Interleukin 1 Alpha Gene Polymorphism in Breast Cancer After Chemotherapy Treatment

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Abstract

Background: The interleukin 1 (IL-1) family of cytokines is essential for triggering and controlling immunological and inflammatory responses. We believe the levels of these cytokines in breast tumor homogenates relate to other known prognosticators of patient survival. **Objectives:** The association between the risk of breast tumors and the IL-1 alpha −889 C>T Promoter Primer polymorphism has been established. Interleukin 1 alpha −889 C>T Promoter Primer has been proven to influence breast tumor susceptibility. Our research aimed to determine whether the IL-1 alpha −889 C>T Promoter Primer gene polymorphism and susceptibility to Breast cancers (BCs) are related. **Materials and Methods:** The genotype frequencies of the IL-1 alpha −889 C>T Promoter Primer polymorphism were compared between 100 BC cases and 50 controls with the assistance of polymerase chain reaction and restriction fragment length polymorphism techniques. Additional multivariate binary logistic regression analyses were carried out to determine the level of IL-1 in patients' blood and to examine the association between the IL-1 alpha −889 C>T Promoter Primer polymorphism and BC risk utilizing the ELISA technique after therapy. **Results:** The concentration of CA 15-3 in patients with BC was 101.107 increase significantly compare with mean of control group was 62.802, in chemotherapy patients genotype frequencies of TT, CT, CC of IL-1 alpha −889 C>T gene polymorphism where it patients with genotype TT were affected by breast tumors approximately one time comparison with patients having genotype CT (odd ratio = 1.50 and 0.91). **Conclusion:** Concentration of CA 15-3 increase in patients after chemotherapy compared with healthy, The IL-1 alpha −889 C>T polymorphism affects breast tumors in women.

Keywords: Breast tumors, chemotherapy, CA 15-3, genetic susceptibility, interleukin 1 alpha polymorphism genetic

Introduction

Breast cancer (BC) is a term used to describe a variety of malignancies that can develop in the mammary glands. Of all the malignancies that affect women worldwide, BC has the greatest incidence.[1] According to the Jordan Cancer Registry, BC was one of the three cancers that people in Jordan with female genders were most likely to develop.[2] Additionally, it was noted to be the second cause of death for females, behind cardiovascular diseases.[3]

On chromosome 2 (q14.1),[4-6] the IL-1 gene, which codes for the interleukin 1 protein, can be located. A cytokine is a type of protein that can affect various cells and organs when present in small quantities. Multiple functions of the cytokine IL-1 are involved in both innate and adaptive immune responses. It has been discovered that a number of genetic variations within the IL-1 gene, including rs16944 (C/T) and rs1143627 (T/C), significantly contribute to the hereditary susceptibility of various cancers.[7]

Interleukin 1 (IL-1) is a member of the cytokine family that is, crucial for initiating and regulating inflammatory and immune responses. The IL-1 family consists of eleven members, including receptor antagonists and cytokines that are both pro- and anti-inflammatory.[8] The two defining members of the IL-1 family, IL-1 alpha (IL-1) and IL-1 beta

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(IL-1), which are each encoded by a separate gene, bind to the type 1 IL-1 receptor (IL-1R1) and have similar biological activities. When IL-1 or IL-1 binds to IL-1R1, the IL-1R accessory protein (IL-1RAcP) dimer sizes with IL-1R1.[9]

In many different cell types, particularly epithelial cells, Interleukin -1α is constitutively expressed in several types of cells at steady state, especially in epithelial cells, whereas its expression is increased in response to proinflammatory and stress-associated stimuli. This intracellular cytokine exerts its function in three ways. First, IL-1α can translocate from the cytosol onto the cell surface where membrane-bound IL-1α activates IL-1 receptor type 1 (IL-1R1) signaling in an intra- and paracrine manner, contributing to local inflammatory responses. Second, by extracellular release after loss of membrane integrity resulting from necrotic-type cell death, IL-1α functions as an alarmin or danger-associated molecular pattern (DAMP) initiating a cascade of inflammatory responses.[10]

Third, like transcription factors, IL-1 can enter the cell nucleus from the cytosol and carry out intracellular functions.[11] Two of IL-1's main biological functions are the induction of T helper 17 (Th17) responses and sterile and pathogen-induced inflammation.[8,12] IL-1 is located in the nucleus, where it acts as a transcription factor to regulate both healthy cell development and cancer cell neoplasia. However, when cells undergo cell death, such as necrosis, IL-1 translocates into the cytosol and is released into the extracellular space to act as a "alarmin."[13,14] Chemotherapy is a key method of treatment for BC and has greatly increased patient survival rates.[15] Patients may experience some degree of behavioral challenges when controlling their symptoms due to the many etiologies of chemo side effects.[16] the Breast MR imaging depicts more accurate dimensions of the tumor and its extensions than digital and sonomammography.[17] Throughout 2016, 897 women died from that disease which is recorded as the first cause of cancer-related mortality among Iraqi females after bronchogenic cancer.[18]

Materials and Methods

One hundred blood specimens from woman with BC after chemotherapy. fifty blood sample from healthy women determine concentration of CA15-3 by using ELISA, DNA extraction from whole blood patients and healthy for polymorphism of IL-1 alpha −889 C>T. The age of women in this study ranging from 14 to 66 years in AL-Hilla-Teaching Hospital, Imam Sadiq Hospital and Marjan Hospital a period from September 2021 to October 2022.

IL-1 alpha −889 C>T genotyping

Genotyping Magna Pure LC was used to extract genomic DNA from 2 mL of peripheral blood leukocytes in accordance with the manufacturer's instructions (Roche, Germany). Using PCR-RFLP (restriction fragment length polymorphism) techniques, all genotypes were determined. Two primers, one forward (5′-TTACATATGAGCCTTC-CATG-3′) and the other reverse (5′-AAGCTTGTTCTACCACCT-GAACTAGGC-3′)[19] were used for IL-1a −889 C>T promoter polymorphism. Each patient's and the control group's DNA samples were amplified in a reaction volume of 50 l containing 200 ng of DNA and 2.5 mmol/L magnesium chloride (MgCl). Taq DNA polymerase (2.5 U/μL), oligonucleotide primers (10 pmol each), and a mixture of deoxyribonucleotide triphosphates (dNTPs) are also included. The following PCR protocol was used: A total of 35 cycles of initial denaturation for 5 min at 94°C, followed by 1 min at 94°C, 1 min at 53.9°C, and 2 min at 72°C for extension. The final exercise was carried out at 72°C for 5 min. The 108-bp PCR product was combined with Nco I restriction enzyme, and the reaction mixture was then incubated at 37°C overnight. To determine different genotypes, 25 L of the digestion result was put HD into a 3% agarose gel electrophoresis and stained with ethidium bromide.

Inclusion criteria

Female Patient with BC after taken chemotherapy

Exclusion criteria

Exclusion patients infected with other types of tumors

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.

Ethical approval

The study was conducted in accordance with the Helsinki Declaration's ethical guidelines. The patient's verbal and written agreement 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 to get this suggestion.

Results

In Table 1 and Figure 1 the mean of CA 15-3 in patients with BC was 101.107 increase significantly compare with mean of control group was 62.

IL-1 alpha −889 C>T promoter primer (IL1 α −889 C>T) genotyping PCR

The PCR product of IL-1 alpha −889 C>T gene was amplified by using specific primer. the PCR product (band) of IL-1 alpha −889g C>T gene was 108 –bp in blood patients [Figure 2].

Detection of genotype frequency of IL-1 alpha −889 C>T gene polymorphism associated with breast tumors by using PCR-RFLP

Genotype of IL-1 alpha −889 C>T gene polymorphism with allele frequency between (the healthy 50 sample and blood patients were 100 sample) were represent TT homozygote (92 pb), CT heterozygote (108 pb and 92 pb) and CC homozygote (108 pb) [Figure 3].

In chemotherapy patients genotype frequencies of TT, CT, CC of IL-1 alpha −889 C>T gene polymorphism where it were 5 (17%), 15 (50%) and 10 (33%) [Table 2] in the breast tumors patients the *P* value of the genotypes frequencies of IL-1 alpha −889 C>T gene were significantly 0.007 for TT ($P < 0.05$) and no significant for CC and CT patients with genotype TT were affected by breast tumors approximately one time comparison with patients having genotype CT (odd ratio = 1.50 and 0.91).

Data of allele frequencies of IL-1 alpha −889 C>T in chemotherapy blood patients and control subjects were presentenced in Table 2. For blood patients, allele frequency of (T and C) were [25 (0.41), 35 (0.58)] for control allele frequency of (T and C) were [54 (46%), 46 (54%)] respectively, according to Hardy–Weinberg equation.

Table 1: Comparison between patients and control on CA15-3 with breast tumors with chemotherapy

	After chemotherapy		
Parameter	M ± SD concentration pg/mL		*P* value
	Patients	Control	
CA 15-3	101.107 ± 28.881	62.802 ± 28.881	0.05*

*($P \leq 0.05$) is considered significant

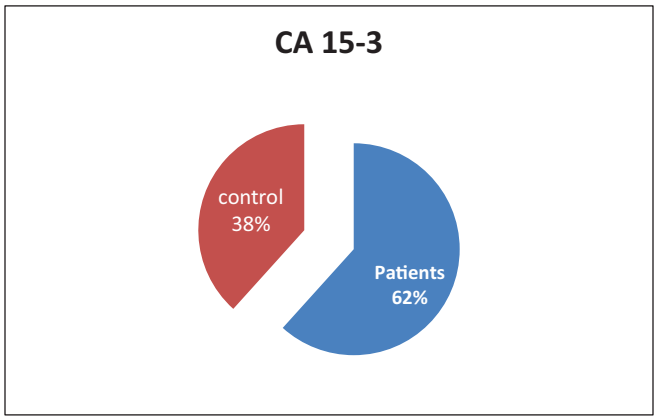

Figure 1: Concentration of CA15-3 in patients and healthy after chemotherapy

Discussion

Breast ductal epithelium from both benign and malignant tumors expresses cancer antigen 15-3. It is a mucin that is, a member of a sizable family of glycoproteins that are heterogeneously expressed on the apical surface of several types of normal epithelial cells, including those found in the breast.[20] For the preclinical diagnosis of tumor recurrence as well as the monitoring of patients with metastatic BC who are receiving therapy, serum CA 15-3 is employed as a surrogate measure of disease mass. A percentage of BC patients with distant metastases have high CA 15-3 levels despite the present American Society of Clinical Oncology.[21]

In Table 1 and Figure 1 the mean of CA 15-3 in patients with BC was 101.107 increase significantly compare with mean of control group was 62.802 this study was agreed with[22] who showed When comparing the adriamycin and cyclophosphamide followed by taxane (AC-T) (mean rank: 144.39) to the adriamycin and cyclophosphamide (AC) (mean rank: 25.55) at late stage but not at early stage ($P = 0.089$), CA 15-3 levels behaved differently ($P = 0.056$).

Using the PCR-RFLP technique, the polymorphism of the IL-1 alpha −889 C>T gene was identified in the DNA samples of the Iraqi population suffering from breast tumors for the current study. The results revealed an association between the polymorphism of the IL-1 alpha −889 C>T gene and breast tumors.

In contrast to study[23] which found no association between the presence of the homozygous mutant genotypes of the IL1A −889 and IL1B +3953 gene polymorphisms and

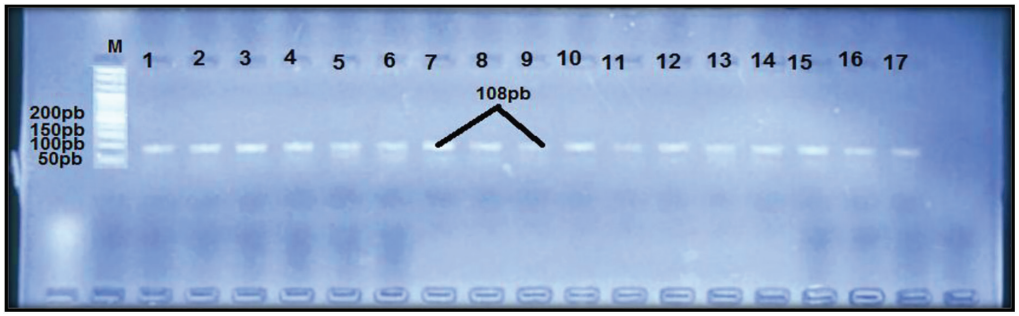

Figure 2: Electrophoreses pattern of PCR product of IL-1 alpha −889 C>T in blood of chemotherapy patients, M: molecular DNA ladder, 1-17 PCR product, the optimum annealing temperature was 53.9

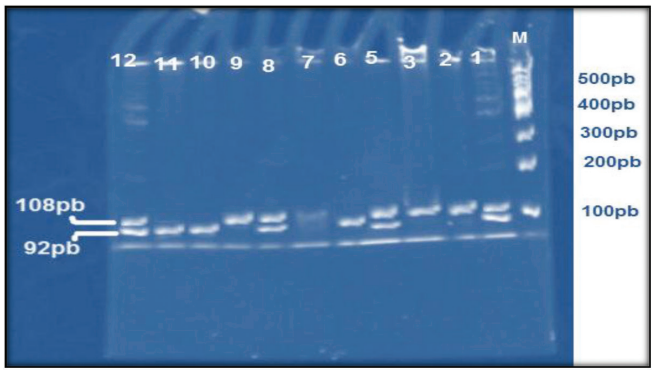

Figure 3: Electrophoresis pattern of IL-1 alpha −889 C>T gene PCR-RFLP by PAGE gel for PCR product (108 pb) with restriction enzyme *NocI*. M: DNA ladder. lane (5,9,10) homozygote TT genotype 92 pb, lane (2,3,8) homozygote (CC) genotype (108 pb) lane (1,4,6,7,11) heterozygote (CT) genotype (108 pb and 92 pb)

Table 2: Genotype frequency of IL-1 alpha −889 C>T gene polymorphism with allele frequency in healthy control and blood breast tumors patients

Genotype IL-1 alpha-889 C>T	Chemotherapy	Healthy	*P* value	OR CL 95%
CC	10 (33%)	16 (32%)		
CT	15 (50%)	22 (44%)	0.86	0.91 (0.32-2.56)
TT	5 (17%)	12 (24%)	0.54	1.50 (0.40-5.55)
Total	30	50		
Alleles frequency				
C	35 (0.58)	46 (54%)		
T	25 (0.41)	54 (46%)	0.59	1.19 (0.62-2.27)

overall survival in the multivariate analysis, the current study demonstrated that alpha −889 C>T was not associated with breast tumors. Overall survival and any conceivable haplotype combinations were not found to be correlated.

The IL1 gene, which is found on chromosome 2q13, contains a number of polymorphisms, but two of them have drawn the most attention: one is in the 5′UTR regulatory region (−889 C>T), and the other is in exon 5 (+4845 G>T). In two separate cohort studies, the IL1 −889 polymorphism has not been linked to BC. This study also contradicts study[24,25] which found that a meta-analysis of 20 publications involving 6.782 case and 7.767 control subjects revealed a significant association between the −889 C/T polymorphism and cancer risk in the Asian population. The variations in results in this study could be attributed to behavioral and environmental factors.

This study was also incompatible with Cheng *et al*.[26] who found that from total of 20 publications involved 6.782 case and 7.767 control, as meta analysis, revealed a significant association between -889 C/T polymorphism and cancer risk, significant association between cancer risk and IL1α in Asian population.

Conclusion

Increase in CA 15-3 concentration in chemotherapy patients compared to healthy individuals IL-1 alpha −889 C>T polymorphism influences female BCs.

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Conflicts of interest

There are no conflicts of interest.

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