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Inflammatory markers and risk of breast tumor

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ABSTRACT

Serum amyloid A have been identified as a potentially useful as a non-invasive biomarker for prognosis of tumors such as breast cancer.The present study aims to evaluate the associations between SAA and hormones in breast tumor, and the possibility use gel electrophoresis as a tool for SAA diagnosis. A total of 77 patient attending center of Breast Cancer, and33 healthy individual as a control were included in this study. The results indicated a significant increase of BMI,SAA,CRP, globulin and a significant decrease in albumin level in sera of benign and malignant groups compared with control group.A significant decrease in E2 level was observed in postmenopausal malignant compared with control.Three bands were observed in gel electrophoresis. Our conclusion that SAA may be useful as a tumor marker and it can be possible to adopt gel electrophoresis as laboratory technique for detection presence of SAA.

Keywords: Tumor, breast cancer, amyloid A, CRP, hormone, electrophoresis

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INTRODUCTION

Breast cancer is one of the most diagnosed life-threatening and deadly cancer in women worldwideand second most common cancer overall [1, 2].Epidemiologic studies have established a handful of risk factors for breast cancer. Life style factors overweight and obesity, as measured by high body mass index (BMI), moderately increases the risk of post-menopausal breast cancer and is one of the few modifiable risk factors for breast cancer [3, 4].Inflammatory status can also be a prognostic factor for breast cancer by promoting mammary tumor development through mechanisms involving chronic activation of humeral immunity and polarized innate inflammatory cells [5]. This inflammatory disordercauses an increase or decrease by at least 25 percent in plasma concentration of some protein called acute phase proteins (APP)which are synthesized by the cytokine mediated induction of the common transcription factor, nuclear factor-IL6 (NFIL6)[6]. APPis large and variedgroup of glycoproteins in serum. All the up-regulated proteins have been called positiveAPPlike C reactive protein (CRP), Serum amyloid A (SAA) who rise as early as 4 hours after inflammatory stimulus and attain their maximum levels within 24 to 72 hours and also decline very rapidly, while APP which are down-regulated called negativeAPP such as albumin [7, 8].Serum amyloid A as anacute phase reactant, it is also a high-density lipoprotein (HDL) associatedlipoproteinand a modulator of inflammation. Its levels may rapidly increase by up to 1000-fold in response to acute inflammation, therefore it is a well-established indicator of inflammation in the body.SAA has been reported in different human malignanciesas a predictor of cancer risk and as a prognosticparameter [9]. Elevated levels of SAA have been identified as a potentially useful as a non-invasivebiomarker for prognosis ofhuman tumorssuchas breast cancer[5].breast cancer is influenced by hormone-related pathways which considered to be the most established risks factors, such as estrogen which believed to increase the risk of breast cancer because it act on proliferation of cells and increasing the risk of errors during deoxyribonucleic acid replication of cell division which can lead to cancer [10, 11]. However testosterone is also believed to indirectly increase the risk of breast cancer due to its strong affinity to sex-hormone binding globulin and binding competitively with this protein it increases the amount of free

estradiol by affecting the fraction of bioavailable estradiol therefore it affects breast tissue and promotes tumor development [11].

Few studies have explored factors that correlate with inflammatory markers in benign and malignant breast cancer and none have examined the correlates of E2 and T with SAA specifically. The aim of the present study was to thoroughly evaluate the associations between markers ofinflammation SAA and hormones in breast tumor.Also our aim is to study the possibility use serum protein electrophoresis (SPE) as a tool for SAA diagnosis, where SPE is a laboratory technique that examines specific serum proteins. Serum (fluid portion of the blood without clotting factors) is placed on special paper treated with agarose gel and exposed to an electric current that separates serum proteins into several fractions by size and electrical charge [12].

EXPERIMENTAL SECTION

A total of 77 patient women (ages 28–71 years), attending center of Breast Cancer in Al-Eluia Hospital for Woman Care, Hospital of Radiotherapy & Nuclear Medicine and Dejla privet hospital were included in the present study .All patients were undergoing surgery for primary invasive breast cancer .The complete physical examination was done to every patient. The final diagnosis was established by aspiration of cysts (FNA) to check cytology, histology (biopsy) and Mammography.All blood samples were taken from patients who had not received any treatment of any kind of therapy that might influence on their hormonal level (this include chemotherapy, radiotherapy and hormonal therapy).As a control of 33 healthy individual with age (23-65year) was included in this study. In order to measure Testosterone and E2 levelswhich affected by the menstrual cycle, the main groups were further divided into sub-groups, premenopausal subjects during the follicular phase of menstrual cycle and postmenopausal subjects as illustrated in Table 1.

Five milliliters of venous blood samples were collected from the patients and the healthy controls groups then immediately transferred into plan tube and allowed to coagulate at room temperature then centrifuged at 3000 rpm for 5 min. The resulting serum was separated and stored at (-20°C) until assay. MI is be calculated directly as weight (in kilograms)/[height (in meters)]^2.Biochemical tests including, SAA, CRP, E2, testosteroneweredetected by using Enzyme Linked Immuno Assay (ELISA) kits and performedaccording to the manufacturer's instructions. Total proteins and Albumin werealso determined for all subjects by Biuret method and BromoCresol Green respectively. Agarose gel electrophoresis analysis was also performed to detect presence of SAA in subject's sera.

Table1: Classified groups according to menopausal: premenopausal andpostmenopausal.

Group	No.	age in year	Sub-Group	No.	age in year
Control	33	23-65	***Pre-menopause***	18	23-40
			Post-menopause	15	49-65
Benign	38	28-68	***Pre-menopause***	18	28-48
			Post-menopause	20	48-68
Malignant	39	28-71	***Pre-menopause***	19	28-45
			Post-menopause	20	45-71

For statistical analysis (SPSS 15.0 Chicago, IL, USA)statistical software for windows was used.Student t-test was used to evaluate the differences in the means values for all biochemical tests between the studied groups. Pearson's rank correlation was also used to study the correlation between SAA concentrations and other parameters in all groups. For all tests,two-tailed analysis $p<0.05$ was considered a statistically significant.

RESULTS AND DISCUSSION

The results presented in Table2 indicated presence a significant increasein BMIofboth benign and malignant groups compared with control group ($p<0.05$).This results were agreement with previous study which demonstrated that obesity is associated with poorer breast cancer survival in pre- and post-menopausal patients [13].Also another study reported thathigh body mass index (BMI) is an established risk factor for postmenopausal breast cancer [14].

A non-significant increase of total protein concentration in benign and malignantgroups were observed in the present study compared to that of control group ($p>0.05$), These results were found to be in the same line with the result obtained by other investigators who found that total serum protein was lower than the normal range in breast cancer women[15]. Furthermore sometimes total serum protein decreased in abnormal level when there was weight loss in breast cancer patients [16].

Table2:Meanvalues±SD of BMI, SAA, CRP, total protein, albumin, globulin, A/G ratio

PARAMETERS	CONTROL MEAN±SD	BENIGN MEAN±SD	*P*	MALIGNANT MEAN±SD	*P*
BMI	26.1635±3.82220	30.0476±4.28572	0.001	28.9231±4.96721	0.022
T.P	7.2974±1.04227	7.5892±1.16582	0.315	7.1691±1.19431	0.649
ALB.	4.0189±0.55310	3.7197±1.03499	0.188	3.3909±0.51317	0.000
GLOB.	3.1523±1.04575	4.0085±1.73225	0.049	3.8643±1.35287	0.043
A/G RATIO	1.4571±1.03139	1.0214±.68767	0.092	0.9843±.44081	0.025
SAA	3446.4438±1211.73222	5102.0562±897.09481	0.000	5379.5343±536.92347	0.000
CRP	2.9779±2.51022	6.0518±5.29572	0.019	8.3058±6.57151	0.001

Albumin as a negative APP were also measured and found presence a non-significant decrease of albumin concentration in benign group compared with control group ($p>0.05$), while a highly significant decrease in albumin concentration of malignant group was observed compared to control group ($p<0.001$).These results were in agreement with those of Al Murri*et*AM *et al* who reported a significant association between inflammatory response (reduced in albumin concentration) and patients with primary operable breast cancer[17]. The decrease in serum albumin may affect the biological functions of circulating albumin including binding and transporting of hormones and growth factors, inhibition of platelet function and thrombosis[18[. High levels of pro inflammatory surrounding cells, alters metabolic homeostasis in the tumor micro environment as a part the systemic inflammatory response against the tumor[19].Therefore albumin concentration can be down regulated by pro inflammatory cytokines such as tumor necrosis factor (TNF) and interleukin 6 (IL-6)which synthesis and increase acute-phase protein production in isolated hepatocytes[20].A significant increase of globulin concentration in benign and malignant groups compared with that of control group ($p<0.05$) were observed in the present study, this significant elevation of globulin in all patients groups was expected due to the decrease in serum albumin with subsistence of serum total protein within the normal range .These results were agreement withresults of Raya KM *et al* who studied serum proteins concentration among several types of cancer including breast cancer [21]. While these results were disagreement with those of Tareq*et al* who reported a non-significant decrease in serum globulin of breast cancer [15].High levels of globulins are caused by elevation of acute-phase proteins and immune globulins, as well as other serum proteins [22].

The A/G was calculated in this study. We indicated presence a non-significant decrease of A/G ratio in benign group compared with control group ($p>0.05$), and a significant decrease in A/G ratio of malignant group compared to control group ($p<0.05$).These results were agreement with previous studies which showed a low A/G ratio and it was predictive for poor survival[23-25]. This decrease in A/G ratio was predictable due to the decrease in serum albumin and increase in globulin for all reasons we have mentioned before.

Human serum amyloid A (SAA) level was measured to evaluate its usefulness in diagnosis and follow up of patients with breast cancer. The results we obtained indicated presence of a highly significant increase of SAA concentration in benign and malignant compared to control group ($p<0.001$).These results were agreement with those Pierce BL *et al*who have been observed elevated SAA levels in breast cancer patients[5]. Similarly of Schaub*et al* and Guojun *et al*who both reported that the blood level of SAA is significantly high in breast cancer patients depending on tumor stage [26,27]. Also JG Rayne*set al* found that in non-malignant disease the concentrations of SAA were higher than the designated upper limit of normal [28]. By contrast, Guojun *et al* found that there was no statistically significant difference in SAA concentrations among the benign breast disease compared to healthy controls [27].TheSAAsynthesis as an acute-phase protein is largely regulated by inflammation- associatedcytokine-peptide hormone signals. Inflammatory status may be an important prognostic factor for breast cancer prognosis; therefore a high concentration of circulating SAA may represent an ideal marker for inflammatory disease tissue injury, infection, and inflammation [5, 29].

The results of CRP concentration indicated presence of a significant increase in benign group compared with control group ($p<0.05$).Also presence of a highly significant increase in CRP concentration of malignant group compared to control group ($p<0.001$).These results were in agreementwith those of O'Hanlon *et al*and Blann*et al*whose reported that breast cancer patients have elevated concentrations of CRP, and these concentrations are higher in women with more advanced stage of disease thus CRP may be an important prognostic factor for breast cancer[30,31]. Another report by Praveen *et al*who reportedelevation of CRP in sera of breast cancer patients[32]. Chelsea *et al* andKatherine W. *et al* supported the elevation of serum CRP in benign breast disease that observed [33, 34]. Women with benign proliferative breast disease canincrease the risk for developing breast cancer by carcinogenic stimuli; breast epithelial-cell carcinogenesis follows the transformation of the cell, once a cell has deviated from its normal function, it emits signals recognized as foreign by the inflammatory-response detection mechanisms [33]. In addition tumor cells in the breast microenvironment can induce the inflammatory cascade; therefore inflammation may be related to early carcinogenic changes [35]. CRP was measured because it is a classical acute-phase protein

displaying rapid and pronounced rise of its plasma concentration in response to acute inflammation, infection, and tissue damage, also circulating levels of CRP are moderately elevated during chronic inflammatory diseases and cancer [36].

Table (3) shows the measurement of Estradiol, Testosterone, and the (T /E2)in sera of control, benign and malignant breast cancer depending on their menopausal statue (pre menopause and post menopause). In agreement with previous studies in Malaysia and Egypt [11,37] our study found increasing serum E2 levels in pre-menopausal malignant breast patients. Unfortunately, it was not statistically significant. On the other hand our results are in full agreement with other previous studies [38-40]that suggested a non-significant increase in mean E2 concentrations among pre-menopausal cases in comparison with controls. Furthermore a study by Fortner et al for women postmenopausal at diagnosis, a significant decrease ($p<0.05$) was observed in E2 which is fully agrees with this study[41]. Estrogens are associated with increased proliferation and decreased apoptosis, and may promote proliferation of cells with genetic mutations [41]. Increased concentrations of circulating estrogens were found to be strongly associated with increased risk for breast cancer in postmenopausal women. Furthermore experimental studies in animals have shown that estrogens can promote mammary tumors and a decrease in exposure reverse this effect [10].

Testosterone levels are strongly supported by previous study that reported similar results and accordingly suggested that there was no statistically significant($p>0.05$) elevation in serum testosterone among premenopausal patients, while in postmenopausal malignant breast patients; serum testosterone levels were significantly elevated[11].Several studies were similar to ours; Ghada et al and Evangeliaobserved that postmenopausal breast cancer subjects had statistically significantly ($p<0.05$) higher median levels of testosterone compared with control subjects[42,44]. Testosterone is also believed to indirectly increase the risk of breast cancer due to its strong affinity to sex-hormone binding globulin and binding competitively with this protein in this way it increases the amount of free estradiol by affecting the fraction of bioavailable estradiol therefor it affects breast tissue and promotes tumor development[11].Also androgens can be converted to estrogens in breast tissue via aromatase, therefore, they are indirectly associated with breast cancer risk[41].Serum T/E2 ratio was calculated as testosterone in ng/ml divided by estradiol in ng/ml, These results indicated presence of a non-significant decrease of T/E2 ratio in pre-menopausal benign group compared with control group ($p>0.05$), also presence of a non-significant increase in T/E2 ratio of pre-menopausal malignant group compared to control group ($p>0.05$). Another non-significant increase for T/E2 ratio appeared in post-menopausal benign group ($p>0.05$). In contrast, a significant increase ($p<0.05$) was observed in post-menopausal malignant breast cancer patients. To the best of our knowledge, this is the first study to specifically focus on the T/E2 ratio among this type of cancer. These results were predictable due to the decrease and increase in serum E2 and testosterone respectively among post-menopausal subjects for all reasons we mentioned before. The results we reveled in this study were in the same line with Tanaka *et al* who indicate that elevated levels of serum T/E2 ratio are predictive of Hepatocellular Carcinoma risk among male cirrhotic patients[44].

The overall results indicated a non-significant difference for all studied parameters between benign and malignant groups ($p>0.05$).

Table3: Measurement of E2, Testosteroneand (T /E2)in sera of control, benign and malignant breast cancer

Group	Sub-Group	E2(Pg/ml) Mean±SD	*P*	Testosterone (ng/ml) Mean±SD	*P*	T/E Mean±SD	*P*
Control	*Pre-menopause*	53.3875±32.31744		0.5579±0.16250		27.3231±42.73193	
	Post-menopause	34.1033±26.05757		0.4933±.20853		23.2841±25.29603	
Benign	*Pre-menopause*	51.4941±45.23274	0.91	0.5713±0.21157	0.858	17.3950±12.22458	0.510
	Post-menopause	24.9382±24.12500	0.372	0.5476±0.24768	0.557	39.9567±30.88397	0.181
Malignant	*Pre-menopause*	59.9220±60.35464	0.428	0.6539±0.38900	0.4	30.9824±30.60667	0.832
	Post-menopause	19.2208±10.80724	0.045	0.7593±0.35050	0.032	49.4804±25.52788	0.016

Table (4) shows general correlations between SAA concentration and other parameters (depending on Pearson's correlations)in sera of control, benign and malignant breast cancer.The current study found four significant correlations: negative significantcorrelation ($p<0.05$) between SAA and albumin was observed to be in agreement with George et al whoobserved the same significantly negative correlation between SAA and serum albumin in dialysis patients with a delay of 1–2 weeks after inflammation considering that serum albumin act as a negative acute-phase protein [45].

The present study also revealed a significant positive association ($p<0.05$) between SAA and BMI and a highly significant positive association ($p<0.001$) for SAA with age and with CRP levels. These findings were supported by Pierce *et al* who also observed a statistically significant positive association for SAA with BMI, age, and CRP

[46].Several studies also suggested that serum SAA concentration correlated positively with BMI [47, 48]. Accordingly obesity is described by chronic low-grade inflammation in the adipose tissue. These adipose tissues produce a wide range of inflammatory molecules as cytokines which are linked to the immune system or acute phase response, such as SAA and other acute phase proteins. The adipose tissue is a major site for SAA production. Results of recent study have demonstrated that higher concentrations of SAA were associated (positive association) with increasing concentrations of CRP [49].

Table 4: Shows general correlationsbetween SAA concentration and other parameters

Parameters	r value	*P* Value
Total Protein	-0.144	0.198
Albumin	-0.262(*)	0.023
globulin	0.005	0.970
Alb/glob	-0.124	0.327
CRP	0.426(**)	0.000
Age	0.349(**)	0.001
BMI	0.247(*)	0.026

** Correlation is significant at the 0.05 level*
*** Correlation is significant at the 0.01 level.*

The mechanism by which chronic inflammation is related to breast cancer prognosis is unclear. It might be due to the promotion of carcinogenesis through complex processes such as polarization of M2 tumor-associated macrophages via cytokines and then production of tumor growth factors or promotion of angiogenesis. In addition, inflammatory status is correlated with several prognostic factors such as body fatness and physical activity, which may affect prognosis through alternate mechanisms. It is also possible that inflammation is a response to the presence of undetected cancer cells, rather than being solely a contributor to tumor promotion [5].
In contrast, non-significant associations($p>0.05$) between SAA and total protein, globulin, and globulin/albumin ratio were observed.

Among all hormonal parameters we only observed a significant negative association ($p<0.05$)between SAA concentration and serum E2 in post-menopausal subjects as shown in table (5).

Table 5:General correlations between SAA concentration and hormonal parameters

Parameters	Group	r value	*P* Value
E2	*Pre-menopause*	0.106	0.598
	Post-menopause	-0.426(*)	0.011
Testosterone	*Pre-menopause*	0.083	0.614
	Post-menopause	0.177	0.280
E2/T	*Pre-menopause*	0.009	0.963
	Post-menopause	0.210	0.325

** Correlation is significant at the 0.05 level.*
*** Correlation is significant at the 0.01 level.*

Meanwhile the rest of parameters had non-significant association with SAA.To the best of our knowledge, this is the first study to specifically focus on the relationship between SAA and E2 among this type of cancer.

Anti-inflammatory as well as pro-inflammatory responses to estrogens have been reported and data have been revealing that estrogens decrease the expression of adhesion and chemokine molecules as a response to inflammation promoters in various experimental systems which may explain the negative association between SAA and E2[50]. Also this result was agreement with previous study which reported that estrogens have both anti-inflammatory and pro-inflammatory roles, and that estrogens do not function in the same manner in all inflammatory diseases, due to the massive variable responses of immune and repair systems [51].

As shown in Fig(1) agarose gel electrophoresis analysis of standard SAA which considered as reference gel revealed that the protein resolved into three bands (A,B,C) which represent the SAA isoforms. We observed that C band was found in gels of malignant and benign between the β and γ globulins regions but was never observed in gel of healthy controls. Also band A was observed in malignant and benign between the albumin and α1 globulins regions but was never observed in gel of healthy controls. On the other hand band B was not clear due to the effect of β-globulin fractions. These results indicated presence of increased levels of SAA concentration in sera of malignant and benign compared with control group, which support the results we obtained from ELISA test.

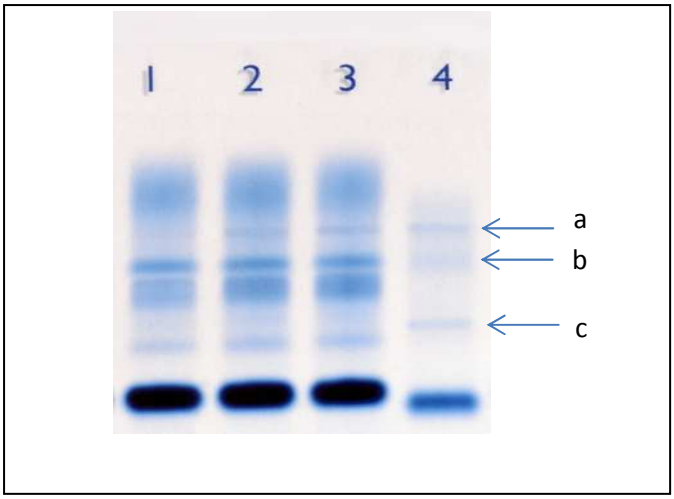

Figure (1). Serum agarose gel electrophoresis pattern of SAA in sera of control (1), benign (2), malignant breast cancer patients, and standard SAA (4)

CONCLUSION

In conclusion, our results suggest that an elevated SAA levels which have been observed insera of benign and malignant breast patients by using both of ELISA kit and agarose gel electrophoresis analysis found to bea significant prognostic factor that may be useful as a tumor markerwhether it's benign or malignant. Also it can be possible to adopt serum protein electrophoresis as laboratory technique for detection of SAA elevated and diagnosis presence of tumor.

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