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Decreased Serum Level of Interleukin-19 in Iranian Patients with Breast Cancer

Fereshteh Mehdipour[*,], Mahyar Malekzadeh[*], Abdolrasoul Talei[***], Abbas Ghaderi[*,**♦]**

**Shiraz Institute for Cancer Research, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran*
***Department of Immunology, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran*
****Department of Surgery, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran*

Abstract

Background: Interleukin-19, a member of the interleukin-10 family of cytokines, contributes to breast cancer pathogenesis. High interleukin-19 expression in breast tumor tissues is associated with poor clinical outcome. This study aimed to assess the changes in serum level of interleukin-19 in breast cancer patients in comparison with normal women and its association with the clinicopathological parameters of this disease.

Methods: Enzyme-linked immunosorbent assay was used to analyze serum levels of interleukin-19 in 116 women with breast cancer before chemotherapy or radiotherapy, and in 60 healthy age-matched women without any acute or chronic diseases or family history of cancer.

Results: There were significantly lower serum interleukin-19 levels in breast cancer patients (median: 27.3 pg/ml; range: 10.5-2443.6 pg/ml) compared to healthy controls (median: 35.1 pg/ml; range: 10.9-13676.6 pg/ml; $P<0.01$). Compared to the healthy control group, the decrease in serum interleukin-19 concentration was seen in all breast cancer stages. However the decrease was only significant for stage III ($P=0.02$). We found no significant association between serum interleukin-19 levels and stage, grade, lymph node involvement or other clinicopathological variables of the disease. However, when compared to the healthy control group, we found significantly decreased serum interleukin-19 levels in patients with involved lymph nodes ($P<0.01$) or tumor size greater than 2 cm ($P=0.01$).

Conclusion: There were significantly decreased interleukin-19 levels in breast cancer patients compared to the healthy control group. We observed no association between serum interleukin-19 levels and clinicopathological parameters in breast cancer patients.

Keywords: Interleukin-19, Serum level, Breast cancer

♦Corresponding Author:
Abbas Ghaderi, PhD
Shiraz Institute for Cancer Research, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran
Tel: +98-71-32303687
Fax: +98-71-32304952
Email: ghaderia@sums.ac.ir

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Introduction

Breast cancer as one of the most fatal cancers that affect women, is a global health problem. Despite tremendous research in the past years, the cause of breast cancer has largely remained undetermined. Inflammation is recognized to contribute to breast cancer pathogenesis.[1,2] Numerous studies have focused on the role of cytokines, as important mediators of inflammation, on breast cancer development and progression. Depending on their direct effects on tumor cells or their role in angiogenesis or cellular immunity, cytokines can both stimulate or inhibit breast cancer growth and invasion.[3,4] Changes in the levels of different cytokines in sera or tumor tissues of breast cancer patients correlate with stage, metastasis or other clinicopathological parameters of the disease.[3,5-9]

Interleukin (IL)-19 is a member of the IL-10 family of cytokines which includes IL-10, IL-19, IL-20, IL-22, IL-24 and IL-26. This cytokine is produced by a variety of immune and non-immune cells such as monocytes, macrophages, B cells, endothelial and epithelial cells.[10] IL-19 exerts its biological effects through the IL-20R1/IL-20R2 receptor complex.[11] IL-19 has been designated in both categories of pro- and anti-inflammatory cytokines. IL-19 was shown to induce TNF-a and IL-6 production by mouse monocytes, while it increased IL-10 and decreased TNF-a in human PBMCs.[12,13] IL-19 was reported to be an anti-inflammatory factor in inflammatory bowel disease[14,15] and involved in post-cardiopulmonary bypass immunosuppression;[16] on the other hand, it was shown to have a role in tissue injury in endotoxic shock.[17] IL-19 was shown to stimulate Th2 cytokine production and contribute to the pathogenesis of asthma.[18]

Several studies have implied an involvement of IL-19 in physiologic or pathologic cell proliferation. IL-19 was suggested to increase keratinocyte proliferation in the process of wound healing and psoriasis via induction of keratinocyte growth factor (KGF) in skin fibroblasts and CD8+T cells, respectively.[19,20] It may also have a role in the formation of synovial hyperplasia.[21]

Numerous carcinoma tissues such as squamous cell carcinoma (SCC), renal cell carcinoma and infiltrating ductal carcinoma (IDC) of the breast

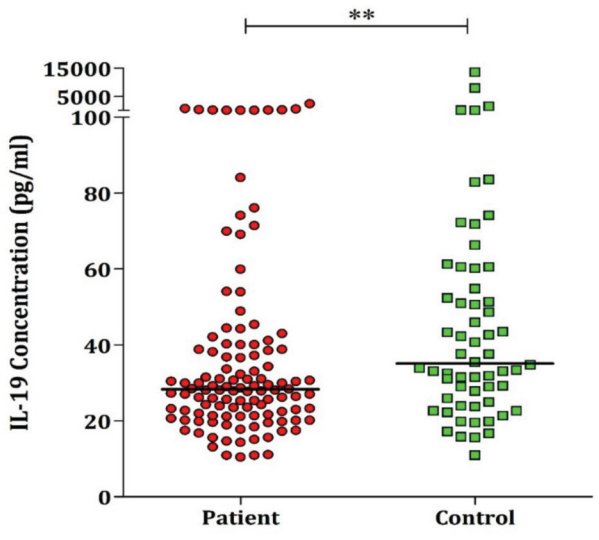

Figure 1. Serum IL-19 level was measured by ELISA and compared in breast cancer patients (n=116) and controls (n=60). Serum level of IL-19 was significantly lower in breast cancer patients (median, 27.3pg/ml; range, 10.5-2443.6 pg/ml) than in healthy controls (median, 35.1pg/ml; range, 10.91-13676.6pg/ml) (P<0.01). Statistical analysis was done using the Mann-Whitney U-test. Medians are shown by horizontal bars.

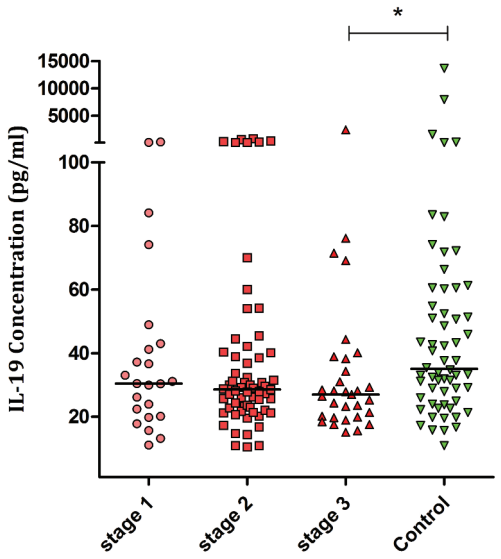

Figure 2. The level of IL-19 in sera of breast cancer patients in each stage was compared with normal group. Compared to normal group (n=60, median, 35.1pg/ml; range, 10.9 -13676.6pg/ml) the decrease in serum IL-19 concentration was only significant in stage III (n=31, median, 27; range, 15.1-2443.6) (P=0. 02). Statistical analysis was performed using the Kruskal-Wallis test. Medians are shown by horizontal bars.

express IL-19.[22] In breast and esophageal cancer, high IL-19 expression in tumor tissues have been associated with advanced tumor stage, high tumor metastasis and poor clinical outcome.[23,24] Oral, breast or esophageal cancer cell lines express IL-19 receptors and IL-19 is capable of directly inducing proliferation in these cell lines.[22-24] IL-19 upregulates molecules such as MMPs, CXCR4 and TGF-β in breast or esophageal cancer cells. These molecules are involved in cancer cell migration and metastasis.[23,24] On the other hand anti-IL-19 monoclonal antibody has been shown to suppress esophageal tumor growth in SCID mice.[24]

In studies that have investigated the role of IL-19 in cancers, including breast cancer, the main focus was on its expression in tumor tissues. Few, if any, data exist in terms of the changes in IL-19 serum level in cancer patients. This study assessed serum levels of IL-19 in breast cancer patients in comparison with normal, healthy women, and examined the association of serum IL-19 levels in breast cancer patients with clinicopathological parameters of this disease.

Materials and Methods

Subjects

This study was approved by the Ethics Committee of Shiraz University of Medical Sciences. All participants were informed about the aims of the study as well as the safety and security measures before their consents were obtained. The patient group included 116 women with breast cancer referred to our laboratory by a collaborating surgeon prior to surgery. None of the patients had any history of chemo- or radiotherapy before sample collection. The control group consisted of 60 age matched females without acute or chronic diseases or family history of cancer. The mean age of the breast cancer patients was 49.3±12.4 years (range: 25-82 years) and the mean age of the controls was 46.5±12.2 years (range: 22-78 years; P=0.16).

Sample collection

We obtained four ml venous blood from each patient and healthy control. Sera were isolated by centrifugation and preserved at-20°C until the day of the experiment. After surgery and pathological evaluation of the tissue specimens, pathological reports that included histological tumor type, tumor size and axillary lymph node (LN) involvement were provided by a collaborating pathologist. Tumor-node-metastasis staging was performed according to the American Joint Committee on Cancer Classification and Stage Grouping 7th edition (cancerstaging.org). Clinicopathological characteristics of the patients are summarized in Table 1.

Enzyme-linked immunosorbent assay (ELISA)

Serum levels of IL-19 were measured using a commercial enzyme-linked immunosorbent assay (ELISA) kit (E91190Hu, Uscn, Life Science, Inc.) according to the manufacturer's instructions. The sensitivity of the assay was 6.1 pg/ml.

Statistical analysis

Data were analyzed using SPSS software (version 11.5, SPSS Inc., IL, USA) and GraphPad Prism 5 was used to prepare the graphs. First normality of data was checked after which non-parametric tests were used for analysis. Mann Whitney rank and Kruskal-Wallis tests were performed to compare IL-19 levels between two and multiple groups, respectively. Comparisons that resulted in P-values <0.05 were considered statistically significant.

Results

Serum IL-19 levels in breast cancer patients compared to normal, healthy women

First we compared the level of IL-19 in sera of patients and normal controls. There was a significantly lower median serum IL-19 level in breast cancer patients (median: 27.3 pg/ml; range: 10.5-2443.6 pg/ml) compared to the healthy control group (median: 35.1 pg/ml; range: 10.9-13676.6 pg/ml; P<0.01; Figure 1).

Table 1. Serum IL-19 level according to clinicopathological parameters of the disease in breast cancer patients.

Parameter	category	Number	IL-19 concentration(pg /ml)	*P* value	Sig.
Stage	I	23	median, 30.4; range, 11.1 -220.6	0.55	ns*
	II	62	median, 28.6; range, 10.5-814.2		
	III	31	median, 27; range, 15.1-2443.6		
Grade	I	23	median, 27.8; range, 14.6-288.8	0.93	ns
	II	53	median, 29.2; range, 10.9-2443.6		
	III	34	median, 27.3; range, 10.5-647.8		
	Unknown	6	range, 23.9-29.9		
Nodal Status	N0	56	median, 30.1; range,10.9-814.2	0.57	ns
	N1	24	median, 26.7; range, 10.5-288.8		
	N2	22	median, 26.7; range, 15.1-2443.6		
	N3	9	median, 27.3; range, 17.5-69.1		
	Nx	5	range, 21.3-40		
Tumor Size	T1	42	median, 29.6; range, 11.1-220.6	0.61	ns
	T2	68	median, 28.2; range, 10.5-2443.6		
	T3	5	median, 24.2; range, 17.5-69.9		
	T4	1	19.9		

Data were analyzed using the Kruskal-Wallis test.; *ns; not statistically significant

Serum IL-19 levels in patients with different breast cancer stages

Our results showed that the level of IL-19 did not significantly differ in sera of patients with different stages of breast cancer. A comparison of IL-19 levels in sera of patients from each breast cancer stage with the healthy control group revealed a decrease in IL-19 level for all disease stages. However the decrease in serum IL-19 concentration was only significant for stage III (median: 27; range: 15.1-2443.6 pg/ml; P=0.02; Figure 2).

Serum IL-19 level in patients with different tumor sizes or lymph node (LN) status

Interleukin-19 serum levels did not significantly differ in breast cancer patients regarding tumor size (T1-T3). We divided the patients into two groups according to their tumor size: those with tumor size ≤2 cm in greatest dimension (categorized as T1 in TNM staging) and those with tumor size >2 cm (categorized as T2, T3 and T4). Next, serum IL-19 levels were compared in patients with tumor sizes ≤2 cm and >2cm to healthy controls. The median IL-19 concentration significantly decreased in patients with tumor sizes >2 cm (P=0.01). In the same manner, IL-19 concentration did not significantly differ in patients with or without LN involvement (LN+ vs. LN-) or those with different numbers of involved LNs (N1-N3). However a comparison of IL-19 serum levels in healthy individuals to patients who were LN+ or LN- revealed that IL-19 levels significantly decreased in sera of LN+ (P<0.01) but not LN- patients.

Level of IL-19 in sera of patients with breast cancer according to other clinicopathological disease characteristics

We compared serum IL-19 levels in breast cancer patients in different age groups (<40, 40-49, 50-59 and ≥60 years).There was no significant difference in the level of this cytokine between these age groups. Serum IL-19 levels were not significantly different in breast cancer patients with different tumor grades. Of 91 patients with known histological tumor type, 86 were diagnosed with IDC and 5 with medullary carcinoma. Hence it was not feasible to compare serum IL-19 levels in patients according to their tumor type.

Discussion

This study showed a significant decrease in IL-19 concentration in sera of patients with breast cancer compared to healthy age matched women. There was no association between serum IL-19 levels in breast cancer patients and their tumor stage, size, grade or other clinicopathological

characteristics. Our results appeared to contrast a study by Hsing et al. which showed upregulation of IL-19 in breast tumors compared to normal breast tissues and an association of high IL-19 expression in tumor tissues with advanced tumor stage.[23] The expression of IL-19 receptors by breast tumor tissues was not studied, however the receptors were expressed on mouse and human breast cancer cell lines and IL-19 induced proliferation in these cell lines.[23] The best explanation for a contradictory change in IL-19 level in tumor tissues and sera of patients with breast cancer might be the binding of this cytokine to its receptor on the tumor and its utilization by tumor cells. Another finding of this study which could support this hypothesis was a significantly decreased serum level of IL-19 in patients with tumor size >2 cm compared to normal controls. IL-19 also induced migration and metastasis in breast cancer cells.[23]

Lymph node involvement is a sign of regional metastasis and our results have indicated that when compared to the healthy group, IL-19 was significantly reduced in patients with involved LN. It is believed that IL-19 can enhance its own production,[13] hence utilizing of local or systemic IL-19 by tumor cells may induce and increase IL-19 production by breast cancer cells which further augments their proliferation and invasion. It should be mentioned that Hsing et al. did not find a significant difference in serum IL-19 levels in breast cancer patients with high or low IL-19 expressions in their tumors.[23] This finding has further supported that changes in serum IL-19 may not mimic its changes in the tumor tissue.

Another explanation for decreased IL-19 concentrations in sera of patients with breast cancer can be the recruitment of a part of IL-19 systemic sources to the tumor site, so whether IL-19 producing monocytes and B cells are recruited from the periphery to the tumor site is another possibility worth evaluating.

Psoriasis and ovarian endometrioma are two other conditions in which decreased serum IL-19 levels have been reported.[20,25] As with breast cancer, chronic inflammation and abnormal proliferation are the hallmarks of these diseases. In the case of psoriasis, IL-19 transcripts, proteins and its receptors upregulate in psoriatic skin.[20,26] In ovarian endometrioma the reduced serum IL-19 level has a correlation with extensiveness of the disease.[25] Decreased serum IL-19 levels were observed in rheumatoid arthritis (RA) patients.[27] Synovial tissues of RA patients expressed IL-19 and its receptors; IL-19 could reduce apoptosis in RA synovial cells via STAT3 activation and contribute to synovial hyperplasia.[21] Collectively, results of these studies may imply that decreased serum IL-19 concentration may be a sign of deregulated epithelial proliferation in the body.

As mentioned, in addition to breast cancer, increased expression of IL-19 has been shown in other tumor tissues such as SCC of the oral cavity, esophagus and lung.[22] Interleukin-19, as with the breast cancer cell line, promoted proliferation and migration of an esophageal SCC cell line.[24] Despite these reports about the expression of IL-19 in tumor tissues and the potential importance of this cytokine in tumor metastasis, there was no study about the systemic changes of IL-19 in patients with these cancers. As a result, evaluation of serum IL-19 level in sera of patients affected with these cancers in comparison with healthy individuals might support the assessment of the possibility of reduced serum IL-19 levels in cases of abnormal epithelial proliferation.

Taken together, this study showed significantly lower median IL-19 concentration in sera of breast cancer patients than in healthy women. We could not find any significant association between serum IL-19 levels and clinicopathological parameters of the disease among breast cancer patients. However compared to normal controls, IL-19 significantly decreased in patients with involved LN or larger tumor sizes. Evaluation of IL-19 receptor expression in breast cancer tissues together with extending the study with larger numbers of cases, and designing the same set of experiments for other carcinomas could help researchers to understand, at least in part, the significance of IL-19 in the cancer setting.

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Conflict of interest

No conflict of interest is declared.

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