ORIGINAL ARTICLE
Iran J Allergy Asthma Immunol
December 2019; 18(6):671-678.

Association of rs1946518 C/A Polymorphism in Promoter Region of Interleukin 18 Gene and Breast Cancer Risk in Iranian Women: A Case-control Study

Nazila Fathi Maroufi[1,2], Elnaz Aghayi[3], Hamid Garshasbi[4], Milad Gholampour Matin[5], Ahmad Babazadeh Bedoustani[6], Fatemeh Firouzi Amoudizaj[4], Saba Hajazimian[7], Alireza Isazadeh[7], Sina Taefehshokr[7], Nima Taefehshokr[8], and Behzad Baradaran[7]

[1] Department of Clinical Biochemistry and Laboratory Medicine, Tabriz University of Medical Sciences, Tabriz, Iran
[2] Student Research Committee, Tabriz University of Medical Sciences, Tabriz, Iran
[3] Department of Molecular Biology, Bonab Branch, Islamic Azad University, Bonab, Iran
[4] Department of Genetic, Tabriz Branch, Islamic Azad University, Tabriz, Iran
[5] Department of Microbiology, Urmia Branch, Islamic Azad University, Urmia, Iran
[6] Biotechnology Research Center, Tabriz Branch, Islamic Azad University, Tabriz, Iran
[7] Immunology Research Center, Tabriz University of Medical Sciences, Tabriz, Iran
[8] Division of Biosciences, Department of Life Sciences, College of Health and Life Sciences, Brunel University London, Uxbridge, Middlesex, United kingdom

Received: 20 November 2018; Received in revised form: 30 June 2019; Accepted: 15 July 2019

ABSTRACT

Breast cancer (BC) is the most frequently diagnosed cancer among women in the world. Genetic polymorphisms in Interleukin (IL) genes are one of the most important risk factors in BC. The aim of this study was to investigate the association of rs1946518 C/A polymorphism in the promoter region of the IL-18 gene and BC risk in Iranian women.

In this case-control study, we recruited 140 women with BC as a case group and 140 age and ethnically matched women as healthy controls from East Azerbaijan, Tabriz in Iran. The genomic DNA was extracted using a salting-out method from peripheral blood leukocytes. Genotyping was performed using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP).

The genotype distribution in BC patients was 37.86% CC, 47.14% CA, and 15.00% AA, whereas in healthy controls these were 40.72% CC, 42.85% CA, and 16.43% AA. Statistical analysis showed that the genotype and allele frequencies of IL-18 rs1946518 C/A polymorphism were not significantly different between BC patients and healthy controls ($p>0.05$). The only significant difference between cases and controls was related to family history ($p=0.023$).

In conclusion, our study indicated that IL-18 rs1946518 C/A polymorphism was not associated with BC in the Iranian women population. However, more studies on different races and geographic areas are required to determine the exact role of rs1946518 C/A polymorphism in prognosis, diagnosis, and risk of BC.

Keywords: Breast cancer; Interleukin 18; Polymorphism

Corresponding Author: Behzad Baradaran, PhD; Immunology Research Center, Tabriz University of Medical Sciences, Gholghasht Ave, Tabriz, Iran. Tel: (+98 41) 3337 1440, Fax: (+98 41) 3337 1311, Email: baradaranb@tbzmed.ac.ir

Published by Tehran University of Medical Sciences (http://ijaai.tums.ac.ir)

INTRODUCTION

Nowadays, breast cancer (BC) is one of the most common diseases in women across the world which is increasing in developed and developing countries.[1] In 2017, about 255180 newly affected cases by BC and about 41070 death causalities of BC were reported in the United States.[2] Although the main cause of it, has not been known yet, the findings of different researches prove that BC can be caused by multiple reasons. There are different factors that may expose a person to this disease including genetic alterations, diet, breastfeeding, and environmental factors.[3] The most significant cause which can lead to BC is genetic alternations in some main genes such as tumor suppressor genes, growth adjusting genes, and oncogenes.[4] Moreover, single nucleotide pymorphisms (SNPs) which are detrimental in phenotype variations among people, seem to play a role in the susceptibility of people to cancer and worsening the disease condition.[5]

Chronic inflammation can be one of the most important factors leading to tumor formation and its further development. In addition, different factors of chronic inflammation are involved in the stimulation of angiogenesis, mutagenesis, invasion, and cell growth. The factors associated with angiogenesis, inflammation, and thrombosis can increase the risk of developing different types of cancer.[6] Interleukin 18 (IL-18) gene is located on 11q22.2-q22.3 chromosome region in human and is a member of IL-1 cytokine family.[7] IL-18 is produced by different cells such as T cells, B cells, and antigen presenting cells (APCs); including active monocytes, dendritic cells, and macrophages which can adjust innate and adaptive immune responses.[8] In addition to the vital role of IL-18 in the immune response, there are pieces of evidence proving a connection between IL-18 and tumorigenesis which indicates the connection between inflammation and cancer.[9] In comparison to normal cells, high available level of IL-18 in various cancer cells has been observed. In a study, it was reported that the IL-18 levels in breast tumor tissue and lymph nodes were increased during tumor progression in mice.[10] In another study, it has been reported that levels of IL-18 were increased in serum of patients with metastatic BC and has a high value for use as an effective marker for prognosis and diagnosis of BC.[11] Moreover, higher IL-18 levels show to be capable of producing angiogenesis, proliferation and escaping from the immune system.[12] IL-18 gene has considerable polymorphisms; especially in the promoter region. Studied reported that some polymorphisms in the promoter of the IL-18 gene are related to differential levels of gene expression and production of protein.[13] The rs1946518 C>A polymorphism in the promoter region of the IL-18 gene is the most common SNP which can change the protein binding site of cyclic adenosine monophosphate and increase the IL-18 transcription. Also, a substitution from C to A at position -607 of promoter region in rs1946518 C>A polymorphism leads to disturbs a potential cAMP-responsive element-binding protein site.[14]

In general, the association of interleukin genes polymorphisms with BC is still unclear. Therefore this study aimed to investigate the association of rs1946518 C/A polymorphism in the promoter region of the IL-18 gene and BC risk in Iranian women.

MATERIALS AND METHODS

Patients and Sample Collection

In the current case-control study, 280 women were recruited from educational hospitals of Tabriz-Iran, from December 2015 to January 2017. All studied women aged 40-60 years. A total of 140 women with BC were selected as case groups with histologically confirmed BC; using physical and histopathologic examination and imaging tests. Also, 140 age- and ethnically-matched healthy women without a familial history of any malignancy were selected as a control group who were referred to as routine physical examination. All participants avoided anti-inflammatory agents within 72 hours before sample collection. Women with kidney, cardiovascular, metabolic, and liver diseases, as well as any systemic disease, were excluded. The information such as clinical characteristics, lifestyle, and demographic data were collected using interviews and questionnaires from case and control groups. The collected information included age (year), Body Mass Index (BMI kg/m2), age at menarche (year), menopausal status, tobacco smoking, alcohol drinking, age at first delivery (year), and family history of BC (Table 1). In order to prevent the epidemiological bias, all selected women in this study were from the East Azerbaijan province of Iran and matched for age and ethnic and were genetically unrelated. All participants were

informed about the study and signed a consent form according to the Declaration of Helsinki ethical standards. The ethical code was IR.TBZMED.REC.1395.366.

DNA Extraction and Genotyping

The peripheral blood (5 mL) was drawn from all participants and collected into vials containing Ethylene diamine tetraacetic acid (EDTA) as an anticoagulant. Genomic DNA extraction was performed using the salting-out procedure from collected peripheral blood leukocytes. The quantity and quality of extracted DNA were investigated according to OD 260/280 ratio using a nanodrop instrument. The ratio between 1.7-1.9 was desirable. Also, electrophoresis on 1% agarose gel was carried out in order to confirm the results. The extracted DNA samples were stored at -20°C until genotype analysis. The genotyping was carried out using polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) method. The respective forward and reverse primer sequences were as follows: 5′-TCAGTGGAACAGGAGTCCAT-3′ and: 5′-GCAGAAAGTGTAAAAATTTTT-3′.[13] The obtained PCR products (150 bp) were digested with the DraI restriction enzymes and then incubated at 37°C. After digestion, the PCR product remained intact and a 150bp fragment was produced in presence of C allele, and also PCR product was cut and two 109bp and 41bp fragments were produced in presence of A allele. The RFLP pattern was CC: 150bp, CA: 109bp+41bp+150bp, and AA: 109bp+41bp for rs1946518 C/A polymorphism. The PCR reaction was carried out in a total volume of 25 μL: forward primer (25pmol), reverse primer (25 pmol), template DNA (1 μg), dNTP (0.1 mmol), PCR buffer (2.5 μL), Mgcl2 (1.5 mmol/L), and Taq DNA polymerase (1.5 unit) in the following condition: initial denaturation (1 cycle in 94°C for 4 minutes), denaturation (40 cycles in 94°C for 40 seconds), annealing (40 cycles in 50°C for 30 seconds), extension (40 cycles in 72°C for 25 seconds), and final extension (1 cycle in 72°C for 5 minutes). The digested fragments were separated using electrophoresis on 3% agarose gel stained by ethidium bromide. A 50bp size marker (ladder) was used to estimate the size of DNA bands. Finally, a gel documentation instrument was used to visualize the bands of digested PCR products.

Statistical Analysis

The statistical analysis of obtained data was carried out using Statistical Package for the Social Sciences (SPSS) software (version 21.0). The logistic regression was used to analyze the association between rs1946518 C/A polymorphism and BC risk. The hardy-weinberg equilibrium (HWE) in genotypes distribution of BC patients and healthy controls were analyzed using the chi-square ($\chi 2$) test and Fisher's exact test. Also, the odds ratio (OR) and 95% confidence intervals (CI) were evaluated. The difference between demographic and clinical features between BC patients and healthy controls were analyzed using independent sample t-test. The statistically significant was considered as $p<0.05$.

Web Base Analysis of Survival

A meta-analysis of gene markers obtained from BC cell microarray database and disease-free survival data collected by the Gene Expression Omnibus (NCBI, Bethesda, MD, USA; http://www.ncbi.nlm.nih.gov/geo) was conducted by the use of analytic tools generated by Gyorffy et al and facilitated by the Kaplan-Meier Plotter (http://www. kmplot.com) in a web-based analysis tool. The all-cause of mortality was compared by inverse probability log-rank test and plotted survival functions and the hazard ratio was estimated.

RESULTS

In the current study, we recruited 140 women with BC (case group) and 140 age- and ethnically matched healthy women (control group) from East Azerbaijan province origin for evaluating the association between rs1946518 C/A polymorphism in the promoter of IL-18 gene and BC in the Iranian population.

The demographic characteristics and clinical features of studied patients and controls are presented in Table 1. The statistical analysis showed that there were significant differences between cases and control groups in terms of family history of BC ($p=0.023$); whereas we did not find any significant differences between case and control groups in term of age, body mass index (BMI), age at menarche, menopausal status, tobacco smoking, alcohol drinking, and age at first delivery ($p>0.05$).

The distributions of genotype and allele frequencies of rs1946518 C/A polymorphism in case and control

groups are presented in Table 2. According to $\chi 2$ tests, the rs1946518 C/A polymorphism was in HWE in the case and control groups (p>0.05).

The obtained results revealed that the frequency of homozygous CC, heterozygous CA, and homozygous AA in patients group were 37.86%, 47.14%, and 15.00%, respectively. Also, the frequency of homozygous CC, heterozygous CA and homozygous AA in patients group were 40.72%, 42.85%, and 16.43%, respectively. The statistical analysis showed that there were no significant differences between BC patients and healthy controls in the genotypes frequencies of rs1946518 C/A polymorphism in the codominant, dominant (p=0.333; OR=0.95; 95% CI=0.88-1.51), recessive (p=0.677; OR=1.18; 95% CI=0.69-1.31), and overdominant (p=0.522; OR=0.99; 95% CI=0.77-1.45) inheritance model. The typical electrophoresis results for genotyping of rs1946518 C/A polymorphism are presented in Figure 1.

Table 1. The clinical features and demographic variables of cases and controls

Variables	Patients (n=140)	Controls (n=140)	*p*-value
Age, year	53.21 ± 9.12	52.95 ± 10.05	0.455
BMI, kg/m	23.34 ± 2.85	22.50 ± 3.20	0.295
Age at menarche, year	12.49 ± 2.13	12.56 ± 2.13	0.395
Menopausal status			
Premenopausal	104 (74.28%)	113 (80.71%)	
Postmenopausal	36 (25.72%)	27 (19.29%)	0.121
Tobacco smoking			
Never	131 (93.57%)	133 (95.00%)	
Ever	9 (6.43%)	7 (5.00%)	0.566
Alcohol drinking			
Never	126 (90.00%)	131 (93.57%)	
Ever	14 (10.00%)	9 (6.43%)	0.449
Age at first delivery, year	25.15±3.55	23.85±3.22	0.288
Family history			
Positive	11 (7.85%)	3 (2.14%)	
Negative	129 (92.15%)	137 (97.86%)	0.023

Data mean (SD) or n (%); Statistically Significant p<0.05; BMI-Body Mass Index

Table 2. Genotype and allele distribution of IL-18 rs1946518 C/A polymorphism in cases and controls

Polymorphisms	Inheritance model	Genotype and Allele	Patients (n=140)	Controls (n=140)	p-value	OR (95% CI)
IL-18	Codominant	CC	53 (37.86%)	57 (40.72%)	Ref	Ref =1
rs1946518 C/A		AC	66 (47.14%)	60 (42.85%)	0.267	1.25 (0.83-2.49)
		AA	21 (15.00%)	23 (16.43%)	0.788	1.29 (0.77-2.50)
	Dominant	CC	53 (37.85%)	57 (40.72%)	Ref	Ref =1
		AC + AA	87 (62.15%)	83 (59.25%)	0.333	0.95 (0.88-1.51)
	Recessive	AA	21 (15.00%)	23 (16.43%)	Ref	Ref =1
		CC + AC	119 (85.00%)	117 (83.57%)	0.677	1.18 (0.69-1.31)
	Overdominant	AC	66 (47.15%)	60 (42.85%)	Ref	Ref =1
		CC + AA	74 (52.85%)	80 (57.15%)	0.522	0.99 (0.77-1.45)
		C normal	172 (61.43%)	174 (62.14%)	Ref	Ref =1
		A minor	108 (38.57%)	106 (37.86%)	0.885	1.09 (0.49-2.31)

Statistically Significant p<0.05; OR-Odds Ratio; CI-Confidence Interval

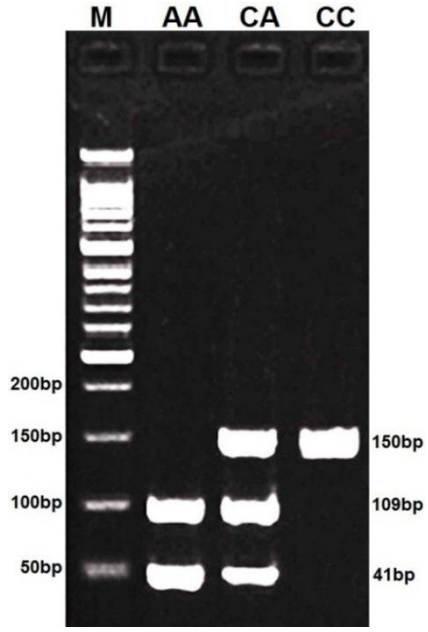

Figure 1. The electrophoresis digested PCR products of IL-18 rs1946518 C/A polymorphism on 3% agarose gel. PCR product (150bp) remains intact if C allele is present and yields two fragments (109bp and 41bp) in the case of A allele.

According to Table 2, the frequency of C allele in patients and healthy controls were 61.43% and 62.14%, respectively. Also, the frequency of A allele in patients was 38.57% and in healthy controls, it was 37.86%. The evaluation of alleles frequencies showed no significant differences between cases and controls (p>0.05).

The web-based analysis of survival revealed that there is no significant difference in survival of patients with BC between the low expression of IL-18 gene and high expression of IL-18 gene (Hazard Ratio-HR=0.84; 95% CI=0.68-1.04; p=0.002 by the log-rank test). However, low expression of IL-18 is associated with better survival in patients with BC (Figure 2).

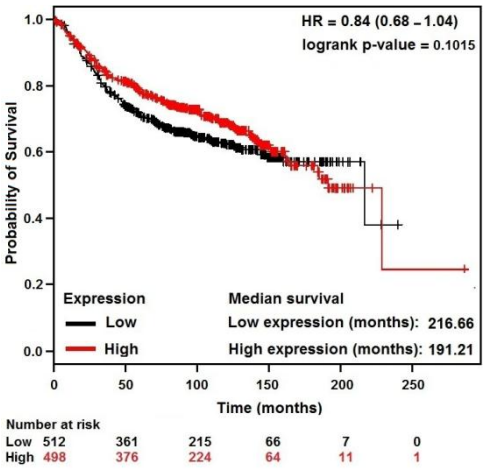

Figure 2. Inverse probability of survival curves among women with breast cancer, according to the expression of IL-18. The women with high expression of IL-18 had shorter overall survival than those with low expression of IL-18 (p=0.1015 by the log-rank test).

DISCUSSION

According to the complex, heterogeneous, and multifactorial entity of BC, many different agents such as proinflammatory cytokines have been studied to be associated with it.[15] The proinflammatory cytokines are involved in altering epithelial tissues and play an important role in the suppression of anti-cancer immune responses and promotion of angiogenesis.[16] The inflammation process also plays a role in suppressing the anti-cancer immune response and promote angiogenesis.[17] Genetic variation in regulatory genes in the immune system can affect anti-tumor immune response.[18] Cytokines derived from tumors and their environmental components, play an important role in growth, invasion, and metastasis in BC.[10] Several studies have demonstrated that polymorphisms of cytokine genes result in upregulation or downregulation of cytokines production.[9,18] Therefore, the aim of this study was to evaluate the association of rs1946518 C/A polymorphism in the promoter region of the IL-18 gene and BC risk in Iranian women. According to results of this study which was performed on 140 BC patients and 140 healthy controls, there was no significant association between rs1946518 C/A polymorphism and BC in Iranian women. Also, according to obtained results from web-based analysis of Kaplan Meier plot for expression of IL-18, median survival in BC patients with low expression of IL-18 was more than BC patients with high expression of IL-18.

Previous studies reported that IL-18 plays a crucial role in tumor formation, invasion, and angiogenesis.[19,20] The higher expression of IL-18 gene have been found in many cancer types in human and animal models.[21] In addition, serum levels of IL-18 are increased in the advanced stages of various tumors.[22] The previous studies showed a significant association of many cancer types in different populations in the world and polymorphisms in the promoter region of IL-18 genes, such as rs1946518 C/A and rs187238 G/C polymorphisms. For example colorectal cancer in a Greek population,[23] ovarian cancer in native Hawaiian populations,[24] prostate cancer and esophageal squamous cell carcinoma oral cancer in Chinese populations,[25] and nasopharyngeal carcinoma risk in the world.[26] Genetic polymorphisms on promoter of IL-18 could change production levels and function of IL-18 and affect in tumorigenesis process.[27]Both of the rs1946518 C/A and rs187238 G/C polymorphisms in IL-18 promoter region play important role in expression and activity of IL-18 gene. Therefore, high promoter activity may increase IL-18 gene expression.[28] Several studies investigated the association of IL-18 polymorphisms and the risk of BC in various populations. A number of these studies have reported a significant association between IL-18 gene polymorphisms and risk of BC,[29,30] whereas various studies did not indicate any correlation between the mentioned gene polymorphisms and risk of BC.[31,32] Khalili-Azad et al (2009) and Taheri et al (2012) reported no significant association between rs1946518 C/A polymorphism and risk of BC in Iranian women.[31,32] In our study, the correlation between rs1946518 C/A polymorphism and risk of BC was investigated in Iranian women and no significant association was observed, which is in agreement with mentioned studies. In two studies by Hasan and Naif (2017) and Bao et al (2015); reporting that IL-18 rs1946518 C/A polymorphism in promoter region does not play a pivotal role in the expression of IL-18 gene. They showed that the expression of IL-18 was increased insignificantly with presence of C allele.[33,34] Therefore, these can confirm the results of our study, due to remained fairly constant expression of IL-18 with different genotypes of rs1946518 C/A polymorphism. However, Back et al (2014) and Zhao et al (2017) reported that AA genotype was increased significantly in Brazilian and Chinese BC patients compared to healthy controls.[30,35] Nevertheless, another meta-analysis study by Li et al (2015) suggested that rs1946518 C/A polymorphism in IL-18 gene promoter is associated with a substantially increased risk of BC.[29] The reasons for the difference of reported results among the above studies could be due to several other related genes and environmental factors, and difference in studied samples size, ethnicity, race, and geographical area.[36-38]

In summary, this study demonstrated a more understanding of BC as a multifactorial disease and suggests that IL-18 rs1946518 C/A polymorphism is not associated with the risk of BC in Iranian women. However, the exact role and effects of IL-18 rs1946518 C/A polymorphism in BC are not fully identified. Therefore, for a better understanding of the association of this polymorphism with BC, further studies are recommended on other populations and races with larger sample sizes.

REFERENCES

1. Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J, Jemal A. Global cancer statistics, 2012. CA Cancer J Clin 2015; 65(2):87-108.
2. De Santis CE, Ma J, Goding Sauer A, Newman LA, Jemal A. Breast cancer statistics, 2017, racial disparity in mortality by state. CA Cancer J Clin 2017; 67(6):439-48.
3. Lichtenstein P, Holm NV, Verkasalo PK, Iliadou A, Kaprio J, Koskenvuo M, et al. Environmental and heritable factors in the causation of cancer-analyses of cohorts of twins from Sweden, Denmark, and Finland. N Engl J Med 2000; 343(2):78-85.
4. Soheilyfar S, Velashjerdi Z, Hajizadeh YS, Maroufi NF, Amini Z, Khorrami A, et al. In vivo and in vitro impact of miR-31 and miR-143 on the suppression of metastasis and invasion in breast cancer. JBUON 2018; 23(5):1290-6.
5. Fathi Maroufi N, Gholampour Matin M, Ghanbari N, Khorrami A, Amini Z, Haj Azimian S, et al. Influence of single nucleotide polymorphism in IL-27 and IL-33 genes on breast cancer. Br J Biomed Sci 2018; 76(2):1-3.
6. Hamed EA, Zakhary MM, Maximous DW. Apoptosis, angiogenesis, inflammation, and oxidative stress: basic interactions in patients with early and metastatic breast cancer. J Cancer Res Clin Oncol 2012; 138(6):999-1009.
7. Chang JT, Segal BM, Nakanishi K, Okamura H, Shevach EM. The costimulatory effect of IL-18 on the induction of antigen-specific IFN-γ production by resting T cells is IL-12 dependent and is mediated by up-regulation of the IL-12 receptor β2 subunit. Eur J Immunol 2000; 30(4):1113-9.
8. Srivastava S, Salim N, Robertson M. Interleukin-18: biology and role in the immunotherapy of cancer. Curr Med Chem 2010; 17(29):3353-7.
9. Yang Y-C, Chang T-Y, Chen T-C, Chang S-C, Lin W-S, Lee Y-J. Genetic variants in interleukin-18 gene and risk for cervical squamous cell carcinoma. Hum Immunol 2013; 74(7):882-7.
10. Jung MY, Kim SH, Cho D, Kim TS. Analysis of the expression profiles of cytokines and cytokine-related genes during the progression of breast cancer growth in mice. Oncol Rep 2009; 22(5):1141-7.
11. Eissa S, Zaki SA, El-Maghraby SM, Kadry DY. Importance of serum IL-18 and RANTES as markers for breast carcinoma progression. J Egypt Natl Canc Inst. 2005; 17(1):51-5.
12. Park S, Cheon S, Cho D. The dual effects of interleukin-18 in tumor progression. Cell Mol Immunol 2007; 4(5):329-35.
13. Bao J, Lu Y, Deng Y, Rong C, Liu Y, Huang X, Song L, Li S, Qin X. Association between IL-18 polymorphisms, serum levels, and HBV-related hepatocellular carcinoma in a Chinese population: a retrospective case-control study. Cancer Cell Int 2015; 15(1):72.
14. Giedraitis V, He B, Huang W-X, Hillert J. Cloning and mutation analysis of the human IL-18 promoter: a possible role of polymorphisms in expression regulation. J Neuroimmunol 2001; 112(1-2):146-52.
15. He Y, Sun S, Liu Y, Tian K. Association of IL-8 genetic polymorphisms and breast cancer risk in a Chinese population. Biomed Res 2017; 28(18):7892-8.
16. Huang Q, Qian X, Fan J, Lv Z, Zhang X, Han J, et al. IL-17 promotes angiogenic factors IL-6, IL-8, and Vegf production via Stat1 in lung adenocarcinoma. Sci Rep 2016; 6:36551.
17. Chechlinska M, Kowalewska M, Nowak R. Systemic inflammation as a confounding factor in cancer biomarker discovery and validation. Nat Rev Cancer 2010; 10(1):2-3.
18. Andersen SW, Trentham-Dietz A, Gangnon R, Hampton J, Figueroa J, Skinner H, et al. Breast cancer susceptibility loci in association with age at menarche, age at natural menopause and the reproductive lifespan. Cancer Epidemiol Biomarkers Prev 2013; 22(3):471–8.
19. Diekstra MH, Liu X, Swen JJ, Boven E, Castellano D, Gelderblom H, et al. Association of single nucleotide polymorphisms in IL8 and IL13 with sunitinib-induced toxicity in patients with metastatic renal cell carcinoma. Eur J Clin Pharmacol 2015; 71(12):1477-84.
20. Kassim SK, El-Salahy EM, Fayed ST, Helal SA, Helal T, Azzam EE-d, et al. Vascular endothelial growth factor and interleukin-8 are associated with poor prognosis in epithelial ovarian cancer patients. Clin Biochem 2004; 37(5):363-9.
21. Srabović N, Mujagić Z, Mujanović-Mustedanagić J, Muminović Z, Čičkušić E. Interleukin 18 expression in the primary breast cancer tumour tissue. Med Glas Ljek komore Zenicko doboj kantona 2011; 8(1):109-12.
22. Sözen S, Coskun U, Sancak B, Bukan N, Günel N, Tunc L, et al. Serum levels of interleukin-18 and nitrite+ nitrate in renal cell carcinoma patients with different tumor stage and grade. Neoplasma 2004; 51(1):25-9.
23. Bushley AW, Ferrell R, McDuffie K, Terada KY, Carney ME, Thompson PJ, et al. Polymorphisms of interleukin (IL)-1α, IL-1β, IL-6, IL-10, and IL-18 and the risk of ovarian cancer. Gynecol Oncol 2004; 95(3):672-9.

24. Guo X-G, Xia Y. The Interleukin-18 promoter-607C> A polymorphism contributes to nasopharyngeal carcinoma risk: evidence from a meta-analysis including 1,886 subjects. Asian Pac J Cancer Prev 2013; 14(12):7577-81.
25. Nikiteas N, Yannopoulos A, Chatzitheofylaktou A, Tsigris C. Heterozygosity for interleukin-18-607 A/C polymorphism is associated with risk for colorectal cancer. Anticancer Res 2007; 27(6B):3849-53.
26. Liang T-J, Ma H, Wang C-X, Liu Y-R, Wang X-G. The-137G> C polymorphism in interleukin-18 promoter region and cancer risk: evidence from a meta-analysis of 21 studies. Tumour Biol 2013; 34(6):3483-90.
27. Hajilooi M, Abasi M, Bazmani A, Ahmadi A, Matini M, Solgi G, et al. Evaluation of interleukin-8-251 T/A polymorphisms in visceral leishmaniasis. J Res Health Sci 2014; 15(1):59-61.
28. Soheilyfar S, Nikyar T, Fathi Maroufi N, Mohebi Chamkhorami F, Amini Z, Ahmadi M, et al. Association of IL-10, IL-18, and IL-33 genetic polymorphisms with recurrent pregnancy loss risk in Iranian women. Gynecol Endocrinol 2019; 35(4):342-5.
29. Li X, Ren D, Li Y, Xu J, Liu C, Zhao Y. Increased cancer risk associated with the -607C/A poly-morphism in interleukin-18 gene promoter: an updated meta-analysis including 12,502 subjects. JBUON 2015; 20(3):902-17.
30. Back LdC, Farias T, Da Cunha P, Muniz Y, Ribeiro M, Fernandes B, et al. Functional polymorphisms of interleukin-18 gene and risk of breast cancer in a Brazilian population. Tissue Antigens 2014; 84(2):229-33.
31. Khalili-Azad T, Razmkhah M, Ghiam A, Doroudchi M, Talei A, Mojtahedi Z, et al. Association of interleukin-18 gene promoter polymorphisms with breast cancer. Neoplasma 2009; 56(1):22-5.
32. Taheri M, Hashemi M, Eskandari-Nasab E, Fazaeli A, Arababi F, Bahrani-Zeidabadi M, et al. Association of-607 C/A polymorphism of IL-18 gene (rs1946518) with breast cancer risk in Zahedan, Southeast Iran. Prague Med Rep 2012; 113(3):217-22.
33. Hasan F, Naif H. Association of Gene Polymorphisms and Serum Levels of IL-18 with the Susceptibility to Infection with Hepatitis B Virus. J Infect Dis Med 2017; 2(3):117-25.
34. Bao J, Lu Y, Deng Y, Rong C, Liu Y, Huang X, et al. Association between IL-18 polymorphisms, serum levels, and HBV-related hepatocellular carcinoma in a Chinese population: a retrospective case–control study. Cancer Cell Int 2015; 15(1):72-80.
35. Zhao Y, Wang S, Zhang Z, Qin C, Yang X, Xie Q. Association of IL-18 genetic polymorphisms and haplotypes with breast cancer risk in a Chinese population. Biomed Res 2017; 28(19):8433-7.
36. Isazadeh A, Azimian SH, Tariverdi N, Rahmani SA, Esmaeili M, Karimkhanilouei S, et al. Effects of coagulation factor XIII (Val34Leu) polymorphism on recurrent pregnancy loss in Iranian Azeri women. LaboratoriumsMedizin-J Lab Med 2017; 41(2):89-92.
37. Isazadeh A, Hajazimian S, Rahmani SA, Mohammadoo-Khorasani M, Samanmanesh S, Karimkhanilouei S. The effects of Factor II (rs1799963) polymorphism on recurrent pregnancy loss in Iranian Azeri women. La Rivista Italiana della Medicina di Laboratorio-Ital J Lab Med 2017; 13(1):37-40.
38. Isazadeh A, Hajazimian S, Rahmani SA, Mohammadoo-Khorasani M, Moghtaran N, Maroufi NF. The effect of factor-xi (rs3756008) polymorphism on recurrent pregnancy loss in Iranian Azeri women. Gene Cell Tissue 2017; 4(1):e43717.