The effect of tamoxifen and luteinizing hormone-releasing hormone (LHRH) analogue on estrogen level in women with breast cancer

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Abstract

Estrogen exposure is a major risk factor for breast cancer. Increased estrogen responsiveness of breast epithelium may enhance this effect.

Surgical or medical castration and antiestrogenic treatment with tamoxifen are common endocrine treatments for premenopausal women with breast cancer.

However, tamoxifen therapy induces high levels of plasma estradiol, with unknown long-term effects. In this study, we investigated the effect of combining the luteinizing hormone-releasing hormone agonist with tamoxifen and measuring estradiol level **. method** : Are taking random samples of breast cancer patients under treatment of patients attending the Institute of Atomic Radiation in Baghdad , and specifically who use Tamoxifen (20 mg/day) . 100 samples and 30 sample as a control there age (20 – 80). Approximately 3ml of blood are collected from each women using standard procedures. And then measuring the level of estradiol E2 hormone ,Measurement of the liver enzymes (GOT,GPT,ALP,LDH and GGT) and billirubin for study the liver health **results :** combined treatment with tamoxifen and LHRH analogue cause reduced in estradiol level (37.3634±45.02893) $P \leq o.o1$ which is consider the main cause of breast cancer in this study group while the control group was in normal range (93.3700±51.07188) and the level of liver enzymes and total

billirubin was in normal range and there was no significant change. **Conclusion :** combining the luteinizing hormone-releasing hormone agonist with tamoxifen leads to reduced the level of estradiol E2 hormone while treatment tamoxifen alone cause elevating these hormone , also these drugs didn't effected on the liver health.

تأثير اقتران العلاج بالتاموكسيفين ونظائر هرمون LHRH على مستوى الاستروجين في النساء المصابات بسرطان الثدي

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الخلاصة :

إن التعرض لهرمون الاستروجين يعتبر من أهم واخطر عوامل الإصابة بسرطان الثدي وكذلك إن زيادة مستوى هرمون الاستروجين قد يحث زيادة الاستجابة والنمو في نسيج الثدي , العلاج بواسطة تثبيط المبيض جراحيا أو باستخدام العقاقير بالإضافة إلى التاموكسيفين هو العلاج الأمثل للنساء المصابات بسرطان الثدي قبل سن اليأس.

أما عند استخدام التاموكسيفين لوحده فيعمل على حث ارتفاع مستوى هرمون الاستروجين في بلازما الدم , في الدراسة الحالية يتم التحري عن العلاج بواسطة التاموكسيفين مع نظائر هرمون LHRH وتأثيرهما على مستوى هرمون الاستروجين .

طريقة العمل : اخذ ت عينات عشوائية من مريضات سرطان الثدي اللاتي يخضعن للعلاج (التاموكسيفين 20 ملغم/يوم) ويراجعن معهد الإشعاع الذري في بغداد .

100 عينة من النساء المريضات و 30 عينة عشوائية من النساء الطبيعيات واللاتي يتراوح أعمارهن (20-80) , اخذ 3مل تقريبا من الدم لكل أمراه باستخدام طريقة قياسية وبعدها تم قياس كل من والبيليروبين الكلي (GOT,GPT,ALP,LDH and GGT) , قياس إنزيمات الكبد E2الاسترادايول لملاحظة صحة الكبد.

النتائج : إن اجتماع كل من التاموكسيفين و نظائر الهرمون LHRH كل منهما لعلاج مرض سرطان الثدي أدى ذلك إلى انخفاض معنوي في مستوى هرمون الاستروجين (45.02893±37.3634) $P \leq$

أما مستوى إنزيمات الكبد (93.3700±51.07188) لمجموعة المرضى مقارنة مع مجموعة السيطرة o.o1 والبيليروبين الكلي فلم يحصل أي تغيير معنوي لهم .

يعمل على خفض مستوى LHRH وبذلك فأن العلاج بمرافقة التاموكسيفين مع نظائر الهرمون بشكل ملحوظ مقارنة مع علاج مرض سرطان الثدي بالتاموكسيفين لوحده فأن الأخير يعمل على رفع E2 مستوى هرمون الاستروجين وان خفض مستوى هرمون الاستروجين يقلل من احتمالية عودة المرض وكذلك الإصابة في عضو أخر كالرحم .

INTRODUCTION

Breast cancer is a major public health problem. It is the most common malignancy in women. Breast cancer accounts for one-third of all cancers in females and 24% of the patients are younger than 55 years of age. The number of women with breast cancer is increasing. Each year, over 1.1 million females worldwide are diagnosed with breast cancer and 410,000 women die of the disease.(Hulka ., 1996)

According to Iraqi Cancer registry 2008 there is an increase in the frequency of breast cancer incidence from 31%in 2005(Iraqi Cancer Registry,2005) to 34% in 2008. Breast cancer occupy the first degree in the commonest ten cancer in Iraq with number of cases about 2729(19.25%)

Endogenous hormones like estrogen are believed to play a central role in breast cancer development **(**Bernstein & Ross **.,** 1993 ; mark *et al* ., 2007).

The predominant form of circulating estrogen in women is estradiol (Stanczyk.,1997) Estradiol(17-beta-estradiol or E_2) a steroid hormone is derived from cholesterol,targets a variety of tissues ,is located in female and male reproductive tracts ,mammary glands skeletal and cardiovascular systems (Hall *et al*.,2001).Among women,it is primarily synthesized in ovarian follicles whereas in among men is produced by the testes and from extraglandular conversion of androgen (Tivis *et al.*,2005).In women estradiol synthesis normally declines after menopause (Manly & Merchant.,2000).

Estrogen is an important steroid hormone involved in regulating the differentiation and proliferation of normal breast epithelial cells(Goldfien & Monroe.,1997 ; Allred *et al* ., 2004).Breast development at puberty and during sexual maturity is stimulated by estradiol hormone(Russo & Russo.,2005).

Circulating estrogen is mainly secreting from the ovary in premenopausal women, however, after menopause ,estrogen is biosynthesizing in peripheral tissues such as adipose tissues ,skin and muscles through conversion of circulating inactive

steroids(Sasano & Harada.,1998 ; Ma H *et al* ., 2006) as represented in estron produced by the peripheral conversion of androstendion ,the precursor of testosterone(Miller.,1990).

Most breast tumor are of estrogen dependent and postmenopausal women with elevated serum estrogen are at an increased risk of developing breast cancer (The Endogenous Hormones and Breast Collaborative Group,2002).Malignant breast tumors produce large amounts of estrogen locally via overexpressing aromatase enzyme compared to their normal counterparts (Bulun *et al*.,2005).

The concentration of estradiol was 2.3 times higher in breast cancer tissues than in the areas as morphologically normal(Chetrite *et al*.,2000).

Biologically, it is known that endogenous estrogen bind specifically to estrogen receptor (ER) and influence tumor growth For instance, women whose tumors are positive for both ER respond better to endocrine therapy compared with those whose tumors are negative for both receptors. **(**Rayter **.,** 1991 ; Habel & Stanford ., 1993).

For premenopausal patients with metastatic breast cancer, the classic treatment is ovariectomy (Beatson ., 1986).

the first clinical study with an LHRH analogue were reported by Klijn and de Jong 1982 Since then, a series of more than 13 phase II studies with various LHRH agonists, such as goserelin, buserelin, and others, have shown an objective response in 161 (38%) of 419 patients (Klijn ., 1992).

Beside the LHRH analogue anti-estrogens are now widely used for the treatment of postmenopausal women with hormone dependent breast cancer anti-estrogens, such as tamoxifen, tamoxifen is now the standard first-line therapy for postmenopausal metastatic breast cancer and is also accepted as an alternative to ovariectomy in premenopausal patients (Fossati *et al* ., 1998).

Tamoxifen block the interaction of estradiol (E2) with the estrogen receptor (ER) (Cole MP *et al* ., 1971; Smith IE & Dowsett M ., 2003).

Tamoxifen is regarded as a pro-drug since two of its metabolites, 4-hydroxytamoxifen (4OHtam) and 4-hydroxy-N-demethyltamoxifen (4OHNDtam, endoxifen), both have estrogen receptor affinity markedly exceeding that of tamoxifen itself (Katzenellenbogen *et al* ., 1984 ; Johnson *et al* ., 2004) .

The 4OHNDtam is considered the main active metabolite of tamoxifen, since it has 100-fold higher affinity for the estrogen receptor (ER) than tamoxifen and

is 10-fold higher in serum levels than 4OHtam (Borgna & Rochefort., 1981; Gjerde *et al* ., 2008).

These potent metabolites are converted from tamoxifen through the cytochrome P450 (CYP) enzymes 2C19, 2D6, and 3A5. They are conjugated and deactivated through sulfotransferase (SULT) 1A1 (Desta *et al* ., 2004) in this research we investigated the effect of tamoxifen and LHRH analogue on estrogen level and the side effect of these drug on some liver enzymes when these drug metabolism in the liver.

Material and methods :

Are taking random samples of breast cancer patients under treatment of patients attending the Institute of Atomic Radiation in Baghdad , and specifically who use (20 mg/day) of tamoxifen . 100 samples of breast cancer and 30 sample as a control aged (20 – 80) years the collection of samples was conducted during the period from April 2013 to July 2013

Approximately 3ml of blood were collected from each women using standard procedures. The blood put in Spain tube allowed to stand at room temperature for at least one-half hour or until it was thoroughly clotted and then refrigerated within 2 hours of collection ,blood was centrifuged and serum was separated and put into sterile apendroff tubes ,the letters were labeled and stored at -70C° (Dorgan *et al.*,2010).

1- Measurement of Estaradiol hormone E2:

Reagents according to Estaradiol E2 hormone kit, Monobind, USA.

2- Measurement of the liver enzymes:

Test for (alkaline phosphate ALP , of gamma glutamyltransferase (GGT) or Ɣ GT , glutathione oxidase transferase GOT , glutathione pyruvate transferase GPT in blood serum plasma with The Reflotron® System.

Measurement of lactate dehydrogenase (LDH) : Testes of LDH with linear chemical S.L , France (kit)

3- Statistical analysis :

The statistical analysis of this study is made by using SPSS program (Version 10) and the statistical processes used here were Means, Standard deviations, One way ANOVA and Chi square .

RESULTES:

1- Age

The highest percentage of breast cancer patients under investigation was recorded in (41-60 years) with 58 cases (%58) followed by 32 cases (%32) were seen (20-40 y), and 10 cases (%10) were seen in (61-80 y), table (1).

Table (1) Distribution patient breast cancer groups according to age .

Studied groups	0-40	1-60	1-80	$\chi 2$ between the Categories for patient
Patient group n = 100	2	8	0	0.000
%	32%	58%	10%	

P ≤ o.o5

2- Estradiol hormone (E_2).

The results of table (2) showed a significant decrease difference in the levels of estradiol hormone E_2 in group of breast cancer (37.3634±45.02893) as compared with the control groups (93.3700±51.07188) .

Table (2) Serum estradiol hormone levels (E_2) (pg/ml) in control and patient groups .

Studied groups	Mean±SD	Significant
Control group	93.3700±51.07188	.000
Patient group	37.3634±45.02893	

P ≤ o.o1

3- Liver function enzymes (Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), Alkaline Phosphate (ALP), glutamyl transpeptidase (GGT), lactate dehydrogenase (LDH-5) , Total Billirubin:

Results showed no significant differences in liver enzymes, as well as total bilirubin between patients group and the control group As in the table (3)

Table (3) the level of liver enzymes U / l, total bilirubin (mg / dl) between patients and the control group

The liver enzymes	Studied Groups	Mean ± Std . deviation	Significant
AST	Control group	27.2100±3.61914	.296
	Patient group	24.1865±15.63097	
ALT	Control group	31.7570 ±7.47237	.554
	Patient group	34.7260± 26.99979	
GGT	Control group	18.1733± 3.98946	.084
	Patient group	50.4226± 101.14239	
ALP	Control group	83.42±22.58	0.620
	Patient group	89.64±67.21	
LDH -5	Control group	168.5333 ±39.23293	.654
	Patient group	164.9100 ±38.58327	
Total billirubin	Control group	.08337 .6473±	.292
	Patient group	.21078 ± .6891	

$P \leq o.o1$

Discussion :

1- Age:

In the present study maximum number of women with malignant breast cancer were observed in 41-60 years (58 cases) followed by 20-40 years (32 cases) .The average age was 50 years .These results agreed with previous studies in Iraq(Waheda, 1998 ; Madhoor ,2002).

Age is the single most important risk factor in breast cancer. women are 10 times as likely to develop breast cancer in their thirties and twenties ,40 times as

likely in their forties ,60 times as likely in their fifties and 90 times as likely after sixties(Forbes, 1997).

And agreed with other study that show in united state the higher percent 20% in age before 50 years and followed 4% in 40 and the higher infect in the third decades (Howlader *et al* ., 2013).

2- **Estradiol hormone (E_2).**

The present study demonstrated that there was a significant lowering in the levels of serum estradiol hormone in women with breast cancer table (2) who were taking tamoxifen drug and LHRH analogue this result was supported by Jan *et al* ., 2000.

Estrogen can cause cancer by stimulating cell proliferation(promotion) and causing genotoxic damage(initiation), estrogen are highly mitogenic in hormone sensitive tissues such as breast ,prolonged exposure of target tissues and cells to excessive mitogenic stimulation by estrogens has been considered an important etiological factor for induction breast cancer (Hiraku *et al*.,2001).Serum estradiol hormone levels were found to be associated with local estradiol levels in normal breast tissue of breast cancer patients, this strengthens the hypothesis that serum estradiol levels influence the gene expression in breast tissue(Lonning *et al*.,2009).

In the two groups of patients treated with buserelin (LHRH analogue) alone or buserelin with tamoxifen, both the median and the mean levels of plasma estradiol dropped to normal postmenopausal values within 6 weeks and remained suppressed throughout treatment in all patients In the group treated with tamoxifen alone, however, plasma estradiol levels increased on average threefold to fourfold in nearly all patients.

The study also agreed with (Forward *et al* 2004) As it was found that giving tamoxifen treatment with giving hormone ((LHRH analogue) lead to a lack of estrogen significantly.
And tamoxifen treatment is competitive with estrogen receptors in breast tissue (Marian *et al* ., 2011).

In another study confirmed that giving tamoxifen alone for women with breast cancer pre-menopause is working to increase the concentration of estrogen 2-3 times compared to women who did not taking of tamoxifen (Sherman ., 1979)

In three published randomized trials have compared combined hormonal therapy using tamoxifen and an LHRH analogue to endocrine monotherapy (the LHRH analogue in all but one of the trials) in premenopausal women with advanced breast cancer A meta-analysis of these trials has been performed, and it suggests that the combination is superiorto monotherapy for all end points, with significant benefits in mortality rate (reduction in estradiol plasma level) , (22% relative reduction), rate of disease progression (30% relative reduction), rate of objective clinical response (39% versus 30%), and duration of response (19 months versus 11 months) (Boccardo *et al* ., 1994; Jonat *et al* ., 1995 ; Klijn *et al* ., 2000).

3- Liver function enzymes (Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), Alkaline Phosphate (ALP), glutamyl transpeptidase (GGT), lactate dehydrogenase (LDH-5) , Total Billirubin:

The liver is important organ that metabolized transformation and elimination different drugs (Miya *et al* ., 1991).

The metabolism of liver in two phases , the first phase include: oxidation , reduction, hydroxylation and mithelation by the enzyme system of Cytochrome P - 450 located in the endoplasmic reticulum which is the most important enzyme in drug metabolism in the liver

While the second phase include: conjunction the chemical materials with aquoes compound like : glucoronide , sulfur compounds and amino acids and thus lead to the formation of intermediate compounds soluble in water and are easy to elimination (Kedderis ., 1996).

The last reaction occurs in the second stage is glutathione that works on conjunction intermediate compounds with glutathione-S transferase covalently that result in elimination of toxic intermediate compounds that may cause hepatotoxicity (Lee ., 1995)

For this reason the test of liver enzymes are necessary for liver health (El-Beshbishya *et al* ., 2010)

In recent study the liver enzymes and billirubin are measured and show there are no significant different and agreed with (Degregorio *et al* ., 1989) the

results of this study show that the high doses of tamoxifen 20 mg twice in a day causes damage in the liver by rising ALT , ALP , AST and total billirubin while GGT and LDH didn't show any change in high doses but when the doses of tamoxifen was reduced all enzymes and billirubin become normal .

Another study appeared that the tamoxifen with normal dose 10 mg twice in day didn't affected in liver enzymes and billirubin (Floren *et al* ., 1998) .

While (El-Beshbishya *et al* ., 2010) appeared the high doses of tamoxifen consider toxic and cause hepatotoxicity and raises sGOT, sGPT, sLDH, sALP and s GGT in rates but this not approved on the human .

And the studies (Sharma R *et al* ., 2003 ; Jakesz R *et al* ., 2002 Jan G. M. Klijn *et al* ., 2000) showed that the treatment with tamoxifen and LHRH analogue didn't affected on the liver.

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