Study the relationship of IFN-γ (+874A/ T) polymorphism, and some cytokines with Breast cancer in a population from Baghdad city,Iraq

Abstract

Background: Breast cancer is still a complicated and common health issue that affects millions of people globally. **Aim**: the current investigation aimed to study the relationship of IFN-γ (+874 A/T) genetic polymorphism, and some cytokines to breast cancer women in a population from Baghdad city/ Iraq.

Materials & methods: In this study, (80) females with an age of (20-60) years were employed; they were attending Oncology Teaching Hospital, Medical City, Baghdad. All these females were notified regarding the purpose of these investigations and agreed to its protocol. 80 healthy women were taken into the current study for comparison purposes.

Results: The findings demonstrated that C3 levels showed a significant ($P \leq 0.05$) elevation in patients compared to healthy females. C4 levels demonstrated significantly ($P \leq 0.05$) elevation in patients that compared to healthy females. IL-6 levels significantly ($P < 0.05$) increased in patients compared with control group (4.15±0.65 pg/ml). IL-17 concentration in serum of patients showed a significant ($P < 0.05$) increased in patients compared with healthy ones. T and A alleles of IFN-γ T / A +874 locus had distinct results when repeated distribution was used to compare women who had breast cancer to healthy individuals. In the patient BC sample, the T allele was 42.4%, while the allele T in the control group was 71.4%. While the allele A in the sample of BC patients, which is 57.6%, compared to the allele A in control group, which was 28.6%.

Conclusions: This study suggests that elevated IL-6 and IL-17 levels, along with specific IFN-γ gene polymorphisms, could serve as potential biomarkers for breast cancer risk and prognosis.

Keywords: Breast cancer, IFN-γ (+874 A/ T), cytokines, SNP.

Introduction

Breast cancer is the most important cancer of the many common life related tumors that mainly affect women. Breast cancer may progress and happen from many different external and/or

internal variables [1-3]. The most well known cause of deaths caused by cancer in women globally is breast cancer, accounting for 670,000 of these deaths in 2022 [4]. This represents about 7% of all cancer-related deaths worldwide. The rates in developed regions of the world are as high as 80 per 100,000 [5]. However, certain developing nation estimates may be the result of underreporting [6]. The differences in rates between countries may explained by differences in the external risk factor exposure as well as changes in the lifestyle features of different demographic groups [7]. Breast cancer progresses and spreads due to a variety of factors [8–9]. One of these factors is the inflammatory tumor microenvironment (TME). TME differs from the tissue and cell microenvironment of healthy cells in that it is reducible, hypoxic, and has low blood vessels density and weak acidity. It is made up of both cellular and non-cellular components, including mast cells, white blood cells, cytokines, and malignant cells [10]. The recognized relationship between the inflammation and the cancer is demonstrated by the varying concentrations of pro- inflammatory cytokines and anti-inflammatory cytokines in cancer. An invasive malignancy and the advanced stages of the disease are related to a poor outcome for tumors with high levels of cytokines. The primary cytokines in the tumor microenvironment include interleukin (IL)-1, 6, and 17, as well as tumor necrosis factor-α. These cytokines are different from one another. These cytokines promote the growth of tumors through multiple signaling pathways, according to studies by Kaur et al. [11] and Landskron et al. [12]. A single nucleotide polymorphism (SNP) which is in the cytokine gene polymorphism has been demonstrated to be linked to alterations in the tumor's cytokine level profile. SNPs related to cytokines and their functional receptors may found in either coding (rare) or non-coding (common) regions, such as promoters and introns [13–14]. So, the current investigation aimed to study the relationship of IFN-γ (+874A/ T) polymorphism, and some cytokines with breast cancer in a population from Baghdad city.

Materials and method

Study design

The current study is a case (women with Breast cancer) -control (healthy women) study.

Subjects

In this study, (80) females with an age of (20-60) years were employed; they were attending Oncology Teaching Hospital, Medical City, Baghdad. All these females were notified regarding the purpose of these investigations and agreed to its protocol. 80 healthy women were taken into the current study for comparison purposes. The subjects were divided into two study groups each group include

- Group (1): 80 females with breast cancer.
- Group (2): 80 healthy females as control.

Inclusion and exclusion criteria

The individuals met eligibility criteria if they were aged 25 years and older, and didn't undergo to surgical operation irrespective of sociodemographic aspects. The individuals with female reported as diabetic or other chronic disease were excluded from the study.

Blood samples

A 5 ml of venous blood was taken from each female for measurements of some cytokines levels and IFN-γ (+874A/ T) polymorphism. The biochemical measurements were performed at the Medical laboratories in Baghdad city.

Extraction of DNA

Wizard® Genomic DNA Purification Kit was utilized for extraction of DNA. After extraction, the DNA samples were kept at -15 °C until needed.

PCR Premix kit

The accompanying document from the Korean company BIONEER states that ARMS-PCR technology experiments were conducted using an AccuPower® PCR PreMix kit.

Primers

The IFN-γ mutant gene was identified using three of the unique primers, according to [15], where the antisense was: 5` -TCAACAAAGCTGATACTCCA-3`. As well as, the Sense primers for T allele was 5`-TTCTTACAACACAAAATCAAATCT-3` and the sequence of A allele was 5`-TTCTTACAACACAAAATCAAATCA-3`.

The recognition of IFN-γ T/A + 874

The method of ARMS-PCR technique was utilized to identify the IFN-γ+874 gene polymorphism, and a small change from the protocol was observed in the preparation of the master mix [15]. The ARMS-PCR technology studies were conducted using an kit known as AccuPower®, in accordance with the protocol of BIONEER. To identify the A allele, specific A and the antisense primer were utilized, and to identify the T allele, specific T and the antisense primer. The samples were put in a thermocycler to amplicate the DNA, and the software was changed to get the best possible circumstances for interaction. The optimum condition of detection recognition of IFN-γ T/A + 874 were appeared in table (1).

Table (1): the optimum condition of detection recognition of IFN-γ T/A + 874

Phase	Tm (0C)	Time	No. of cycle
Initial denaturation	95°C	15 sec	1 cycle
Second denaturation	72 °C	50 sec	30 cycle
Initial Denaturation	96 °C	30 sec	
Second Denaturation	95°C	50 sec	
Initial Annealing	65 °C	50 sec	
Second Annealing	95°C	50 sec	
Initial Extension	72 °C	40 sec	
Second Extension	55°C	50 sec	

Statistical analysis

Utilizing the SPSS statistic program for analysis, PCR product data were compared utilizing Fisher's at P <0.05 to determine which averages differed significantly. The Compare 2 software Ver.3.04, developed by J. H. Abramson between 2003 and 2017, was used to evaluate genotypes, allele frequencies, confidence intervals (CI) and odds ratios (OR) [16]. The Hardy-Weinberg equilibrium rule was used to examine the results, as stated on the www.had2know.com website.

Results

Complements

Table (2) show the concentrations of C3 and C4 in BC females and healthy females, C3 levels revealed a significant (P≤0.05) elevated in patients compared to healthy females. C4 levels demonstrated a significant (P≤0.05) increased in patients compared with healthy females.

Table (2): the concentrations of some complements

Groups / Parameter	Control (35)	Patients (80)	P-Value
C3 (mg\dl)	95.11±4.65	162.91±5.03*	0.001
C4 (mg\dl)	24.73±1.93	42.58±2.17*	0.001

Interleukins

The activities of some interleukins in breast cancer patients serum showed in Table (3), where IL-6 levels showed a significant (P <0.05) increase in patients compared with control group. IL-17 concentrations in breast cancer patient's serum showed a significant (P <0.05) increase in patients compared with healthy ones.

Table (3): IL-6 and IL-17 (pg/ml) concentrations in studied groups

Groups / Parameter	Control (35)	Patients (80)	P-Value
IL-6 pg/ml	4.15±0.65	17.23± 0.93*	0.001
IL-17 pg/ml	20.14 ±1.05	37.62 ±4.51*	0.001

IFN-γ gene

The IFN-γ gene in BC patients was investigated and compared with specimens from healthy women. The IFN- mutant gene electrophoresis results revealed two alleles, T and A. Based on these findings, three types of genotypes have been determined in the samples of BC individuals and the healthy women: TT, AT, and AA. The genotype is TT when appears and is absent of the domain A; AA is identified when appears in domain A but is absent in T; and the genotype is AT appears when the two alleles are displayed in both of the domains T and A, as shown in Figure (1). In the patient BC sample, the T allele was 42.4%, while the allele T in the control group was 71.4%. While the allele A in the sample of BC patients, which is 57.6%, compared to the allele A in control group, which was 28.6%, displayed in table (4).

Table (4): The repeats of two alleles A and T.

Gene	Type of allele	BC women (80)	Healthy women (35)	OR (95%CI)	P value
IFN-γ	*T*	36(42.4%)	25(71.4%)	0.39(0.52-0.12)	*0.000
	P.F	51.5%			
	A	49(57.6%)	10(28.6%)	3.43(1.62-6.85)	
	E.F	41.7%			

PF = Preventive faction, CI = Confidence Intervals, EF = Etiological faction, * = significant difference, OR = Odds ratio.

Utilizing the Hardy-Weinberg equilibrium equation, IFN-γ T / A +874 revealed three genotypes: TT, AT, and AA in the healthy and BC women. The genotype frequency between BC individuals and normal individuals differed, according to the outcomes. The AA genotype was more prevalent in the BC individuals than in the healthy women, with percentages of 40% and 17.1%, respectively, and a significant variation ($P<0.05$). In addition, the AA genotype was shown to be related with a high risk of contracting the BC, as evidenced by the disease-causing risk of 27%. The Odds Ratio value was (3.21). The analysis revealed that the TT genotype was substantially connected with the preventive element in the risk of preventive fraction (PF) of developing breast cancer , with a greater rate of cases in the healthy women than to the breast cancer sample (60%) and 22.5%, respectively. TT genotype is more prevalent significantly among BC specimens than in the healthy women. Its protective value (46.2%) demonstrated a significant frequency in healthy women with higher rates compared to the BC patients.

The AT genotype was also linked to the side which increases the breast cancer risk (16.9 %). However, this relationship was non-significant difference between the control samples and BC samples. As seen in Figure (1) Table (5), genotype AT revealed a higher proportion of BC individuals than the control group; the rate were 37.5% and 22.9%, respectively.

Table (5): The frequency of IFN-γ mutant gene genotypes

Gene name	The genotype	BC samples (80)	healthy samples (35)	(95%CI) OR	P value
IFN-γ	TT	18(22.5%)	21(60%)	0.22(0.07-0.54)	*0.001
	P.F	46.2%			

	AT	30(37.5%)	8(22.9%)	1.92(0.72-5.15)	0.151
	E.F	16.9%			
	AA	32(40%)	6(17.1%)	3.21(9.91-1.04)	*0.001
	E.F	27%			

EF = Etiological faction, CI = Confidence Intervals, OR = Odds ratio, PF = Preventive faction.

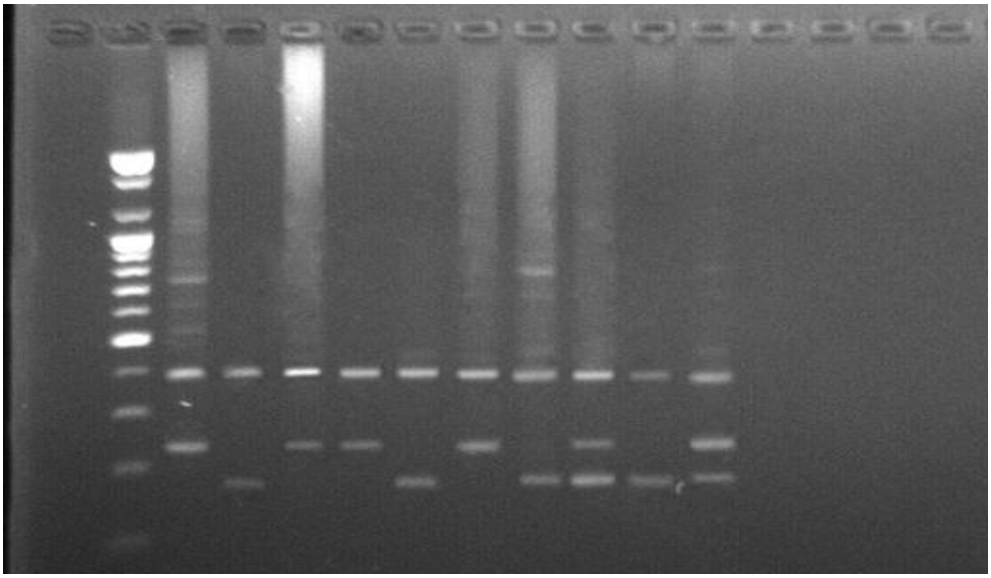

Figure (1):The IFN-γ gene electrophoresis to demonstrates the presence of both A and T alleles, in BC samples.

Discussion

Breast cancer samples that include C3, C4, and other related compounds show that the conventional pathway has triggered the complement system [17]. Caragine et al. used a rat model of human breast cancer. [18] to study the effect of a tumor-expressed inhibitor of the lytic pathway in its early complement, not the late complement of the lytic pathway, on tumor progression. Consistent with the findings of Vijayakumar et al. [19], the current study's results show a considerably higher amount of C3 in all illness phases compared to the controls. Additionally, the outcomes concurred with Ferda et al.'s findings [20], supporting the theory that malignant tumors raise complement element levels. Many tumor tissues include complement components. For example, C4 has been found in follicular and mucosal-associated lymphoid tissue lymphomas [22] and oropharyngeal squamous cell carcinomas [21]. In a similar vein, other researchers have demonstrated that, in contrast to non-malignant controls, C3 is widely deposited in the tumor tissue of glioblastoma multiforme patients [23].

In addition to activation of lymphocyte and the boosting of hematopoiesis, interleukin-6 is recognized to govern the invasion, genesis, and spread of cancer. Inflammation relationship to BC has been associated. A poor prognosis for survival is associated with elevated blood interleukin-6 levels, and the expression of BC IL-6 increases with tumor grade [24–25]. Excessive levels of interleukin-6 associated with poor overall survival and tumor formation in several of different types of malignancies [25]. The development and spread of cancer, the incidence of humoral hypercalcemia and the process of osteolysis and the levels of estrogen contol in breast cancer cells and tissues are all potential effects of interleukin (IL)-6 [26]. However, its specific purpose is still unclear and varies. It suggests that the kind of tumor cell involved may affect IL-6's capacity to promote tumor cell development [27]. When compared to controls, it was demonstrated that patients significantly overexpressed IL-6 (P 0.01). The dysregulated inflammatory responses caused by IL-6 can lead to chronic inflammation and even cancer. Prognosis for BC is correlated with IL-6 expression [28]. This study shows the impact of breast cancer infection on the levels of IL-17, thus the patients had a greater concentration of IL-17 than the control group. Interleukin-17's significance in the genesis of breast cancer remain not distinctly recognized although its precise mechanisms, it has been proven that Interleukin-17 both suppresses and promotes tumor activity. Chemoresistance, tumor proliferation, tumor metastasis angiogenesis/angiostasis, and tumorigenesis are among the many interconnected processes that IL-17 may be crucial to [29]. Individuals with breast cancer might exhibit a higher blood level of Interleukin-17 than those in the control category for a several reasons, including the hypothesis that proinflammatory cytokines are generally associated with cancer and interleukins and their receptors, part of the most prevalent cytokines, promote the angiogenesis and development of tumors, generate neutrophils to inflammation site, and intensify the process of inflammation [39].

The optimization of PCR for IFN-γ +874 A /T detection was displayed in (Figure 1), and was 60°C during the annealing process. When minimizing unspecific fragments and exhibiting the thickest DNA fragment at a given size, these ideal conditions were identified. About one-third of all cancer-related deaths are caused by BC, the most common cancer type known in women [31]. The immune system displayed as one of the important factors because it has two roles in cancer, because this system encourages the growth of cancer cells and their ability to spread and infect the other tissues of the body, but it also suppresses the angiogenesis and proliferation of cancer

cells [32–33]. IFN-γ coding gene located in the 12q24 region of chromosome 12, it consists of four exons with three introns [34]. The studied SNP is at the position +874 in intron 1, which discovered lately. This region is bound by kappa (a nuclear factor) light chain enhancer of activated B cells (NFkB) that plays an important role in the IFN-γ. IFN-γ production which upregulated in the TT, AT, and AA genotypes, and decreased in the AA genotype due to the transversion of A to T in +874 region [35]. Kamali-Sarvestani et al. found that the genotype frequency of TT was higher in patients compared to healthy women in a study involving both. Conversely, it was discovered that the significant correlation between the occurrence of BC in women and the IFN-γ +874 A/T polymorphism is absent [36]. Furthermore, Gonullu et al. looked at the connection between the disease and polymorphisms in IFN-γ, IL-10, TGF, and TNF-α. The formation of BC has been associated with mutations in IL-10 coding gene, but the pathophysiology of BC has not been connected to mutations in IFN-1, or TNF-α coding gene region [37]. Karakus et al., [38] discovered in their study that in female with breast cancer, AT and AA genotypes coming from IFN- γ +874 A/T locus were larger than in healthy females.

Conclusions

The outcomes of this study demonstrated a significant association between BC and genotype frequency, with patients carrying the IFN-γ+874A/T genotype having a high risk of developing into a malignant stage, and the AA genotype is linked to a better prognosis for the breast cancer.

Authors' contribution

The final manuscript draft was reviewed by all authors, who also gave their approval.

DISCLAIMER

NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

Ethical approval and consent

These investigations were accepted, according to the native ethics group, and all participating patients provided informed consent and knowledge about the purpose of the study.

References

1. Obeagu EI, Babar Q, Vincent CC, et al.. Therapeutic targets in breast cancer signaling: a review. J Pharm Res Int. 2021;33:82–99.
2. Ibekwe AM, Obeagu EI, Ibekwe CE, et al.. Challenges of exclusive breastfeeding among working class women in a teaching hospital South East, Nigeria. J Pharm Res Int. 2022;34:1.
3. Aizaz M, Khan M, Khan FI, et al.. Burden of breast cancer: developing countries perspective. Int J Innov Appl Res. 2023;11:31–7.
4. WHO. Breast-Cancer. Available online: https://www.who.int/news-room/fact-sheets/detail/breast-cancer (accessed on 25 March 2024).
5. Arnold, M.; Morgan, E.; Rumgay, H.; Mafra, A.; Singh, D.; Laversanne, M.; Vignat, J.; Gralow, J.R.; Cardoso, F.; Siesling, S.; et al. Current and future burden of breast cancer: Global statistics for 2020 and 2040. Breast 2022, 66, 15–23.
6. Cumber, S.N.; Nchanji, K.N.; Tsoka-Gwegweni, J.M. Breast cancer among women in sub-Saharan Africa: Prevalence and a situational analysis. South. Afr. J. Gynaecol. Oncol. 2017, 9, 35–37.
7. Francies, F.Z.; Hull, R.; Khanyile, R.; Dlamini, Z. Breast cancer in low-middle income countries: Abnormality in splicing and lack of targeted treatment options. Am. J. Cancer Res. 2020, 10, 1568–1591.
8. Feng, Y., Spezia, M., Huang, S., Yuan, C., Zeng, Z., Zhang, L., et al. (2018). Breast cancer development and progression: risk factors, cancer stem cells, signaling pathways, genomics, and molecular pathogenesis. Genes Dis. 5 (2), 77–106.
9. Park, M., Jaiswal, V., Kim, K., Chun, J., Lee, M. J., Shin, J. H., et al. (2022). Breast cancer metastasis: mechanisms and therapeutic implications. Int. J. Mol. Sci. 23, 15215.
10. Zarrilli, G., Businello, G., Dieci, M. V., Paccagnella, S., Carraro, V., Cappellesso, R., et al. (2020). The tumor microenvironment of primitive and metastatic breast cancer: implications for novel therapeutic strategies. Int. J. Mol. Sci. 21, 8102.
11. Kaur R.P., Vasudeva K., Singla H., Paramjeet R., Benipal S. Analysis of pro- and anti-inflammatory cytokine gene variants and serum cytokine levels as prognostic markers in breast cancer. J. Cell. Physiol. 2018;233:9716–9723.

12. Landskron G., De la Fuente M., Thuwajit P., Thuwajit C., Hermoso M.A. Chronic inflammation and cytokines in the tumor microenvironment. J. Immunol. Res. 2014;2:1–19.
13. Dondeti M.F., El-Maadawy E.A., Talaat R.M. Hepatitis-related hepatocellular carcinoma: Insights into cytokine gene polymorphisms. World J. Gastroenterol. 2016;22(30):6800–6816.
14. Aqbi H.F., Wallace M., Sappal S., Payne K.K., Manjili M.H. IFN- γ orchestrates tumor elimination, tumor dormancy, tumor escape, and progression. J. Leukoc. Biol. 2018;103:1219–1223.
15. Chong, W.P., Ip, W.E., Tso, G.H.W. et al. The interferon gamma gene polymorphism +874 A/T is associated with severe acute respiratory syndrome. BMC Infect Dis., 2006; 6(82): 1-7.
16. Dhabaan A. A. The Allelic and Polymorphism Association of Tumor Necrosis Factor-alpha Gene (-308 G/A Genotype) in Some Iraqi Rheumatoid Arthritis Patients. International Journal of Sciences: Basic and Applied Research (IJSBAR . 2017; 36(5): 302-309
17. Niculescu F, Rus HG, Retegan M, et al. Persistent complement activation on tumor cells in breast cancer. American Journal of Pathology 1992;140:1039-1043.
18. Caragine TA, OkadaN, FreyAB, et al. Tumor-expressed inhibitor of the early but not late complement lytic pathway enhances tumor growth in a rat model of human breast cancer. Cancer Research 2002;62:1110-1115
19. Vijayakumar T, Ankathil R, Remani P, Beevi VM, Vijayan KK, Panicker CK, et al. Total hemolytic complement (CH50) and its fractions (C3 and C4) in the sera of patients with carcinoma of the oral cavity, uterine cervix, and breast. J Clin Immunol 1987;7:300-3.
20. Ferda O,İsmail S, Numan N. Immunoglobulins and complement components in patients with lung cancer. TüberkülozveToraksDergisi2004;52:19-23.
21. Ajona D., Pajares M.J., Chiara M., Rodrigo J.P., Jantus-Lewintre E., Camps C., Suarez C., Bagán J., Montuenga L., Pio R. Complement activation product C4d in oral and oropharyngeal squamous cell carcinoma. Oral Dis. 2015;21:899–904.

22. Bu X., Zheng Z., Wang C., Yu Y. Significance of C4d deposition in the follicular lymphoma and MALT lymphoma and their relationship with follicular dendritic cells. Pathol. Res. Pract. 2007;203:163–167.
23. Bouwens T.A.M., Trouw L.A., Veerhuis R., Dirven C.M.F., Lamfers M.L.M., Al-khawaja H. Complement activation in Glioblastoma Multiforme pathophysiology: Evidence from serum levels and presence of complement activation products in tumor tissue. J. Neuroimmunol. 2015;278:271–276.
24. Nyati KK, Hashimoto S, Singh SK, Tekguc M, Metwally H, Liu Y-C, et al. The novel long noncoding RNA AU021063, induced by IL-6/Arid5a signaling, exacerbates BC invasion and metastasis by stabilizing Trib3 and activating the Mek/Erk pathway. Cancer Lett. 2021;520:295–306.
25. Park Y, Kim J. Regulation of IL-6 signaling by miR-125a and let-7e in endothelial cells controls vasculogenic mimicry formation of BC cells. BMB Rep. 2019; 52(3): 214.
26. Kurebayashi J. Regulation of interleukin-6 secretion from BC cells and its clinical implications. BC. 2000;7:124–9.
27. Conze D, Weiss L, Regen PS, Bhushan A, Weaver D, Johnson P, et al. Autocrine production of interleukin 6 causes multidrug resistance in BC cells. Cancer Res. 2001;61(24):8851–8.
28. Chen J, Chen J, Wei Y, Yang W, Huang Q, Chen Y, et al. IL-6: The link between inflammation, immunity and BC. Front Oncol. 2022; 3472.
29. Huang CK, Yang CY, Jeng YM, Chen CL, Wu HH, Chang YC, et al. Autocrine/paracrine mechanism of interleukin-17B receptor promotes breast tumorigenesis through NF-κB-mediated antiapoptotic pathway. Oncogene 2014; 33: 2968-77.
30. Lin Y, Xu J, Su H, Zhong W, Yuan Y, Yu Z, Fang Y, Zhou H, Li C, Huang K. Interleukin-17 is a favorable prognostic marker for colorectal cancer. Clin Transl Oncol. 2015;17(1):50–56.
31. Siegel R, Naishadham D, Jemal A. Cancer statistics, 2013. CA Cancer J Clin. 2013;63(1):11–30.
32. Colotta F, Allavena P, Sica A, Garlanda C, Mantovani A. Cancer-related infammation, the seventh hallmark of cancer: links to genetic instability. Carcinogenesis. 2009;30(7):1073–81.

33. Hanahan D, Folkman J. Patterns and emerging mechanisms of the angiogenic switch during tumorigenesis. Cell. 1996;86(3):353–64.

34. Calvo J, Martínez N, Etxagibel A, Calleja S, Sáez-Torres C, Sedeño M, et al. Allelic frequencies of polymorphic variants of cytokine genes (IL1A, IL1B, IL1RN, IL6, IL10, IL12p40, and IFNG) in a Spanish population. Inmunologia. 2002;21(2):76–86.

35. Pravica V, Perrey C, Stevens A, Lee J-H, Hutchinson IV. A single nucleotide polymorphism in the frst intron of the human IFN-γ gene: absolute correlation with a polymorphic CA microsatellite marker of high IFN-γ production. Hum Immunol. 2000;61(9):863–6.

36. Kamali-Sarvestani E, Merat A, Talei A-R. Polymorphism in the genes of alpha and beta tumor necrosis factors (TNF-α and TNF-β) and gamma interferon (IFN-γ) among Iranian women with breast cancer. Cancer Lett. 2005;223(1):113–9.

37. Gonullu G, Basturk B, Evrensel T, Oral B, Gozkaman A, Manavoglu O. Association of breast cancer and cytokine gene polymorphism in Turkish women. Saudi Med J. 2007;28(11):1728–33.

38. Karakus N, Kara N, Ulusoy AN, Özaslan C, Bek Y. Tumor necrosis factor alpha and beta and interferon-gamma gene polymorphisms in Turkish breast cancer patients. DNA Cell Biol. 2011;30(6):371–7.