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RESEARCH ARTICLE

Higher circulating levels of chemokine CCL22 in patients with breast cancer: evaluation of the influences of tumor stage and chemokine gene polymorphism

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Abstract The receptor for CCL22 is named CCR4 that preferentially is expressed on the regulatory T cells (Treg), and accordingly, CCL22 acts as a chemoattractant for the intratumoral Treg migration. The aim of this study was to evaluate the serum CCL22 levels and a single nucleotide polymorphism (SNP) in chemokine gene, [2030 G/C (rs223818)], in patients with breast cancer. Blood samples were collected from 100 women with breast cancer before receiving chemotherapy, radiotherapy, or immunotherapy and 100 age-matched healthy women as a control group. The serum CCL22 levels were measured by ELISA. The DNA extracted and the SNP rs223818 determined by amplification refractory mutation system–polymerase chain reaction (ARMS–PCR) technique. The mean serum CCL22 levels in patients with breast cancer (2398.5±123 Pg/mL) was significantly higher in comparison to healthy control group (974.2± 39.9 Pg/mL; $P<0.001$). According to the tumor stages, the mean serum levels of CCL22 were 999.8±85.0 Pg/mL in stage I, 1718.8±82.3 Pg/mL in stage II, 2846.8±118.0 Pg/mL in stage III, and 3954.5±245.2 Pg/mL in stage IV. There was significant difference between tumor stages regarding the serum CCL22 levels ($P<0.001$). In patients with breast cancer, the frequencies of CC genotype (63 %) and C allele (79 %) at rs223818 were significantly higher as compared to healthy controls (31 and 52 %, respectively; $P<0.001$). In both patients and control groups, the mean serum levels of CCL22 in subjects with CC genotype or C allele at rs223818 were also significantly higher as compared to subjects with GG genotype or G allele ($P<0.001$). Higher serum CCL22 levels were observed in patients with breast cancer that is increased with advanced stages. These findings represent that the CCL22 may contribute in tumor development. The CC genotype and C allele at rs223818 were more frequent in breast cancer patients. The serum CCL22 levels were affected by genetic variations at SNP rs223818. Accordingly, SNP rs223818 may play a role in the susceptibility to breast cancer.

Keywords Chemokine · CCL22 · Breast cancer · Gene polymorphism · rs223818

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Introduction

Breast cancer is one of the most important health problems worldwide, with more incidence rates in women with age ≥40 years [1]. In 2013, breast cancer has accounted for 29 % of all new cancer cases and 14 % of the total cancer-related deaths among women, worldwide [2]. One of the main functions of the immune system is defense against tumors, and it

has been reported that the breast cancer risk is associated with impaired immune responses [3]. The modulation of both innate and adaptive immune parameters such as B cells, T cells, macrophages, dendritic cells (DC), and natural killer (NK) cells is important for the initiation and progression of breast cancer [4, 5].

The helper T (Th) cell responses, especially, exert a prominent role in the immune responses to tumor antigens. The Th cells were divided into several subsets including Th1, Th2, and regulatory T (Treg) cells which release different cytokines [6]. The Th1-related cytokines such as interferon gamma (IFN-γ) and TNF-α increase immune responses to tumor cells, leading to tumor regression, whereas the Th2-related cytokines (such as interleukin (IL)-4 and IL-10) and Treg-related cytokines (TGF-β and IL-10) reduce the immune responses to tumors [7, 8].

Chemokines belong to a superfamily of small molecules (8–14 kDa) that interact with their cell-surface receptors and induce directional cell migration, particularly the leukocytes, during inflammation. Chemokines can be divided into four subfamilies (CXC, CC, CX3C, or C), depending on the situation of their cysteine residues near the N-terminus, in which X represents any amino acid [9, 10]. Chemokines are produced by many cell types including leukocytes (such as macrophages, dendritic cells, monocytes, and neutrophils), endothelial cells, fibroblasts, epithelial cells, and tumor cells [9].

In addition to their role in the immune system, chemokines and their receptors also played an important role in the tumor initiation and progression [9]. In cancer, chemokines play a crucial role in the trafficking of cells into and out of the tumor microenvironment, regulating the behavior of the tumor [9]. Chemokines induce the directional cell migration of leukocytes into inflammatory sites, and extended inflammation can promote carcinogenesis by providing a desirable microenvironment around the tumor for its development [11]. Chemokines may influence tumorigenesis indirectly by controlling angiogenesis and tumor–leukocyte communication and/or directly by regulating tumor transformation, growth, invasion, and metastasis [9]. However, the roles of chemokines and their receptors in the cancer development are complex because some chemokines promote conditions suitable for tumor growth, while others have antitumor activity [12]. In addition, chemokines can also directly regulate the development, priming, and effector functions of T lymphocytes [13]. In the viewpoint of cancer immunosurveillance, chemokines manage the intratumoral distribution of leukocytes which is crucial for the induction of antitumor immunity [14].

Macrophage-derived chemokine (CCL22) is a member of the CC family of chemokines that is produced by monocyte-derived macrophages and DCs, upon the activation with microbial products or anti-CD40 antibody [15]. Moreover, the CCL22 production is upregulated by Th2-related cytokines, such as IL-4 and IL-5, but is downregulated by Th1-related cytokines, such as IFN-γ [15]. Furthermore, it has been reported that some tumor cells may also produce chemokines, such as CCL22 [16]. The CCL22 interacts with its receptor, CCR4, and is preferentially expressed on Th2- and Treg cells [15]. Accordingly, CCL22 has important effect on suppressing immune responses to microbial infections and tumor cells by its ability to recruit Th2- and Treg cells, thereby enhancing microbial persistence and tumor development [17]. The higher expression of CCL22 has been implicated in some cancers, including colorectal adenocarcinomas [18], lung cancer [19] breast cancer [20], lymphoma [21], and head and neck squamous cell carcinoma [22].

It should be also noted that the genetic variations such as polymorphisms of the genes which are involve in inflammation and immunity, may confer susceptibility to cancers [23]. Indeed, the association of the several inflammatory or immune response-related gene polymorphisms such as CXCL12, IL-18, IL-21, IFN-γ, and TNF-α with breast cancer have been reported in different populations [24–27]. It has been reported that chemokines genetic variations may influence the susceptibility to some infectious diseases and also to a variety of noninfectious diseases such as cancer, autoimmune, and cardiovascular diseases [28]. It has been also indicated that the cytokine synthesis is controlled by genetic factors and certain single nucleotide polymorphisms (SNPs) in the cytokine-related genes that are associated with high or low cytokine production, which in turn may influence the susceptibility to certain diseases [29]. The chemokine CCL22 gene has been located on the chromosome 16q13 with several SNPs in both coding and noncoding sequences that may influence the chemokine expression [30]. Several polymorphisms have been reported in the promoter, intron, and exon regions of the CCL22 gene, and the SNP rs223818 is among of the polymorphisms located within the intron 2 of the chemokine gene [31]. The results of a study have demonstrated an association between SNP rs223818 in the CCL22 gene with susceptibility to atopic dermatitis [31]. It is presumable that this SNP may also have an association with breast cancer disease. Moreover, the CCL22 changes and its gene polymorphisms have not been investigated in breast cancer patients, adequately. Accordingly, this study was conducted to evaluate the serum levels of CCL22 and a single nucleotide polymorphism [2030 G/C (rs223818)] in the chemokine gene in patients with breast cancer to clarify any association.

Materials and methods

Subjects

In a total 100 women, patients (age 45.73±10. 52 years) with breast cancer were selected among patients who referred to the hospitals affiliated to Shiraz Medical School since October

2012 up to August 2013. None of the patients had received chemotherapy, radiotherapy, or immunotherapy before sample collection. Breast cancer was verified by oncologists according to the surgical and pathological reports. The breast cancer staging has been done according to the criteria of the Sixth Edition of the American Joint Committee on Cancer (AJCC) [32]. The distribution of patients according to tumor stages was described as follows: 14 patients having stage I, 29 patients having stage II, 39 patients having stage III, and 18 patients having stage IV. Moreover, 100 healthy women (44.23±10.50 years) were enrolled into the study as a control group. The healthy subjects were recruited among blood donors and interviewed with regard to malignancy, and none of them had any history of malignancy disease. All control subjects were basically healthy, with no acute or chronic illnesses. Indeed, each individual with disease such as history of recurrent infections, asthma, allergy and atopic diseases, any suspected immunological disorders, cigarette smoking, and use of any drugs were all excluded from the study. The other exclusion criteria were surgery and major trauma within 6 months prior to blood collection. This study was evaluated and approved by the Ethical Committee of Kerman University of Medical Sciences. Moreover, patients were recruited if they agreed for blood sampling. A peripheral blood sample (3–5 ml) was obtained from all participants and the sera were separated and stored at −70 °C until analysis.

DNA extraction

The DNA samples were extracted from peripheral blood leukocytes by salting out method as previously described by Miller et al. [33]. The quantity and the purity of extracted DNA for each sample were determined by detecting the optical density at 260 and 280 nm wavelengths using spectrophotometry (Ependorf, Germany). DNA samples were stored at −20 °C until use.

Polymorphism genotyping

The CCL22 gene polymorphism at rs223818 was determined with allelic discrimination by amplification refractory mutation system–polymerase chain reaction (ARMS–PCR) method. The PCR reaction mixture was prepared by addition of the following reagents to a 0.2-ml microcentrifuge tube on ice: 2.5 μl of Taq DNA polymerase buffer (10×), 0.5 μl of MgCl2 (stock concentration 1.5 mM), 0.5 μl of each dNTP [dATP, dCTP, dGTP, and dTTP (stock concentration of 10 mM)], 1 μl of each primer, 1 μl of prepared DNA, and sterile double-distilled water to a final volume of 25 μl. The CCL22 primer sequences were as follows: CCL22 common primer: 5-CTGG AAGAGATGGCTCAGTTCA-3; CCL22 primer G allele (for wild SNP): 5-CTACCTACTGCCATACCCACAATC-3; CCL22 primer C allele (for mutant SNP): 5-CTACCTACTG CCATACCCACAATG-3; internal control primer 1: 5-CCTG TGCCAACTCTCTGCATTC-3; and internal control primer 2: 5-CAAAGACCCAGAAGTGGGATGTG-3. The protocol used for PCR program was as follows: initial denaturation at 95 °C for 5 min and followed by 37 cycles of denaturation at 95 °C for 30 s, annealing at 61 °C for 30 s, and extension at 72 °C for 40 s. Final extension at 72 °C for 10 min was done to complete the PCR. The amplified PCR product of CCL22 gene covers rs223818 with a molecular size of 169 bp. For internal control, the amplified PCR produce a product with molecular size of 340 bp. The amplified products were monitored by electrophoresis on a 2 % agarose gel containing ethidium bromide (0.5 mg/ml) (Fig. 1) [34].

Measurement of the serum levels of CCL22 and CXCL10

The serum concentrations of CCL22 and CXCL10 were measured by using commercial ELISA kits (R&D Systems, Minneapolis, USA) according to the manufacturer guidelines. The serum levels of chemokine were quantitated by using standard samples with known concentrations of chemokine and expressed as picogram per milliliter, provided by the manufacturer.

Statistical analysis

Hardy–Weinberg equilibrium was assessed using genotype data. Differences in variables were analyzed using Student *t*, ANOVA, and χ^2 tests as appropriate, and *P* values of less than 0.05 were considered significant. The data were analyzed by SPSS statistical software (version 15, Chicago, IL, USA).

Results

Serum levels of chemokine CCL22

The mean serum levels of CCL22 in patients with breast cancer and healthy women demonstrated in Fig. 2. The mean serum levels of CCL22 in patients with breast cancer (2398.5±123 Pg/mL) was significantly higher than that in healthy control group (974.2±39.9 Pg/mL; $P<0.001$). In patients, the mean serum levels of CCL22 was 999.8±85.0 Pg/mL in stage I, 1718.8±82.3 Pg/mL in stage II, 2846.8±118.0 Pg/mL in stage III, and 3954.5±245.2 Pg/mL in stage IV of disease. The serum levels of CCL22 increased with advancing of tumor stages so that there was significant difference between cancer stages regarding the mean serum levels of CCL22 ($P<0.001$) (Fig. 3). The mean serum levels of CCL22 in patients having stage II, stage III, and stage IV were significantly higher as compared with healthy women ($P<0.001$, $P<0.0001$, and $P<0.0001$, respectively). No significant difference regarding the mean serum levels of CCL22 was observed between patients having stage I and

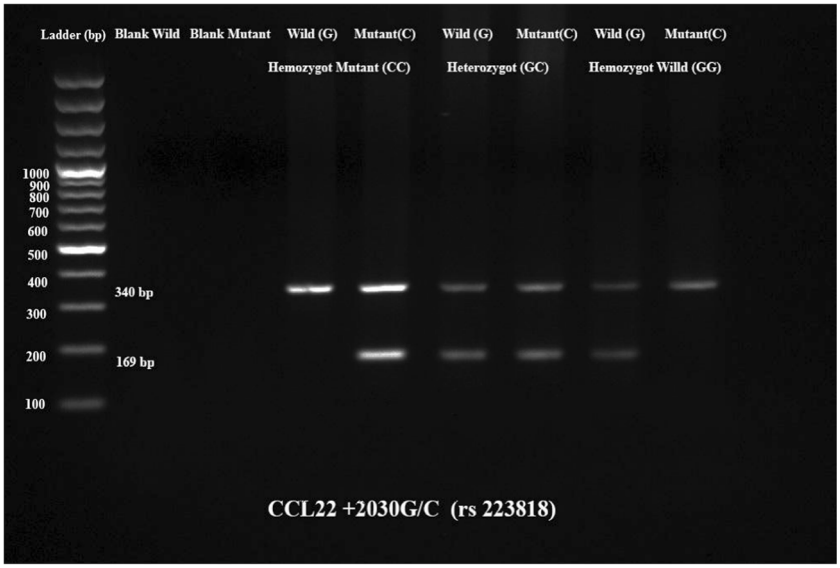

Fig. 1 Amplification refractory mutation system–polymerase chain reaction (ARMS–PCR) of genetic variation at SNP rs223818 in the CCL22 gene. The *first column* shows ladder pattern. The *2 and 3 columns* are blanks. The *4 and 5 columns* represent CC homozygous, *6 and 7 columns* represent GC heterozygous, and *8 and 9 columns* represent GG homozygous. The *first row* shows internal controls gene and *second row* indicates the type of gene polymorphisms based on primer extension

healthy group. Statistical comparison of the serum CCL22 levels between patients with breast cancer, according to their stages, has been summarized in Table 1.

The CCL22 gene polymorphism in breast cancer and control groups

The frequencies of genotypes and alleles in patients with breast cancer and healthy control group at rs223818 have been demonstrated in Table 2. Statistical analysis showed no deviation of genotype frequencies from Hardy–Weinberg equilibrium, neither in the patients nor in the controls. The frequencies of GG, GC, and CC genotypes were 5, 32, and 63 % among patients and 27, 42, and 31 % among healthy control group, respectively. Statistical analyses showed that the frequency CC genotype was significantly higher in patients as compared with healthy subjects ($P<0.001$). On the other hand, the frequencies of GG and GC alleles were significantly higher in healthy control group in comparison to breast cancer patients ($P<0.001$ and $P<0.02$, respectively). Multivariate logistic regression analysis demonstrated that individuals with the CC genotype at rs223818 had a >11-fold increased risk for developing breast carcinoma as compared with individuals with the GG genotype. The heterozygous GC genotype had also a >4.1-fold increased risk for developing breast carcinoma (Table 2).

Moreover, the frequency of C allele was significantly higher, whereas the frequency of G allele was significantly lower in patients than healthy subjects ($P<0.001$). Multivariate logistic regression analysis demonstrated that individuals with the C allele at rs223818 had a >3.7-fold increased risk for developing breast carcinoma in comparison with individuals with the G allele (Table 2).

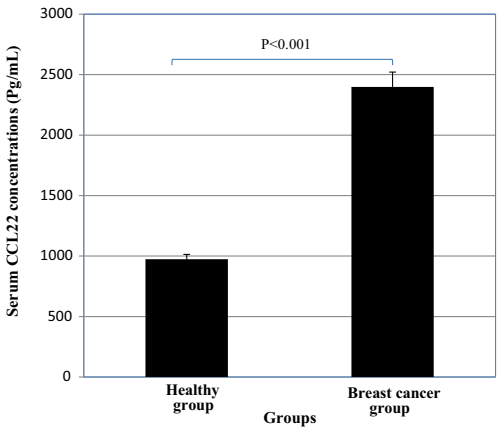

Fig. 2 Comparison of the serum CCL22 levels between patients with breast cancer and healthy group. The mean serum levels of CCL22 in patients with breast cancer was significantly higher than that in healthy group (P<0.001)

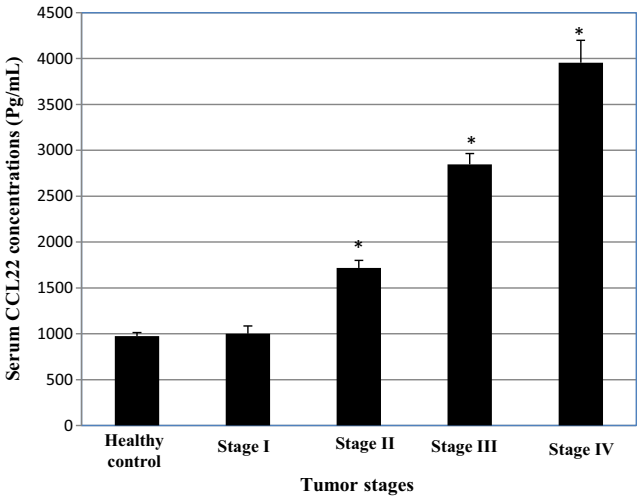

Fig. 3 Comparison of the serum levels of CCL22 in healthy group and patients with different stages of breast cancer. The serum levels of CCL22 increased with advanced stages. The mean serum levels of CCL22 in patients having stage II, stage III and stage IV was significantly higher as compared with healthy women (P<0.001, P<0.0001 and P<0.0001, respectively)

Table 1 Statistical comparison of serum CCL22 levels between patients with breast cancer according to their stages

Tumor stages	Stage I	Stage II	Stage III	Stage IV
Stage I	–	0.001[a]	0.0001	0.0001
Stage II	0.001	–	0.001	0.0001
Stage III	0.0001	0.001	–	0.001
Stage IV	0.0001	0.0001	0.001	–
Healthy control	0.81	0.001	0.0001	0.0001

[a] The symbol represents *P* values

The levels of CCL22 according to chemokine gene polymorphism at rs223818

The serum levels of CCL22 according to chemokine gene polymorphism have been demonstrated in Table 3. In both breast cancer and control groups, the mean serum levels of CCL22 were significantly higher in subjects with CC genotype as compared to subjects with CG or GG genotypes ($P<0.01$ and $P<0.001$, respectively). In both patients and healthy groups, the mean serum levels of CCL22 were also significantly higher in individuals with CG genotype in comparison to individuals with GG genotype ($P<0.05$). Furthermore, the mean serum levels of CCL22 were also significantly higher in subjects with C allele as compared to subjects with G allele at rs223818 ($P<0.001$).

Similar patterns of differences were also observed between levels of CCL22 in total subjects (patients plus healthy controls) according to genetic variations at rs223818 (Table 3). In both healthy and patient groups, the presence of the C allele at rs223818 was associated with higher levels of CCL22 whereas the presence of G allele was related with lower levels of chemokine.

Serum levels of chemokine CXCL10

We have also measured the serum levels of CXCL10 in 50 randomly selected healthy subjects and in 50 randomly selected patients with breast cancer. The mean serum levels of CXCL10 in patients with breast cancer (498.6±24.5 Pg/mL) were significantly higher than that observed in healthy control group (184.9±9.9 Pg/mL; $P<0.001$). We have also calculated the ratio of CCL22/CXCL10 concentration for each subject. We have observed that the mean ratio of CCL22/CXCL10 levels was also significantly higher in patients with breast cancer as compared to healthy subjects (6.45±0.27 vs 5.32±0.33, $P<0.01$).

Discussion

The results of the present study showed that the mean serum levels of chemokine CCL22 in patients with breast cancer was significantly higher than that in healthy women. The serum levels of CCL22 were also increased with advanced tumor stages. These data represent that the elevation of the CCL22 levels may contribute in the breast cancer progression and development. The exact mechanisms which are responsible for the elevation of the CCL22 levels in patients with breast cancer remain to be clarified. CCL22, also known as macrophage-derived chemokine (MDC), was originally found to be released by macrophages and dendritic cells (DC) [15]. The chemokine CCL22 production by macrophages is upregulated by Th2-type cytokines, such as IL-4 and IL-5, whereas is downregulated by Th1-type cytokines, such as IFN-γ [15]. Indeed, higher levels of Th2 cytokines have been reported in the tumor microenvironment and peripheral blood of patients with breast cancer [35] which could be considered as an account for CCL22 changes in these patients. CCL22 is not only produced by different immune cell types but is also expressed by several types of tumor cells [36–38]. It has been demonstrated that a reciprocal communication between tumor cells and immune cells may lead to CCL22 production by tumor cells. The NK cells, in particular, may have an important role in CCL22 production by tumor cells. Tumor-infiltrating NK cells detect tumor cells and secrete IFN-γ. IFN-γ promotes macrophage activation that will produce TNF-α and IL-1β after interaction with breast tumor cells. Finally, the combined action of IFN-γ, IL-1β, and TNF-α will induce strong CCL22 secretion by tumor cells that will induce the recruitment of $CCR4^+$ Treg from periphery [38, 39]. Other

Table 2 The frequencies of the genotypes and alleles at rs223818 in patients with breast cancer and control group

Genetic variations		Patient no. (%)	Control no. (%)	*P* value	Odds ratio (OR)	95 % confidence interval (CI) for OR
Genotypes	GG	5 (5 %)	27 (27 %)	0.001	1.00 (Ref)	
	GC	32 (32 %)	42 (42 %)	0.02	4.11	1.43–11.87
	CC	63 (63 %)	31 (31 %)	0.001	11.00	3.85–31.26
Alleles	G	42 (21 %)	96 (48 %)	0.001	1.00 (Ref)	
	C	158 (79 %)	104 (52 %)	0.001	3.47	2.24–5.39

Table 3 Serum levels of CCL22 in breast cancer and healthy groups according to the genotypes of chemokine gene polymorphisms at rs223818

Groups	Genotype/allele	CCL20 levels[a] (mean±SEM)	*P* value
Patients	GG	764.95±64.53	0.001
	GC	1340.12±75.36	
	CC	2977.46±124.24	
	G	1200.68±72.25	0.001
	C	2682.98±90.44	
Healthy	GG	584.60±27.30	0.001
	GC	929.35±22.94	
	CC	1374.53±74.71	
	G	735.43±22.84	0.001
	C	1194.75±38.95	
Total	GG	607.8±27.2	0.001
	GC	1082.6±39.7	
	CC	2412.8±117.7	
	G	854.45±30.7	0.001
	C	2046.04±72.0	

[a] The serum levels of chemokine expressed as picogram per milliliter

investigation on a murine model of lung cancer has confirmed that the CCL22 production by tumor cells is a NK cell-dependent process [40].

Interestingly, the results of the present study also showed that the serum levels of chemokine CCL22 increase with advancing of tumor stages. The reason for these observations remains to explain. These results suggest that the elevated levels of chemokine CCL22 may contribute in the progression of breast cancer. A number of studies have indicated that CCL22 secretion by tumor cells is responsible for intratumoral accumulation of Tregs in ovarian cancer [41], prostate cancer [42], gastric cancer [43], esophageal carcinoma [44], and breast carcinoma [45]. However, it may be concluded that the CCL22 production by tumors cells and/or tumor-infiltrating macrophages induces the intratumoral accumulation of Tregs. The Tregs expand in response to tumor antigens and produce cytokines, such as TGF-β or IL-10, which are presumably responsible for the suppression of antitumor immune responses and for tumor escape [46].

Apart its function in leukocyte recruitment, CCL22 has been reported, in a murine model, to reduce the antigen-specific proliferation of $CD4^+$ T cells. Accordingly, high CCL22 production within the tumor environment may directly inhibit the specific responses of $CD4^+$ T cell to tumor-associated antigens [39, 47]. Collectively, higher levels of CCL22 (which observed in higher tumor stages) may directly (via the inhibition of leukocytes responses) and/or indirectly (via the accumulation of Treg into tumor environments) lead to more immunosuppression and in turn more tumor development. Indeed, higher regulatory T cells have been observed in breast cancer, especially in the stage IV cancer patients that were associated with lower immune function [48]. The results of the other studies have also demonstrated an association between the increased number of intratumoral accumulation of Tregs and tumor stages which are in consistence with our findings [49, 50].

The results of the present study also showed that the serum levels of CXCL10 were significantly higher in patients with breast cancer as compared to healthy control group. CXCL10 also called interferon-γ-inducible protein 10 (IP-10) is released from neutrophils, eosinophils, monocytes, keratinocytes, epithelial, and endothelial cells in response to IFN-γ [51, 52]. CXCL10 binds to its receptor, CXCR3, which is mainly express on Th1 cells. Therefore, CXCL10 contributes to the selective recruitment of Th1 cells into the intratumoral and inflammatory sites [51, 53]. The tumor-specific Th1 cells play an important role in antitumor-related immune responses, in part through the secretion of IFN-γ. IFN-γ induces the tumoricidal activity of tumor-infiltrating macrophages and triggers the macrophages to secrete the angiostatic chemokines such as CXCL10 which may stop tumor progression by inhibiting angiogenesis [54]. Treatment with recombinant CXCL10 was also shown to delay tumor growth in mice by inducing vascular damage and tumor necrosis [54, 55]. The tumor-infiltrating macrophage-derived CXCL10 selectively attracts Th1 cells and may thus function as a positive feedback loop in the Th1-related antitumor immune response [54]. It has also demonstrated a relation between intratumoral expression of CXCL10 and tumor infiltration by Th1, $CD8^+$ T, and NK cells which induce cancer cell killing and tumor regression [56]. Thus, the higher expression levels of CXCL10 have been associated with higher density of Th1 cells in tumors that may contribute in the protection through the induction of a Th1-relate immune response [56]. Accordingly, the higher levels of Th1-related chemokines such as CXCL10 have been related with better outcome in malignant diseases [53, 57]. Although, we have observed higher serum levels of CXCL10 in patients with breast cancer, but the mean ratio of CCL22/CXCL10 levels was significantly higher in patients with breast cancer as compared to healthy subjects. These observations represent an imbalance in the Th1/Th2 and Th1/Treg-associated immune responses with a tendency toward the Th2 and Treg responses that may contribute in the tumor development and progression.

The results of the present study also showed that the frequencies of the CC genotype and C allele at rs223818 were significantly higher in patients with breast cancer as compared with healthy subjects. Accordingly, the presence of the CC genotype or C allele at rs223818 may confer a susceptibility to breast cancer. There is no previous study regarding the association of rs223818 in the CCL22 with cancers. However, the mechanism(s) by which genetic variations at rs223818 may

influence the susceptibility to breast cancer remain to be clear in further studies. The SNP rs223818 may influence the breast cancer development through its regulatory effects on the CCL22 production. Indeed, the results of the present study showed that the serum levels of CCL22 were significantly higher in subjects with CC genotype or C alleles as compared to subjects with GG genotype or G allele. Therefore, the presence of the C allele at SNP rs223818 may contribute in the susceptibility to breast cancer through the upregulation of the CCL22 production and induction of the prominent Th2- and/or Treg-related responses in the tumor microenvironment.

Several polymorphisms have been reported in the promoter, intron, and exon regions of the CCL22 gene [31]. Introns may possess functional polymorphisms that can influence the expression of the genes that host them. Some of the intronic functional polymorphisms may directly confer susceptibility to disease through a genotype–phenotype relationship. A direct genotype–phenotype relationship may be characterize solely according to the observed association between a specific allele and a related serum protein level, enzymatic activity, or clinical/laboratory finding. In the majority of situation, however, it is unclear whether such polymorphisms directly perform the functional effects or being in linkage disequilibrium with another functional SNP in their vicinity. The SNP rs223818 is among of the polymorphisms that are located within the intron 2 of the human CCL22 gene [31]. Whether the SNP rs223818 is itself a functional polymorphism that exerts a direct effect on CCL22 gene expression or whether it is in linkage disequilibrium with another functional SNP remains to be clear.

However, the SNP rs223818 may influence the CCL22 expression through the effects on the chemokine gene promoter activity, enhancer activity, transcriptional process, and the splicing efficiency. It has also been reported that the introns may react with functional elements, including intron splice enhancers and silencers that regulate splicing [58]. The presence of the C allele at SNP rs223818 may be functional and lead to enhance CCL22 expression through the decreasing of the binding of transcriptional repressor and therefore increasing the binding affinity of transcription factors. On the other hand, the presence of the G allele at SNP rs223818 may lead to the reduction of CCL22 expression through inducing the loss of the binding of some transcription factors to the chemokine gene promoter. Accordingly, the presence of G allele at SNP rs223818 may lead to the markedly reduction of CCL22 expression and therefore cause to the reduced Th2 and/or Treg cells activity in the tumor microenvironment.

Probably, an unidentified nuclear factor (s) may also interact with the genomic region at intron 2 of CCL22 (consisting the SNP rs223818). This factor might have an allele-specific effect on the chemokine expression through changing the affinity for a transcription factor. However, SNP rs223818 may directly or indirectly alter the level of the CCL22 protein expression in tumor microenvironment and contribute in tumor development.

In conclusion, the results of the present study showed higher serum levels of CCL22 in patients with breast cancer. The levels of CCL22 also increased with advanced stages which represent that the chemokine may contribute in the tumor development. The CC genotype and C allele at rs223818 were more frequent in breast cancer patients. The serum levels of CCL22 were affected by genetic variations in SNP rs223818. Accordingly, the SNP rs223818 may play a role in the susceptibility to breast cancer.

Conflicts of interest None

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