The Effect of TNFα -308G/A Gene Polymorphism with Breast Cancer Risk in Iraqi Population

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ABSTRACT

The study included two hundred and thirty samples of which 130 patients women of breast cancer in Iraqi population,, their ages ranged from 29 to 71 year (ages mean 42.95 ± 1.5) and 100 controls (healthy women), their ages ranged from 25 to 65 year (ages mean 31.37 ± 1.9). We confined the frequency of TNF-α gene -308G/A polymorphism by TARMS PCR technique (Tetra-amplification refractory mutation system-polymerase chain reaction technique). Also, we determined the association of TNF-α gene -308G/A polymorphism with breast cancer of Iraqi women population. Statistical results showed significant difference in genotype frequency of TNF-α gene -308G/A polymorphism with breast cancer women patients and control (healthy women). The A allele showed high frequency in breast cancer patients comparison with control (healthy women) and present with etiological fraction risk (EF) of breast cancer patients in Iraqi women, and its ratio 64.23% in patients while in control 50.50%. The G allele shows high frequency in control comparison with breast cancer patients was 35.77% and 49.50% respectively, and present related with protective fraction (PF) with breast cancer patients was (0,21.4). The genotypes of AA and GG (homozygotes) shows high frequency in breast cancer patients was 58.46% and 30% respectively, comparison with control was 16% and 15% respectively, also AA and GG homozygotes genotypes showed relationship with etiological fraction risk of breast cancer in women patients, while the GA heterozygote genotype show high frequency in control (healthy) was 69% comparison with breast cancer patients was 11.54%, and show related with preventive fraction of breast cancer patients. Our findings demonstrate that the TNFα -308G/A gene polymorphism may represent a risk factor for breast cancer development of patient's women in Iraqi population.

Keywords: TNFα -308G/A Gene, Breast cancer, Polymorphism, TARMS PCR

INTRODUCTION

Breast cancer is the most common cancers and the leading cause of mortality between women in population (Singel et al. 2016). The tumor necrosis factor- alpha gene (TNF-α gene) is pro-inflammatory cytokine which is produced by activating macrophages (Sfikakis, 2010), and plays an important role in malignant diseases of which breast cancer (Leek et al. 1998). The lymph node activation in breast cancer is correlated with tumor necrosis factor alpha, and plays a role in enhancing of tumors cells metastasis. The concentration of TNF-α in the circulation of breast cancer patients has a poor prognosis, thus tumor necrosis factor alpha gene is a useful biomarker in breast cancer (Garcia et al. 2006). There is an association between breast cancer risk and TNF-α gene 308G/A polymorphism. The polymorphisms regions of promoter TNF-α gene have been implicated and identified in the regulation of TNF-α transcription, such as polymorphisms of TNF-α in codon -308G/A; it has been found as a risk factor for breast cancer (Qing et al. 2017). The tumor necrosis factor alpha gene is located on sixth chromosome in position 21.3 for small arm (chromosome 6p21.3) with the major histocompatibility complex (Maha et al. 2015). The HLA (human leukocyte antigen) class III genes locus and contains several sites of single nucleotide polymorphisms (SNP), which modify gene expression. Polymorphic locus –308G/A in the promoter region of TNF-α gene is a portion of a sequence, and binds transcription factor AP-2. G →A substitution at –308 site increased TNF-α gene expression by 6 to 9 factor (Hajcer et al. 2001). Most studies have a relationship between a TNF-α -308G/A gene and developing breast cancer risk (Ostashkin et al. 2008; Ahmad et al. 2020), but few studies have shown contradictor results between association TNFα –308G/A gene polymorphism and Breast cancer risk (Mestiri et al. 2001; Scol et al. 2006). There are genetic variations in TNF-α gene Polymorphism can be diagnosed using partial technologies such as Amplification refractory mutation system (ARMS), these variations effect on TNF-α gene expression. Polymorphism of TNF-α gene in codon -308 G/A has been reported in development of breast cancer risk (Ostashkin et al. 2008). The study showed presence of association between TNF-α -308 G/A gene with breast cancer risk in Iraqi women population by using ARMA-PCR Technique. The variation of association TNF-α -308 with cancer resulted from genetic diversity of population. The genetics factor such as family history and closed mating in the Iraqi population may lead enhanced genetic predisposition of some disease like breast cancer risk (Hasan, 2016). The study aimed to the finding association between TNF-α gene of codon -308 G/A genotype polymorphism and risk of Breast cancer development in women Iraqi population.

MATERIALS AND METHODS

Study samples of Population

The total of Study samples was of 230 sample, of which 130 breast cancer patients women, there ages range from 29 to 71 year, and 100 control (healthy women), there ages range 25 to 65 year of Iraqi population. All the samples of breast cancer patients were collected from The Breast Cancer Early Detection center in Baghdad. They had an established

diagnosis of breast cancer by the laboratory and clinical examination.

Genotyping of TNF-α 308G/A gene polymorphism

The genomic of DNA was extracted by take two ml of blood from each breast cancer patients women and control (healthy women) by using venipuncture, later, 2.5 ml was added into EDTA tubes then DNA was extracted by DNA isolation kit (Pro-Mega USA, the according to manufacture instructions manual). DNA purity was qualified by Nano drop and it was about 1.6 ±1.9. All samples were kept at <20 Cº for further study. TNFα –308G/A gene polymorphism were examined by using TARMS-PCR technique (Tetra amplification refractory mutation system-polymerase chain reaction). The TARMS-PCR reaction was carried out on a DNA template with a pair of specific primers (Alpha DNA, Canada) that designed according to (Solhjoo et al. 2014) in table (1), and 20 µl was the total volume of reaction mix (PioNeer, Korea), and the molecular marker size (Pro-Mega, USA) 100-1500 base pair. TARMS-PCR programs of TNFα –308G/A gene polymorphism were summarized in table (2), according to (Solhjoo et al. 2014). The genotypes were established by analyzing electrophoresed 2% gel of agarose stained with diamond dye (Pro-Mega).

Table 1: primer sequences of TNFα –308G/A gene polymorphism by TARMS PCR technique.

Target Gene	primer	Primer sequences (5`→ 3`)	Size (bp)
TNFα -308G/A gene	Forward outer	5′-AGGACTCAGCTTTCCGAAGCCCCTCCCA-3′	304
	Reverse outer	5′-TTCTGTCTCGGTTTCTTCTCCATCGCGG-3′	
	Reverse inner (G allele)	5′-GGAGGCAATAGGTTTTGAGGCGCAGGG-3′	197
	Forward inner (A allele)	5′-GTAGGACCCTGGAGGCTGAACCCCGTACT-3′	162

Table 2: The program of TNFα –308G/A gene polymorphism by using Tetra-ARMS PCR technique for breast cancer patients and control (healthy) samples.

Target gene	steps	Temperature (co)	Number of cycles	Time (seconds)
TNFα –308G/A gene	Pre-denaturation	95		300
	Initial denaturation	95	30	30
	Annealing	68		20
	Extension	72		15
	Final Extension	72		5

Statistics

Differences in the frequencies of TNFα –308G/A gene polymorphism for breast cancer patients in this study with control groups were analyzed with a value P<0.05 by Fisher's exact test. Odds ratios (OR) and confidence intervals (CI) were calculated using Compare 2 Ver.3.04 software J. H. Abramson (2003-2013). Preventive Fraction (PF) and Etiologic Fraction (EF) results were compared with Hardy-Weinberg equilibrium and according to the software within the following website www. had2know.com.

RESULTS

The genetic polymorphisms study of TNFα –308G/A gene in 130 breast cancer patients was ages mean 42.95 ± 1.5 year, and 100 control samples (healthy women) was ages mean 31.37±1.9 year. Notably, the two alleles G/A are more present for TNFα –308G/A gene polymorphism with GG, GA and AA genotypes in breast cancer patients women and control (healthy women) (figure 1 and 2), by using TARMS PCR technique in study. The allelic and genotypes frequency distribution for each polymorphisms study of control and breast cancer patients, the TNFα –308G/A gene polymorphisms was a significance in breast cancer patient compare with control samples (P>0.05 by using Fisher's test). The A and G alleles were different in frequency of breast cancer patient compare with control, so A allele frequency was 64.23% in breast cancer patient while allele G allele frequency was 35.77%. The A allele frequency in control group was 50.50% and G allele 49.50% are presented in figure (1). The confidence intervals (CI) 1.21 to 2.56 at 95 %, and odds ratio (OR) for A allele was 7.39, and it was 0.27.7 as an etiological fraction (EF), while confidence intervals (CI) 0.39 to 0.83 at 95 % and odds ratio (OR) was 0.57 for G allele, and it was 0.21.4 as an preventive fraction (PF) are presented in table (3). The allele A was significance in breast cancer patient comparison with control *0.000 (P<0.05 by Fisher's). The previous report on polymorphisms of TNFα -308G/A gene show that A allele be an etiological fraction and it's describe that the G allele be a preventive fraction that correlated with the risk of breast cancer patients.

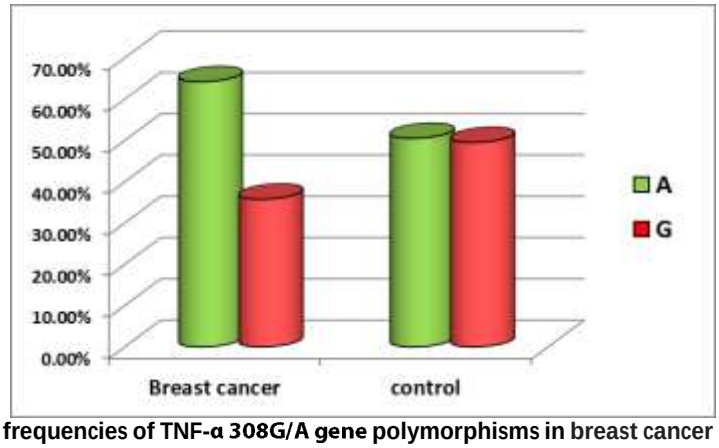

Figure 2: The allelic frequencies of TNF-α 308G/A gene polymorphisms in breast cancer patients and control (healthy) samples.

The genotyping polymorphisms frequency for TNFα –308G/A gene by using TARMS PCR technique, are significance in breast cancer patients, so AA and GG genotypes showed the high frequency in breast cancer patients as compared with control group, and it was 58.46 % and 30 % respectively are presented in figure table (3), also the OR for AA and GG genotypes was 7.39 and 2.43 respectively are presented in figure (2), with CI 3.91 to 13.9 and 1.25 to 4.71respectively (table 3). The genotypes of GG and AA present association with etiological fraction for breast cancer risk, while for GA genotype the frequency was 11.54% and 69% for breast cancer patients and control group respectively (Figure 4), also the OR was 0.06 and CI was 0.03 to 0.12 and the value of GA genotype as protective fraction (PF) was 0.65 (table 3). Briefly, the result showed that AA and GG genotypes were correlated with the risk of breast cancer for women Iraqi population, while GA genotype was correlated with the protective fraction (PF) of breast cancer in women Iraqi Population. The results correspond to (Ostashkin et al. 2008), also another study showed association between TNFα –308G/A gene polymorphism and risk of breast cancer in North European population and It was found that genetic variants AG and AA genotypes of TNFα –308G/A gene polymorphism were associated with a decreased risk of breast cancer (Azmy et al. 2004).

Table 3: The allelic and genotypes frequency of TNFα –308G/A gene polymorphism in Breast cancer patients and control (healthy) samples.

Gene	Genotype & Allele	Breast cancer (%)Number	Healthy (%)Number	OR (CI 95%)	P-value
TNFα –308G/A gene	AA	76 (58.46%)	16 (16%)	7.39(3.91 to 13.9)	* 0.000
		0.51			
	GA	15 (11.54%)	69 (69%)	0.06 (0.03 to 0.12)	*0.000
	P.F	0. 65			
	GG	39 (30.00%)	15 (15%)	2.43 (1.25 to 4.71)	*0.008
	E.F	0.18			
	A allele	167(64.23%)	101(50.50)	1.76(1.21 to 2.56)	*0.003
	P.F	0.21			
	G allele	93(35.77%)	99(49.50%)	0.57(0.39 to 0.83)	
		0.28			

Notes: OR =Odds ratio, CI =Confidence Interval, E.F =Etiological fraction, P.F =Preventive fraction, and P<0.05 by Fisher's

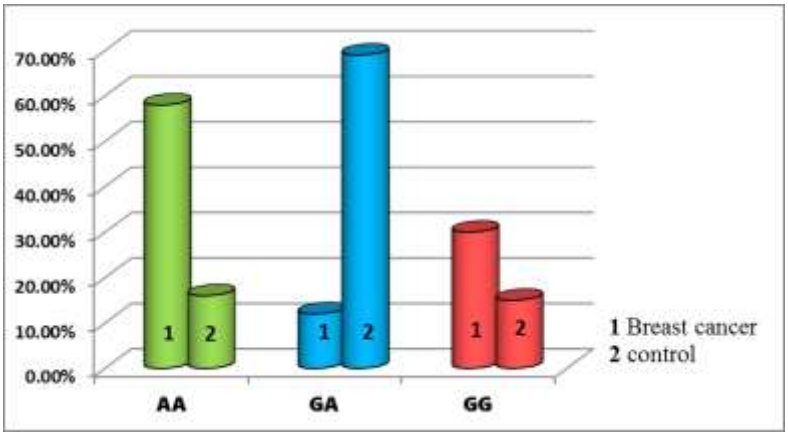

Figure 3: The genotypes frequencies of TNF-α 308G/A gene polymorphisms in breast cancer patients and control (healthy) samples

DISCUSSION

The present study showed association between polymorphisms of TNF-α gene with breast cancer patients for women Iraqi population. The frequency of allelic and genotype for TNF-α–308G/A gene polymorphisms was higher significantly in breast cancer patients compared to healthy women's. Al Hasnawi (2011) Study shows that breast cancer in the first rank of cancer types in Iraq and accounted for 16% of all women Iraqi patients (Hasnawi, 2011). The genotype is significantly associated with the development of breast cancer disease. The breast cancer enhanced by genetic and environmental factors such as family history and endogamy (Hasan, 2016). The cytokines genetics plays a role in cancer risk of which breast cancer such as interferon-γ, TGF-β, IL-6, and TNF-α (Vela et al. 2015). Tumor necrosis factor alpha (TNF-α) is an inflammatory cytokine that is highly expressed in breast carcinomas of women (Leek et al. 1998). The position -308 G/A polymorphism in TNF-α has been implicated in breast cancer risk (Fang et al. 2010). The variation in association between TNF -308 G/A gene in cancer resulted from environmental and genetic diversity of population of which closed mating in the family lead to enhanced genetic predisposition of some diseases like cancer in Iraq (Hasan, 2016). Cytokines responsible for inflammation and metabolism activates and have special role cancer genetics of which breast cancer (Balasubramanian et al. 2006). Inflammatory cytokines play critical roles at different stages of tumors development and progression, including invasion and metastasis, although several studies have reported an association between the TNF-α-308G> A polymorphism and breast cancer risk (Li et al. 2013). Inflammatory responses contribute in tumor development, including pathogenesis; invasion, and metastasis, thus, inflammatory cytokines are important components of tumor progression (Xu et al. 2014). Cytokine genes are important for researching cancer predisposition to cancers that elicit anti-tumor immune response (Karakus et al. 2011), these genes interaction regulate inflammatory response, invasive activity and metastatic potential of tumor cells (Xu et al. 2014). In study with Caucasians and Asians women population, these findings indicate that TNF-α Cytokine gene might play a distinct role in the progression of cancer, especially in distant tumor metastasis of breast cancer (Li et al. 2013). The allele and genotype of TNF-α 308G/A gene polymorphism have effect breast cancer risk. The A allele is associated with increased of breast cancer risk in Indian Population (Ahmad et al. 2020), and GG genotype associated with increased risk of Breast Cancer in Turkish Patients (Karakus et al. 2011). The study of Hasan (2016) concluded that TNFα -308 G/A gene polymorphism have association with breast cancer in Iraqi women (Hasan, 2016).

CONCLUSION

The statistical analysis data of present study demonstrate the association between of TNFα-308 G/A gene polymorphism and breast cancer risk, and indicate that the TNFα-308 G/A gene polymorphism may represent a significant risk factor in patients of breast cancer women of Iraqi population.

CONFLICT OF INTEREST

None

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