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Association Of Interleukin1 Beta C31T Polymorphism With Breast Tumour Patients

Wurood Ali hathal[1]
Frial Gemeel Abd[2]
Azhar Ammran Al-Thahab[3]
Wala Noori Majeed Barem[4]

[1]*Babylon Technical Institute, Al-Furat Al-Awsat Technical University, Babylon / Iraq*
[2,3]*University of Babylon – Collage of Sciences, Babylon, Iraq*
[4]*Consultant in general Surgery Babylon, Iraq*

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*Corresponding Author: Wurood Ali hathal,

Email: wurhathal@atu.edu.iq

Abstract: A mass of aberrant tissue is called a tumor. Breast tumors can be classified as either "malignant" or "benign," depending on whether they are malignant or non-cancerous. **Method:** seventy (70) breast tumor biopsies patients who were not receiving chemotherapy were included in the study. As a control group, fifty (50) blood samples were taken from healthy women. The total number of patients with breast tumor(130) as (100) blood samples of breast tumor patients before treatment and thirty samples after treatment were detected for the polymorphism of Interleukin 1 beta C31T by RFLP-PCR .**Results**: the IL-1 beta C31 T gene demonstrated a substantial difference between blood benign and malignant cells, with allele C being a dangerous allele in benign cells at (p – 0.07*). Additionally, the genetic influence of TT was shown to be significantly different in malignant versus benign tissue, with a *P* value of 0.02*.

Keywords :- Breast ,Tumor, , RFLP-PCR , IL1 beta. C31T

1. Introduction

Breast soft tissue lesions frequently present a diagnostic problem in day-to-day practice. These are generically classified as benign and malignant tumor lesions, encompassing a wide range of histopathological abnormalities [1]. Analyze the age-specific distribution of diseases. As a result of demographic disparities, 30-45 more than other age groups [2]. Focusing on Iraqi patients in a comparative study involving 1,940 Iraqi and British women diagnosed with invasive breast cancer, it was displayed that infiltrative invasive Ductal carcinoma was the most frequent histological type (86.5%) followed by Lobular carcinoma (8.8%) [3].The majority of individuals with breast lesions are female, In most countries, benign breast disorders are ten times more common than breast cancer and are the most common cause of breast issues in women [4]. Interleukin-1β (IL-1β), which is involved in inflammatory and immunological responses and how its involvement with breast cancer had been investigated. Two signals are needed for the tightly regulated production and processing of IL-1β: the "priming" signal, which initiates transcription of the IL1β gene, and the activation signal, which triggers the inflammatory caspases and inflammasome complexes to cleave pro-IL-1β into mature IL-1β [5]. Interleukin 1β is thought to play an important role in cancer invasiveness, progression, and metastases via inflammation in the tumor microenvironment [6]. Moreover, IL-1β is up regulated in breast neoplasm initiation and development [7], while IL1R and IL-1β variations have also been related to breast tumorigenesis [8] .

One way to initially assess the significance of IL-1β is to look at how it affects the onset or spread of cancer. The cancer genome atlas (TCGA) database can be used to measure gene polymorphisms that may affect the expression of IL1B mRNA or IL-1β protein. Patients

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with breast cancer who have high levels of IL1B mRNA expression have been shown to have a better prognosis than those with low levels [9]. Variations in IL-1β expression can be linked to polymorphisms on the IL1β gene. (rs1143627) IL-1β-31 The T allele is linked to higher production of IL1B. The T/T genotype has been linked to an increased risk of breast cancer [10,11]. The aim of this study investigated the role of IL-1 beta polymorphisms and their correlation with breast tumor.

2. Methodology

Samples collection

A130 specimens were collected from November 2021 and November 2022, Seventy (70) breast tumor biopsy patients who were not receiving chemotherapy were included in the study. As a reference group, fifty blood samples were taken from healthy women. A total of one hundred (100) blood samples from breast tumor patients at age ranged between (14-66) years and thirty samples following treatment at AL-Fayhaa National Hospital and AL-Hilla Teaching Hospital are located in Babylon Provinces . All of the women underwent lumpectomies or mastectomies to remove benign or malignant tumors; some had never had breast cancer before. Five centimeters beyond the tumor's edge, the tissue obtained for analysis for female patients with tumors. Within half an hour of the tissue being harvested, it was circucisioned, homogenized, and sliced with wooden sticks and a sterile medical knife. A normal saline solution was then poured into the sterile pee cup or plane tube.

DNA extraction

Favorgen (Taiwan) kit was used to isolate genomic DNA from the blood samples and G-spin total DNA (Korea) was used to isolated genomic DNA from tissue.

Genotyping of Interleukin 1 beta (IL1β) C31T

IL1β - C31 were genotyped by using the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method. A segment of the (IL1β) C31gene About 30 ng of DNA, 125 mM of deoxynucleotide triphosphates, 500 U of Taq DNA polymerase (MBI Fermentas, Vilnius, Lithuania), 20 mM PCR buffer of (NH4)2SO4, 2.5 mM of MgCl, and 50 pmol of each primer (forward and reverse) were included in the 23 µL PCR mixture. The PCR amplification process involved five minutes of 94°C melting, twenty-five cycles of one minute at 94°C, one minute at 54°C, and one minute at 72°C, and a final five minutes of 72°C elongation. The 240 bp PCR products were diagested in 3.0% agarose gel stained with ethidium bromide after being digested with AluI restriction enzyme (MBI Fermentas) during an overnight incubation at 37°C. The IL-1β T-31C was broken down into the three predicted results: one band with 240 bp for the CC genotype, two bands with 138 bp and 103 bp for the TT genotype, and three bands with 240 bp, 138 bp, and 103 bp for the CT genotype
F 5′-AGA AGC TTC CAC CAA TAC T-3′R 5′-TAG CAC CTA GTT GTA AGG A-3′[10].

Statistical analysis

SPSS software (version 23 SPSS) was used for the statistical presentation and analysis of the current study. Statistical significance was defined as p 0.05 [16].

3.Results and discussion

IL-1 beta C31 T genotyping by PCR
A- The PCR product in patients

The PCR product of IL-1 beta C31 T gene was amplified by using specific set of primer . the PCR product (band) of IL-1 beta C31 T gene was 240 –bp in blood patients and control Figure (1) and (2). This study agree with study [11] showed that product of PCR was 240 bp The Hardy-Weinberg equation can help to estimate allele frequencies in a population. Dominant (p) and recessive (q) allele frequencies and genotype frequencies [17].

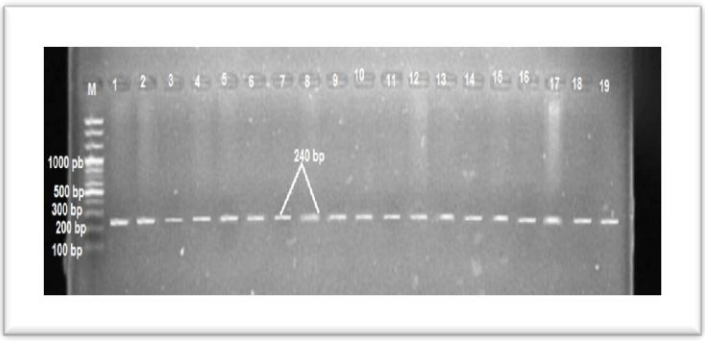

Figure 1. Electrophoresis pattern of PCR product of IL-1 beta C31 T patients, M : molecular DNA ladder , 1-19 PCR Product , Electrophoresis condition : agarose 1.5% , volt 85 , for 1 hour , red safe stain

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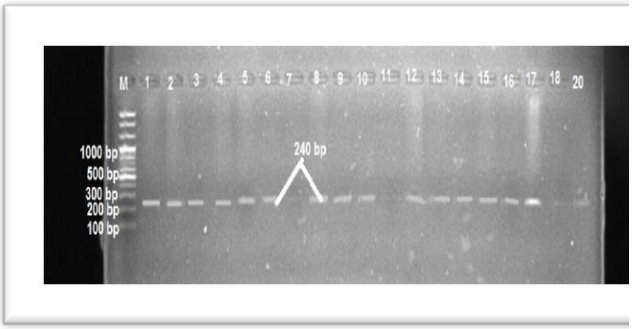

Figure 2. Electrophoresis pattern of PCR product of IL-1 beta C31 T in control case , M : molecular DNA ladder , 1-17 PCR product . Electrophoresis condition : agarose 1.5% , volt 85 , for 1 hour , red safe stain.

Detection of genotypes frequencies of IL-1 beta C31 gene in breast cancer patients compared to controls Patients without chemotherapy (blood and tissue)

The genotypes of the IL-1 beta C31 T gene polymorphism were identified along with alleles frequencies in patients (no. of samples and type of these samples) compared to healthy women (type of samples and number of samples tested. Result from Figure (3) showed that genotype of IL-1beta C31 T gene polymorphism with allele frequency between (the healthy 50 sample and blood patients were 100 sample) were represent TT homozygous (138pb and 103pb), CT heterozygous (240pb ,138pb and 103pb) and CC homozygous (240pb).

Figure 3. Electrophoresis pattern of IL-1 beta C31 T gene PCR-RFLP by PAGE gel for PCR product (240pb) with restriction enzyme *AluI* . M : DNA ladder 100 bp . lane (3,7) homozygous CC genotype 240 pb , lane (1,2) TT homozygous (138pb and 103pb) lane (3,4,6,9,10) CT heterozygous (240pb ,138pb and 103pb).

Conditions for electrophoresis were 30% PAGE, 100 volts, 1 hour, and ethidium bromide stain. In blood patients, the genotype frequencies of TT, CT, and CC of the IL-1 beta C31 T gene polymorphism were 28(28%), 52(52%), and 20(20%), whereas in healthy groups, they were 17(34%), 20(40%), and 13(26%) Table (1).The P-values for the IL-1 beta C31 T gene genotype frequencies were despite not reaching significant. individuals with genotype TT were more impacted by breast tumors than individuals with genotype CT (odd ratio = 0.93 and 0.59), with values for CT and TT being 0.23 and 0.88, respectively. According to a study conducted in Turkey [11], allele frequency frequently changes significantly between healthy and disease groups. The current investigation found no correlation between CC and CT and breast cancer. In the Turkish population, the T allele was found to be 47.9% frequent, but the frequency in [10] was found to be 55.4%. It's interesting to note that the control group's T allele frequency (0.509) matched that of the Asian population from Korea [12].

T allele frequency was determined to be 0.541 in an Asian population from China [13], which was comparable to the frequency noted in a Turkish community [10]. Prior research on this polymorphism's allele frequency in the Asian population from Japan found that it was 0.849 [14], a much higher value than that found in other Asian populations, such as the Chinese [13] and Koreans [12]. Individuals from different geographical regions have variable genotype rates for the IL-1-C317 polymorphism, as previously mentioned. The small sample size in these several research may account for the variations in the genotype distribution.

Allele frequencies of IL-1 beta C31 T in people who were healthy and those who were ill were presented. Table 1. According to the Hardy-Weinberg equation, the allele frequency of the variant alleles for T and C was 54(0.54) and 46(0.46) for healthy control patients, respectively, and 108(0.54) and 92(0.46) for blood patients.

Table 1. Genotype of IL-1 beta C31 T gene polymorphism with allele frequency in patients' blood and healthy.

Table (2) shows the genotype frequencies of TT, CT, and CC of the IL-1 beta C31 T gene polymorphism in tissue patient samples, which were 19(28%), 31(43%), and 20(29%) in patients with breast cancers. Patients with genotype CT were found to have a lower incidence of breast cancers compared to those with genotype TT (odd ratio = 1.17 and 1.03). The P-value of the genotype

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frequencies of the IL-1 beta C31 T gene was not substantially different for TT patients ($p<0.05$) and not significantly different for CC and CT patients.

Table (2) presents information on the allele frequencies of IL-1 beta C31 T in patients with blood and tissue. According to the Hardy-Weinberg equation, the allele frequencies of T and C for blood patients were (108(54), 92(0.46)) and for tissue patients, they were (71(0.51), 69(0.49)) correspondingly.

Table 2. Genotype of IL-1 beta C31 T gene polymorphism with allele frequency in patients' blood and tissue

Genotype IL-1 beta C31 T	**Patients (Blood)**	**Patients (tissue)**	**P value**	**Odd ratio**	**CI 95%**
CC	20(20%)	20(29%)	0.31	0.70	**(0.35 - 1.40)**
CT	52(52%)	31(43%)	0.56	1.17	**(0.68 - 2.01)**
TT	28(28%)	19(28%)	0.92	1.03	**(0.53 - 1.99)**
Total	100	70			
Alleles frequency					
C	92(0.46)	71(0.51)			
T	108(54)	69(0.49)			

Genotype frequencies of TT, CT, and CC of the IL-1 beta C31 T gene polymorphism were 12(21%), 21(36%), and 25(43%) in blood benign patients, but 13(31%), 22(36%), and 25(43%) in malignant patients .Table 3 shows that the P-value of the IL-1 beta C31 T gene genotype frequencies was substantially higher in individuals with breast cancers who had genotype CC.
Allele frequencies of IL-1 beta C31 T in the blood of patients who were either malignant or benign were reported in.

Table (3). According to the Hardy-Weinberg equation, the allele frequencies of T and C for malignant blood patients were 48(0.57), 36(0.42), and for benign patients, they were 45(0.39), 7(0.61), respectively.

Table 3. Genotype frequency of IL-1 beta C31 T gene polymorphism with allele frequency in malignant and benign blood breast tumors patients

Genotype IL-1 beta C31 T	**Malignant Blood**	**Benign Blood**	**P value**	**Odd ratio**	**CI 95%**
CC	7 (17%)	25(43%)	0.04*	0.39	(0.15 - 0.98)
CT	22(52%)	21(36%)	0.31	1.45	(0.71 - 2.97)
TT	13(31%)	12(21%)	0.36	1.50	(0.62 - 3.6)
Total	42	58			
Alleles frequency					
C	36(0.42)	71(0.61)			
T	48(0.57)	45(0.39)			

Genotype frequencies of TT, CT, and CC of the IL-1 beta C31T gene polymorphism were 10(22%), 20(43%), and 16(35%) in tissue benign patients, but 17(68%), 5(20%), and 3(12%) in malignant patients. Table 4 shows that among patients with breast tumors, the P-value of the genotype frequencies of the IL-1 beta C31 T gene were significantly for patients with TT genotype ($p<0.05$) but not significantly for patients with CT or CC genotype. In contrast, patients with CT genotype were more likely to have breast tumors than patients with TT genotype (Odd ratio 0.46, 0.26).
The results of the current study contradicted those of study [12], which found that the IL1 beta 31C polymorphism affects breast susceptibility, particularly in women, and study [15], which found a significant association between the IL-1 beta -31T/C polymorphism and hepatocellular cancer but not breast cancer. The current study found no significant association between the IL1 beta gene in patients' blood, tissue, or healthy was associated with breast tumors.

Table (4) displays the allele frequencies of IL-1 beta C31 T in the tissue samples of patients who were either malignant or benign. The Hardy-Weinberg equation shows that the allele frequencies of T and C for malignant blood patients were (11(0.22), 37(0.77)), and for benign patients, they were (40(0.43), 52(0.57)], respectively.

Table 4. Genotype frequency of IL-1 beta C31 T gene polymorphism with allele frequency in malignant and benign blood breast tumors patients

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Genotype IL-1 beta C31 T	**Malignant Tissue**	**Benign Tissue**	**P value**	**Odd ratio**	**CI 95%**
CC	3(12%)	16(35%)	0.13	0.36	(0.09 - 1.36)
CT	5(21%)	20(43%)	0.18	0.48	(0.16 - 1.44)
TT	16(67%)	10(22%)	0.02*	3.07	(1.2 - 7.78)
Total	24	46			
Alleles frequency					
C	11(0.22)	52(0.57)			
T	37(0.77)	40(0.43)			

Genotypes frequencies in chemotherapy-treated patients

Genotype frequencies of TT, CT, and CC of the IL-1 beta C31 T gene polymorphism were 9(30%), 14(47%), and 7(23%) in chemotherapy patients. Table (5)The *P*-value for the genotype frequencies of the IL-1 beta C31 T gene in patients with breast tumors was not substantially different for TT, CC, or CT. Patients with genotype TT were more affected by breast tumors than patients with genotype CT (odd ratio = 1.01 and 1.01). [16.17]Table (5) presents the allele frequencies of IL-1 beta C31T in chemotherapy blood patients and control persons. According to the Hardy-Weinberg equation, the allele frequencies for T and C in blood patients were 32(0.53), 28(0.47), and for the control group, they were 54(0.54), 46(0.46) [18].

Table 5. Genotype of IL-1 beta C31 T gene polymorphism with allele frequency in patients with chemotherapy and healthy

Genotype IL-1 beta C31 T	(Chemotherapy)	Healthy	P value	Odd ratio	CI 95%
CC	7(23%)	13(26%)	0.83	0.90	0.32 - 2.50)(
CT	14(47%)	20(40%)	0.71	1.17	0.51 - 2.65)(
TT	9(30%)	17(34%)	0.97	1.01	(0.29-3.45)
Total	30	50			
Frequency Alleles					
C	28(0.47)	46(0.46)			
T	32(0.53)	54(0.54)			

Conclusion

The result IL-1 beta C31 T gene were significantly between blood and malignant and blood benign shoed that allele T was risky allele in benign at (p – 0.005*). also the result was significantly between malignant and benign tissue the TT was genotype affect in malignant and T allele was risky allele in malignant at (p = <0.001*) .

Ethical approval

The study was conducted in accordance with the Helsinki Declaration's ethical guidelines. The patient's verbal and written consent was obtained before any samples were taken. The study protocol, subject information, and consent form were reviewed by a local ethics committee, which authorized them in accordance with document M220106 (which has the reference and date of 17/1/2022).17/1/2022) to get this suggestion.

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