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## Evaluation ESR1 Single Nucleotide Polymorphisms rs106577 and Susceptibility for Breast cancer Risk in Young Women in Dhi Qar Governorate.

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### Abstract:

The current study was conducted in Dhi Qar Governorate on young women suffering from Breast Cancer (BC), where their ages ranged exclusively between (20-40) years. It was the first study in the governorate to address young ages only, to highlight the spread of this serious disease in young groups and to know its relationship with genetic causes and genotypes.

The aim of the current study is to investigate genetic mutations of the estrogen receptor gene (ESR1 rs1062577) and its relationship to breast cancer risk in young women.

This study included (100) randomly collected samples, divided into two groups: the first group consisted of ( 80 ) of blood samples from patients undergoing surgical intervention, breast biopsy, and follow-up at Al-Haboubi Hospital/Oncology Unit/Dhi -Qar province, which is a specialized department for treating cancer patients. The second group consisted of (20) samples from healthy young women. The study was conducted from February 2023 to July 2023.

The results of the Tetra ARMS-PCR analysis for the estrogen receptor gene ESR1 rs1062577 revealed the presence of two alleles ‘A and T and three genotypic patterns, AA, AT, and TT, in both the breast cancer patients and the control group. The allele A was recorded at a percentage of 58.75% in the patient sample compared to the allele T , ‘which was recorded at a percentage of 41.25% In contrast, the allele A was present at a percentage of 100% in the control sample, while the allele t did not appear and had a percentage of 0%. No significant differences were found at a significance level of  $p < 0.05$ .

Increased the incidence of breast cancer among the young age group in Iraq and in our province ( Dhi-qar) so at limited time of our study we can collect good number of patient diagnosed with invasive breast cancer in age between (20-40) year ,These results can predict and give idea about prognostic staging and fate of this dangerous cancer especially in young age group who may be lived for long time after diagnosis of cancer, Study the type of genetic mutation for estrogen receptors as important cause for incidence of breast cancer cases.

**Key word:** Breast cancer , ESR1, rs 1062577 , Tetra –primer – ARMS –PCR.

### Introduction:

Breast Carcinoma (BC) is a type of cancer that appears in breast tissue. It arises from the uncontrolled growth of abnormal breast cells. It usually affects the tissues responsible for producing milk (ducts and

lobes). It is also more common in women and is the main cause of death. Associated with cancer, the death rate is about (15%) annually around the world [1].

It is still one of the greatest health threats facing women around the world ,This disease is one of the most common types of cancer in women, second after lung cancer, and represents (10.4%) of all cancer cases, and the fifth most cancer deaths[2]. It is the highest cancer among Iraqi women, as it constitutes about (34%) of all women's cancers. This disease is recorded in Iraq, representing a third of the total number of cancers [3].

Breast cancer is also considered a multifactorial disease that results from the association between environmental factors, lifestyle, hormonal and genetic factors [4] . Only about (10%) of breast cancers are caused by genetic factors. While the other 90% of breast cancers are caused by non-genetic factors. Common risk factors for breast cancer include exposure to radiation, lifestyle, age, estrogen medications, and diet, in addition to some genetic mutations [5].

Estrogen plays an important function in the development of certain types of cancers, such as breast and ovarian cancer , When estrogen levels are affected by common polymorphisms in genes, it may cause breast cancer [6], and the estrogen receptor gene rs1062577 (ESR1) has a close association with breast cancer, as shown by previous studies in the Chinese population [7] and the Iranian population [8] They noticed that there is a relationship between the estrogen gene and the risk of breast cancer.

Therefore, more population studies are needed to prove the relationship between the estrogen receptor gene ESR1 (rs1062577) and the risk of breast cancer. To achieve this goal, this current study was established to investigate the relationship between this gene and Breast cancer. in Dhi -Qar Governorate..

## **Materials and methods:**

### **-Collection of Samples:**

The current study included one hundred samples peripheral blood samples, with 3 ml of blood collected from each woman and preserved in EDTA-coated tubes for DNA extraction. The samples were divided into two groups: the breast cancer (BC) group, consisting of (80) samples, and the control group, consisting of (20 )samples. The age range for both groups was between (20 – 40 )years. Each step was performed simultaneously for the control group alongside the breast cancer group samples. Tissue samples were collected from patients undergoing breast surgery and biopsy at Al-Imam Al-Hussein Teaching Hospital. Blood samples were collected from the laboratory of Al-Habboubi Hospital/Cancer Unit in Thi Qar Governorate, which is a specialized department for treating cancer patients. The collection period starting from February 2023 to July 2023.

As for the molecular study, DNA was extracted from blood samples of breast cancer patients and healthy women using the Geneaid DNA extraction kit from Taiwan.

### **DNA isolation**

To isolation the genomic DNA from the blood samples , prime prep Genomic DNA Isolation Kit (Geneaid DNA extraction kit,Taiwan) was recruited .the yielded DNA was diluted in 0.5 M and stored at -20 °C degree.

## Primers

The primer software was recruited to design the Tetra ARMS PCR primers ( <http://primer.soton.ac.uk/primer.html>), as shown in Table (1). Primers were respectively used to amplify rs1062577 A and T alleles.

**Table 1:** Primer sequences used for Tetra-Primer-ARMS- PCR

Primers	Primers Sequences	Length	Tm(c)
<b>OUTER</b>	F-5GTTAATTATGCTCTGTTTCCAAC-3	24	54.2
	R-5GCACAGCTCTAAACACACACA-3	21	58.8
<b>INNER</b>	F-5TTGAGATTCAAGAAAATTTCTATACA-3	27	51.8
	R-5CACAATTGGATGCAAAAATAA-3	21	50.6

After adding all the materials, the samples were mixed centrally using a PCR tube centrifuge and transferred to a PCR Thermal cycler. , primers were respectively used to amplify rs 1062577 A and T alleles. The generated product sizes for the A allele, T allele, and two outer primers were 119, 178 and 251 bp respectively.

The working method was carried out in a volume of 25 microliters, based on the instructions provided with the Green Master Mix manufactured by Macrogen. The following materials were mixed in a PCR tube.

### -Statistical analysis:

Statistical analysis was performed for all the studied samples using the SPSS Statistical Packages for Social Sciences software, employing the chi-square  $X^2$  test to determine significant differences between the studied groups .

## Results:

The genetic polymorphism of the estrogen receptor gene( rs1062577)in young female breast cancer patients was studied and compared to a healthy sample usingTETRA ARMS-PCR technology..

### Polymorphism of the estrogen receptor gene rs1062577

The results of electrophoresis of the amplified estrogen gene using the TETRA ARMS-PCR method showed the presence of two alleles, a and t, and the presence of three genotypes, AA, AT, and TT, in the sample of women with breast cancer and the sample of the control group, as in Figure (1) and (2).

Allele A was recorded in the patient sample at a rate of (84,4%)compared to allele T , which recorded a rate of (15,6%) as shown in Table(2) while allele A recorded a rate of (4%)in the control sample compared to allele T,The variable that did not appear and had a percentage of (0%) as

shown in Table (3) and there were no significant differences at A probability level of 0.05 for both tables.

**Table (2) Frequencies of two alleles A,T of the estrogen receptor gene rs 1062577 in blood samples from a group of young women with BC**

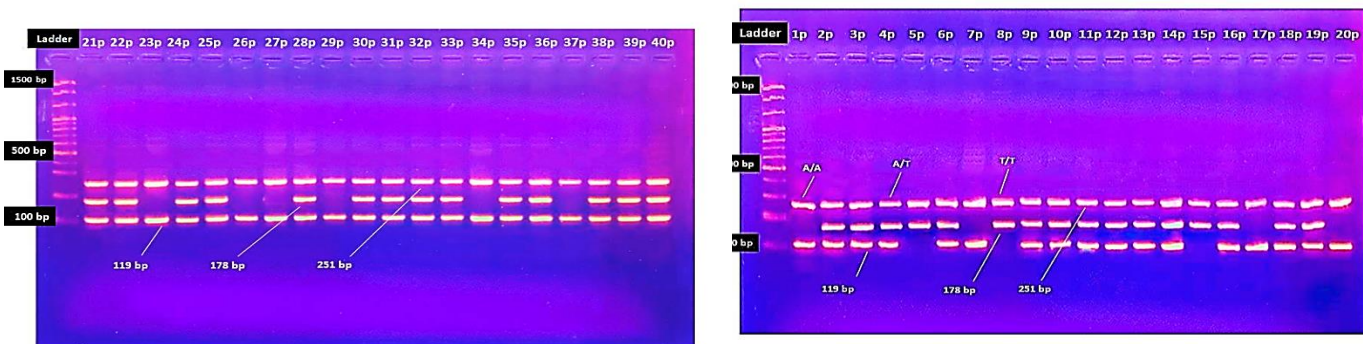
Genotype	Patients	Percentage
AA	19	23.75%
AT	56	70%
TT	5	6.25%
A	94	58.75%
T	66	41.25%

$X^2=180.20$  ,  $df=4$  ,  $p - value < 0.05$

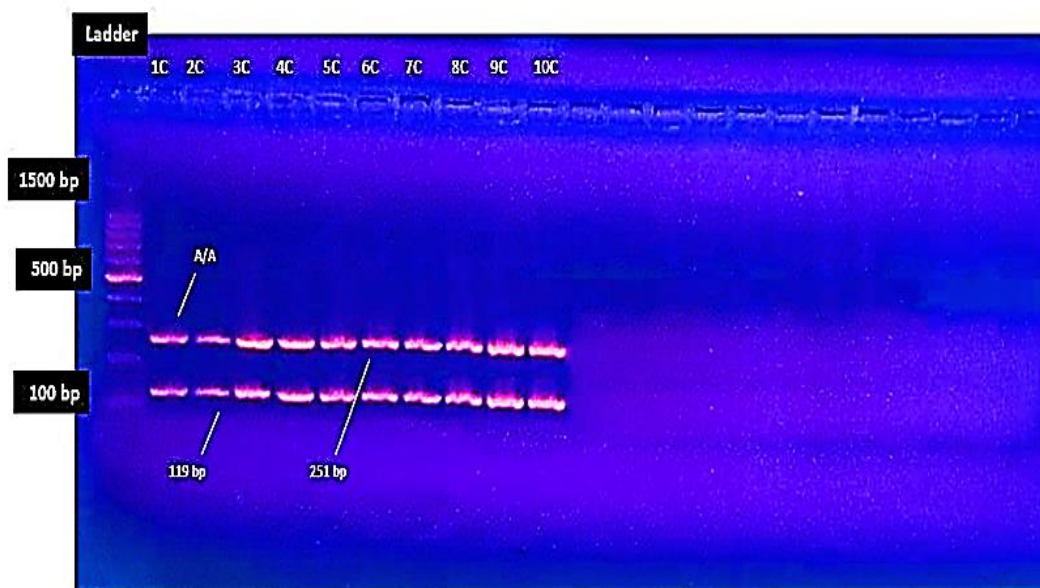
**Table (3) Frequencies of two alleles A , T of the estrogen receptor gene rs 1062577 in blood samples from a group of control**

Genotype	Control	Percentage
AA	20	100%
AT	0	0%
TT	0	0%
A	40	100%
T	0	0%

$X^2=106.66$  ,  $df=4$  ,  $p - value < 0.05$



**Figure (1) Estrogen receptor gene packages rs 1062577 for agarose electrophoresis at concentration 1.5% shown AA,AT,TT respectively 251bp,178bp,119bp**



**Figure (2) Estrogen receptor gene packages rs 1062577 for agarose electrophoresis at concentration 1.5% shown AA from a group of control**

#### **Discussion:**

The genetic polymorphism of the estrogen receptor gene ESR rs1062577 was studied in young breast cancer patients and compared with a healthy sample using the ARMS-PCR technique) Tetra-primer.

#### **Polymorphism of the estrogen receptor gene ESR1 rs1062577**

Breast cancer is closely associated with the estrogen receptor gene rs1062577 and its presence considers a potential biomarker for breast cancer. The polymorphism test of the estrogen receptor gene was used to predict the disease, prevent its development, and determine appropriate treatment. Therefore, our study aimed to evaluate the role of the polymorphism of the estrogen receptor gene and its association with the risk of breast cancer in young women in Dhi Qar province. This is the first study was conducted to examine this gene and its relationship with breast cancer in Iraq.

The results of the ARMS-PCR method for the amplification of the estrogen receptor gene revealed the presence of two alleles, A and T, and three genetic patterns, AA, AT, and TT, in the samples of young women with breast cancer and the control group. The AT genetic pattern had the highest frequency among the other patterns, accounting for (70%). From these results, we can infer that the presence of the AT genetic pattern increases the risk of breast cancer. On the other hand, the AA genetic pattern accounted for only (23%) of the patient group, which was significantly lower than the healthy control group, where it accounted for (100%). The other genetic patterns did not appear. This genetic pattern may indicate protection against the disease, which is consistent with the findings of [7]. They found that genotype AA was higher in control percent (100% ), than AT percent (0%). The environment contains numerous carcinogenic and mutagenic substances, including physical and chemical agents, which have become a significant risk for living organisms, including humans. These pollutants



directly cause changes and effects on living organisms, either immediately or after a long period of exposure to these substances [9].

Due to the contamination of the Iraqi environment with large amounts of chemical and radiation substances, the risk of breast cancer has increased. These results can be explained by the exposure of both rural and urban areas to the same pollutants or by the interaction between genetic and environmental factors in the occurrence of breast cancer [10] mentioned that environmental factors affect genes, leading to mutations. As a result of the accumulation of these acquired and inherited mutations, abnormal cell division is activated.

### **Conclusions:**

This study demonstrated that rs 1062577 A allele is significantly and dramatically associated with the increased risk of breast cancer. Increased the incidence of breast cancer among the young age group in Iraq and in our province ( Dhi-qar) so at limited time of our study we can collect good number of patient diagnosed with invasive breast cancer in age between (20-40) year .These results can predict and give idea about prognostic staging and fate of this dangerous cancer especially in young age group who may be lived for long time after diagnosis of cancer, Study the type of genetic mutation for estrogen receptors as important cause for incidence of breast cancer cases.

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