

INTERLEUKIN-18 GENE POLYMORPHISM AND SOME RISK FACTORS IN IRAQI PATIENTS WITH BREAST CANCER

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ABSTRACT

Objective: Breast cancer is the most diagnosed cancer in women, which leads to death in a lot of women with breast cancer. The major risk factors associated with breast cancer risk related to family history, age, clinical history, lifestyle factors, long-period hormonal exposure, and single nucleotide polymorphisms in many genes showed possible links with breast cancer incidence risk in different people populations. Our study aimed to figure out the correlation between smoking, lodging and family history, and other factors with the risk of breast cancer.

Methods: Blood sample from female patients with breast cancer and healthy individuals were collected and subjected to tetra-amplification refractory mutation system-polymerase chain reaction (T-ARMS-PCR) technique for -607 C/A mutation of an interleukin (IL-18) gene and SPSS 18 software analyzed the results statically.

Results: Results showed no association between lodging and smoking with risk of breast cancer, ($p > 0.05$), while the association between the risk and family history were obvious ($p < 0.05$).

Conclusion: The results obtained by T-ARMS-PCR technique did not show the association between -607 C/A alternation of IL-18 gene and breast cancer ($p > 0.05$) in the individuals examined in our study.

Keywords: Interleukin-18, Gene, Polymorphism, Tetra-amplification refractory mutation system-polymerase chain reaction, Mutations.

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INTRODUCTION

Breast cancer begins when normal cells in the portions of breast multiply and divide to compose new cells, as the body needs. When regular cells grow obsolete or damaged, it dies, and the new cells replace it [1]. Occasionally, this operation goes erroneous. New cells compose once the body does not need them, and obsolete or damaged cells do not die duly. The accumulation of additional cells often composes a mass of tissue named a lump, growth, or tumor [1,2]. It is the most frequently diagnosed cancer in women and the cause that leads to cancer death in women.

The main risk factors associated with breast cancer risk has been related to family history, age, clinical history, lifestyle factors, late first pregnancy, and long-period hormonal exposure [3].

Raised expression of a number of genes due to single nucleotide polymorphisms (SNPs) increases the breast cancer incidence risk [4,5]. Many researchers have demonstrated that genetic polymorphisms are one of the reasons for the individual difference in the incidence of cancer [6].

Interleukin-18 (IL), a protein of the IL-1 family, can activate immune cells without or with IL-12 [7]. IL-18 can organize both adaptive and innate immune responses during its impacts on natural killer (NK) cells, dendritic cells, monocytes, B-cells, and T-cells [8]. It can increase the immune resistance toward tumor cells by energizing and stimulating the interferon (IFN- γ) production and therefore has a key role in Th1 response [8].

The human IL-18 gene is situated on chromosome 11q22.2-22.3 and composed of "6 exons and 5 introns" [9]. Three types of SNPs of the IL-18 gene in the promoter nucleotides was determined, called

-137 G/C (rs187238), -656 G/T or rs1946518, and -607 C/A or rs1946519 [9]. IL-18 gene polymorphisms have shown correlation with different diseases such as oral cancer [10], colorectal cancer [11], thyroid cancer [12], bladder cancer [13], lung cancer [14], and nasopharyngeal carcinoma [15]. On the other hand, other studies found there were no correlations between -607 C/A polymorphism of IL-18 gene and head and neck squamous cell carcinoma [16] or breast cancer risk [17]. Therefore, our study aims to determine whether there is a link between -607 C/A polymorphism of IL-18 gene with the risk of breast cancer or in our Iraqi sample of the study.

METHODS

Blood samples were collected from 34 females with breast cancer who admitted AD Diwaniya Teaching University Hospital and 21 healthy females as control group through study, groups were illustrated in Fig. 1.

IL-18 (rs1946518) polymorphisms were detected using (T-ARMS PCR), as mentioned by Taheri *et al.* [17]. Polymerase chain reaction (PCR) was performed using commercially available PCR premix (AccuPower PCR PreMix, BIONEER, South Korea) as described by manufacturer; 1 μ l template DNA (50 ng/ μ l), 1 μ l of each primer (10 μ M), and 15 μ l PCR-water were added to each reaction. The PCR program were as follows: Initial denaturation at 95°C/5 minutes followed by 35 cycles; consisting of denaturation at 95°C/30 seconds, annealing at 54°C/30 seconds, and extension at 72°C/45 seconds with a final extension at 72°C/5 minutes. The PCR products were analyzed by 1.5% agarose gel electrophoresis containing 0.5 μ g/ml ethidium bromide and visualized under ultraviolet light. Product sizes were 208 bp for the C allele, 278 bp for the A allele, and 440 bp for control band.

The statistical analysis between the alleles frequencies and genotype distributions of the groups were confirmed by the binary logistic regression at $p < 0.05$ using the SPSS 18 software. The correlation between some variables as a risk factor and breast cancer susceptibility were conducted by t-test at $p < 0.05$ and as a significant variation.

RESULTS AND DISCUSSION

The variables and the risk of breast cancer

The risk of breast cancer incidence with some variables such as smoking, lodging, and family history was estimated by the odd ratio and their confidence interval at $p < 0.05$, the matching in ages between cases and controls were demonstrated by p value as shown in the Table 2.

In our study and as obvious from the results, there was not an association between lodging and smoking with risk of breast cancer occurrence (p value were higher than 0.05). While the association between the risk and family history were obvious ($p < 0.05$), the same result obtained from a previous study [18].

Association between polymorphisms and risk of breast cancer

Our study examined the correlation between 2124 and -607 C/A (rs1946519) polymorphisms of IL-18 gene with breast cancer risk. The study was performed using tetra-amplification refractory mutation system-PCR (T-ARMS-PCR) [17]. Fig. 2 shows the PCR product of IL-18 gene on agarose gel using (100 bp DNA Ladder), the results were shown the bands of extracted DNA samples for the four groups (G1, G2, G3 and G4), and the PCR product is C allele (208 bp), an allele (278 bp) and control band (440 bp).

Genotype frequencies and allele distributions

Related individuals to patients group (G2) with control group (G1)

The genotype frequencies and allelic distributions of -607 C/A polymorphism of IL-18 in related (G2) and control (G1) groups were summarized in Table 3. The outcomes demonstrate that the frequency of CC is 6.7%, AC is 80%, and AA is 13.3% in G1. The frequency of CA -607 polymorphism in G2 were 16.6% for CC, 83.3% for AC, and 0% for AA. There were no significantly difference in -607 C/A polymorphism of IL-18 between G2 and G1 ($p = 0.488$). As shown in Table 3 in G1, an allele frequency was 53.3% and the C allele frequency were 46.7%, and in the G2 the frequencies were 58.33% for C allele and 41.66% for A allele. The frequency of allele was not different between G1 and G2 ($p = 0.496$).

Treated (G3) with control (G1) groups

The genotype frequencies with allele distributions of -607 C/A alternation of IL-18 in treated (G3) and control (G1) groups explained in Table 4. The results show that the frequency of CC is 6.7%, AC is 80%, and AA is 13.3% in G1. The frequency of CA -607 alternation in G3 were 23.8% for CC, 71.4% for AC, and 4.8% for AA. There were not significantly difference in -607 C/A polymorphism of IL-18 between G3 and G1. As shown in Table 3 in G1, an allele frequency were 53.3% and the C allele frequency were 46.7%, and in the G3 the frequencies were 59.5% for an allele and 41.66 % for C allele. Logistic regression analyses indicated that the

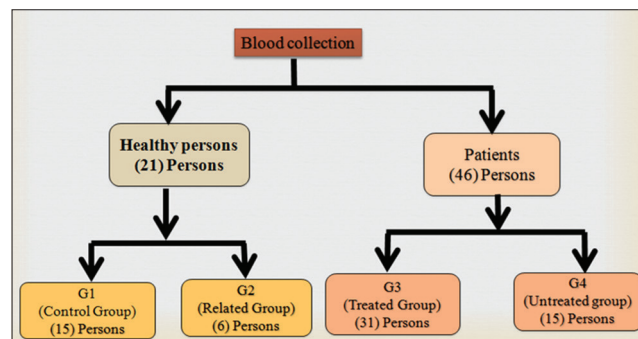


Fig. 1: Study groups and subgroups

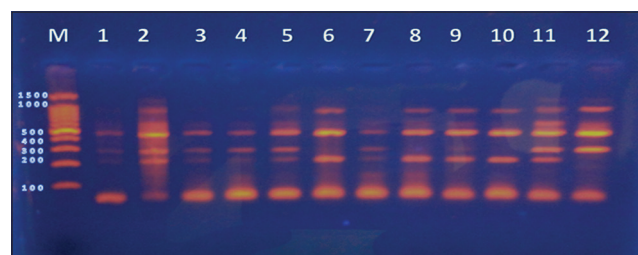


Fig. 2: Electrophoresis pattern of tetra-amplification refractory mutation system-polymerase chain reaction products for polymorphisms for interleukin-18 gene -607 C/A (rs1946519). M: 100bp DNA Ladder; Lane 1: to G4 (Untreated group), Lane 2-9: G3 (Treated), Lane 10: G2 (Related-sister of the patient No. 9) and Lane 11, 12: G1 (Control)

Table 1: IL-18 -607 C/A (rs1946518) T-ARMS-PCR primers

Primers	Sequence 5' → 3'	Temperature (°C)
Forward outer primer	CCTACAATGTTACAACACTTAAAT	53
Reverse outer primer	ATAAGCCCTAAATATATGTATCCTTA	
Forward inner primer	GATACCATCATTAGAATTTGTG	
Reverse inner primer	GCAGAAAGTGTAATAATATCA	

T-ARMS-PCR: Tetra-amplification refractory mutation system-polymerase chain reaction, IL-18: Interleukin-18

Table 2: Clinical characteristic and breast cancer risk

Variables	Cases (G3+G4) N=46 (%)	Control (G1) N=15 (%)	OR (95% CI)	p value
Lodging				
Urban	28 (60.9)	8 (53.33)	0.563 (0.174-1.821)	0.334
Rural	18 (39.1)	7 (46.77)		
Smoking				
Non smoker	43 (93.5)	15 (100)	1.070 (0.991-1.155)	0.31
Smoker	3 (6.5)	0 (0)		
Family history				
Without family history	34 (71.9)	15 (100)	1.353 (1.140-1.606)	0.027*
With family history	12 (26.1)	0 (0)		
Age (mean±SE)	46.73±3.54	47.93±3.05		0.988

*The value is statically significant, G3 treated, G4 untreated, G1 control. SE: Standard error, OR: Odd ratio, CI: Confidence interval

-607 C/AAC and CC genotypes did not correlate with breast cancer risk in comparison with the AA genotype (p=0.476 and p=0.161). Alike results noticed when comparing the C allele with the reference A allele (p=0.233).

Untreated (G4) with control (G1) groups

The genotype frequencies and allele distributions of -607 C/A polymorphism of IL-18 in untreated (G4) and control (G1) groups were shown in Table 5. The results show that the frequency of CC is 6.7%, AC is 80%, and AA is 13.3% in G1. The frequency of CA -607 polymorphism in G4 were 72.7% for AC, 9.1% for AA, and 18.2% for CC. There was no significantly difference in -607 C/A polymorphism of IL-18 between G4 and G1 (p>0.05). As shown in Table 4 in G1, An allele frequency was 53.3% and the C allele frequency were 46.7%, and in the G4 the frequencies were 40.9% for An allele and 59.1% for C allele. Logistic regression analyses confirmed that the "-607 C/AAC and CC genotypes have not associated with breast cancer risk compared with the AA genotype (p=0.826 and p=0.711). Alike results were noticed as comparing the C allele with the reference A allele (p=0.574)."

Untreated (G4) with treated (G3)

The genotype frequencies and allele distributions of -607 C/A alternation of IL-18 in untreated (G4) and control (G1) groups were shown in Table 6.

The results show that the frequencies were 23.8% for CC, 71.4% for AC, and 4.8% for AA in G3. The frequency of CA -607 polymorphism in G4 were 18.2% for CC, 72.7% for AC, and 9.1% for AA. There was no significantly difference in -607 C/A polymorphism of IL-18 between G4 and G3 (p>0.05). As shown in Table 5 in G3, an allele frequency was 59.5% and the C allele frequency were 40.5%, and in the G4 the frequencies were 40.9% for An allele and 59.1% for C allele. Logistic regression analyses indicated so as to the -607C/AAC and "CC" genotypes were compared with the AA genotype there was an insignificant difference (p=0.467 and p=0.368). In a similar way, the results were registered when comparing the "C allele with the reference A allele (p=0.621)."

DISCUSSION

The family background of breast cancer occurrence is one of the most significant risk factors for the progression of breast cancer [19]. Aside from age, women with one influenced the first-degree relative are approximately twice as likely to promote breast cancer compared with women who have no influenced relatives, and risks are higher when more than one first-degree relative is influenced or the relative is youthful at diagnosis [19,20].

In our study and as obvious from the results, there is an association between family background and the risk of breast

Table 3: The genotypes and allelic distribution of IL-18 gene-607 C/A (rs1946519) in G2 and G1 groups

Polymorphisms IL-18 (-607C/A)	G1 (Control) N=15 (%)	G2 (Related) N=6 (%)	χ^{2a}	p ^a	OR (95% CI) ^b	p ^b
AA	2 (13.3)		1.433	0.488	1.0 ^{ref} (1.0 ^{ref})	
AC	12 (80.0)	5 (83.3)			0.000 (0.000)	
CC	1 (6.7)	1 (16.7)			0.000 (0.000)	0.488
A allele	16 (53.3)	5 (41.66)	0.468	0.494	1.0 ^{ref} (1.0 ^{ref})	
C allele	14 (46.7)	7 (58.33)			1.600 (0.413-6.193)	0.496

^aValue for genotype distribution, ^bValue was calculated as relative to subjects with the A/A genotypes and An allele. IL-18: Interleukin-18

Table 4: The genotypes and allele distribution of IL-18 polymorphism in G3 and G1

Polymorphisms IL-18 (-607 C/A)	G1 (Control) N=15 (%)	G3 (Treated) N=21 (%)	χ^{2a}	p ^a	OR (95% CI) ^b	p ^b
AA	2 (13.3)	1 (4.8)	2.58	0.275	1.0 ^{ref} (1.0 ^{ref})	
AC	12 (80.0)	15 (71.4)			0.400 (0.320-4.960)	0.476
CC	1 (6.7)	5 (23.8)			0.100 (0.004-2.504)	0.161
A allele	16 (53.3)	25 (59.5)	1.434	0.231	1.0 ^{ref} (1.0 ^{ref})	
C allele	14 (46.7)	17 (40.5)			1.786 (0.689-4.631)	0.233

^aValues for genotype distribution, ^bValue was calculated as relative to subjects with the A/A genotypes and An allele. IL-18: Interleukin-18

Table 5: The genotypes and allele distribution of IL-18 polymorphism in G4 and G1

Polymorphisms IL-18 (-607 C/A)	G1 (Control) N=15 (%)	G4 (Untreated) N=10 (%)	χ^{2a}	p ^a	OR (95% CI) ^b	p ^b
AA	2 (13.3)	1 (9.1)	0.138	0.933	1.0 ^{ref} (1.0 ^{ref})	
AC	12 (80.0)	7 (72.2)			0.750 (0.580-9.719)	0.826
CC	1 (6.7)	2 (18.2)			0.500 (0.013-19.562)	0.711
A allele	16 (53.3)	9 (40.9)	0.316	0.574	1.0 ^{ref} (1.0 ^{ref})	
C allele	14 (46.7)	13 (59.1)			1.371 (0.455-4.136)	0.574

^aValue of genotype distribution, ^bValue were calculated as relative to subjects with the A/A genotypes and An allele. IL-18: Interleukin-18

Table 6: The genotypes and allele distribution of IL-18 polymorphism in G4 and G3

Polymorphisms IL-18 (-607 C/A)	G3 (Treated) N=21 (%)	G4 (Untreated) N=10 (%)	χ^{2a}	p ^a	OR (95% CI) ^b	p ^b
AA	1 (4.8)	1 (9.1)	1.088	0.581	1.0 ^{ref} (1.0 ^{ref})	
AC	15 (71.4)	7 (72.2)			0.533 (0.029-9.708)	0.476
CC	5 (23.8)	2 (18.2)			0.200 (0.006-6.664)	0.368
A allele	25 (59.5)	9 (40.9)	0.243	0.622	1.0 ^{ref} (1.0 ^{ref})	
C allele	17 (40.5)	13 (59.1)			0.768 (0.269-2.190)	0.621

^aValues about genotype distribution, ^bCalculated as relative to subjects with the A/A genotypes and An allele. IL-18: Interleukin-18

cancer occurrence, the same result obtained from a previous study [18].

Our current study aims to identify the effect of IL-18 -607 C/A mutation on the predisposition of breast cancer in our population. We found no correlation between IL-18 -607 C/A polymorphism and predisposition to breast cancer. This result similar to a previous study [17].

IL-18 can be organized both adaptive and innate immune responses during its impacts on NK cells, dendritic cells, monocytes, B-cells, and T-cells [8]. It can raise the immune protection against cancer cells by energizing and stimulating the IFN- γ creation and therefore has a key role in Th1 response [8]. It has been made known that IL-18 has a major role in the progression of the tumor. IL-18 expression and serum level have been shown to elevate in different cancers in the blood stream of metastatic patients compared to healthy controls and patients with no metastasis [21]. Because of the double roles of IL-18 expression and serum level in both tumor metastasis and drug resistance, IL-18 may denote a helpful drug target for therapy of breast cancer [22]. It was clear that two SNPs in the promoter area of the IL-18 gene organized the gene expression levels at the transcriptional stage and changed the IL-18 production level [17]. The alteration from the "C allele to the "A" allele at site -607C/A and at site -137 G/C alternation from the G allele to the C allele in the (promoter area) were prophesied to be the molecular factor linking sites for the cyclic adenosine monophosphate responsive element between protein and H4TF-1 factor, respectively in addition, polymorphisms of the two positions have been correlated with the activity of IL-18 gene promoter transcription, which can impact the IL-18 expression and, may be, of IFN- γ ; these changes in alleles, potentially be the essential mechanism of IL-18 involvement in different diseases [23,24]. IL-18 polymorphism has been shown correlated with different diseases such as oral cancer [10], colorectal cancer [11], thyroid cancer [12], bladder cancer [13], lung cancer [14], and nasopharyngeal carcinoma" [15].

CONCLUSION

Our study showed no significant correlation between -607 C/A polymorphism of IL-18 gene and breast cancer risk in the Iraqi individuals subjected to this study.

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