studied groups and this agree with previous studies that emphasized that the most important application of CA15.3 is in predicting outcome and monitoring therapy in patients with advanced breast cancer [23–25].

The angiogenic factor VEGF, showed a highly significant increase in breast cancer cases compared to normal group, this supports the finding of previous studies which concluded that, expression of VEGF and its intensity, are associated with a significantly lower outcome of early breast cancer [26, 27]. Also VEGF was found to be the only factor expressed throughout the entire tumor life cycle of a breast tumor [6]. Our results showed also that in lymph node-positive breast cancer patients; the level of VEGF was significantly increased than in lymph node-negative patients. This observation agree with the previous studies reported that higher levels of VEGF protein in breast tumors have been shown to be associated with poor prognosis in breast cancer patients [28–31].

The results of immunohistochemistry showed that the expression of both VEGF and CD31 was significantly increased in cancerous tissue compared to adjacent normal tissue and benign tissue in the high risk group. These results confirmed the enhanced angiogenesis in breast cancer cases, and seem to agree with previous study concluded that in breast cancer, immunohistochemical stain of VEGF, CD31 and D2-40 microvessel density was correlated significantly with several prognostic factors, including lymph node metastasis [9].

The results of caspase-3, in this study showed that the enzyme concentration in the tissue homogenates were statistically higher in the adjacent normal tissues than the malignant tissues. This decrease in the level of caspase-3 reflect a down regulation and inhibition of the apoptotic feature in the malignant breast tissue. This result seem to agree with previous studies which emphasized that caspase-3 plays an important role in apoptotic process, and down regulation of caspase-3 has been reported in breast cancer and other types of cancers [32, 33].

The result for TNF- α showed only *0.59-fold* increase in breast cancer cases compared to control, this slight elevation may be contributed in part to the enhanced angiogenesis presented by the elevated level of VEGF in breast cancer cases, and this supported by previous study who reported that, tumor angiogenesis is promoted by enhanced expression of the endogenous TNF-α and Interleukin-1β (IL-1β) [34].

Human have evolved a highly sophisticated and complex antioxidant protection system to control and neutralize free radicals, among them are sulfur containing amino acids such as methionine, cysteine, and taurine whose represent one of the most important antioxidant systems in human [35–38].

The most impressive observation in our work is the results of the antioxidant taurine which showed significant decreases in its serum levels in both breast cancer and high risk groups when compared to normal control group.

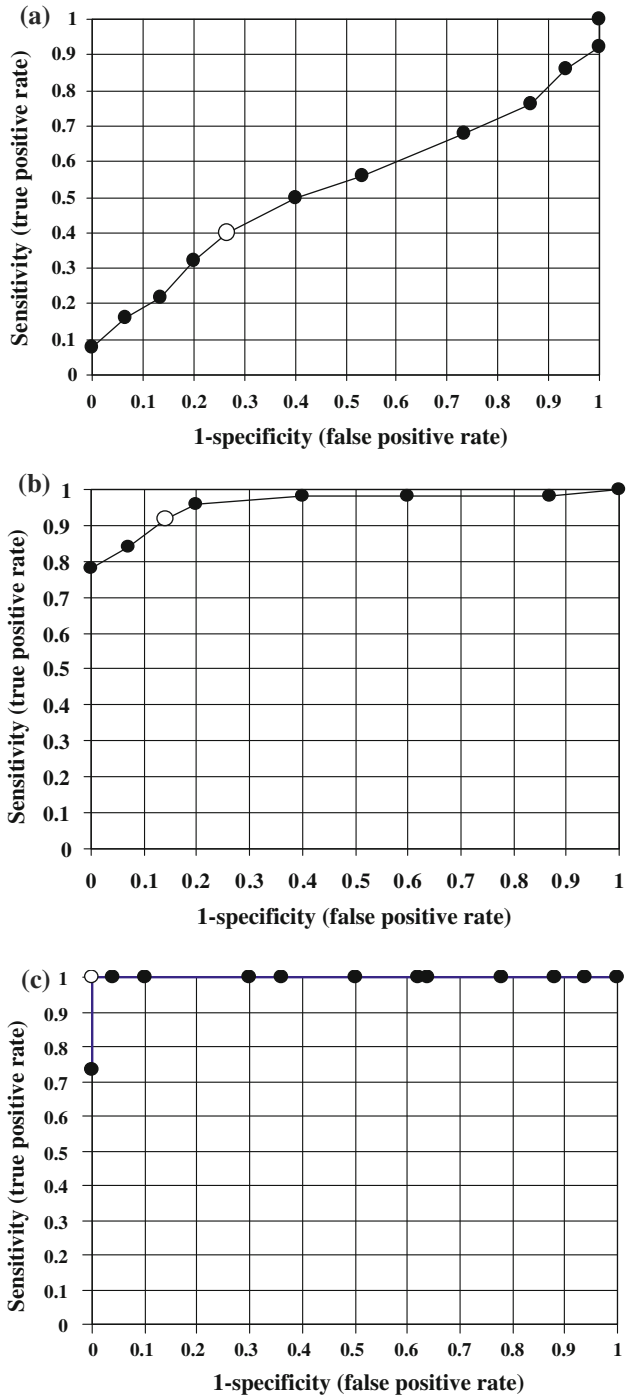

Fig. 1 ROC curve analysis for **a** CA15.3, **b** VEGF and **c** for Taurin

This marked decrease in serum taurine levels in breast cancer cases may be contributed to the down regulation of the apoptosis process in malignant breast tissue, presented by the lower values of caspse-3 activity. This finding supported the previous researches postulated that taurolidine inhibit the growth of human tumor cell lines in vitro through the induction of apoptosis in tumor cells [39–41].

This diminished level of serum taurine in breast cancer group may explain the enhancement of tumor angiogenesis, as there is a reciprocal relationship between taurine and VEGF clearly observed in our results, confirmed also by the angiogenic/antiangiogenic ratio calculated as VEGF/taurine which showed 69.06-fold increase in cancer patient than the control.

The most interesting finding in our results is the level of taurine in the high risk group which recorded also significant decreased level than that of normal group (30.7 μmol/l vs. 53 μmol/l). Meanwhile among high risk group, the group of patients having benign breast lesion showed a significantly decreased level of taurine than the group with positive family history and having no breast lesion (22 μmol/l vs. 47.4 μmol/l). It is clear that, even for cases having only positive family history without any sign of benign or malignant tumor their taurine level didn't exceed the lower limit recorded in control healthy group (46 μmol/l).

It remain of interest, to highlight the range of serum taurine levels among the group of high risk patients having benign breast lesion, as the maximum and minimal values was 31 and 18 μmol/l, respectively, and in group having only positive family history was 57 and 40 μmol/l, respectively, while in control group this range was 70 and 46 μmol/l. So we could spiculate tumor transformation when taurine level exhibited a value lower than 30 μmol/l. One can also notice that there was a safe margin for serum taurine level at 40 μmol/l which is the lower value recorded in high risk with only family history.

The use of the area under Receiver Operating Characteristic curve (ROC) curve was useful in the elucidation of the validity of a specific marker in the early detection of breast cancer. The calculated area for each of taurine and VEGF in our results proved the validity of using them as an early marker in diagnosis of breast cancer. While that for CA15.3 revealed the rejection of this marker for early diagnosis, and this finding agree with previous finding by several studies emphasized that CA15.3 lacking sensitivity for early disease, and also lacks specificity for breast cancer [8]. The impressive finding is the sensitivity of taurine which recorded 100% sensitivity, at its optimal cutoff value (18 μmol/l), as we have no false positive or false negative result for serum taurine levels among breast cancer cases.

Based on these finding we can suggest that taurine measurement is more efficient in predicting highly susceptible women for breast cancer as its level was clearly decreased even before the malignant transformation occurs and in high risk women having benign breast lesion.

VEGF, has been suggested to play a role in the development of cancer, and many studies revealed that the expression of VEGF could be driven by oxidative stress [42, 43]. We can suggest that the decreased level of serum taurine may results on oxidative stress which involved in

Table 8 Angiogenic/apoptotic (VEGF/TNF-α) and angiogenic/antiangiogenic (VEGF/taurine) ratios in patient groups and control

Groups	N	VEGF/TNF -α (Mean ± SE)	VEGF/Taurine (Mean ± SE)
Control	20	1.23 ± 0.36 b	0.16 ± 0.02 b
High risk group	15	2.21 ± 0.58 b (0.79)	0.82 ± 0.08 b (4.12)
Cancer patients	50	13.55 ± 2.033 a (10.01)	11.21 ± 1.09 a (69.06)
F-ratio		11.27	33.64
P-value		<0.001	<0.001

The different letters indicate statistically significant means according to Duncan multiple range test. The numbers in between brackets indicate the folds of change from control

$P < 0.05$: significant, $P < 0.001$ highly significant

the initiation of the malignant transformation of the breast tissue. Moreover this oxidative stress result in an over production of the angiogenic factor VEGF, and this supported by several studies emphasized that oxidative stress can cause angiogenesis within breast carcinoma through increments of cell production of the angiogenic factor VEGF and IL-8 and also by promoting secretion of matrix metalloproteinase-1(MMP-1), and by other mechanisms. Also other studies found that regulation of VEGF may be achieved by ameliorating the oxidative stress through the administration of antioxidants [44–46]. Furthermore other studies emphasized that using taurine as a potent free radical scavenger, could ameliorate methotrexate (MTX)–induced oxidative injury and modulate immune response, and hens alleviating the systemic side effects of chemotherapy, as well as nephrotoxcicty of tamoxifen on treatment for breast cancer [47, 48].

Finally we can suggest that taurine by its role as antioxidant, antiangiogenic and apoptotic not only act as anticarcinogens but it also can be used as chemo-preventive in case of cancer therapy, especially in breast cancer [4, 38, 44, 48]. Recently, a lot of researches through light on the use of taurilidine as antineoplastic drug in human bladder carcinoma [49] gastro-intestinal cancer [50] to prevent the development of lung metastasis [51], and it may offer additional therapeutic option in patient with colon adenocarcinoma [51], as a useful modulator to enhance the therapeutic index of some antitumor agents [16], in the treatment of oesophageal cancer [14], in treatment of malignant mesothelioma [52] and as a biomarker in non-muscle invasive bladder cancer [53]. In conclusion and based on the present study we can strongly suggest the assessment of taurine level in sera of patients with high risk of breast cancer and in the early diagnosis of any malignant changes in the breast.

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