Study the Effect of Anastrozole on Estradiol and Cytochrome P450 (Aromatase Enzyme) in Postmenopausal Breast Cancer Patients

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Abstract

In this study sixty patients were selected according to a positive estrogen receptor. All of 60 naturally or surgically postmenopausal women that had a diagnosis of breast cancer verified by histology breast cancer. Subdivided to with hormone treatment or without any treatment, Forty patients (group P1) with the ages range from 46 to 75 years, and were being treated with an aromatase inhibitor, Arimidex (1 mg) once daily, and another twenty (group P2) as newly diagnosed of breast cancer with ages ranging from 47 to 75 years. While the age of control ranging from (48-75) years old, used as a reference. Recent investigations have challenged the hypothesis that aromatization of androgens into estrogen is the sole production pathway for estrogens in postmenopausal women. The finding that estradiol persists in the plasma of patients receiving anastrozole treatment despite a near total inhibition of the aromatase enzyme suggests that alternative pathways for estrogen synthesis exist, which appeared in our results, aromatase and estradiol with treatment (452.34pg/ml) and (13.09pg/ml) respectively. An although evidence a positive and strong correlation between aromatase enzyme and estradiol (r=0.573, P=0.00).

Keyword: Breast cancer, Aromatase, Anastrozole, Estrogen receptor.

Introduction

Hormonal therapy for breast cancer is the first targeted therapy used in any type of cancer. It was used successfully without a known target for more than 50 years before Jensen described the estrogen receptor (ER) in the 1960s [1]. Subsequently, it was found that hormone therapy was effective only in those patients whose tumors expressed the ER, Currently, all breast cancers are tested for expression of Estrogen Receptor (ER), Progesterone Receptor (PR) and human epidermal growth factor receptor 2 (HER2/neu) proteins. ER and PR tests are usually done by immunohistochemistry whereas HER2/neu is accessed by FISH. This protein profiling of tumors helps to predict the eventual prognosis and can assist in the determination of the most appropriate treatment for the individual [2].

ERs are over-expressed in around 70% of breast cancer cases, and are referred to as "ER-positive" tumors. Binding of estrogen to ER stimulates proliferation of mammary cells, with the resulting increase in cell division and DNA replication and increases mutation rate.

This causes disruption of the cell cycle, apoptosis and DNA repair processes eventually leading to tumor formation [3].

About 65% of ER-positive breast cancers are also PR-positive and about 5% of breast cancers are ER-negative and PR-positive. If cells have receptors for both hormones or receptors for one of the two hormones, the cancer is considered hormone-receptor positive [4].

In postmenopausal women, estrogens are synthesized in most of the body compartments, including the liver, muscle, connective tissue, and skin [5]. While one single aromatase gene exists, the gene contains at least ten different promoters [6], with different promoters and ligands regulating estrogen synthesis across different tissue types [7] .However, proteins coded for by the different promoters are similar. The aromatase *(CYP19* gene), an enzyme catalyzing the conversion of testosterone into estradiol (E2) and androstenedione into estrone (E1) [8]. This process called aromatization is inhibited by aromatase inhibitors (AIs), is first-line systemic treatment for the majority of postmenopausal breast cancer patients with estrogen receptor (ER)-positive primary tumor [9].

Aromatase
Androgen ----------> Estrogen

its work by blocking the enzyme aromatase, a cytochrome p450, decreasing in this way the level of circulating estrogen which stimulates the growth of estrogen-receptor positive breast cancer cells, aromatase enzyme are available in different tissues such as adipose tissue, liver, muscle, brain, skin, bone, endometrium, and breast tissue [10].

Estrogens are the end-products of a sequence of steroid transformations [11]. Blockade of any conversion in the pathway potentially leads to decreased estrogen production, but more specific suppression will result from inhibition of the final step that is unique to estrogen biosynthesis. This reaction that changes androgens into estrogens is complex [12].

Three generations of AIs have been developed [13]. Each successive generation has been associated with higher specificity for the aromatase enzyme, fewer adverse events, and greater suppression of aromatase activity. The utility of first and second-generation AIs was limited by adverse events, such as rash, fatigue, dizziness, ataxia, nausea and vomiting, as well as by a lack of enzyme selectivity, whereas third-generation AIs are superior to earlier versions because they are associated with fewer adverse events and greater suppression of aromatase activity [14].

Third-generation aromatase inhibitors are currently recommended for adjuvant endocrine treatment as primary, sequential or extended therapy with tamoxifen, for postmenopausal women diagnosed with estrogen receptor positive breast cancer [15-17].

Arimidex (anastrozole), chemically known as 2,2'-(5-((1H-1, 2,4-triazol-1-yl)methyl)-1,3-phenylene)bis(2-methylpropanenitrile), is the first-line endocrine therapy for postmenopausal HR-positive breast cancer patients. Anastrozole has been shown to significantly reduce the rates of breast cancer recurrences. This trial led to the approval of anastrozole by the US Food and Drug Administration for adjuvant treatment of HR-positive early stage breast cancer [18].

Material and Method

Patient

The Study participants were naturally or surgically postmenopausal women, who had a diagnosis of breast cancer verified by histology. Sixty patient were selected according to positive estrogen receptor .The (mean± SD) age of the patients (58.15±7.45) years old; ranging from (46-75) years old. This subdivided to with hormone treatment or without any treatment. Forty patients (group P1) were identified in the Medical Oncology service/medical city/Baghdad, with the ages ranges from 46 to 75 years, and were being treated with an aromatase inhibitor, Arimidex (1 mg) once daily, and another twenty (group P2) as newly diagnosis of breast cancer were identified at Al Yarmouk teaching hospital, with ages ranging from 47 to 75 year. While the (mean ±SD) age of control (58.55±6.85) ranges from (48-75) years old.

Breast cancer patients and control were characterized in terms of age, family history, duration of treatment, BMI, WHR, WHtR. As shown in Table (1).

Table (1)
Mean (±SD) level of Age, BMI, WHR and WHtR in group (C), group (P1) and group (P2).

Parameter	*Group (c) (n=20)*	*Group (p1)(n=20)*	*Group (p2)(n=40)*	*P- value*
Age (year)	58.55±6.85	59.00±7.87	57.73±7.29	N.S
Weight (Kg)	66.90±8.29	82.15±16.07 a**	78.25±15.78 b*	0.003
Duration of treatment(month)	———	———	12.82±9.39	
Height (cm)	161.90±4.81	158.75±3.04 a*	158.55±3.67 b**	0.006
BMI (Kg/m^2)	25.44±2.24	32.56±6.24 a***	30.98±5.43 b***	0.000
Waist (cm)	102.8±8.78	106.30±12.79	105.27±11.65	N.S
Hip(cm)	106.65±7.04	110.75±9.78	109.92±10.67	N.S
WHR	0.96±0.06	0.96±0.11	0.95±0.06	N.S
WHtR	0.63±0.04	0.66±0.07	0.66±0.06	N.S
Family history No. (%)	———	11(55%)	30(75%)	———

$P^* \leq 0.05$; $P^{**}<0.01$; $P^{***}<0.001$; no asterisk: $P > 0.05$.

a) indicate significant difference between groups (C) and (P1)
b) indicate significant difference between groups (C) and (P2)
c) indicate significant difference between groups (P1) and (P2)

Blood sample

Four milliliters of Blood samples were collected from each patient and controlled by vein puncture using 5ml disposable syringe between 8 am and 11 am. The blood samples were put in tubes with gel (activator clotting tubes) then was centrifuged at (3000rpm) for 10 min after allowing the blood to clot at room temperature, the sera was frozen at (-20 c°) until the assay day.

Assays

Human cytochrome P450 19A1 (CYP19A1) analysis was measured by an enzyme immunoassay for the quantitative assay using kit.cusabio.com. China. This assay has a detection limit of 26.7 pg/ml-1000 pg/ml. the sensitivity of the assay was 6.7 pg/mL.

Serum estradiol concentration was measured by an enzyme immunoassay for the quantitative invitro assay using kit.demeditec.com. Germany. The range of measurement was 0 to 2000 pg/mL and the sensitivity of the assay was 9.714 pg/mL.

Statistical analysis

Statistical package for the social sciences (SPSS), version 17.1 for windows software (SPSS inc., Chicago, USA) was used for statistical analysis. The data normally distributed and were expressed as mean ± standard deviation (SD). One-way analysis of variance (ANOVA) hoc test was used to compare the parameters among groups (C), (P1) and (P2) followed by post hoc test. A difference among groups was defined to be statistically significant if the corresponding p-value was less than 0.05. Correlations between variable were determined by person correlation coefficients (r-value).

Results and Discussion

Some breast cancers require estrogen for growth and, if deprived of these hormones, will regress. Consequently, estrogen deprivation therapy is a major treatment strategy for hormone-dependent breast cancer. There are various forms of endocrine therapy, but recently inhibiting the aromatase enzyme, which catalyzes the conversion of androgens to estrogen, have been increasingly used [19]. Among these aromatase inhibitors available,

anastrozole has a well emphatic role in clinical practice for advanced disease and first-line treatment. In this paper, analyzes the biological profile of anastrozole by evaluating the serum cytochrome P450 and serum estrogen level in the patient. The results were confirmed the efficacy and selectivity of anastrozole in estrogen suppression, as a noticeable change in aromatase concentration.

The results of this study show that serum aromatase was significantly evaluated in breast cancer patients group (P) compared with non-breast cancer control group (C) and so that for estradiol, as shown in Table (2).

Table (2)
Mean (±SD) level of serum aromatase of group (C) and group (P).

Parameter	*Group (c) (n=20)*	*Group (p) (n=60)*	*P – value*
Aromatase pg/ml	347.97± 73.17	487.133± 214.39	0.006
Estradiol pg/ml	12.29± 1.86	14.76± 3.4	0.004

Serum aromatase correlates positively and strongly with serum estradiol (r =0.79, P=0.00) and BMI (r=0.529, P=0.00) show in Fig.(1). While it negatively correlate and significant with WHR (r= -0.38) with P-value (0.01) for group (P1).

For group (P2) Serum aromatase positively correlates and strongly with serum estradiol (r =0.821, P=0.00), BMI (r=0.617, P=0.00) as shown in Fig.(2) and weight (0.33, P=0.00) [20-21].

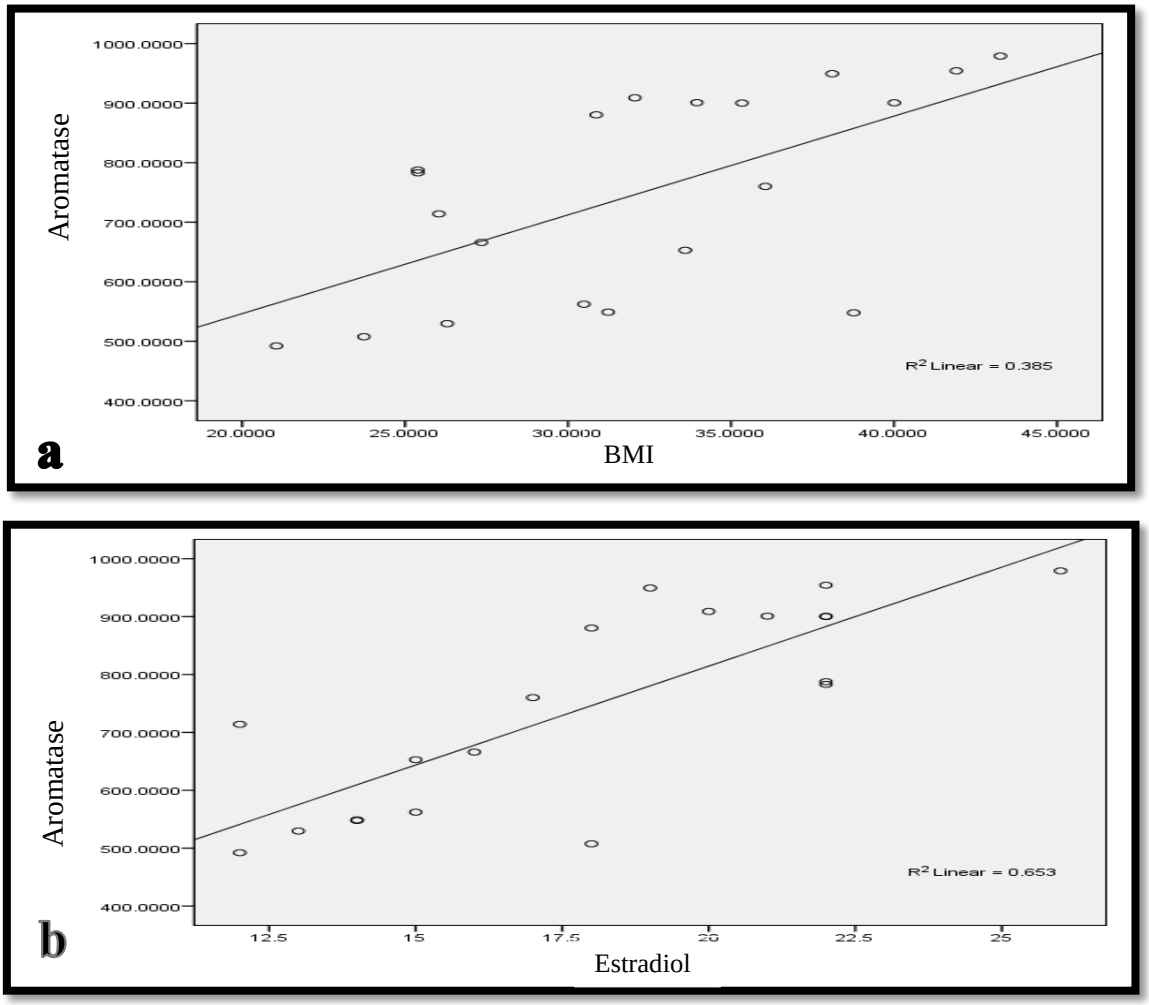

Fig.(1): The significant correlation between aromatase enzyme and a) BMI and b) Estradiol for group (P1).

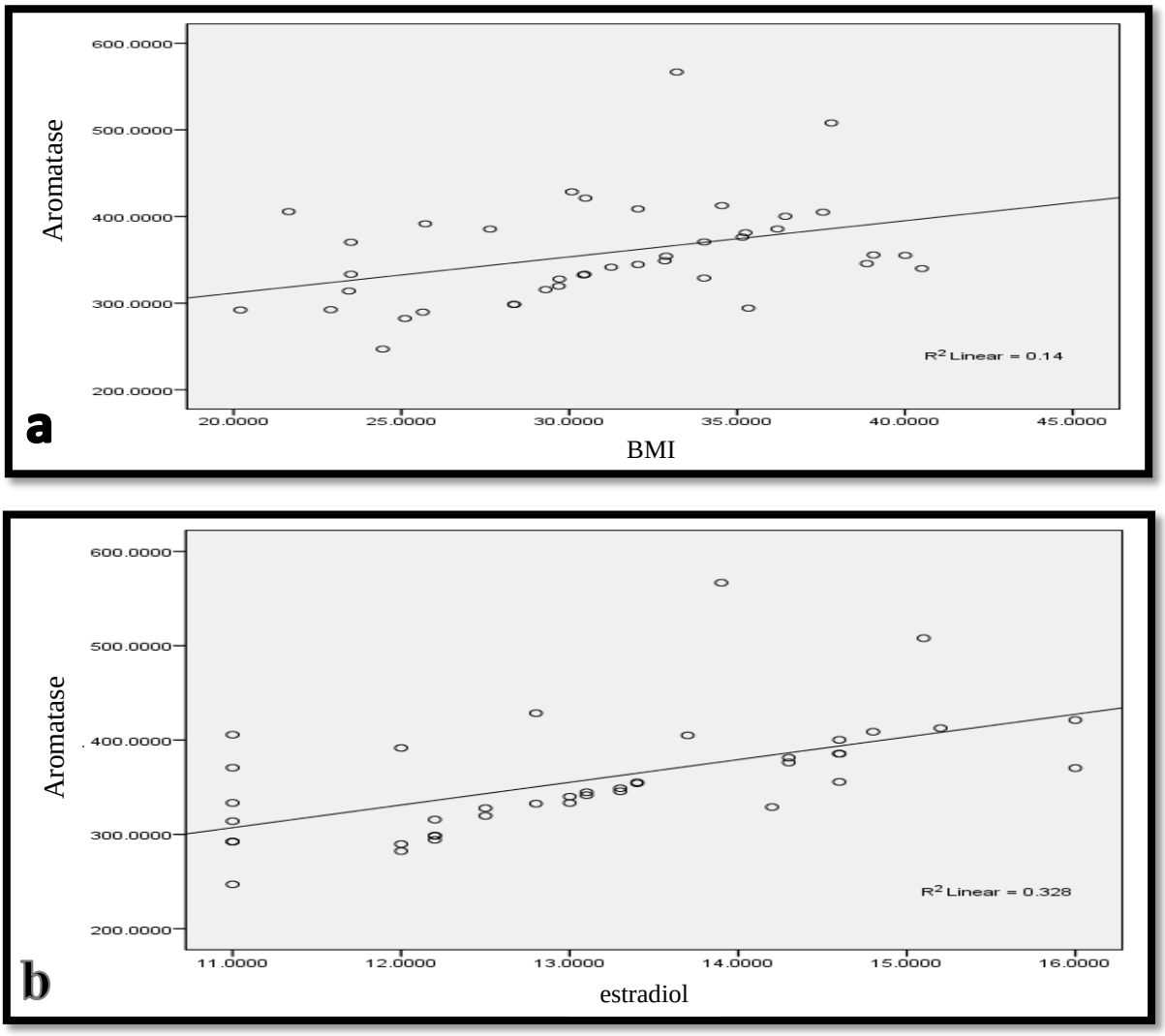

Fig.(2): The significant correlation between aromatase enzyme and a) BMI and b) estradiol for group (P2).

when was included body mass index at the time of blood collection in each of the models, because, in postmenopausal women, body mass index is a major determinant of estrogen levels[22] and that agree with the results that show high significant of BMI between the groups. As shown in Table (1) and Fig.(3).

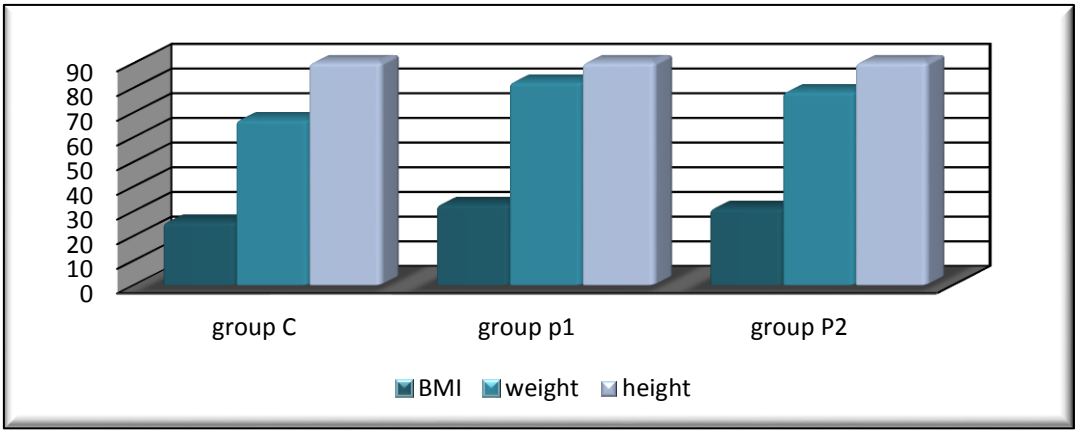

Fig.(3): Mean distribution of weight, BMI, waist and height in studied groups (P1),(P2) and control.

Rates of obesity have increased markedly in the recent past, and aromatase and estrogen levels are higher in obese postmenopausal women. The relationship between risk of postmenopausal breast cancer and BMI has been found to be largely (but probably not entirely) explained by the higher levels of estradiol [23-24] .Given that AIs act by the suppression of estrogen synthesis and withdrawal of estrogenic growth support to ER-positive breast cancer [25], it was reasonable to hypothesize that the efficacy of anastrozole appeared in group (p2) in Table(3). The results are in good agreement with previous literatures regarding the role of aromatization in postmenopausal breast cancer patients [26].

Table (3)
Mean (±SD) level of serum aromatase and estradiol of group (C), group (P1) and group (P2).

Parameter	*Group (c) (n=20)*	*Group (p1) (n=20)*	*Group (p2) (n=40)*	*P – value*
Aromatase pg/ml	347.97±73.17	746.24±170.86 a***	452.34±198.22 c***	0.00
Estradiol pg/ml	12.29±1.86	18.10±3.9 a***	13.09±1.44 c***	0.00

The concentration of the aromatase enzyme and of its substrates (testosterone and androstenedione) provides the primary determinants of estradiol production [27]. That explain the direct proportional for both estradiol and cytochrome levels which evident in the Table (3), Through lowering estrogen production by using aromatase inhibitors accompanied a decrease in stimulation of aromatase enzyme plus the positively and strongly correlation between them, Fig.(2-b) and (3-b).

this study revealed a significant biochemical efficacy of anastrozole by measuring the level of estradiol and cytochrome p450 with regard to invivo aromatase inhibition as well as estrogen level suppression in postmenopausal breast cancer woman it has proved aromatase inhibitor action at the limits of quantification ,and comparing residual levels of E2 and cytochrome P450 after AI treatment (13.09pg/ml) and (487.133pg/ml) respectively, and newly diagnosed patients (18.1pg/ml) and (746.24pg/ml) represented for p1 and p2 in Table (3), by dependence control group as a reference. Recent investigations have challenged the hypothesis that aromatization of androgens into estrogen is the sole production pathway for estrogens in postmenopausal women. The finding that estradiol insists in the plasma of patients receiving anastrozole treatment despite a near total inhibition of the aromatase enzyme suggests that alternative pathways for estrogen synthesis exist, which apparent in the results as shown in Table (3), despite that shown evidence of positive and strong correlation between aromatase enzyme and estradiol. As shown in Fig.(2-b) and (3-b)**.**

Conclusion

The results show that increased weight and to some extent increased BMI exhibit a trend towards increased serum cytochrome P450 and E2 levels, and appear positive and strong correlation between them, all that confirms the hypothesis of aromatization but the finding that estradiol persists in the plasma of patients receiving anastrozole treatment despite a near

total inhibition of the aromatase enzyme suggests that alternative pathways for estrogen synthesis exist.

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الخلاصة

في هذا البحث تم اختيار (60) مصابة بسرطان الثدي ذات مستقبلات ايجابية لهرمون الاستروجين .جميع المصابات تم تشخيصهم بسرطان الثدي والتحقق من الاصابة بالزرع النسيجي. تم تقسيمهم الى مجموعتين: 40 مريضة (مجموعة P1) أعمارهم تتراوح (46–75) سنة، يتلقون علاج Arimidex (١ ملغ) مرة واحدة يوميا، و المجموعه الثانيه تشمل (20) مريضة (مجموعة P2) تم تشخيصهم حديثا بسرطان الثدي وتراوحت اعمارهم (47–75) سنة. في حين تم استخدام مجموعة اخرى شملت (20) سيدة يتمتعن بصحة جيدة , تراوحت اعمارهم ما بين (48–75) سنة واستخدمت كمجموعه تحكم. وقد تحدت الدراسات الأخيرة الفرضية القائلة بأن أرمتة الأندروجين إلى إستروجين هي السبيل الوحيد لإنتاج هرمون الاستروجين في النساء بعد سن اليأس, بالنتائج التي مفادها أن استمرار الاستراديول في بلازما المرضى الذين يتلقون العلاج اناستروزول على الرغم من تثبيط شبه الكامل للانزيم الاروماتيز تشير إلى أن مسارات بديلة لتخليق هرمون الاستروجين موجودة، والتي ظهرت في نتائجنا ايضا ، حيث ان تركيز الاروماتيز والاستراديول بوجود العلاج (13.09pg/ml)(452.34pg/ml) تباعا, على الرغم من أدلة وجود علاقة إيجابية وقوية بين انزيم الاروماتيز واستراديول (P = 0.0 ،r=0.573).