ARTICLE / INVESTIGACIÓN

Evaluation of Sex Hormone in Benign and Malignant Breast Cancer in Iraqi Women

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Abstract. Elevated levels of circulating estrogens and androgens are linked to higher breast cancer risk among postmenopausal women; however, little is known about hormone levels within the breast. Hormone concentrations within the breast may not be reflected in the blood and are likely important contributors to breast carcinogenesis. The present study investigated the sex hormone (Estrogen, progesterone, Prolactin and testosterone). Female patients are divided into three groups (Benign, malignant and control). Benign (B)(34 patients) is divided into sub-groups including Benign premenopausal stage (B1)(17 patients) Benign postmenopausal stage (B2) (17 patients) and Malignant (M)(34 patients), Malignant premenopausal stage (M1) (17patients) and Malignant postmenopausal stage (M2)(17patients), and control group (C) include (11) premenopausal stage (C1) and (11) postmenopausal stage (C2). The expression level of soluble sex hormone (Estrogen, progesterone, Prolactin and testosterone) in serum was determined by an ELISA. Technique. The patients attended the Center for Early Detection of Breast Tumor at an oncology teaching hospital in Medical City. The study was conducted on 15/February (2021) to 20/July (2021). The values of Estrogen hormone in premenopausal malignant M1 (34.76 ±4.26 pg/ml) decreased significantly (P≤0.05) in comparison with C1, but it was non-significant in comparison with C2 and B1. M2 (64.28 ±4.17 pg/ml) shows a non-significant increase compared with C1, but it increased significantly with C2, B2 and M1. The values of progesterone hormone B1(12.75 ±3.34ng/ml) and B2(13.06 ±2.98 ng/ml) was non-significant(P≤0.05) in comparison with C1 (8.17 ±2.87 ng/ml) and C2(6.28 ±2.87 ng/ml).M1 (14.30 ±4.29 ng/ml) and M2 (15.76 ±4.34 ng/ml) show non-significant difference in comparison with C1,C2,B1and B2. The values of Prolactin hormone in the M1(32.07±3.56(µIU/ml)) and M2(29.42±3.16) show non-significant difference(p>0.05) with C1,C2,B1 and B2.levelsTestosterone hormone show a significant in M1(1.462 ±0.11(ng/ml)) increased (p<0.05) in comparison with C1,C2,B1,B2. M2(1.392 ±0.10(ng/ml)) increase significantly(p<0.05) in comparison with C1,C2,B1,B2 and M1. concluded from this study that the levels of estrogen increased significantly in postmenopausal malignant M2 women with breast cancer, and the levels of testosterone hormone significant increase in pre and postmenopausal breast cancer women, the levels of Prolactin and progesterone hormone showed non-significant differences in comparison with other groups.
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Keywords: Sex Hormone, Benign, malignant, Breast Cancer,

Introduction

Breast cancer is the most common public health problem and the leading cause of cancer-related death worldwide [1]. Women whose mother was diagnosed before 50 have an increased risk of 1.7, and those whose mother was diagnosed at age 50 or after have an increased risk of [2]. More than half of the incidence of breast cancer and)60%(of deaths occur in low and middle-income countries (LMICs). The ovarian hormones progesterone (P), and Estrogen (E) play a critical role in the growth and proliferation of the breast during normal development and ovarian activity by signals through the progesterone receptor (PR). This signaling pathway, along with its synthetic analogs, has also been implication in the etiology and pathogenesis of breast cancer[3]. Estrogen has an essential role as breast carcinogens have long been suspected and recently confirmed by epidemiological studies. Previous studies proved that estrogen is related to mammalian tumorigenesis, ovarian carcinogenesis and endometrial cancer[4]. Prolactin, a hormone involved in normal breast development and lactation, has been hypothesized to be important in the etiology of breast cancer[5]. Prolactin is a polypeptide hormone composed of 199 amino acids prolactin plays an essential role in initiating and promoting breast cancer. That well-defined lactogenic hormone encourages the proliferation of breast epithelial cells and the differentiation of alveoli [6]. Testosterone (T) is referred to as a 'male' hormone; however, it is the most abundant biologically active hormone in women. It is produced in the ovaries, adrenal gland, and abundantly (more than 50%) at the cellular level from androgen precursors[7]. There is much evidence that androgens are

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breast-protective. In vitro breast cell cultures and in vivo primates' studies support that T's direct effect on the AR is anti-proliferative, pro-apoptotic, and decreased ER activity and breast cancer (BCA) cell growth [8]. The relationship between high T levels and BCA may reflect the correlation between high androgen levels and higher estrogen levels, as evidenced by studies that adjusted for estrogen and no longer found an association between T and BCA.

Materials and methods

A questionnaire in appendix form was used for recording the necessary information concerning with subject groups which risk factor data were collected using a short structured questionnaire that included information on age, weight, height, marital status, number of pregnancies and children, age at first childbirth, average lactation term, family history of breast cancer or other cancers (first and second-degree relatives), age at menarche, age at marriage, menopausal status and age at onset. The study was conducted on(90) female patients coming to do an early examination for breast cancer; after the examination and making sure that she is infected, whether it is a benign or malignant injury, samples were taken from the patients and conduct tests on it. Their ages ranged from 23-70 years. Female patients were divided into three groups (Benign, malignant and control). Benign B(34 patients) is divided into sub-groups, including Benign premenopausal stage B1(17 patients) and Benign postmenopausal stages B2 (17 patients) and Malignant(34 patients), Malignant premenopausal stage M1 (17 patients) and Malignant postmenopausal stage M2(17 patients). Control group C includes (11) premenopausal stage C1 and (11) postmenopausal stage C2. The patients were referred to the Center for Early Detection of Breast Tumor at an oncology teaching hospital in Medical City. Between 15/February /2021 and 20/July/ 2021.

The consultant medical staff made the diagnosis based on a Triple Assessment Technique (such as physical breast examination, ultrasonography, with or without mammography and fine needle aspiration cytology). Concerning blood, 5 ml were collected in a 5ml disposable syringe under aseptic conditions. The blood was put in a plain tube and left for 15 minutes at 4ºC to clot. Then, it was centrifuged at 5000 rpm for 5 minutes. Sera were separated and kept in Eppendorf tubes and then stored in deep freeze at(-20C˚) till examination for biochemical assay.

Measurement of Estrogen hormone

E hormone concentrations were measured in the serum using CUSABIO(USA) ELISA Kits. At the end of the assay, results are automatically calculated by an ELISA reader highly sensitive. Each well's optical density was determined within 10 minutes using a microplate reader set to 450 nm.

Normal values:

for Estrogen 41.395-68.882 pg/mL for premenopausal women.

29.417- 56.904 pg/mL for postmenopausal women.

Measurement of progesterone hormone

Progesterone hormone concentration was measured in the serum using CUSABIO(USA) ELISA Kits. At the end of the assay, results are automatically calculated by an ELISA reader highly sensitive. Optical density was determined for each well within 10 minutes using a microplate reader set to 450 nm.

Normal values:pre-menopausal=3.907-57.330 ng/ml

Post-menopausal=2.974-56.346ng/ml

Women at the beginning of their menstrual cycle: 1 ng/mL or under.

Women in the middle of their menstrual cycle: 5 to 20 ng/mL.

Pregnant women in their first trimester: 11.2 to 90 ng/mL.

Measurement of prolactin hormone

Prolactin hormone concentration was measured in the serum by using CUSABIO (USA)ELISA Kits. At the end of the assay, results are automatically calculated by an ELISA reader with high sensitivity. Each well's optical density was determined within 10 minutes by using a microplate reader set to 450 nm.

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Normal values

Premenopausal 3.526- 8.272 ng/mL, postmenopausal 1.922- 6.375 ng/mL

Measurement of Testosterone hormone

(T) hormone concentration was measured in the serum using CUSABIO (USA)ELISA Kits. At the end of the assay, results are automatically calculated by an ELISA reader highly sensitive. At 450 nm was read with a microtiter well reader within 15

Normal values: premenopausal 10.54-0.93ng/ml,
postmenopausal 0.30-0.83 ng/ml

Statistical Analysis:

The Statistical Analysis System [9] program was used to detect the effect of different factors on study parameters. The least significant difference –LSD test (Analysis of Variation-ANOVA) was used to compare between means significantly. Estimate the correlation coefficient between variables in this study level of p< 0.05.prbability.

Results

Table(1) shows the comparison between different groups in levels of hormones.The values of Prolactin hormone in B1(38.77 ±3.38(µIU/ml)),B2(36.19 ±4.37(µIU/ml)) show non-significant difference (p>0.05) with C1(30.54 ±4.89(µIU/ml)) , and C2(30.84 ±5.65(µIU/ml)). The M1(32.07±3.56(µIU/ml)) and M2 (29.42±3.16(µIU/ml)) show non-significant difference(p>0.05) with C1,C2,B1 and B2. The values of Estrogen hormone in B1(38.77 ±3.38(pg/ml)), B2(42.51 ±5.5(pg/ml)) was non-significant (p>0.05) difference when compared with C1(54.17 ±2.83(pg/ml)) and C2 (42.19 ±2.83(pg/ml)). B2 shows a non-significant (p>0.05) difference in comparison with C1and C2. M1 (34.76 ±4.26(pg/ml)) decreased significantly (P≤0.05) in comparison with C1, but it was non-significant in comparison with C2 and B1. There was a non-significant (p>00.05) decrease between B2 and M1.M2 (64.28 ±4.17(pg/ml)) shows a non-significant increase in comparison with C1, but it was increased significantly with C2, B2 and M1. Estrogen was increased significantly in postmenopausal malignant M2 compared to the other

groups.The values of progesterone hormone B1(12.75 ±3.34(ng/ml)) and B2(13.06 ±2.98(ng/ml)) was non significant(P≤0.5) in comparison with C1 (8.17 ±2.87(ng/ml)) and C2(6.28 ±2.87(ng/ml)).M1 (14.30 ±4.29(ng/ml)) and M2 (15.76 ±4.34(ng/ml)) show non significant difference in comparison with C1,C2,B1and B2. This study present that the progesterone levels increased in B1, B2, and M2 compared to C1 and C2. The values of testosterone hormone in B1(0.530 ±0.07ng/ml) and B2(0.741 ±0.07ng/ml) show non significant difference in comparison with C1(0.734 ±0.03ng/ml) and C2(0.627 ±0.06ng/ml).M1(1.462 ±0.11ng/ml) increased significantly($p<0.05$) in comparison with C1,C2,B1,B2. M2(1.392 ±0.1ng/ml) increase significantly($p<0.05$) in comparison with C1,C2,B1,B2,M2.But M1 and M2 were non-significant.

Table 2 shows the negative correlation (NS) between prolactin hormone (-0.01) with age while showing a significant correlation with BMI. Fig(1).Estrogen(0.02) shows non-significant ($p>0.05$) correlation with age and BMI(-0.11).Progesterone(0.06)show non-significant($P>0.05$) correlation with age and BMI(-0.15). Testosterone (0.15) shows non-significant ($P>0.05$) correlation with age and BMI(0.04).The following Prolactin can explain the positive relationship between BMI and Prolactin (PRL) and promotes (visceral) fat accrual in various animal models.

Group	Mean ± SE			
	Prolactin (µIU/ml)	Estrogen (pg/ml)	Progesterone (ng/ml)	Testosterone (ng/ml)
pre-control C1	30.54 ±4.89	54.17 ±2.83ab	8.17 ±2.87	0.734 ±0.03 b
post-control C2	30.84 ±5.65	42.19 ±2.83bc	6.28 ±2.87	0.627 ±0.06 b
pre-benign B1	38.77 ±3.38	53.04 ±4.75ab	12.75 ±3.34	0.530 ±0.07 b
post-benign B2	36.19 ±4.37	42.51 ±5.56bc	13.06 ±2.98	0.741 ±0.07 b
pre-malignant M1	32.07 ±3.56	34.76 ±4.26 c	14.30 ±4.29	1.462 ±0.11 a
post-malignant M2	29.42 ±3.16	64.28 ±4.17 a	15.76 ±4.34	1.392 ±0.10 a
LSD value	11.631 NS	13.047 *	10.684NS	0.255 *
This means having the different letters in the same column differed significantly. * (P≤0.05).				

Table 1. Comparison between different groups in Hormones levels

Parameters	Correlation coefficient-r	
	Age	BMI
Prolactin	-0.01 NS	0.21 *
Estrogen	0.02 NS	-0.11 NS
Progesterone	0.06 NS	-0.15 NS
Testosterone	0.15 NS	0.04 NS
*** (P≤0.05), NS: Non-Significant.**		

Table 2. The correlation coefficient between Age and BMI with Hormones

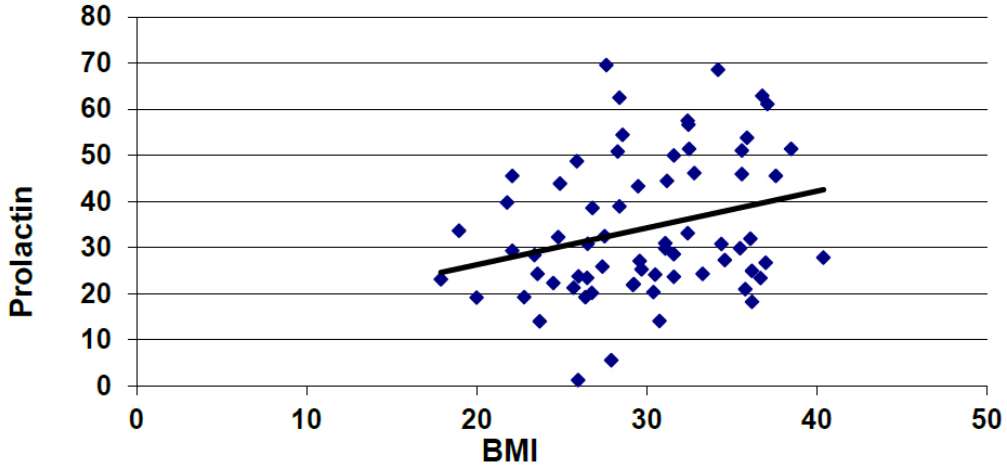

Figure 1. Relationship between BMI & Prolactin

DISCUSSION

Prolactin is critical in enhancing angiogenesis and cell migration, which may contribute considerably to cancer metastases. Increased plasma prolactin levels are primarily found in patients with developed and late/stage cancers. Shreds of evidence suggested the role of the PRL in breast cancer, such as high levels of serum PRL were related to the risk of breast cancer in postmenopausal women[10]. In addition, PRL is involved in breast cancer since it induces proliferation, survival, migration, and angiogenesis in the breast cancer cells. Many studies showed that expression levels of Prolactin and (PRLR) from the breast cancer cells and tissues were much higher than in normal tissues. Additional work also reported that, other than the robust tumor-promoting function, Prolactin can serve to augment the number of (S phase-cells), boost cell proliferation frequency and raise levels of cyclin D1 in breast cancer cell lines [11,12]. One of the reports showed no significant relationship between serum prolactin and breast cancer risk among either pre- or postmenopausal women. Still, they did not provide any information regarding the hormone receptor status of the tumors [13]. Another study was referred at the Breast Cancer Center of Peymanieh Hospital (Jahrom University of Medical Sciences) in two groups of breast cancer (25 menopausal and 25 premenopausal women) and a control group (25 menopausal and 25 premenopausal women), and the result show no significant relationship between increased prolactin level and breast cancer in menopausal women ($p = 0.425$). There was no meaningful relationship between increased prolactin hormone and breast cancer in premenopausal women ($p = 0.867$). Estrogen was increased significantly in postmenopausal malignant (M2) compared to the other groups. The estrogen hormone plays an important role in resisting many diseases. The most important of these diseases is cancerous tumors, so most of these diseases are shown in the menopause group(M2), during which this hormone decreases[14].

The hormone 17-estradiol (E2) stimulates breast development during puberty and sexual maturity, which is the prevalent circulating ovarian steroid and the most biologically active hormone in the breast tissue [15]. Considerable epidemiological and clinical evidence link estrogens are considered to play an essential role in promoting the proliferation of both the normal and the neoplastic breast epithelium. Their role as breast carcinogens has long been suspected and recently confirmed by epidemiological studies[16]. Physiologic and Pharmacologic concentrations of estradiol are linked with the increased mitogenic activity of the breast epithelial cells. The possible association between progesterone and breast cancer risk continues to be debated, which agrees with our result[17-19].

The progesterone hormone is regularly and naturally in these C1 and C2 groups. At the same time, the study showed that all groups that suffer from tumors, whether benign or malignant tumors, this hormone begins to increase its secretion due to the decrease and decline of estrogen due to the nature of the inverse relationship between them. Therefore it is an important indicator of a hormonal imbalance in women.The role of endogenous progesterone in the development of breast cancer is still largely unexplored to date, primarily owing to assay sensitivity limitations and decreased progesterone concentrations in postmenopausal women[19]. Recently identified progesterone metabolites may provide insights as experimental data suggest that 5α-dihydro-progesterone (5αP) concentrations reflect cancer-promoting properties and 3α-dihydro-progesterone (3αHP) concentrations reflect cancer inhibiting properties. The study by [19] showed the higher Progesterone concentrations in the women were higher circulating progesterone at an increased risk for breast cancer. In this case-cohort study of postmenopausal women, increased circulating progesterone levels were associated with a (16%) increase in the risk of breast cancer.This study agreed with our results that progesterone levels increased in B1, B2, and M2 compared with C1 and C2. In this study, testosterone levels increased in the M2 group compared with the other groups. Because this hormone increases dramatically in women who suffer from mental disorders and tension, these symptoms appear clearly in group M2 who suffer from cancerous tumors and therefore suffer from tension and thus affecting the psychological state and the increase of this hormone. The role of androgens in breast cancer is an old-debated topic. Many studies consistently demonstrated that high testosterone levels are linked with an increased risk of developing breast cancer, particularly in ER-positive tumors [20]. Conversely, some studies have investigated the potential predictive value of the testosterone hormone [21,22]. More recently, prospective studies have provided evidence for a raised rate of progression in postmenopausal patients with high circulating levels of testosterone [23]. It is known that, in postmenopausal women, adiposity, particularly abdominal fatness, is linked with high levels of circulating testosterone and estradiol. A positive correlation has also been described between the sex hormones levels and Body Mass Index (BMI), the parameter used as a proxy for adiposity [24]. Some evidence suggested the presence of a coordinate mechanism whereby, when estrogens decrease, testosterone promotes a redistribution of fat deposits that preferentially accumulate in the abdomen [25-27].This excess of visceral fat, known as central obesity, plays an important role in favoring the onset of insulin resistance and related dysmetabolism, such as hyperinsulinemia (IGF-I) hyper-production and metabolic syndrome[28]. Some studies also reported an association between the increased risk of breast cancer and high serum levels of the estrogens, a finding consistent with increased production of the estrogens fueled by high androgen levels, Prospective studies in women with ER-positive breast cancer demonstrate that patients with high testosterone levels had a higher risk of relapse, underlining the significance of androgen excess in disease development [21-23]. The release of the PRL by the pituitary is tonically inhibited by dopamine by activating the dopamine D2 receptor (D2R) of lactotroph cells, and obese humans appear to have reduced D2R binding sites in their brains [29]. Prolactin (Prl) is a single-chain polypeptide involved in several actions, such as lactation, luteal function, reproduction, appetite, osmotic balance suppression of fertility, homeostasis, immunity, and coagulation. Prolactin receptor (Prl-R) gene expression has already been described in the adipose tissue, and an increase in this expression during lactation has been documented in humans and rats. Prl-R deficient mice have shown decreased abdominal fat and leptin concentration compared to the controls; because Prl is inhibited by activation of the dopamine D2 receptor (D2 R), increased Prl secretion may occur due to reduced D2 R availability in the brain, which makes these people more likely to have increased Prl secretion [30]. In a study considering hyperprolactinemia as a result of the overweight, increased Prl secretion in obese women was significantly reduced after

a loss of (50%) overweight [31]. In that study, the authors suggested that improvement of deficit (D2R) mediated neurotransmission and decreased circulating leptin-estrogen levels might be involved in this phenomenon. Weight reduction, with accompanying reduction in insulin levels, has been shown to lead to a normalization of Prolactin response in most, but not all, circumstances. Lima and his coworkers had concluded that the prevalence of obesity was shown to be high in hyperprolactinemic patients, regardless of obesity level or reason for hyperprolactinemia. It is important to observe BMI in patients with elevated Prl levels to introduce measures aiming to maintain a healthy weight and decrease associated comorbidities[32-34].

Conclusions

The levels of estrogen increased significantly in postmenopausal malignant M2 women with breast cancer, and the levels of testosterone hormone significant increase in pre and postmenopausal breast cancer women, the levels of Prolactin and progesterone hormone showed non-significant differences in comparison with other groups.

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