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Comparison of Interleukin-18 Serum Levels in Patients with Breast Cancer and Idiopathic Granulomatous Mastitis

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Abstract

Background: Breast cancer (BC), as a global challenge, is one of the most prevalent malignant diseases among women. Idiopathic granulomatous mastitis (IGM) is a rare, chronic inflammatory breast disease that primarily affects women of fertility age. IGM mimics symptoms and radiographic patterns of BC. IL-18 plays a dual role in cancer, as it can promote tumor growth or reduce tumor growth. Therefore, this study aimed to compare the serum levels of IL-18 in BC and IGM patients.

Method: This case-control study was conducted on 45 patients with BC, 25 with IGM (I), and 30 healthy individuals (C) with normal screening tests as the control group. The BC group consisted of 25 newly diagnosed BC patients (N), and 20 patients with metastatic BC (M). Specialized pathologists confirmed the histopathological pattern of BC and IGM. Enzyme-linked immunosorbent assay (ELISA) sandwich technique was used for the measurement of IL-18 serum levels. All statistical analyses were performed using SPSS-23, and GraphPad Prism. P <0.05 was considered statistically significant.

Results: The serum level of IL-18 showed statistically significant higher values in the three patient groups than the control group ($P < 0.001$). In addition, the IL-18 levels in the M group were significantly higher than in the N and I groups ($P < 0.01$). There was no statistical significance between N and I groups ($P > 0.05$).

Conclusion: IL-18 levels were significantly elevated in BC compared with the IGM and control groups. IL-18 has a potential role as a prognostic indicator in BC, particularly for patients with metastasis.

Keywords: Breast neoplasms, Granulomatous mastitis, Interleukin-18, Metastasis

Introduction

Breast cancer (BC), as a global challenge, is one of the most prevalent malignant diseases among women aged 20-50 years.[1-2] BC is divided into three major groups based on molecular markers for progesterone (PR) or estrogen receptors (ER) and human epidermal growth factor 2 (Her2-neu) (ERBB2), including; hormone receptor positive/ERBB2 negative (more common), ERBB2 positive, and triple-negative (less common).[3] Contrary to all advanced medical therapies and their progression, BC is acquainted as the second cause of mortality worldwide. Thus, the appropriate method for screening and early detection is more helpful for the successful treatment of BC.[4] Therefore, the use of new diagnostic and prognostic biomarkers can have a great advantage for patients.[5]

Idiopathic granulomatous mastitis (IGM) is a disease that has a clinical manifestation and radiographic pattern similar to BC .[6] IGM is a rare, chronic inflammatory breast disease that primarily affects women of fertility age. The exact cause of IGM is not well understood, which is why it is referred to as "idiopathic." It is characterized by the formation of non-caseating granulomas, which are inflammatory nodules in the breast tissue.[7] These granulomas can cause pain, swelling, abscess and fistula formation in the affected breast which is difficult to distinguish from BC.[8] The gold standard method of diagnosing IGM is through percutaneous needle biopsy and based on histopathology findings.[9] Misidentifying these illnesses can negatively affect patients' lives, leading to: expensive and unnecessary treatments, reduced quality of life, improper medication usage, metastasis in BC patients diagnosed with IGM, and potentially even death as a result of the rising BC mortality rate.[10]

Rudolf Virchow, in the 19th century, declared that leukocytes exist in tumors, and there is a possibility of a relation between cancer and inflammation. The accepted evidence is that inflammation causes tumorigenesis.[11] Meanwhile, cytokines and their receptors are key factors in inflammation caused by cancer, which affect the recruitment cell proliferation, angiogenesis, survival, tumor invasion and metastasis.[12] Some of pro-inflammatory cytokines include TNF-α, IL-1, IL-6, IL-8, IL-10 and IL-18.[12-13] In various pathological conditions, IL-18 has wide biological activities and is remarkably high in infections. The exact role of IL-18 in tumors is still not fully understood and there is room for debate.[14] It has been reported that IL-18 plays a dual role in cancer, as it can promote tumor growth, progression, migration, invasion, and metastasis. On the other hand, it can enhance antitumor immunity and reduce tumor growth.[15-17] Regarding the role of IL-18 in different forms of BC, and the importance of these two-challenging disease, this study was conducted with the aim of comparing serum levels of IL-18 in patients with BC and IGM.

Material and Methods

Study design

The case-control study was confirmed by the Ethics Committee of Jahrom University of Medical Sciences (IR.JUMS.REC.1401.140), and conducted in accordance with the Declaration of Helsinki. This study was carried out from 22 June 2023 to 20 May 2024. Before recruiting volunteers, and after explaining the purpose of the study, a written consent form was signed. A total number of 100 women who were referred to Khatam-Al-Anbia Clinic (Paymaneh Hospital, Jahrom University of Medical Sciences, Jahrom, Iran) participated in the study, including 45 patients with BC, and 25 patients with IGM (I). The BC group consisted of 25 newly diagnosed BC patients (N), and 20 patients with metastatic BC (M). In this study, BC and IGM were histo-

pathologically confirmed (through core needle biopsy) by a specialized pathologist.
In addition, 30 sex- and age-matched healthy individuals who were referred for screening and whose screening results were negative for BC or IGM were included in the control group (C). The participants with an active infection or underlying diseases and those with a history of smoking or drug abuse were excluded from the study.

Data collection

The participants' information was collected using a specific checklist. Demographic data including age, marital status, body mass index (BMI), smoking, and menopausal status, underlying diseases, and cancer history were collected. Clinical and paraclinical variables comprising tumor size, tumor grading, lymph node involvement, metastasis, histology, and immunohistochemistry were also collected from each patient's medical records.

Sample collection and preparation

After obtaining written consent from the volunteers, 8 mL of venous blood samples was taken from their antecubital vein and collected in an anticoagulant-free tubes. The serum samples for IL-18 analysis were then separated by centrifuging clotted blood samples at 1500 RPM for 10 minutes and subsequently stored at -20 °C (preferably -70°C) until the time of testing.

Measurement the serum level of IL-18

Enzyme-linked immunosorbant assay (ELISA) sandwich technique was used for measuring human IL-18 (eBioscience high performance assay, Vienna, Austeria), and compared with the standard graph that had been validated for measuring IL-18 according to the manufacturer's guidelines. Then, the serum levels of IL-18 in BC patients were investigated based on tumor grading, tumor histology type, TNM staging [tumor size (T), lymph node involvement (N), metastasis (M)], and immunohistochemistry markers.

Statistical analysis

All statistical analyses were performed using Statistical Package for Social Sciences version 23 (IBM SPSS Statistics version 24, USA), and GraphPad Prism (version 8.0). Quantitative variables were tested for normality using the Kolmogorov-Smirnov test. Data revealed normal distribution was represented as mean ± standard deviation. The data showed deviation from normal distribution represented as median and range. Variance analysis (ANOVA) was used as the data were normally distributed for comparison between more than two mean values. Qualitative data was expressed using number and percent. Spearman's correlation test was used to investigate the relationship between non-parametric quantitative variables. *P*-value <0.05 was considered statistically significant.

Results

In this study, the mean age of the participants in N, M, I, and C groups was 46.12 ± 9.53, 45.42 ± 10.31, 42.37 ± 10.12, and 44.16 ± 9.83 years, respectively, which was not statistically significant (P = 0.068). The majority of the participants were married, with a history of underlying diseases and a history of cancer in their family. The statistical analysis showed that the BC, IGM and control groups were similar in terms of age (P = 0.58), marital status (P = 0.32), smoking (P = 0.27), underlying diseases (P = 0.88), and cancer history (P = 0.61). Meanwhile, there was a significant difference in terms of menopausal status between the four groups (N, M, I, and C; P = 0.001) (Table 1).

The mean concentration of IL-18 in the N, M, I, and C groups was 213 ± 44, 251 ± 57, 196 ± 50, and 135 ± 38 pg/mL, respectively. The IL-18 levels showed statistically significant higher values in the three patient groups than those of the control group (P < 0.001). In addition, the IL-18 levels in the M group

were significantly higher than in the N and I groups ($P < 0.01$). There was no statistical significance between N and I groups ($P > 0.05$). Statistical analysis of the studied parameters in patients and control groups was shown in table 2 and figure 1.

Correlation analysis

The association between the serum level of IL-18 with the immunohistochemistry markers including ER, PR, Her-2 and Ki-67 in the BC groups are given in table 3. The results show that IL-18 levels were significantly associated with Ki-67 marker in N+M groups ($P = 0.04$).

Spearman correlation analysis was carried out to determine the relationship between the serum levels of IL-18 with tissue-based biomarkers. In BC patients (N+M groups), IL-18 strongly correlated with both tumor grade ($r = 0.73$; $P < 0.001$) and metastasis ($r = 0.76$; $P < 0.001$). IL-18 levels were also significantly associated with tissue-based biomarkers in N+M groups (ER: $r = 0.51$, $P < 0.03$; PR: $r = 0.56$, $P < 0.01$, Her-2: $r = 0.62$, $P < 0.002$, Ki-67: $r = 0.66$, $P < 0.001$), whereas did not correlate in I and C groups (Table 4).

Discussion

Our findings showed that age, smoking, underlying diseases history, and family history of BC were not statistically different between the BC, IGM, and control groups.

A significant difference in menopausal status among the groups warrants further investigation regarding its potential influence on IL-18 levels. Estrogen has been shown to regulate IL-18 production.[18-19] Studies suggest that post-menopausal women may have altered cytokine profiles compared with pre-menopausal women. This hormonal shift could influence IL-18 levels and potentially contribute to the observed variations between the groups. Future studies should explore this interaction in detail, potentially stratifying participants by menopausal status to obtain clearer results.[20]

While our study does not establish a definitive link between IL-18 levels and BC or IGM, it lays the groundwork for further investigation. IL-18 is a pro-inflammatory cytokine with complex roles in the immune system. Some studies suggest that it may play a part in tumorigenesis and inflammation.[21] In BC, elevated IL-18 levels have been observed in some patient groups.[10] For IGM, the role of IL-18 is less clear, although its involvement in inflammatory processes is well-established.[22]

In this study, the significantly elevated IL-18 levels in all patient groups compared with the control group support a potential role for IL-18 in inflammatory processes associated with both BC and IGM. This aligns with previous researches in BC and IGM, highlighting the potential of IL-18 as a marker of inflammation in these diseases.[23-24]

The observation that metastatic BC patients exhibited the highest IL-18 levels is particularly interesting. This finding aligns with the known pro-inflammatory nature of IL-18 and its potential role in tumor growth and metastasis.[25] IL-18's ability to stimulate angiogenesis (blood vessel formation) and promote tumor cell survival could contribute to the aggressive nature of metastatic BC.[26]

While both BC and IGM showed elevated IL-18 compared with the control group, the lack of a significant difference between these patient groups is intriguing. This could indicate that IL-18 plays a similar role in the inflammatory response of both diseases. However, it is also possible that the underlying mechanisms differ. BC is primarily driven by uncontrolled cell proliferation, while IGM involves chronic inflammation within breast tissue.[27]

The strong positive correlation between IL-18 levels and both tumor grade ($r = 0.73$; $P < 0.001$) and metastasis ($r = 0.76$; $P < 0.001$) suggests a potential link between IL-18 and

aggressive BC phenotypes. This aligns with our previous observation of higher IL-18 levels in the metastatic BC group as compared with newly diagnosed patients.[28] These findings support the notion that IL-18 may play a role in promoting tumor progression and metastasis.[16] As discussed earlier, IL-18's ability to induce angiogenesis and enhance tumor cell survival aligns with this possibility.[22]

The observed correlation between IL-18 and aggressive BC features strengthens the case for investigating IL-18 as a potential therapeutic target. It suggests the IL-18's involvement in tumor angiogenesis and suppression of anti-tumor immune responses, both of which contribute to a more aggressive tumor phenotype.[15] Our findings support the notion that IL-18 may be a key player within this inflammatory tumor microenvironment in BC.

The specific mechanisms by which IL-18 contributes to aggressive BC remain to be fully elucidated. However, several possibilities based on existing research are worth exploring. First, IL-18's ability to induce the production of other pro-inflammatory cytokines and chemokines could create a microenvironment that fosters tumor cell proliferation and survival.[29] Second, IL-18 may directly stimulate the growth and invasiveness of BC cells through specific signaling pathways.[17] Third, as mentioned earlier, IL-18's role in angiogenesis could be crucial for supplying tumors with the nutrients they need to grow and metastasize. Further research investigating these potential mechanisms and their relative contributions to IL-18's impact on BC progression is essential.

The significant correlation between IL-18 and established BC biomarkers, including estrogen ER, PR, HER2 status, and Ki-67 proliferations, provides valuable insights into the potential role of IL-18 in BC biology. These biomarkers offer valuable information on a tumor's hormone receptor status, growth rate, and potential for aggressiveness. The observed correlations suggest that IL-18 levels may be linked to these different aspects of BC development. The positive correlation between IL-18, ER and PR suggests a potential link with hormone-driven BC growth. Studies have shown that chronic inflammation can alter estrogen receptor signaling, potentially contributing to BC development.[30] Our findings raise the possibility that IL-18, as a pro-inflammatory cytokine, could be involved in this process.

The association between IL-18 and HER2 positivity, a marker for a more aggressive cancer subtype, aligns with our previous observation of elevated IL-18 in metastatic BC. This finding strengthens the notion that IL-18 may contribute to the aggressive behavior of certain BCs. Preclinical studies suggest that IL-18 can activate signaling pathways involved in HER2-positive BC cell proliferation and metastasis.[31]

This study has some limitations. The sample size was small, and further research with larger cohorts is needed for stronger generalizability of the results. Additionally, the study design was observational, precluding the establishment of cause-and-effect relationships. Future studies employing larger, well-controlled designs with longer follow-up periods could provide a more comprehensive understanding of the interplay between IL-18, BC, and IGM. Furthermore, delving into the biological mechanisms underlying potential associations between IL-18 and these conditions could be crucial for developing targeted therapies.

Conclusion

We found that IL-18 levels were significantly elevated in all BC groups (N, M) compared with the control group. Furthermore, in BC patients, IL-18 levels exhibited strong positive correlations with tumor grade,

metastasis status, and established BC biomarkers (ER, PR, Her-2, and Ki-67). These findings suggest a potential role for IL-18 as a prognostic indicator in BC, particularly for patients with an advanced disease. This finding highlights the need for further investigation into the dynamic changes of IL-18 levels throughout BC progression.

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Authors' Contribution
A.HT, M.H, and F.A: Study design; data acquisition; data analysis and interpretation; drafting and critical reviewing of the manuscript. A.SJ, and MR.H: Study design, and reviewing the manuscript. All authors read and approved the final manuscript version and agree with all parts of the work in ensuring that any queries about the accuracy or integrity of any component of the work are appropriately investigated and handled.

Conflict of Interest
None declared.

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Table 1. Demographic variables in breast cancer patients, IGM, and control groups

		Patients, n (%)			**C**, n (%) (n=30)	*P*-value
		N (n=25)	**M** (n=20)	**I** (n=25)		
Age		46.12±9.53	45.42±10.31	42.37±10.12	44.16±9.83	0.06
BMI	Normal	9 (36.0)	6 (30.0)	5 (20.0)	9 (36.0)	0.35
	Overweight	9 (36.0)	9 (45.0)	13 (52.0)	13 (43.3)	
	Obese	7 (28.0)	5 (25.0)	7 (28.0)	8 (26.7)	
Marital status	Single	2 (08.0)	0 (0.0)	0 (0.0)	1 (03.4)	0.51
	Married	23 (92.0)	20 (100)	25 (100)	29 (96.6)	
Smoking	Yes	3 (12.0)	3 (15.0)	2 (08.0)	4 (13.3)	0.11
	No	22 (88.0)	17 (85.0)	23 (92.0)	26 (86.7)	
Menopause	Yes	5 (20.0)	4 (20.0)	1 (04.0)	2 (06.6)	0.01
	No	20 (80.0)	16 (80.0)	24 (96.0)	28 (93.4)	
BC history	Yes	4 (16.0)	5 (25.0)	2 (08.0)	0 (0.0)	0.04
	No	21 (84.0)	15 (75.0)	23 (92.0)	25 (100)	
Underlying disease[*]	Yes	4 (16.0)	4 (20.0)	3 (12.0)	4 (13.3)	0.88
	No	21 (84.0)	16 (80.0)	22 (88.0)	26 (86.7)	

[*]Cardiovascular diseases, Diabetes, Dyslipidemia, Hypertension, Liver disease, Lung disease, Renal disease; BC: Breast cancer; BMI: Body mass index; IGM (I): Idiopathic granulomatous mastitis; M: Metastatic breast cancer patients; N: Newly diagnosed breast cancer patients; C: Control

Table 2. Characteristics of the breast cancer patients based on the tumor size, tumor grading, lymph node involvement, metastasis, histology, and immunohistochemistry

Characteristics		Breast cancer patients	
		N	M
		N = 25 (%)	N = 20 (%)
Tumor size	T0	0 (0.0)	0 (0.0)
	T1	6 (24.0)	3 (15.0)
	T2	14 (56.0)	12 (60.0)
	T3	5 (20.0)	5 (25.0)
Lymph node			
	N0	5 (20.0)	1 (05.0)
	N1	10 (40.0)	5 (25.0)
	N2	7 (28.0)	11 (55.0)
	N3	3 (12.0)	3 (15.0)
Metastasis			
	M0	19 (76.0)	1 (05.0)
	M1	6 (24.0)	19 (95.0)
Tumor grading			
	G1	7 (28.0)	0
	G2	15 (60.0)	14 (70.0)
	G3	3 (12.0)	6 (30.0)
Histology			
	IDC	17 (68.0)	15 (75.0)
	DCIS	4 (16.0)	1 (05.0)
	ILC	4 (16.0)	2 (10.0)
	Mixed IDC & ILC	0	2 (10.0)
Immunohistochemistry			
	ER		
	Positive	21 (84.0)	15 (75.0)
	Negative	4 (16.0)	5 (25.0)
	PR		
	Positive	20 (84.0)	14 (70.0)
	Negative	4 (16.0)	6 (30.0)
	HER2neu		
	Positive	10 (40.0)	14 (70.0)
	Negative	15 (60.0)	6 (30.0)
	Ki-67		
	<20 %	11 (44.0)	4 (20.0)
	>20 %	14 (56.0)	16 (80.0)

DCIS: Ductal carcinoma in situ; ER: Estrogen receptors; Her2 neu: Human epidermal growth factor 2; IDC: Invasive ductal carcinoma; ILC: Invasive lobular carcinoma; M: Metastatic breast cancer patients; N: Newly diagnosed breast cancer patients; PR: Progesterone receptor; T: Tumor size

Table 3. Association between serum levels of IL-18 with ER, PR, Her-2 and Ki-67 markers in the breast cancer patients

Patients		n	IL-18 Odd ratio (95% CI)	*P*-value
ER	Positive	36	172 (19.83-599.12)	0.79
	Negative	9	215 (35.31-425.03)	
PR	Positive	35	169 (78.40-265.90)	0.89
	Negative	10	201 (35.22- 389.08)	
Her-2	Positive	24	256 (88.25-599.07)	0.21
	Negative	21	192 (19.83-327.25)	
Ki-67	>20 %	15	299 (78.49-625.11)	0.04
	<20 %	30	182 (45.01-380.41)	

ER: Estrogen receptors; Her2 neu: Human epidermal growth factor 2; IL-18: Interleukin-18; PR: Progesterone receptor

Table 4. Spearman correlations between tumor markers with serum levels of IL-18 in breast cancer (N +M) and IGM (I) patients

		IL-18	
		N+M	**I**
ER	p	0.03*	0.09
	r	0.51	0.40
PR	P	0.01*	0.10
	r	0.56	0.39
Her-2	p	0.00*	0.15
	r	0.62	0.33
Ki-67	p	0.00*	0.06
	r	0.66	0.41
Tumor grade	p	0.00*	
	r	0.73	
Metastasis	p	0.00*	
	r	0.76	

ER: Estrogen receptor; Her2 neu: Human epidermal growth factor 2; IGM (I): Idiopathic granulomatous mastitis; IL-18: Interleukin-18; M: Metastatic breast cancer patients; N: Newly diagnosed breast cancer patients; PR: Progesterone receptor; r: Spearman coefficient; *, Statistically significant at $P \leq 0.05$

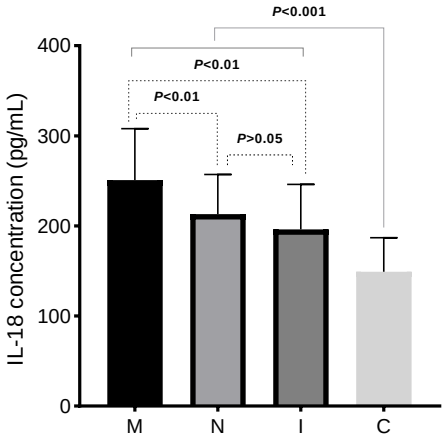

Figure 1. This figure compares IL-18 serum levels in the patients and control groups. There was a significant difference between IL-18 level in patients compared with the control group ($P < 0.001$; by ANCOVA). The IL-18 level in M group were significantly higher than other groups ($P < 0.01$; by ANCOVA).

Results are mean ± SD; C: Control; I: Idiopathic granulomatous mastitis; IL-18: Interleukin-18; M: Metastatic breast cancer patients; N: Newly diagnosed breast cancer patients;