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Association of CYP19 Gene Polymorphism with Breast Cancer in Iraqi Women

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علاقه تعدد الاوجه لجين (CYP19) مع سرطان الثدي في النساء العراقيات

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ABSTRACT

The basic enzyme in estradiol synthesis is, Aromatase, it is encoded by the gene called CYP19A1. Given the importance of estrogen in the development of Breast cancer risk, we studyt genetic polymorphisms in the CYP19A1 gene the, including rs10046, in a group of 45 patient women in Kerbala province, Iraq. Our aim to investigate CYP19AI polymorphism its impact on patients of Breast cancer in Iraqi women. We found in our study, that in Iraqi postmenopausal women,(rs10046) single nucleotides and polymorphism (SNPs) was prognostic of different levels of estrone and Estradiol. Depending on the BMI scale reveals 22.25% with the breast cancer group in Comparison with 30% of healthy women. In case of BMI with(20-24) in the breast cancer group were 22.2% compared with the healthy group 20%., While BMI of >24 we found 33.4% in case of Breast cancer affected group in comparison with 20% of the unaffected group (Healthy). While in case of BMI >30 there were 22.2% in case of the Breast cancer group in comparison with 30% of unaffected (healthy) group. The molecular analysis of CYP19 gene ,reveals that A allele frequency was (0.6333) and G allele (0.3667) upon that, we found that the number of patients which have had breast cancer were 28 and 17 ,respectively. In summary, our data show that polymorphisms in the CYP19A1 gene may contribute to Breast carcinogenesis in Iraqi women.

Keyword: (Iraqi women, SNPs, Breast cancer,CYP19 gene)

الخلاصة

الانزيم الاساسي في عمليه تصنيع هرمون الاستروجين بواسطه انزيم الاوروماتيز والذي يكون مسيطر عليه من قبل جين السي واى بى 19 اي وان, وعليه فان نشاط هذا الهرمون له علاقه مباشره بحالات سرطان الثدي. من خلال البحث الذي اجريناه فى مجموعه مكونه من 45 امرأه في محافظه كربلاء اظهر بان جين السي واي جي 19المتعدد الاوجه من النيكليوتايد وهذا ناتج عن النشاط المتزايد من هرمون الاستروجين فى نسيج الثدي. بالاعتماد على قياس البي ام اى فان المجموعة ذات <20 يظهر بان 22.2% مصابه بسرطان الثدي مقارنه ب 30% غير مصابه . في حاله البي ام اي (20–24) فان22.2% اصابه بسرطان الثدي مقارنه ب20% غير مصابه . اما في البي ام اي>24 فان 22.2% اصابه بسرطان الثدي مقابل 30% غير مصابه .التحليل الجزيئى يظهر بان اليل اي التابع لجين سي واي بي جي 19 كان تردده (0.6333) مقارنه ب اليل ج بتردد (0.3667) , وهذا يعنى بان عدد الاصابات بسرطان الثدي يكون 28 و17 بالتتابع ولكلا المجموعتين من اصل 45 حاله . وهذا دليل على اهميه الاوجه المتعددة لجين السي واي بي جي .

الكلمات المفتاحية: النساء العراقيات، سرطان الثدي، جين .CYP19.

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INTRODUCTION

A close relation was shown to exist There is a relationship between increased estrogens and cancer, as the latter increased mitotic cell division. Also, many studies connected of the exogenous estrogens administration and the growth Breast cancers [1]. Increased levels of estrogen play an important role in that leads to a propagation of several types of cancers, especially breast cancer. It was shown that some tissues which is sensitive to estrogen behaves as intracrine organs, in production of estrogen
and later increase hormone levels locally, which enhanced development and propagation of cancer tissue [2].

There are four main estrogens naturally present , estradiol (E2), estrone (E1), estetrol (E4) and estriol (E3), these are a pregnancy hormones. Estradiol has the greatest attraction to the estrogen cell receptors, with many physiological body requirements during all life periods in both sex females and males [3] Estrogens are manufactured from androgens by Aromatase enzymatic activity, [4]. *CYP19A1*gene is an essential member of the cytochrome group it catalyzes the aromatization of androgens and has rate-limiting step during this process [5]**.** Aromatase gene expression has shown by many studies is (estrogen-dependent) tissues of breast cancer (BC)[6]

Tumor,HER2 status which is a receptor of Breast cancer and aromatase enzymatic inhibitors ability has a relation with CYP19A1 genetic variants [7-8].

Aromatase enzyme is essential for estrogen synthesis. The aromatase gene polymorphic forms appear to give rise a propagation of multiple types of Breast cancer**.** It was shown that some tissues which are sensitive to estrogen act as intracrine organs, estrogens producing tissues and elevating hormone levels locally, consequently accelerating proliferation and development of cell cancer [2].

Many deviations acquired in coding seems to be a character of *CYP19A* gene which have, noncoding, and control sequences [9]. Incidences of alleles in many peoples vary significantly, variances in blood levels of some sex hormones are also shown that the may be because of the presence of the variant alleles, mainly in women at postmenopausal period . A positive feedback mechanism is reported by Several studies suggesting a link between breast cancer and the activity of aromatase enzyme in the local breast parenchymal tissue [15]. Some suggest that the aromatase enzyme produces local tissue estrogens, within the breast tissue, might affects growth and progression of breast cancer.[9].
The study investigated the genetic SNPs of the CYP19A gene and its relationship with Breast cancer in Iraqi women.

Materials and Methods:

-How Samples was collected : Samples of the blood were withdrawn of 55 patients (tubes with EDTA). 45 samples from women with breast cancer and 10 samples from Healthy Women , who was admitted at Imam Hussein Medical City in Kerbala province\Oncology Department. All blood samples were placed in a sterile plastic bag inside portable coolers at 4 C˚, labeled and transported to the laboratory of the Al-safwa University College in order to be processed within 3 – 4 hours of collection . This study was conducted in a period from December 2020 Until May 2021.

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- DNA extraction and purification

As stated by the manufacturer Promega kit recommendations (Promega/ USA), DNA from the samples above was extracted and purified.

2-3-**Oligonucleotide primer sequences used for PCR**

For PCR, The sequences of Oligonucleotide primer were used, amplification (Alpha DNA /Canada) were listed in table (1).

Table (1) : Oligonucleotide primer sequences

Primer	Sequence (5′→ 3′)	Alzahid
Forward	**5- TAGATAAACCCTTCCACTCTGTGCTGT**	
Reverse	**5- CACTGAAACCTAAAGAAGCAGTCTGAA**	
Forward	**5-TCTTCTCCCTTTCACTTTGTTTGCG-3**	
Reverse	**ATACAAGACAAAGAGGGGGCATAGA-3**	

-Method of PCR for CYP19 gene detection:

This step was done in a private Lab. Outside the Al-Safwa University College. Breast cancer was detected by PCR using specific primers (CYP19 gene).

The PCR tubes containing the amplification mixture were moved to a thermocycler. that had been preheated, and the program was begun as shown in the table:

Table (2) : PCR amplification program for CYP19 gene detection

Step	Temperature (C°)	Period of Time (second)	NO .of cycle
Denaturation Step /Initial	94	sixty	one
Denaturation Step	94	sixty	Thirty five
Annealing Step	62	sixty	
Extension Step	Seventy two	60	
extension Step (Finally)	72	Six hundred	one

- Agarose gel electrophoresis:

The production of agarose gels was done with two concentrations of (one percent) and (two percent). After the DNA extraction process, one percent concentration agarose was used in the electrophoresis process, and two percent agarose was employed in the PCR detection process. Then came the next set of steps. **I. Agarose gel Casting process:** Assembling gel was on one side of the tray, next to casting tray and the comb. **II. Loading DNA & DNA running in an agarose gel** After mixing with 3 liters of bromophenol blue (loading buffer) and 9 liters of DNA, the mixture was loaded into the wells of a 2 percent agarose gel. In the well side unit the cathode was linked, and on the opposite side was the anode. Running the Gel at 75 Volt till the tracking dye bromophenol blue traveled toward the gel end . DNA Observation by gel staining with ethidium bromide and viewing under the Ultraviolet trans illuminator device.

-Result of PCR analysis:

After the amplification process, the PCR results were performed. Amplification sample running it for 1 hr with 75 V, the products then were seen by the UV trans illuminator device .

- Statistical analysis

The statistical significance determination between diverse variables , Using SPSS (Program for Social Sciences statistics). Application of the Chi square to test the gained results .

4. Results and Discussion

The cases of our study were 45 women with breast cancer and another group with 10 healthy women. From peripheral blood cells a genetic analysis of CYP19A1 gene was performed .

In the table (3) 22.2% Breast cancer cases compared with 30% Healthy cases at the age of 45-55 years. The ages of (56 – 65) Years old 22.25% cases affected in comparison with 20% Healthy Women of the same age group. The ages of (66-75)Years 33.3% affected cases compared with 20% of Healthy cases. At The age of 76-85 Years Old 22.2% of the cases were affected in comparison with 30% cases Healthy women .

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Table (3): Percentage of different Patient Ages positive for breast cancer in comparison with the control (Healthy Cases).

Patients and healthy collection samples				
Ages(Year)	**Patient (45)**		**Control (10)**	
	+ve	**Percentage (%)**	**+ve**	**Percentage (%)**
45-55 Y	10	22.2	3	30
56-65 Y	10	22.2	2	20
66-75 Y	15	33.4	2	20
76-85 Y	10	22.2	3	30

In Table (4) showed that in January, 22.2 % of the samples Breast cancer women in comparison with the 30% healthy cases. While 22.2% of cases were collected in February compared with 20% healthy cases. In March, 33.4% of the Blood samples were breast cancer cases in comparison with 20% healthy cases, and in April, 22.2 % Blood sample cases were affected with breast cancer in comparison with 30% healthy cases.

Table (4): Percentage of seasonal months Blood Samples Collection for breast cancer in comparison with the control (Healthy Cases).

Patients and healthy collection samples				
Month study	**Patient (45)**		**Control (10)**	
	+ve	**Percentage (%)**	**+ve**	**Percentage (%)**
January	8	22.2	3	30
February	12	22.2	2	20
march	15	33.4	2	20
April	10	22.2	3	30

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In Table (5) the BMI of<20 in case of Breast cancer cases were 22.25% in Comparison with 30% of healthy women. In case of BMI with(20-24) in the breast cancer group were 22.2% compared with the healthy group 20%. There were 33.4% with the BMI of >24 in case of Breast cancer affected group in comparison with 20% of the healthy unaffected group. In a group of BMI >30 there were 22.2% of Breast cancer category in comparison with 30% healthy unaffected group.

Table(5): Percentage of BMI in cases Blood Collected Samples from the breast cancer in comparison with the control (Healthy Cases).

Patients and healthy collection samples				
BMI	Patient (45)		Control (10)	
	+Ve	Percentage(%)	+Ve	Percentage (%)
<20	8	22.2	3	30
20-24	12	22.2	2	20
>24	15	33.4	2	20
>30	10	22.2	3	30

-CYP19 Gene polymorphism, a molecular analysis:

There is an increased AA genotype frequency found in our result (71.1%) , followed by genotype GG and GA as (24.4%) and (24.4%) respectively.

Table(6) Showed odd ratio of gene CYP19 polymorphism with women have breast cancer.

Genotype frequency	Subjects		Odd ratio CI
	Patient N= 45	Health N= 10	
GG	11	3	
GA	11	3	1
AA	23	4	0,637 0,12 -3.35

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The present study showed the most common CYP19 gene polymorphism in the patient have AA genotype as showed in the figure (4-5)**,** our result found the A allele frequency was (0.6333) and G allele (0.3667) therefore the number of patients that have breast cancer were 28 and 17 ,respectively.

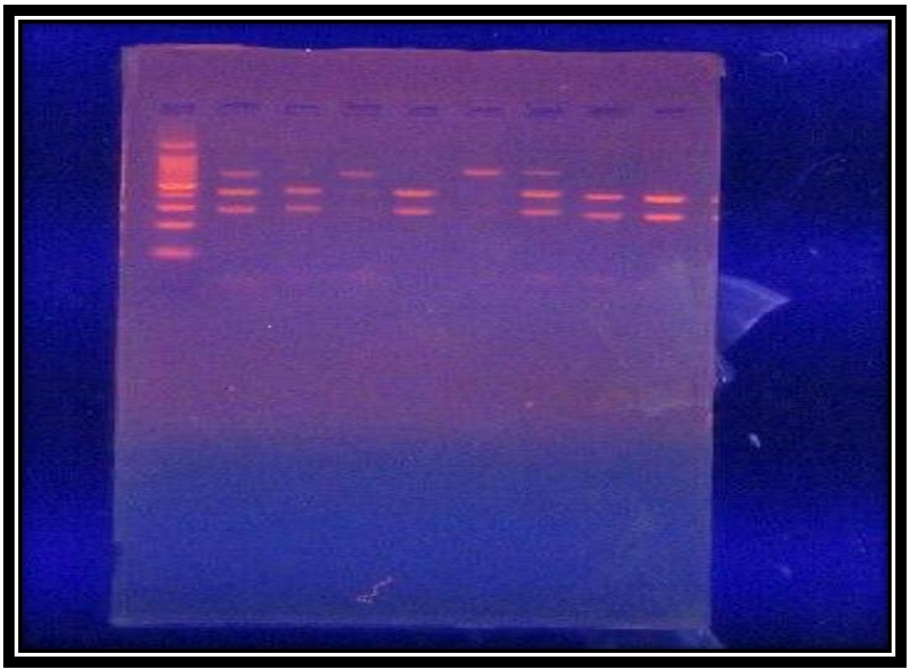

Figure (4-6) : Amplification of a of CYP19 gene by means of Polymerase chain reaction of Lan M: 100 bp marker, Lan 1,6 ,: Hetrozygote allele GA., Lan 2 , 4, 7 and 8 : GG allele while Lane 3 and 5 represented AA allele.

Generally, synthesis of estrogen in postmenopausal women happened primarily in fat tissue, an aromatase action variation might be of higher significance for the threat of breast cancer. Our findings suggest that the variation of CYP19A1 may augment the growth and development of breast cancer in Iraqi women.

Endogenous estrogen is largely produced in adipose tissue during and after menopause [10]. As a result, CYP19A1 polymorphisms are more likely to have an effect on postmenopausal women, especially those BMI levels with higher values [11], which is supported by our findings.

The (rs10046) allele has been thoroughly investigated, including its probable gender-specificity connection with increased estrogen levels[16] and breast cancer risk among Japanese

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populations[17]. This is consistent with our findings. Also, in another study of breast cancer(BC) done in China, an allele was discovered. [11],and it is agreeing with our findings.

However, the findings of another study contradicted this idea, indicating the genotype is related with a reduced threat of breast cancer in Caucasian postmenopausal [9],and it does not agree with our findings.

Adjuvant endocrine therapy lowers the chances of relapse and mortality. In women with positive breast cancer hormone receptor (BCs)[12]. Aromatase is an enzyme complex that biosynthesizes estrogens from androgens. Intratumoral aromatase is the local estrogen source essential in breast cancer (BC) tissues in postmenopausal women. As a result, inhibiting aromatase is a key strategy for lowering estrogen's growth stimulatory effects in estrogen-dependent BC [13].

In conclusion, our findings demonstrate that SNPs in the CYP19A1 gene are linked to elevated blood levels of estrone in postmenopausal women, as well as a close relationship in overweight or obese postmenopausal women and its relation with estradiol plasma levels. This study is a step and further studies is necessary.

Conflict of interests.

There are non-conflicts of interest.

References

[1]. Watanabe S, Kobayashi Y. Exogenous hormones and human cancer. Japanese Journal of Clinical Oncology.;**23**(1):1-13.**(1993)**.

[2]. Sasano H, Harada N. Intratumoral aromatase in human breast, endometrial, and varian malignancies. Endocrine Reviews.;**19**:593-607.**(1998)**.

[3]. Nelson LR, Bulun SE. Estrogen production and action. Journal of the American Academy of Dermatology.;**45**(3):S116-S124.**(2001).**

[4]. Harada, N. Structure, regulation and polymorphisms of the aromatase gene. In Resistance to aromatase inhibitors in breast cancer (pp. 13-31). Springer, Cham**. (2015).**.

[5]. Warsy, A. S., Almukaynizi, F. B., AlDaihan, S., Alam, S., & Daghastani, M. Genetic polymorphisms in aromatase(CYP19)gene and cancer. Genetic polymorphisms, 27**.(2017).**

[6]. Dunning AM, Dowsett M, Healey CS et al. Polymorphisms associated with circulating sex hormone levels in postmenopausal women. J Natl Cancer Inst 96:936–945.**(2004).**

[7]. Colomer R, Monzo M, Tusquets I et al. A single-nucleotide polymorphism in the aromatase gene is associated with the efficacy of the aromatase inhibitor letrozole in advanced breast carcinoma. Clin. Cancer Res 14:811–816.**(2008).**

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info@journalofbabylon.com | jub@itnet.uobabylon.edu.iq | www.journalofbabylon.com

[8]. Fasching PA, Loehberg CR, Strissel PL et al. Single nucleotide polymorphisms of the aromatase gene (CYP19A1),HER2/new status, and prognosis in breast cancer patients. Breast Cancer Res Treat 112:89–989**.(2008).**

[9]. Haiman, C. A., Dossus, L., Setiawan, V. W., Stram, D. O., Dunning, A. .,Thomas, G. et al. Genetic variation at the CYP19A1 locus predicts circulating estrogen levels but not breast cancer risk in postmenopausal women. Cancer Res.67, 1893–1897. **(2007).**

[10].. Ma X, Qi X, Chen C et al. Association between CYP19 polymorphisms and breast cancer risk: results from 10,592 cases and 11,720 controls. Breast Cancer Res Treat 122:495–501.**(2010).**

[11]. Long JR, Shu XO, Cai Q, et al. CYP19A1 genetic polymorphisms may be associated with obesity-related phenotypes in Chinese women. Int. J. Obes (Lond);31:418 – 23**.(2007).**

[12]. Baum M. Polychemotherapy for early breast cancer. Lancet. 7;352(9139):1554 Nov(1998). doi: 10.1016/S0140-6736(05)60364-9.

[13]. Brueggemeier RW, Hackett JC, Diaz-Cruz ES. Aromatase inhibitors in the treatment of breast cancer. Endocr Rev 26: 331–345**(2005)**.

[14]. Sambrook, J., Fritsch, E. R., & Maniatis, T. Molecular Cloning: A Laboratory Manual (2nd ed.). Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press**(1989).**

[15]- Schmidt, M., & Löffler, G. Induction of aromatase in stromal vascular cells from human breast adipose tissue depends on cortisol and growth factors. FEBS letters, 341(2-3), 177-181**(1994).**

[16]. Haiman, C. A., Dossus, L., Setiawan, V. W., Stram, D. O., Dunning, A. M.,Thomas, G. et al. Genetic variation at the CYP19A1 locus predicts circulating estrogen levels but not breast cancer risk in postmenopausal women. Cancer Res. 67, 1893–1897**(2007).**

[17]. Zhang, L., Gu, L., Qian, B., Hao, X., Zhang, W., Wei, Q. et al. Association of genetic polymorphisms of ER-alpha and the estradiol-synthesizing enzyme genes CYP17 and CYP19 with breast cancer risk in Chinese women. Breast Cancer Res. Treat. 114, 327–338**(2009).**

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