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Association between estradiol, estrogen receptors, total lipids, triglycerides, and cholesterol in patients with benign and malignant breast tumors

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Abstract

This study addresses the correlation between the levels of estradiol (E_2), total lipids, triglycerides, and cholesterol in serum and tissue samples of age-matched patients with benign (40 cases; 16 were premenopausal and 24 were postmenopausal) and malignant (50 cases; 17 were premenopausal and 33 were postmenopausal) breast tumors. Estradiol levels were determined in serum and cytosol, estrogen receptors (ER) were assayed in cytosol, and total lipids, triglycerides and cholesterol were determined in serum and membrane fractions of all benign and malignant breast disease patients. Serum E_2 was significantly higher in malignant cases than benign ones ($P < 0.05$) with a significant reduction (40%) in postmenopausal than premenopausal women. ER-positive tumors were significantly higher in postmenopausal women with malignant breast tumors than benign cases ($P < 0.05$). Tissue levels of total lipids, triglycerides, and cholesterol were highly significantly increased in breast cancer women than women with benign breast diseases ($P < 0.05$, $P < 0.005$ and $P < 0.05$ respectively) and they were also significantly correlated with estradiol levels. It could be concluded that the uptake of lipids from plasma by the tumor tissue is greatly correlated to estradiol and it may confirm the possible role of lipids as risk factor in breast cancer.

Keywords: Estradiol; Estrogen receptor; Total lipids; Triglycerides; Cholesterol; Benign breast disease; Breast cancer

1. Introduction

Many epidemiological evidences suggest that dietary and endocrine factors alter the risk of breast cancer. Hence, several experimental and clinical attempts have been made to clarify the role of estrogens in the etiology of female breast cancer [1,2]. In pre-menopausal women, the major source of endogenous estrogens is the ovary. Menopause signals a marked decline in the amount of circulating estrogens and this decline is at least part of the explanation for the decreased risk of breast cancer associated with early menopause. In post-menopausal women, the major source of estrogens is the peripheral conversion of androstenedione in fat tissues [3–8]. This offers the most probable explanation for the association of obesity with increased risk of breast cancer in postmenopausal women [9]. Obesity has been associated with breast cancer by many researchers. It has been found that body fat distribution is an important factor in breast cancer development and prognosis [10–12].

Human mammary tissue metabolizes lipids from plasma, a process affected by female gonadal hormones. Both benign and malignant proliferation of breast tissue in women has been associated with changes in plasma lipids and lipoprotein levels [13,14].

Lane et al. [13] reported that prior to diagnostic biopsy, serum lipid and apolipoprotein components of low-density lipoproteins were increased in women with fibrocystic disease and early stage breast cancer but decreased in women with early recurrence.

Estradiol receptors (ER) are regarded to predict a likely success of hormonal therapeutic efforts and the

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prognosis of breast cancer patients. Post-recurrence survival is significantly longer in ER-positive than ER-negative patients and also in patients treated by anti-estrogen therapies. This prognostic advantage of ER-positive patients is interpreted by estradiol receptors as intrinsic parameters of breast cancer tissue characterizing its biological behavior than therapeutic accessibility [15–18].

This study was constructed to measure the differential distribution of estradiol in serum and cytosolic fractions of both benign and malignant breast tumor in association of estrogen receptors. Additionally, to study the influence and correlation of this distribution on the corresponding distribution of total lipids, triglycerides, and cholesterol.

2. Material and methods

2.1. Subjects

In this study, 50 newly diagnosed women with primary localized breast cancer and 40 age-matched women with different benign breast diseases were recruited. All patients were diagnosed at the Department of Surgery, Ain Shams University Hospitals and the National Cancer Institute, Cairo University between April 1997 and July 1998. Women with breast cancer were subdivided according to the menopausal status into pre- and postmenopausal subgroups and they were compared parallely with their corresponding subgroups of women with benign breast tumors. Both benign and malignant postmenopausal women included in this study had not used postmenopausal hormones for at least 3 months before collecting blood and tumor tissue, and they were defined as postmenopausal if they reported having a natural menopause or a bilateral oophorectomy.

2.2. Methods

2.2.1. Blood sample collection

Early morning fasting blood samples (10 ml) were collected from all patients with benign and malignant breast tumors pre-operatively. Samples were collected into sterile tubes without anticoagulant or preservative. After centrifugation, the serum was separated and stored in aliquots at -20°C till assayed. Serum samples were saved for the assay of estradiol (E_2), total lipids, triglycerides and cholesterol.

2.2.2. Tissue collection

Breast tumor tissue samples were obtained from both patients with benign and malignant breast diseases at the operating theater directly after surgical removal of the tumor mass. Tissue samples were transported to the laboratory in liquid nitrogen and then kept stored at -120°C till processing. For preparation of cytosolic and membrane fractions, tissue samples were dissociated away from fat and necrotic tissues, then washed with cold saline, cut into small pieces.

Cytosolic and membrane fractions were prepared from each tumor sample by the method described by Grimaux et al. [19].

2.2.3. Laboratory analyses

Estradiol (E_2) was determined in serum samples and cytosolic fractions of all benign and malignant breast disease patients according to the method of Reed et al. [20] using IMx-MEIA kit (Abbott Diagnostics, USA). Estrogen receptors (ER) were determined in cytosolic fractions using ER-EIA kit (Abbott Diagnostic, USA) according to the method of Greene et al. [21].

Total lipids were determined in serum samples and membrane fractions according to the method of Frings and Dunn [22]. Triglycerides were determined in serum and membrane fractions according to the method of McGowan et al. [23]. Cholesterol was determined in serum samples and membrane fractions according to the method of Stein [24]. Protein concentration was determined in cytosol and membrane fractions according to the method of Bradford [25].

The results were statistically analyzed using SPSS package (Echo Soft Corp., USA, 1993). First-order Spearman correlation coefficients controlling for data set were calculated to assess the linear association between the studied hormone level and the lipid components. Difference was considered significant at the $P < 0.05$ level.

3. Results

As shown in Table 1 this study included 40 women with different benign breast diseases, 16 (40%) of which were premenopausal at different phases of menstruation and 24 (60%) were postmenopausal. This benign group of patients was conducted parallel with a malignant group composed of 50 women diagnosed as having primary breast cancer, 17 (34%) were premenopausal at different phases of menstruation and 33 (66%) were postmenopausal. The mean age of premenopausal women with benign breast tumors was 32.6 years while that of malignant cases was 37.3 years, which is non-significantly different (Table 1). Also, there was not a significant difference in mean age between postmenopausal women with benign and malignant breast tumors (48.6 and 56.4 years respectively). Postmenopausal women with benign breast tumors had been menopausal for at least 1 year and up to 26 years (mean 13.1) while those with malignant breast tumors had been menopausal for at least 1 year and up to 31 years (mean 15.6).

The results of all the subgroups of premenopausal populations (follicular and luteal) in both benign and malignant patients were not significantly different (data not shown), their mean levels were calculated and shown as commulative groups.

Regarding the serum levels of E_2 in both groups (Table 2), there was a non-significant increase in postmenopausal women with benign breast diseases compared to premenopausal women, which was not the case among patients with malignant breast cancer as there was a significant decrease in E_2 level in postmenopausal women compared to premenopausal women ($P < 0.05$). On the other hand, in patients with malignant breast cancer E_2 showed a remarkable significant increase (in both pre- and postmenopausal) in comparison to the patients with benign breast diseases ($P < 0.05$).

Additionally, a significant correlation ($r_s = 0.88$, $P < 0.04$) was obtained in serum E_2 levels between postmenopausal women with benign breast diseases and postmenopausal women with breast cancer, and between both pre- and postmenopausal patients with breast cancer ($r_s = 0.96$, $P < 0.02$).

On analyzing the levels of E_2 in cytosolic fractions of benign and malignant breast tumors, the same pattern of distribution among the four populations was nearly obtained as in serum (Table 3). It seems that there was a significant decrease in E_2 level in postmenopausal women than premenopausal women in benign and malignant cases ($P < 0.05$). Generally, E_2 seemed to be increased non-significantly in malignant than benign breast diseases.

The selected cut-off for ER positive was >15 fmol/mg protein. According to this cut-off, ER-positive population was remarkably increased in malignant group (41.2 and 72.7% for pre- and postmenopausal respectively) than benign group (18.7 and 43.7% for pre- and postmenopausal respectively). Also, ER-positivity was increased significantly in postmenopausal women with malignant breast tumors and non-significantly in women with benign breast tumors ($P > 0.05$ and $P < 0.05$ respectively) (Table 3).

When the Spearman correlation coefficient and the two-tailed tests of significance were calculated between the levels of E_2 in the two different compartments (serum and cytosol), it revealed a significant correlation

Table 1
Age and menopausal status of benign and malignant breast tumor patients

Variable	Benign group $N = 40$		Malignant group $N = 50$		P
	No. (%)	Mean $\pm$ S.D.	No. (%)	Mean $\pm$ S.D.	
Age (years)					
Premenopausal	16 (40)	32.6 ± 3.4	17 (34)	37.6 ± 1.9	N.S.
Postmenopausal	24 (60)	52.8 ± 4.9	33 (66)	58.4 ± 3.2	N.S.
Years (menopause)		13.1 ± 5.4		15.6 ± 6.1	N.S.

Table 2
Estradiol levels (pmol/l) in serum of benign and malignant breast tumor patients

Variable	Benign group ($N = 40$)		Malignant group ($N = 50$)		P
	No.	Mean $\pm$ S.D.	No.	Mean $\pm$ S.D.	
Premenopausal	16	147 ± 9	17	334 ± 17	<0.05
Postmenopausal	24	159 ± 5	33	238 ± 20	<0.05
P		N.S. (>0.05)		<0.05	

Table 3
Estradiol (E_2) and estradiol receptor (ER) in benign and malignant breast tumor tissues

Variable	E_2 (pmol/g) (mean $\pm$ S.D.)			Number of ER $+$ve (>15 fmol/mg P)		P*
	Benign	Malignant	P	Benign (%)	Malignant (%)	
Premenopausal	6.51 ± 0.19	7.60 ± 0.11	>0.05	3 (18.7)	7 (41.2)	N.S.
Postmenopausal	4.65 ± 0.28	5.21 ± 0.07	>0.05	7 (29.2)	24 (72.7)	<0.05
P	<0.05	<0.05		N.S.	<0.05	

* $P < 0.05$ is significant.

Table 4
Lipid profile in serum and membrane fractions of benign and malignant breast tumors

	Total lipids (mean ± S.D.)		Triglycerides (mean ± S.D.)		Cholesterol (mean ± S.D.)	
	Serum (g/dl)	Tissue (g/100 g)	Serum (g/dl)	Tissue (g/100 g)	Serum (g/dl)	Tissue (g/100 g)
Benign group ($N=40$)	0.69 ± 0.06	3.29 ± 0.05	87.42 ± 6.82	522.12 ± 28.3	122.71 ± 7.9	196.59 ± 6.58
Malignant group ($N=50$)	0.73 ± 0.04	6.34 ± 0.33	102.94 ± 8.1	976.8 ± 20.69	135.88 ± 7.27	328.71 ± 14.43
P	>0.05	<0.05	>0.05	<0.005	>0.05	<0.05

between serum E_2 in postmenopausal women with benign breast diseases and cytosolic E_2 in premenopausal women with benign breast diseases ($r_s = 0.89$, $P < 0.04$); between serum E_2 in premenopausal women with benign breast diseases and cytosolic E_2 in premenopausal women with malignant breast cancer ($r_s = 0.86$, $P < 0.05$); between serum E_2 in premenopausal women with benign breast diseases and cytosolic E_2 in postmenopausal women with malignant breast cancer ($r_s = 0.9$, $P < 0.04$).

The most remarkable observation concerning the lipid profile analysis is that we did not obtain any significant difference in serum levels of total lipids, triglycerides and cholesterol between benign and malignant breast disease patients. However, there was a highly significant increase in their membrane levels in patients with breast cancer than those with benign breast diseases ($P < 0.05$, $P < 0.005$ and $P < 0.05$ respectively) (Table 4).

The Spearman correlation revealed a significant correlation between membrane total lipids in benign and malignant groups ($r_s = 0.88$, $P < 0.04$); membrane triglycerides in benign and malignant groups ($r_s = 0.99$, $P < 0.005$); serum cholesterol and serum total lipids in benign group ($r_s = 0.85$, $P < 0.05$); serum total lipids and membrane triglycerides in malignant group ($r_s = 0.93$, $P < 0.03$).

Also the correlation coefficient between serum and cytosolic levels of E_2 and their corresponding levels of the lipid moieties in both benign and malignant groups was calculated. Obviously, there were persistent correlation between E_2 and the lipid profile among all the patients description but the most pronounced significance was between serum cholesterol in benign patients and cytosolic E_2 in postmenopausal malignant breast cancer patients ($r_s = 0.99$, $P < 0.002$) and between membrane total lipids in malignant group and cytosolic E_2 in premenopausal breast cancer women ($r_s = 0.95$, $P < 0.02$).

4. Discussion

It has been believed that breast cancer has a hormonal origin. In particular, because of its profound stimulatory influence on breast ductal epithelium, it was thought that estradiol must play a central role. Differences in the total concentration of E_2, however, are generally not apparent between patients with breast cancer and normal controls particularly for premenopausal women [20,26,27].

Several studies have evaluated serum E_2 concentration in breast cancer patients and normal controls [3,28–32]. These studies have suggested that postmenopausal breast cancer patients have higher endogenous E_2 levels than normal controls. From a hormonal point of view, it has been suggested that some benign breast diseases and breast cancer may share common epidemiological factors or even represent different stages of one process [33,34]. Because benign breast diseases occur earlier in life than breast cancer, it may be rewarding to focus on the influence of hormonal status.

The results of the present study provides additional evidence that higher serum E_2 concentrations are detected in breast cancer patients than patients with benign breast diseases. Additionally, we noticed a non-significant difference in serum E_2 levels between pre- and postmenopausal patients with benign breast diseases, however, there was a significant reduction (42%) in serum E_2 levels in postmenopausal breast cancer patients than premenopausal cases ($P < 0.05$). These findings are greatly supported by those reported by Wu et al. [35] who obtained a 23% reduction in serum E_2 levels among postmenopausal breast cancer women.

Also serum E_2 levels were highly significantly increased in both pre- and postmenopausal women with breast cancer than their corresponding women with benign breast diseases ($P < 0.05$), which in accordance with the results of Berrino et al. [36].

Yue et al. [37] reported that E_2 stimulates the growth of breast tumor cells in both pre- and postmenopausal women. Following the menopause, the levels of E_2 breast tumor tissues are similar to those from tumors obtained prior to cessation of ovarian function, even though plasma E_2 levels are 10–50 fold lower in postmenopausal than in premenopausal women. Actually, the present study did not revealed such difference in E_2 levels, which may be due in part, to the oscillation in E_2

levels during the menstrual cycle and also the small number of cases included.

In the present study the attention has been extended to measure the distribution of E_2 in tumor tissues. The same pattern of differential distribution of E_2 among the four different populations (pre- and postmenopausal benign and malignant breast disease women) has been obtained with a non-significant increase generally in patients with breast cancer than those with benign breast diseases. It seems that these findings go in line with Yue et al. [37] who suggested the possibility of enhanced E_2 uptake from plasma or in situ synthesis in postmenopausal women.

In conclusion, it would be suggested that higher tissue E_2 levels in breast cancer patients are mirrored in the serum by higher serum E_2 levels and this may postulate the existence of an efficient system of transport to be reflected in serum.

Several epidemiologic and animal studies provide strong evidence for a causal relationship between postmenopausal estradiol levels and development of breast cancer. However, these studies seem to be contradicting to the data reported in the present study concerning the decrease in both serum and tissue E_2 levels in postmenopausal women with benign and malignant breast diseases. Actually, taken together, this contradiction could be get in accordance and explained on the basis of the results reported by Yu et al. [37] who demonstrated that breast tumor tissue deprived of estradiol developed mechanisms rendering them more sensitive to estrogen and could be adapted to be responsive to four log lower amounts of estrogen.

Regarding the estradiol receptors (ER), their prognostic importance is controversial. They considered as either reflecting intrinsic property of the tumor tissue or better therapeutic accessibility of receptor positive tumors. In the present study, the obtained significant increase of ER-positive women with breast cancer than benign cases are confirmed by the previous results obtained by Brawisbery et al. [38], Carpenter et al. [39], Pertschuk et al. [40], and King [41]. On the other hand our findings are not in accordance with those of Zeleniuch-Jacquotte et al. [42], who found that in postmenopausal women the association of endogenous E_2 with breast cancer is independent of the ER status of the tumor. However, it may be obvious that our results are most compatible with the hypothesis that ER status identifies two distinct types of breast cancer. Additionally, the increased population of ER-positive among postmenopausal women with benign breast diseases suggest that this population may be more likely to develop breast cancer and is greatly supported by the hypothesis that benign breast diseases are hormone-dependent.

One of the major purposes of this study was to examine the association of E_2 and the lipid alterations in serum and tumor tissues. According to the obtained results, there was not any significant difference between benign and malignant populations regarding the serum levels of total lipids, triglycerides and cholesterol. However, there was a pronounced significant association between cytosolic E_2 and tissue levels of total lipids, triglycerides, and cholesterol since there was a highly significant increase in their tissue levels in patients with breast cancer than those with benign breast diseases ($P < 0.05$, $P < 0.005$, and $P < 0.05$, respectively).

Extensive previous studies have reported a significant increase in serum lipid moieties in breast cancer patients (Boyd and McGuire [43]; Potischman et al. [44]; Lane et al. [13]; Ooodwin et al. [45]; Aqurs-Collins et al. [46]). These studies are consistent with the obtained results in the present study. However, the present study, additionally, reports a more actual and reliable profile for the lipid moieties, which is the intra-tumoral content.

The obtained significant correlation between serum and cytosolic E_2 and the distribution of total lipids, triglycerides and cholesterol in both compartments (serum and tissue) may postulate an influence of E_2 on this distribution.

As both benign and malignant breast tissues have been shown to be associated with increase in lipid moieties and highly correlated with E_2, further studies are required to explore the influence of E_2 on the uptake of lipids from plasma by the tumor tissues. The present study also may confirm the role of lipids as risk factor in breast cancer.

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