Investigating the asoociation between breast cancer and polymorphism A / T 251 of the IL-8 gene: A systematic review and meta-analysis

Abstract

Background. Breast cancer is the most common cancer in women. In recent research works, the role of cytokines in development of cancer has attracted attention. Regarding the relationship between breast cancer and polymorphism A / T 251 of the IL-8 gene, several preliminary studies have been conducted and there are contradictions between the results of these studies. Therefore, this paper intends to comprehensively assess the association between breast cancer and polymorphism A / T 251 -from the IL-8 gene-using meta-analysis.
Method. In this study, to find articles published electronically between 2004 and 2020, national and international databases of SID, MagIran, Embase, ScienceDirect, Scopus, PubMed and Web of Science (ISI) with the keywords ‘Interleukin-8’ , ‘IL-8’, ‘A / T 251’, ‘Interleukin’, ‘Breast Cancer’, and ‘Cancer’ were searched. The heterogeneity between the results of the articles was determined using Cochran's C (Q) and I^2 tests, and data analysis was performed using the Comprehensive Meta-Analysis software.
Findings. In this meta-analysis and systematic review, finally, 9 articles fulfilled the criteria to enter the study. Among the initial research works included in the meta-analysis, the number of patients were 2429 and 2367 in the patients and control groups respectively. The odds ratio of AA genotype in patients with breast cancer based was obtained as 1.42 (1.11-1.82: 95% confidence interval), which shows the increasing effect of AA genotype by 0.42, the odds ratio the AT genotype in patients with breast cancer based on a meta-analysis of studies was obtained as 1.01 (0.84-1.22: 95% confidence interval), which demonstrates the increasing effect of the AT genotype by 0.01, the odds ratio of the TT genotype in patients with breast cancer, was similarly obtained and was 0.95 (1.08-0.24: 95% confidence limit), which shows the decreasing effect of TT genotype by 0.05.
Analysis. According to this meta-analysis, allele A has a significant relationship with breast cancer, which can be used as a predictor of the course of the disease and the clinical outcome of breast cancer. Identifying polymorphism A / T 251 from the IL-8 gene, as a genetic marker and predictor, can also be effective in treating breast cancer.

Keywords: IL-8, T / A 251, breast cancer, meta-analysis.

Introduction

Breast cancer in women is a commonly developed cancer and is a serious problem in today’s world. It is the second most common cancer in human (men and women) and the first most common cancer in women worldwide. Breast cancer accounts for 23% of all new cancers and 14% of all cancers. Risk factors for breast cancer include: family history of breast cancer, genetic status, personal history of breast cancer, formation of abnormal cells in the lobules or mammary glands in breast

tissue, breast volume, androgen levels, menstrual cycles, pregnancy, breastfeeding baby, bone density, lifestyle factors such as postmenopausal hormone use, obesity and overweight, physical activity, diet, alcohol and tobacco use, birth control pills and other risk factors such as radiation, use of certain medications, infections, and environmental and occupational pollutions and and genetic factors associated with cancer [2, 3]. Breast cancer is a highly heterogeneous disease caused by the interaction of hereditary and environmental risk factors and leads to the progressive accumulation of genetic and epigenetic changes in breast cancer cells. Although epidemiological evidence emphasizes on the existence of specific risk factors (such as age, obesity, and alcohol consumption), having a family history of breast cancer is the greatest risk factor for development of this cancer [4].

Lately, the role of cytokines in cancer has been discussed in research works. Cytokines are glycoproteins that are secreted by the innate and acquired immune system and other cells and mediate many functions of these and other cells [5]. It has recently been reported that at the tumor site, the internal secretion of some cytokines decreases through the interaction with the tumor cells, leading to a weakening of the immune system, leading to tumor proliferation, metastasis, and tumor malignancy [5]. The importance of interleukin-8 receptor signaling pathways and this commocaine itself has been shown to boost the progression of malignant cancers [6, 7].

The IL-8 gene is located on chromosome 4q12-q13 [8] and the host's ability to produce IL-8 can be controlled by polymorphism A / T 251 in the promoter region of this chemokine gene [9]. Allele A has been shown to be associated with high levels of IL-8 production in this single nucleotide polymorphism [9]. Further increase in IL-8 gene expression in tumor cells is of great importance in the survival of this type of tumor through the role of CXCR2, CXCR1 gene receptors (Subfamily of chemokines 1, 2 genes) in cancer cells, endothelial cells and neutrophils, and tumors associated with macrophages [10]. The importance of IL-8 is related to the modulation between different cell types in tumors and microenvironments [6]. Tumor cells have been shown to maintain their mesenchymal state using continuous autocrine signaling rings [11]. IL-8 stimulates the tumor by activating various signaling pathways that ultimately affect the transcription factors associated with the tumor [12].

Studies on the effects of IL-8 and breast cancer have shown that there is a significant relationship between the metastatic potential of breast cancer cells and the expression of IL-8 gene expression. Based on these studies, it is shown that high-metastatic cell lines express more IL-8 genes than low-metastatic cells. This could be due to epigenetic changes such as an inappropriate methylation pattern in the IL-8 gene, which may be responsible for the difference in metastatic cells with other cells [13]. Recent research works have demonstrated that there is an association between polymorphism in IL-8 and CXCR2 genes with an increased risk of breast cancer in the Chinese female population [14-17]. According to clinical studies, serum levels of this interleukin in the serum of patients with breast cancer were higher than in healthy individuals; especially patients who were in the process of developing the disease. Single-nucleotide polymorphisms are important in cytokine genes and affect the expression of these genes and cell activity. The

polymorphism in region 251 of the IL-8 gene promoter plays an important role in the production of IL-8, or its protein expression, both in the living organism and in the laboratory. Previous studies have shown that polymorphism A / T 251 of the IL-8 gene in various populations is associated with an increased risk of developing cancerous tumors [14, 15, 18, 19, 20, 21].

With regards to the relationship between breast cancer and polymorphism A / T 251 of the IL-8 gene, several preliminary studies have been conducted and there are contradictions between the results of these studies. This meta-analysis attempts to clarify these contradictions and inconsistencies; therefore, the aim of this study is to determine the relationship between breast cancer and polymorphism A / T 251 of the IL-8 gene.

Materials and Methods

Method for searching articles

The study searched the SID and MagIran Persian databases, and the international databases of Embase, ScienceDirect, Scopus, PubMed and Web of Science (ISI) to find resources between 2004 and 2020. The lists of references within all searched articles and reports were also manually evaluated to find other possible sources. The keywords used to search for resources were selected from the MeSH medical topics database. The keywords used were Interleukin-8, IL-8, A / T 251, Interleukin, Breast Cancer, and Cancer.

Article selection criteria

Articles with the following characteristics were considered for selection of articles for our meta-analysis: 1) Original research articles, 2) Clinical trial studies, 3) Availability of the full text of the article and 4) Studies that had assessed the association between breast cancer and polymorphism A / T 251 of the IL-8 gene.

Article exclusion criteria

The collected research works were examined in more details. Articles that were review papers, articles in which samples were not selected from breast cancer patients, and research works that only examined previous secondary data were excluded from the meta-analysis. Finally, 17 studies entered the quality evaluation stage.

Article quality evaluation

The quality of the articles was evaluated based on the criteria used within the CONSORT checklist (i.e. study plan, background and review of texts, place and time of study, consequence, entry criteria, sample size and statistical analysis). Articles that fulfilled 6 or 7 criteria were considered as high-quality articles, articles that achieved 3 to 5 criteria, and articles that only fulfilled 2 or less criteria were catogorised as medium and low quality articles respectively [22]. In this study, 9 papers entered the systematic review and meta-analysis as high-quality and medium-quality studies, and 8 papers were classed as low quality articles and they were removed.

Data extraction

All final papers entered into the meta-analysis process were prepared by another checklist. This checklist included fields of: the title of the article, the name of the first author, the year of publication, the place of study, the sample size of the patient and control group, the frequency of the patient group genotype and the probability controls for case and patients groups, the frequency of A and T alleles and the mean age.

Statistical analysis

Since the focus of this study was to assess the relationship between breast cancer and polymorphism A / T 251 of the IL-8 gene, frequency and percentage, as well as the standardized mean difference index in each study were used to amalgamate the results of various studies. To investigate the homogeneity between the studies, the I^2 index was used to measure the percentages of variation, and since heterogeneity was found among the studies, the random effects model was used to combine studies and perform the meta-analysis. When the I^2 index was less than 25%, the heterogeneity was considered as low, between 75-25% as moderate heterogeneity and more than 75% as high heterogeneity. P levels less than 0.05 were considered significant. The funnel diagrams and the Egger's test were also employed to investigate the publication bias. Data analysis was performed using the Comprehensive Meta-Analysis software.

Findings

In this study, all research works that has investigated the relationship between breast cancer and polymorphism A / T 251 of the IL-8 gene were systematically reviewed without time constraints, and in accordance with the PRISMA guidelines. In the initial search, 383 articles were identified; after inputing all the articles in the classification database, all duplicate articles, articles with irrelevant contents, and abstracts of articles published in conferences were further removed. A total of 81 articles was fed into the second stage for further examination. At this stage, while reviewing the titles and abstracts, 64 unrelated articles were excluded from the study. In the next step, the full text of 17 articles were reviewed. At this stage, 8 low quality articles were removed. Finally, 9 articles that were published between 2004 and April 2020 entered the final analysis stage (please see Figure 2).

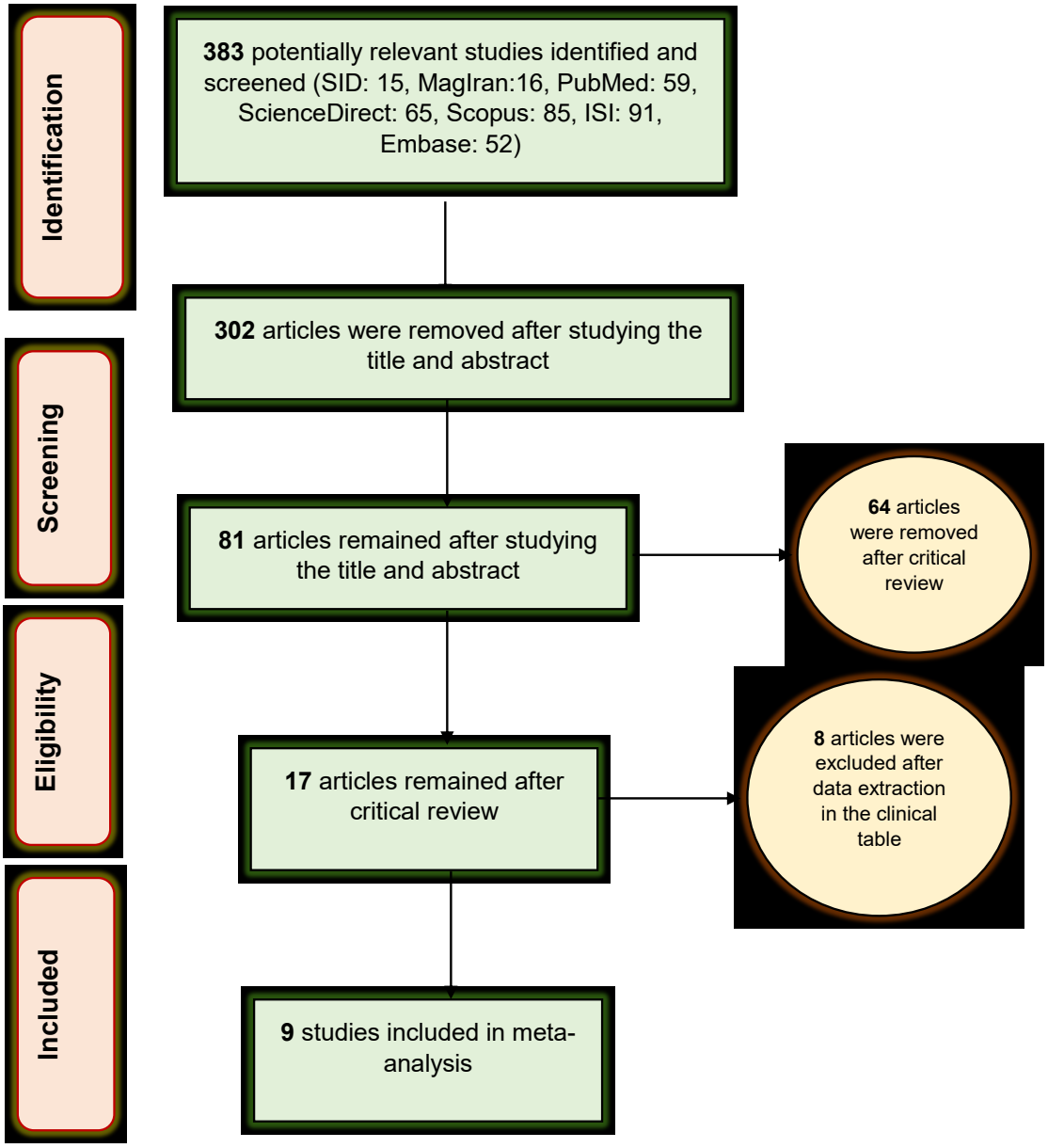

Figure 1: PRISMA flow diagram for article selection

Among the initial studies included in the meta-analysis, the samples were 2429 and 2367 in the patients and control groups respectively. The characteristics of the studies that were systematically reviewed are provided in Table 1. Noet that, all of the final studies were of the clinical trial type, and out of 9 final articles, one article was published in Persian and 8 articles were published in English (please see Table 1).

Table 1: Specifications of studies entered into the meta-analysis

Author, year, Reference	Country	Age (year) of patients	sample size patients	sample size Control	Gene	Patient group number (%)	Control group number (%)	*P*	OR (95% CI)	Genotyping methods	Quality
	Iran	-	50	50	AA	6 (12%)	0 (0%)		14.75 (0.8-269.3)	ARMS-PCR[1]	Medium
					AT	27 (54%)	44 (88%)		0.16 (0.05-0.44)		

Siasi, 2019, (23)					TT	17 (34%)	6 (12%)		4.55 (1.62-12.71)		
					A	20 (39%)	22 (44%)	0.68			
					T	30 (61%)	28 (56%)				
Taheri, 2012, (15)	Iran	44.8±11.6	72	93	AA	11 (15.3%)	8 (8.6%)	0.813	1.91 (0.72-5.04)	ARMS-PCR	High
					AT	32 (44.4%)	45 (48.4%)	0.257	0.85 (0.46-1.58)		
					TT	29 (40.3%)	40 (43%)		3.94 (2.21-7.03)		
					A	54 (37.5%)	61 (32.8%)	0.532			
					T	90 (62.5.5%)	125 (67.2%)	0.002			
Zhang, 2017, (16)	China	49.52±11.0	442	447	AA	78 (17.65%)	43 (9.62%)	0.002	2.01 (1.35-2.99)	PCR–RFLP[2]	High
					AT	174 (39.37%)	191 (42.73%)		0.87 (0.66-1.13)		
					TT	190 (42.99%)	213 (47.65%)		0.72 (0.55-0.94)		
					A	330 (37.3%)	277 (30.98%)				
					T	554 (62.7%)	617 (69.02%)				
He, 2017, (17)	China	57.47±9.69	411	411	AA	73 (17.76%)	42 (10.21%)	<0.001	1.89 (1.26-2.85)	PCR–RFLP	High
					AT	179 (43.55%)	162 (39.41%)		1.18 (0.89-1.56)		
					TT	159 (38.68%)	207 (50.36%)		0.46 (0.35-0.62)		
					A	325 (39.53%)	246 (29.92%)				
					T	497 (60.46%)	576 (70.08%)				
Kamali-Sarvestan, 2007, (24)	Iran	-	257	233	AA	79 (30.7%)	48 (20.6%)	0.016	1.71 (1.13-2.58)	ASO-PCR[3]	Medium
					AT	114 (44.4%)	106 (45.5%)		0.95 (0.66-1.36)		
					TT	64 (24.9%)	79 (33.9%)		0.87 (0.59-1.28)		
					A	272 (52.9%)	202 (43.3%)	0.003	1.47 (1.13-1.91)		
					T	242 (47.1%)	264 (56.7%)				
Snoussi-1, 2010, (25)	Tunisia	48.0 ± 24.0	409	301	AA	124 (30.3%)	71 (23.6%)	0.002	1.4 (1.0-1.97)	AS-PCR[4]	High
					AT	201 (49.2%)	138 (45.8%)	0.01	1.14 (0.84-1.53)		
					TT	84 (20.5%)	92 (30.6%)		0.001 (0.0-0.023)		
					A	449 (54.9%)	280 (46.5%)	0.001	1.4 (1.13-1.74)		
					T	369 (45.1%)	322 (53.5%)				
Smith, 2004, (26)	UK	-	119	235	AA	19 (16.0%)	54 (23.0%)		0.63 (0.35-1.13)	ARMS-PCR	Medium
					AT	63 (52.9%)	105 (44.7%)		1.39 (0.89-2.16)		
					TT	37 (31.1%)	76 (32.3%)		0.97 (0.60-1.56)		
					A	101 (42.43%)	213 (45.31%)				
					T	137 (57.56%)	257 (54.68%)				
Snoussi-2, 2007, (27)	Tunisia	-	308	236	AA	86 (27.92%)	54 (22.88%)	0.338	1.03 (0.75-1.42)	AS-PCR	
					AT	157 (50.97%)	110 (46.61%)		1.19 (0.84-1.67)		High
					TT	65 (21.1%)	72 (30.5%)		1.01 (0.69-1.47)		
					A	329 (53.4%)	218 (46.18%)				
					T	287 (46.59%)	254 (53.82%)				
Vogel, 2007, (28)	Denmark	-	361	361	AA	116 (32.13%)	113 (31.3%)	0.221	1.03 (0.75-1.42)	Taqman	High
					AT	167 (46.26%)	160 (44.32%)		1.08 (0.80-1.45)		
					TT	78 (21.6%)	88 (24.37%)				
					A	399 (55.26%)	386 (53.46%)				
					T	323 (44.74%)	336 (46.54%)				

1- amplification refractory mutation system-polymerase chain reaction
2- polymerase chain reaction-restriction fragment length polymorphism
3- allele-specific oligonucleotide-polymerase chain reaction
4- allele-specific polymerase chain reaction

Investigating heterogeneity and publication bias (AA Genotype)

The heterogeneity of the studies was investigated using the I^2 test and based on this test (I^2 = 59.8%) the heterogeneity in the studies was evident; therefore, the random effects model was used to amalgamate the results of the studies together.

Publication bias was measured using the Egger's test (please see Figure 2), and this was not statistically significant (P = 0.444).

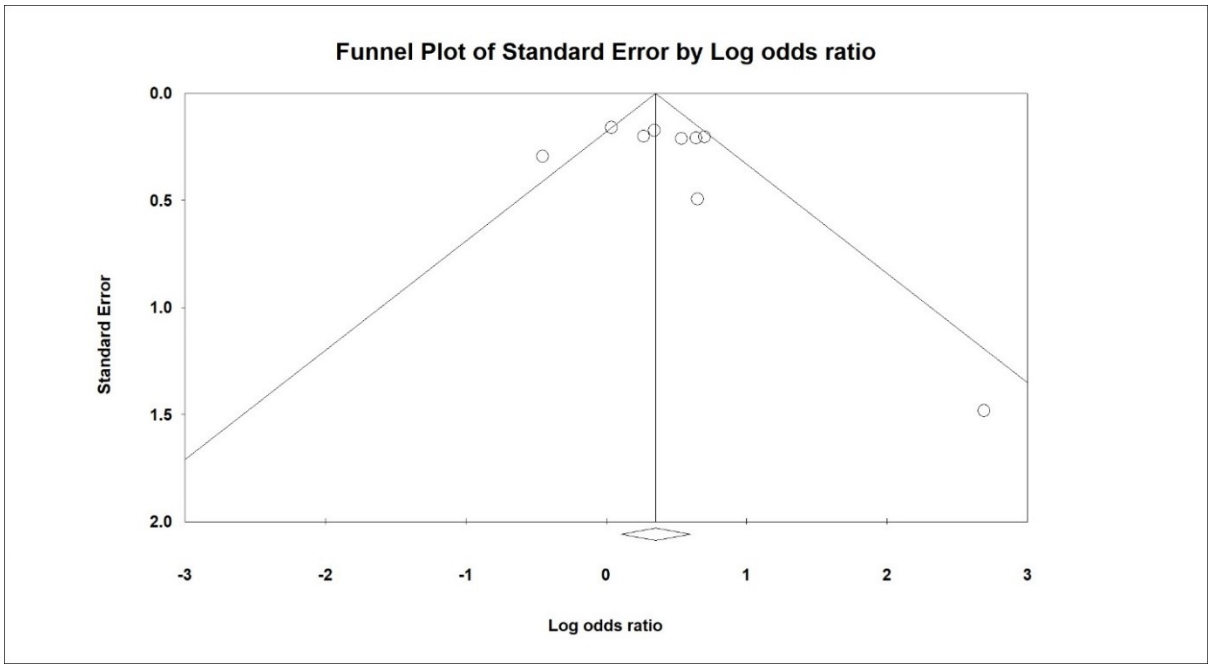

Figure 2: Funnel plot representing the odds ratio of AA genotype in patients with breast cancer.

The total number of samples entered in the study was 2429 in the group of patients and 2367 in the control group. The odds ratio of AA genotype in patients with breast cancer based on meta-analysis of studies was obtained as 1.42 (1.11 ± 1.82: 95% confidence interval), which indicates the increasing effect of AA genotype by 0.42. This means that people with this genotype are 42% more likely than others to have breast cancer (please Figure 3). In Figure 3, the odds ratios based on the random effects model are illustrated. Each black square represents the odds ratio and the length of the line on which the square is located demonstrated the 95% confidence interval in each study. The diamond symbol represents the odds ratio for all studies put together.

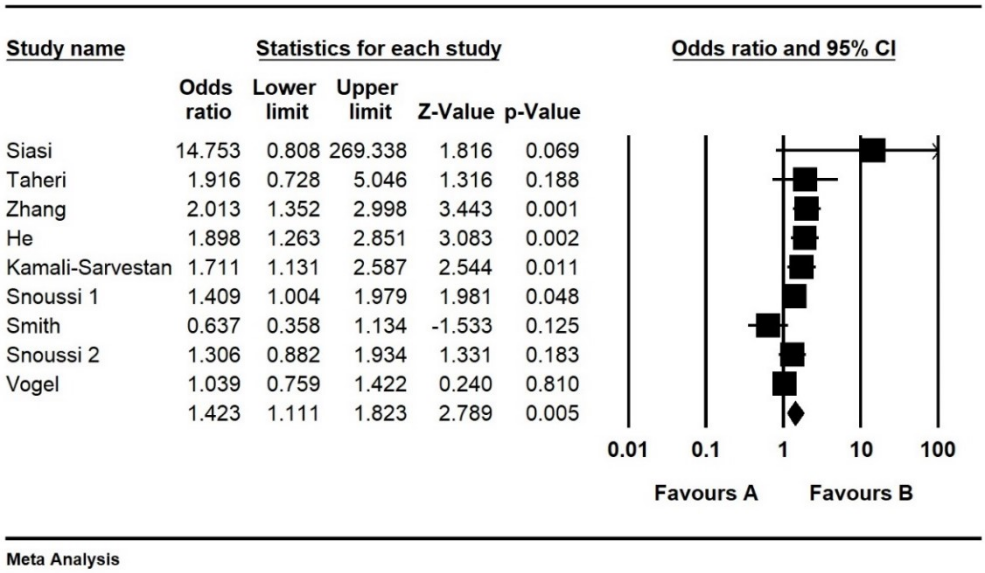

Figure 3: The odds ratios random effect model for AA genotype in patients with breast cancer.

Investigating heterogeneity and publication bias (AT Genotype)

The heterogeneity of the studies was investigated using the I^2 test and based on this test (I^2 = 57.6%) the heterogeneity in the studies was evident; therefore, the random effects model was used to amalgamate the results of the studies together. Publication bias was measured using the Egger's test (please see Figure 4), and this was not statistically significant (P = 0.094).

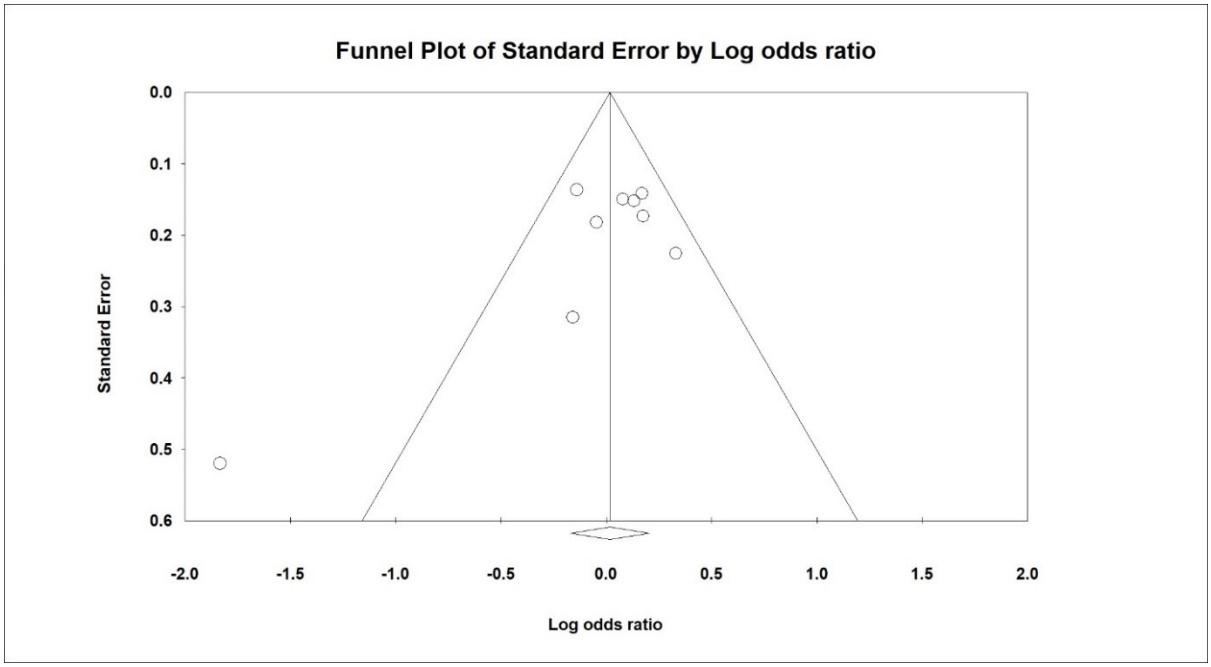

Figure 4: Funnel plot representing the odds ratio of AT genotype in patients with breast cancer

The total number of samples entered in the study was 2429 in the group of patients and 2367 in the control group. The odds ratio of AT genotype in patients with breast cancer based on meta-analysis of studies was obtained as 1.01 (0.84 ± 1.22: 95% confidence interval), which indicates the increasing effect of AT genotype by 0.01. This means that people with this genotype are 1% more likely than others to have breast cancer (please Figure 5). In Figure 5, the odds ratios based on the random effects model are illustrated. Each black square represents the odds ratio and the length of the line on which the square is located demonstrated the 95% confidence interval in each study. The diamond symbol represents the odds ratio for all studies put together.

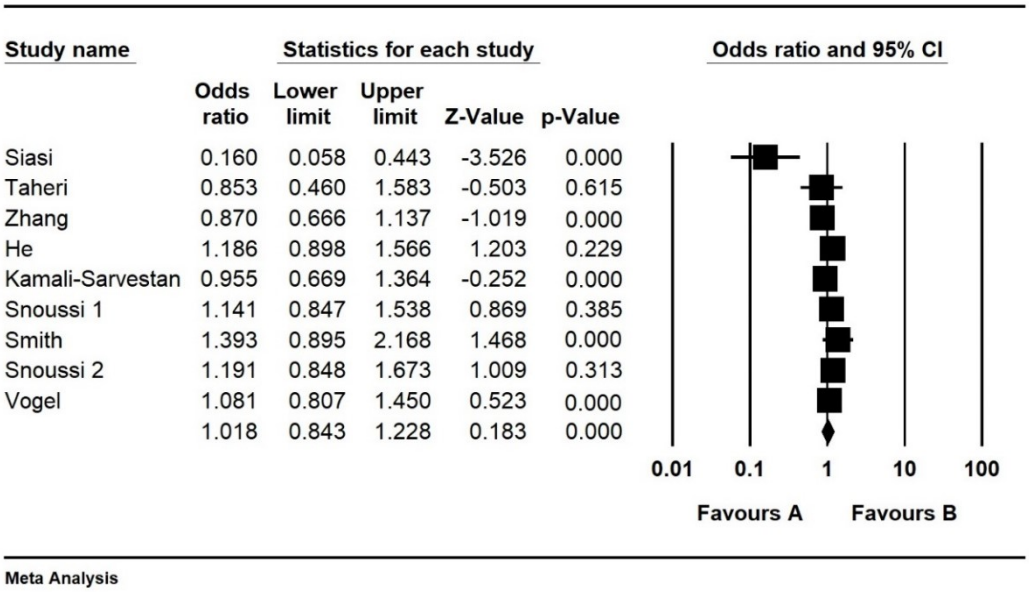

Figure 5: The odds ratios random effect model for AT genotype in patients with breast cancer

Investigating heterogeneity and publication bias (TT Genotype)

The heterogeneity of the studies was investigated using the I^2 test and based on this test (I^2 = 90.8%) the heterogeneity in the studies was evident; therefore, the random effects model was used to amalgamate the results of the studies together. Publication bias was measured using the Egger's test (please see Figure 6), and this was not statistically significant (P = 0.705).

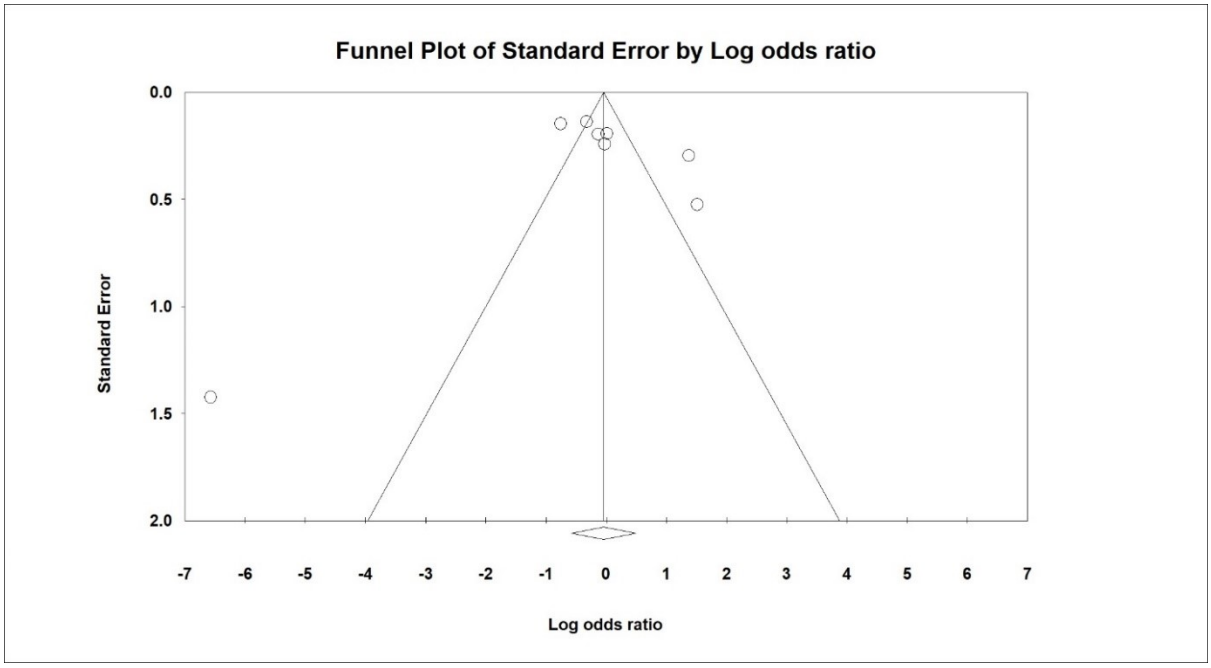

Figure 6: Funnel plot representing the odds ratio of TT genotype in patients with breast cancer

The total number of samples entered in the study was 2068 in the group of patients and 2006 in the control group. The odds ratio of TT genotype in patients with breast cancer based on meta-analysis of studies was obtained as 0.95 (1.08 ± 2.04: 95% confidence interval), which indicates the decreasing effect of TT genotype by 0.05. This means that people with this genotype are 5% less likely than others to have breast cancer (please Figure 7). In Figure 7, the odds ratios based on the random effects model are illustrated. Each black square represents the odds ratio and the length of the line on which the square is located demonstrated the 95% confidence interval in each study. The diamond symbol represents the odds ratio for all studies put together.

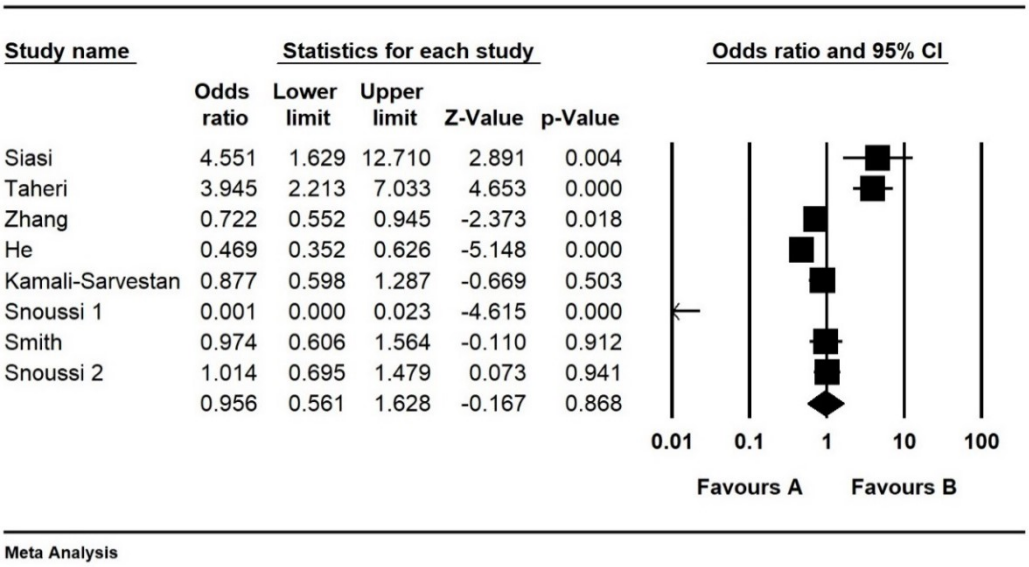

Figure 7: The odds ratios random effect model for TT genotype in patients with breast cancer

Discussion

Cancer is one of the major health threats worldwide, and is one of the leading causes of death and illness in children and adults. Cancer is a term that is refered to a variety of diseases (more than 200 diseases) [29]. Breast cancer is the most common type of cancer, and is the foremost health threat for women today, accounting for about one-third of all cancers in the Western countries. The results of many existing research works suggest that single-nucleotide polymorphisms can increase the susceptibility to cancer by altering the DNA sequence. A number of epidemiological studies have found links between A / T 251 polymorphism in the IL-8 gene and the risk of breast cancer. This polymorphism, which is located in the 251 region of the IL-8 gene promoter, plays an important role in the production of IL-8, and it has been shown that this polymorphism in different populations is associated with tumor formation and metastasis in various cancers [17-15]. Therefore, this study was conducted to investigate the relationship between A / T 251 polymorphism of IL-8 gene and breast cancer using meta-analysis.

According to our systematic review and the meta-analysis, the odds ratio of AA genotype in patients with breast cancer based on meta-analysis of studies was obtained as 1.42 (1.1 ± 11.82: 95% confidence interval), indicating an increasing effect of the AA genotype with the value of 0.42; the odds ratio of the AT genotype in patients with breast cancer based on a meta-analysis of studies was 1.01 (0.84 ±1.22: 95% confidence interval), indicating an increasing effect of the AT genotype by 0.01; and the odds ratio of TT genotype in patients with breast cancer based on meta-analysis of studies was obtained as 0.95 (1.08 ± 2.04: 95% confidence interval), demonstrating the decreasing effect of TT genotype by 0.05. In other words, with the presence of heterozygous and homozygous genotypes in this polymorphism in the IL-8 gene, the factors for projecting the prognosis of the disease and the potential clinical outcome of breast cancer can be predicted. Given the

prevalence of breast cancer in women and the threat to the patients, identifying IL-8 gene polymorphism as a genetic marker and in preventing the desease and diagnosing patients can be effective in restraining the progression of breast cancer.

Cytokines are small molecular weight regulatory proteins or lipoproteins that play an important role in regulating one's immune function [31]. Such immunity effects can be achieved through stimulation, restricting activation, and proliferation or cell differentiation of the target cells. Different types of cytokines play different roles in the start or the spread of cancer and can, on one hand, pave the way for the occurrence and even proliferation and metastasis of cancer, yet on the other hand, prevent the development of cancer through their anti-inflammatory and anti-tumor effects.

Research works in recent years have shown the role of interleukins in breast cancer, where high levels of inflammatory cytokines are found in the serum and tumor tissue of breast cancer patients. The high levels of some of these cytokines in the serum of cancer patients are associated with the progression of the disease stage, the invasion of cancer cells and the development of metastasis [25-28]. Research have also shown that the progression and development of several types of breast cancer is associated with inflammation and the irregular and improper production of chemokines, especially IL-8, which appears to be a key step in metastasis of the cancer cells [28]. Previous research works has demonstrated that IL-8 controls the activating apoptotic pathways. Therefore, decreased expression of the IL-8 gene by tumor cells can stimulate chemokine receptors and thus impair the function of neutrophils and macrophages in suppressing inflammation [32]. It also alters IL-8 gene expression due to the presence of polymorphism in the gene's promoter area, which causes angiogenesis, resulting in metastasis and tumor malignancies, especially in breast cells, which can be directly linked to breast cancer [15]. IL-8 is one of the most important regulatory cytokines that has a central function in initiating and regulating cellular immune responses. IL-8 is expressed in macrophages and fibroblasts derived from intercellular cells and is known as a mediator derived from macrophages that play a role in angiogenesis. This cytokine is a chemotactic factor that can activate white blood cells [33]. The role of this interleukin has also been demonstrated in a range of diseases such as psoriasis, rheumatoid arthritis, and ribosomal fibrosis. Numerous studies have demonstrated that IL-8 is an activating factor for angiogenesis, and can directly or indirectly cause tumor cell proliferation and the formation of new blood vessels through the superficial receptor of tumor cells in endothelial cells, and this can ultimately result in tumor's growth and metastasis [34].

IL-8 is produced by breast cancer cells. It aids breast cancer cells in the Epithelial Mesenchymal Transition (EMT) phenotype, which stimulates, augments and enhances angiogenesis. IL-8 increases the proliferation and survival of endothelial cells and breast cancer, and enhances the migration of breast cancer cells, endothelial cells, and penetrating neutrophils at the tumor site. Thus, IL-8 expression is associated with angiogenesis, tumor formation, and breast cancer metastasis. In addition, IL-8 resultsin the resistance to chemotherapy in breast cancer cells (please see Figure 8).

Huang et al. (2011) investigated genetic variation in the IL-8 gene in relation to the risk of development and prognosis of breast cancer. In the study, they studied 308 patients with breast cancer and 236 healthy people. The results of the study showed that polymorphism in the IL-8 gene promoter is a risk factor for breast cancer. Different types of cytokines play different roles at the start or during the spread of cancer and can, on one hand, pave the way for the occurrence and even propagation and metastasis of cancer, and on the other hand, prevent the development of cancer through anti-inflammatory and anti-tumor effects [35]. Numerous studies have shown an association between IL-8 gene polymorphism and human diseases, all of which focus on the role of A / T 251 polymorphism in the IL-8 gene at the top of the transcription site. It has also been suggested that the identifying the presence of this polymorphism may also be useful in the prognosis of colorectal, prostate and gastric cancers [36].

One of the limitations of this research work is that some samples were not based on random selection. Moreover, many articles were not selected due to the lack of uniform reporting in articles, the non-uniformity of the implementation methods, the unavailability of the full text of the articles, and articles presented in the conferences.

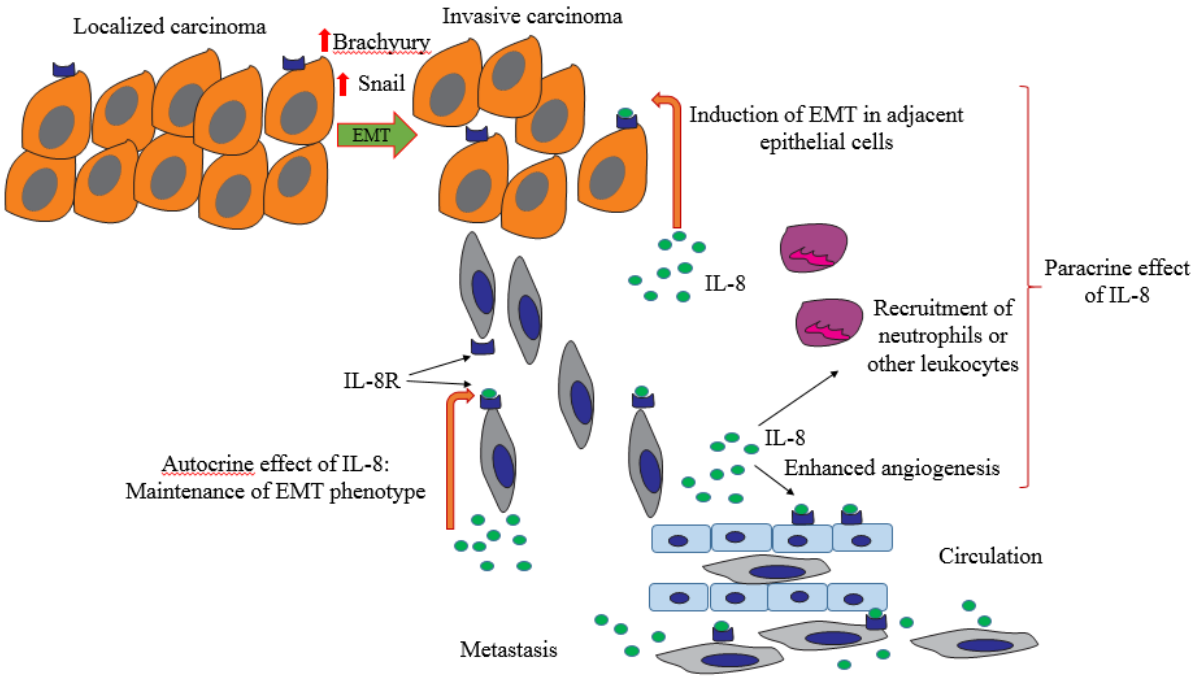

Figure 8: The relationship between IL-8 and breast cancer

Conclusion

Considering the findings of this meta-analysis, allele A has a significant relationship with breast cancer, which can predict the progression course of the disease and the potential clinical outcome of breast cancer. Identifying polymorphism A / T 251 from the IL-8 gene, as a genetic marker and predictor, can also be effective in treating breast cancer.

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