Original Article

Iranian Journal of Immunology

December 2024; 21(4):316-327.

# Blood Cytokine Profile in Breast Cancer: Focusing on Differences among Molecular Subtypes

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## ABSTRACT

**Background:** Breast cancer is the leading cause of cancer-related deaths in women. Cytokines have been linked to various cancers, and both benign and malignant breast diseases are associated with inflammation. However, there is limited understanding of how the immune system's cytokine response varies among different subtypes of breast cancer.

**Objective:** To assess cytokine levels in breast cancer patients according to their subtypes and investigate the potential role of these cytokines in treatment.

**Methods:** Patients with stage 1-2 breast cancer and healthy volunteers were included in the study. The breast cancer patients were classified as luminal A, luminal B, and triple negative based on ER, PR, HER2 receptor status, and Ki67 score of trucut biopsy results. Multiplex assay and flow cytometry were used to quantify the concentrations of IL-1β, IFN-α2, IFN-γ, TNF-α, MCP-1, IL-6, IL-8, IL-10, IL-12p (p70), IL-17A, IL-18, IL-23, and IL-33 in serum samples collected from all participants. Age, menopausal status, and hematologic parameters were also compared between groups.

**Results:** The study involved 19 luminal A, 20 luminal B, 18 triple-negative patients and 21 healthy volunteers. TNF-α, IL-6, IL-8, IL-10, IL-12p (p70), IL-18, and IL-23 cytokines were significantly higher in breast cancer patients than in healthy volunteers. Significant differences in IFN-γ, IL-6, IL-8, IL-10, IL-12p (p70), IL-17A, IL-18, and IL-23 were observed between subtypes, with triple-negative patients showing lower cytokine levels, except for MCP-1.

**Conclusion:** The decreased levels of cytokines in triple-negative breast cancer indicate lower immunogenicity leading to more aggressive tumor progression as a result of an insufficient immune response.

**Keywords:** Breast cancer, Cytokine, Triple negative, Inflammation

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*Cite this article as:*
Demir A, Sevik H, Guler M, Turkoglu F, Cakir C, Sevinc MM, Kinaci E, Idiz UO. Blood Cytokine Profile in Breast Cancer: Focusing on Differences among Molecular Subtypes. *Iran J Immunol.* 2024; 21(4):316-327, doi: 10.22034/iji.2024.103582.2848.

Received: 2024-07-31
Revised: 2024-09-12
Accepted: 2024-09-30

## INTRODUCTION

On a global scale, breast cancer remains one of the most commonly diagnosed forms of cancer, with more than two million new cases reported annually and, is one of the leading causes of cancer-related deaths in women (1). Characterized by its diversity, breast cancer encompasses multiple molecular subtypes, each exhibiting distinct biological and clinical traits. Over the last thirty years, significant progress in targeted and adjuvant therapies has substantially improved the outlook for early-stage breast cancer. However, therapeutic options for advanced metastatic breast cancer remain limited. Median overall survival rates are four to five years for the luminal subtype and only one year for the triple-negative subtype (2).

Cytokines which are small polypeptides or glycoproteins are key regulators of cellular functions like differentiation, proliferation, apoptosis, and survival (3). Upon binding to receptors, they initiate signaling pathways that modify gene transcription and impact biological processes. Imbalances in cytokine profiles are known to drive cancer initiation and progression by promoting chronic inflammation and immune evasion (4). While cytokines such as interferon (IFN)-α and interleukin (IL)-2 have been approved by the Food and Drug Administration (FDA) for various cancers, their clinical use has been largely replaced by more effective and safer immunotherapies like immune checkpoint blockade (ICB) (4, 5). However, interest in cytokines is resurging, particularly when used in combination with other immunotherapies and advancements in drug delivery and protein engineering (6).

Conversely, tumors can exploit certain cytokines to enhance their growth, metastasis, immune evasion, and resistance to treatment. Targeting these pro-tumor cytokines through different strategies, such as neutralizing antibodies and small-molecule inhibitors, shows promise for improving cancer immunotherapy (7). Some antagonists, such as anti-TGF-β and anti-VEGF antibodies, have already demonstrated potential in enhancing ICB and overcoming treatment resistance (4).

Changes in cytokine levels have been observed in various cancers, such as liver and stomach cancers. However, it is still unclear whether serum cytokine levels are associated with tumor stage (8). Both benign and malignant breast diseases have been linked to inflammation. Previous research has suggested that inflammation and proinflammatory cytokines play a crucial role in the development and progression of breast diseases (9). Cytokines such as IL-23, IL-17, IL-33, monocyte chemoattractant protein (MCP)-1, IL-18 along with commonly recognized tumor necrosis factor (TNF)-α, IL-8, IL-1, and IL-6 are found to be elevated in breast cancer patients (10-14). Recent research has shown that IL-10 suppresses the immune system's anti-tumor response and promotes tumor cell growth and proliferation in the tumor microenvironment. Furthermore, levels of IL-10 in the blood are higher in breast cancer patients compared to healthy individuals, with significantly elevated levels in estrogen receptor (ER)-negative breast cancer compared to ER-positive types (15).

The literature contains a few studies that evaluate breast cancer molecular subtypes and blood cytokine levels. These studies have looked at changes in a limited number of cytokines in breast cancer molecular subtypes. In this study, we assessed blood cytokine levels across a wide panel in breast cancer cases with different subtypes and identified potential inflammatory mechanisms associated with each molecular subtype.

## MATERIAL AND METHOD

The study began after approval from the Local Human Clinical Trials Ethics Committee (number: 2364, date: 22.05.2020). Patients showing signs of breast mass and nipple discharge were enrolled between 01.08.2020 and 01.12.2021. Diagnostic modalities such

as breast ultrasonography, mammography, and/or magnetic resonance imaging were used to detect breast masses. Breast cancer diagnosis was confirmed through a Tru-cut biopsy. A public trial system was registered (NCT04540224). Patients with Stage 1 and Stage 2 breast cancer were included, staged using radiological imaging. Histological types, ER and progesterone receptor (PR) status, Her2 receptor positivity and Ki67 score were determined post-biopsy.

*Patient Classification*

Patients were classified as Luminal A, Luminal B or triple negative following the classification system by Hariharan et al. (16). Participants were enrolled based on their order of admission to the outpatient clinic. The control group was comprised of volunteers from the same clinic who did not have a family history of breast cancer or detectable breast masses, and agreed to participate. At admission, we recorded age, menopause status, blood leukocytes, monocytes, neutrophils, lymphocytes, platelets, and hemoglobin were recorded. Informed consent was obtained.

Exclusion criteria comprised recent Covid-19 diagnosis, pregnancy, history of immunodeficiency, age <18 or >80, and refusal to participate. Blood samples were collected in gel biochemistry tubes, prior to surgery or neoadjuvant treatment; both from patients and healthy volunteer samples. The blood samples were centrifuged at 1500 g for 10 minutes to obtain serum which was then stored in cryovials at -80°C until analysis.

*Measurement of Soluble Inflammatory Markers*

The concentrations of IL-1β, IFN-α2, IFN-γ, TNF-α, MCP-1, IL-6, IL-8, IL-10, IL-12p (p70), IL-17A, IL-18, IL-23, and IL-33 proteins in the serum were measured with a multiplex assay (LEGENDplex™ Human Inflammation Panel 1, Biolegend, USA, Cat no: 740808) and analyzed using a flow cytometer (CytoFLEX™, Beckman Coulter, Brea, CA, USA). The serum aliquots that have been previously frozen were thawed, and the analysis was performed according to the manufacturer's protocols. The concentrations were determined by calculating the mean fluorescence intensity (MFI) in the PE-FL2 channel and processed using the LEGENDplex™ Data Analysis Software Suite.

*Statistical Analysis*

Data processing was conducted using SPSS version 26.0. The Kolmogorov-Smirnov test was used to assess variable distributions. The mean±standard deviation (SD) was used to present normally distributed continuous variables, while the median and interquartile range (IQR) were used for those with a non-normal distribution. The assessment of variance homogeneity was performed using Levene's test. A one-way ANOVA was applied for independent data with normal distribution and homogeneous variances, while Welch's ANOVA was utilized for normally distributed data with unequal variances. The Kruskal-Wallis test was conducted for independent data with non-normal distribution, the. Post-hoc analysis of non-normally distributed data used Dunn's Z test. Categorical data were displayed as counts and percentages and analyzed with the Chi-square test. Correlations between cytokine levels and Ki-67 ratios were examined in RStudio using Pearson's correlation coefficient. Statistical significance was defined as a p-value less than 0.05.

## RESULTS

This study included 57 breast cancer patients and 21 healthy volunteers. Among the patients, 14 (24.6%) underwent modified radical mastectomy, 8 (14%) underwent sentinel lymph node sampling (SLNB) with mastectomy, and 35 (61.4%) underwent SLNB with breast-conserving surgery. Tru-Cut biopsy results showed 19 cases classified as Luminal A, 20 as Luminal B, and 18 as triple-negative breast cancer. During the

preoperative period, neoadjuvant treatment was required for 5 patients (26.3%) in the Luminal A group, all cases in the Luminal B group, and 15 cases (83.33%) in the triple-negative group. Molecular subtype evaluation based on histopathological typing revealed that 16 cases (84.2%) were Luminal A, 17 cases (85.0%) were Luminal B, and 15 cases (83.3%) were triple-negative breast cancers, all of which identified as invasive ductal carcinoma. Among the breast cancer cases, three instances of Luminal A, Luminal B, and triple-negative subtypes were identified as invasive lobular carcinoma, with no statistically significant difference (P=0.990). No significant difference was observed between the breast cancer subgroups and the control group in terms of menopausal status (P=0.809).

Comparison of evaluated parameters between breast cancer patients and healthy volunteers (Table 1) revealed that breast cancer patients had significantly higher concentrations of TNF-α, IL-23, IL-6, IL-12p70, IL-8, IL-10 and IL-18. When breast cancer patients were grouped by molecular subtype (Fig. 1, Tables 2 and 3), significant differences were observed in IL-23, IFN-γ, IL-6, IL-12p70, IL-8, IL-18, IL-10 and IL-17A concentrations. Subgroup analysis revealed significant differences among parameters. Specifically, there was a significant difference between the triple-negative and Luminal B subtypes (P=0.024), as well as between the control and Luminal B (P=0.033) for IFN-γ. Differences were also observed between the Luminal B and control groups (P=0.006), as well as between the Luminal A and control groups (P=0.007) for IL-6. Additionally, variations were found between the Luminal A and control groups (P=0.008), as well as between the Luminal B and control groups (P=0.001) for IL-8. Further differences were observed between the triple-negative and Luminal A (P=0.043), triple-negative and Luminal B (P=0.014), as well as between the control and Luminal B groups (P=0.038) for IL-10.

**Table 1. Comparison of the cytokine levels, demographic and laboratory parameters in breast cancer patients and healthy volunteers**

| | Breast Cancer Group (n:57) | Control Group (n:21) | P value |
|---|---|---|---|
| Age (year) (mean±SD) | 56.23±12.51 | 53.39±5.99 | 0.182 |
| Tumor size (mm) (median-IQR) | 17.00-10.00 | - | NA |
| WBC ($10^9$/L) (median-IQR) | 7.19-2.83 | 6.93-1.10 | 0.897 |
| Hgb (g/dL) (median-IQR) | 12.97-1.70 | 12.80-0.80 | 0.379 |
| Lymphocyte ($10^9$/L) (mean±SD) | 2.19±0.65 | 2.26±0.49 | 0.664 |
| Neutrophil ($10^9$/L) (median-IQR) | 4.25-2.36 | 4.16-1.11 | 0.640 |
| Neutrophil/Lymphocyte ratio (median-IQR) | 2.03-1.00 | 1.82-0.58 | 0.154 |
| IL-1β (pg/ml) (median-IQR) | 14.87-57.13 | 68.21-114.78 | 0.057 |
| IFN-α2 (pg/ml) (median-IQR) | 2.10-3.38 | 2.10-0.37 | 0.131 |
| IFN-γ (pg/ml) (median-IQR) | 1.64-11.68 | 1.64-1.90 | 0.122 |
| TNF-α (pg/ml) (median-IQR) | 17.80-40.94 | 2.11-17.08 | 0.037* |
| MCP-1 (pg/ml) (median-IQR) | 340.59-218.01 | 303.09-148.71 | 0.061 |
| IL-6 (pg/ml) (median-IQR) | 7.16-8.07 | 3.32-0.85 | 0.001* |
| IL-8 (pg/ml) (median-IQR) | 31.48-34.41 | 7.67-12.51 | 0.000* |
| IL-10 (pg/ml) (median-IQR) | 6.61-9.97 | 3.75-4.88 | 0.048* |
| IL-12p70 (pg/ml) (median-IQR) | 5.01-8.86 | 2.56-0.60 | 0.001* |
| IL-17A (pg/ml) (median-IQR) | 0.71-0.03 | 0.71-0.00 | 0.155 |
| IL-18 (pg/ml) (median-IQR) | 315.14-170.65 | 217.90-257.95 | 0.036* |
| IL-23 (pg/ml) (median-IQR) | 11.72-12.34 | 8.82-7.67 | 0.028* |
| IL-33 (pg/ml) (median-IQR) | 77.89-194.08 | 32.02-109.97 | 0.071 |

WBC: White Blood Cell, Hgb: Hemoglobin, IL: Interleukin, IFN: Interferon, TNF-α: Tumor Necrosis Faktor- α, MCP-1: Monocyte Chemoattractant Protein-1

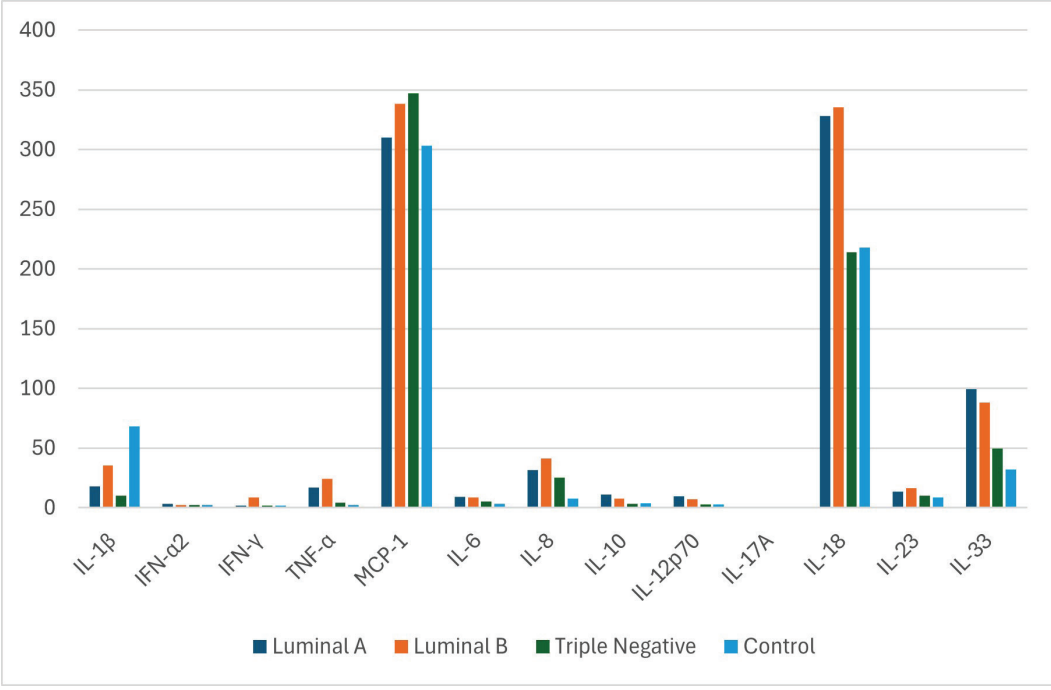


**Fig. 1.** The cytokine profiles of the breast cancer subgroups and healthy volunteers

**Table 2. Evaluation of the patients' age and hemogram in patients with different subtypes of breast cancer, (WBC: White Blood Cell, Hgb: Hemoglobin)**

| | Luminal A (n:19) | Luminal B (n:20) | Triple Negative (n:18) | Control (n:21) | P value |
|---|---|---|---|---|---|
| Age (year) (mean±SD) | 54.95±11.00 | 52.80±11.23 | 61.39±14.24 | 53.39±5.99 | 0.178 |
| Tumor size (mm) (median-IQR) | 17.00-10.00 | 21.00-11.00 | 23.00-23.00 | - | 0.224 |
| WBC ($10^9$/L) (median-IQR) | 7.49-3.45 | 7.22-3.40 | 7.03-2.36 | 6.93-1.10 | 0.816 |
| Hgb (g/dL) (median-IQR) | 13.10-1.60 | 13.05-1.60 | 12.60-1.90 | 12.80-0.80 | 0.692 |
| Lymphocyte ($10^9$/L) (mean±SD) | 2.37±0.77 | 2.18±0.61 | 2.00±0.52 | 2.26±0.49 | 0.315 |
| Neutrophil ($10^9$/L) (median-IQR) | 4.17-2.87 | 4.36-3.12 | 4.33-1.88 | 4.16-1.11 | 0.971 |

**Table 3. Evaluation of the levels of various cytokines in patients with different subtypes of breast cancer and healthy individuals**

| | Luminal A (n:19) | Luminal B (n:20) | Triple Negative(n:18) | Control (n:21) | P value |
|---|---|---|---|---|---|
| IL-1β (pg/ml) (median-IQR) | 18.14-52.18 | 35.42-139.33 | 10.05-13.42 | 68.21-114.78 | 0.060 |
| IFN-α2 (pg/ml) (median-IQR) | 3.24-3.60 | 2.10-14.37 | 2.10-0.80 | 2.10-0.37 | 0.176 |
| IFN-γ (pg/ml) (median-IQR) | 1.64-15.83 | 8.41-18.14 | 1.64-1.31 | 1.64-1.90 | 0.013* |
| TNF-α (pg/ml) (median-IQR) | 17.15-41.46 | 24.15-37.88 | 4.15-36.79 | 2.11-17.08 | 0.132 |
| MCP-1 (pg/ml) (median-IQR) | 309.90-205.89 | 338.27-222.37 | 347.17-214.78 | 303.09-148.71 | 0.267 |
| IL-6 (pg/ml) (median-IQR) | 9.07-6.58 | 8.63-24.16 | 5.32-3.93 | 3.32-0.85 | 0.002* |
| IL-8 (pg/ml) (median-IQR) | 31.48-42.09 | 41.17-55.15 | 25.22-24.08 | 7.67-12.51 | 0.001* |
| IL-10 (pg/ml) (median-IQR) | 11.00-15.78 | 7.75-28.04 | 3.12-3.28 | 3.75-4.88 | 0.002* |
| IL-12p70 (pg/ml) (median-IQR) | 9.59-10.77 | 6.98-18.05 | 2.56-1.43 | 2.56-0.60 | 0.000* |
| IL-17A (pg/ml) (median-IQR) | 0.71-1.68 | 0.71-0.19 | 0.71-0.00 | 0.71-0.00 | 0.036* |
| IL-18 (pg/ml) (median-IQR) | 327.99-138.31 | 335.15-335.73 | 213.90-181.65 | 217.90-257.95 | 0.008* |
| IL-23 (pg/ml) (median-IQR) | 13.42-13.50 | 16.32-38.07 | 10.02-5.73 | 8.82-7.67 | 0.034* |
| IL-33 (pg/ml) (median-IQR) | 99.11-177.96 | 88.25-473.61 | 49.72-90.23 | 32.02-109.97 | 0.085 |

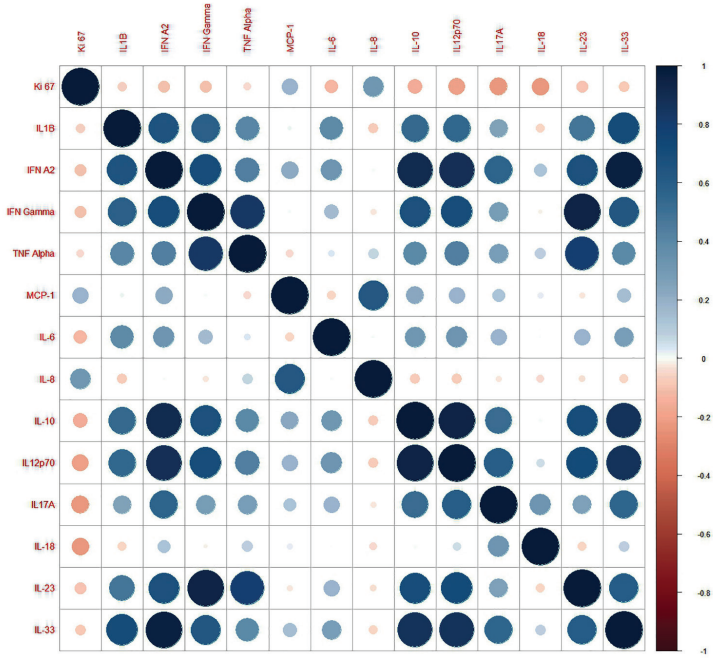


**Fig. 2.** Correlation heatmap of cytokines according to the Ki67 score.

Moreover, variations were found between the Luminal B and control groups (P=0.002), Luminal A and control groups (P=0.001), triple-negative and Luminal A (P=0.007), as well as triple-negative and Luminal B (P=0.014) for IL-12p70. While IL-17A, IL-18, and IL-23 levels showed significant differences across the groups, no significant differences were observed in pairwise subgroup comparisons. Although MCP-1 and IL-33 levels did not differ significantly, breast cancer patients had higher levels than healthy controls. Correlation analysis of blood cytokine values in breast cancer patients based on Ki-67 ratios revealed no significant positive or negative correlation for any parameter (Fig. 2).

## DISCUSSION

Research has indicated that the risk of breast cancer grows as individuals get older (1). Kulkarni et al. reported that young women aged 20-39 have a higher risk of breast cancer with triple-negative and luminal B subtype compared to women aged 50-64 (17). However, a study from Jamaica found that while the incidence of the luminal A subtype increased with age, no significant association was observed between age and other breast cancer subgroups (18). We found no significant relationship between age and molecular subtypes.

The World Health Organization has identified more than 20 histological types of breast cancer. Among all these types, invasive ductal carcinoma is the most frequent accounting for 76% of all breast cancer cases (19). In our study, consistent with the existing literature, over 80% of breast cancer patients in each subgroup were diagnosed with invasive ductal carcinoma, with no significant differences between the groups.

The interaction between tumor cells and the tumor microenvironment and is well known to impact proliferation and metastasis. Among these, inflammatory cells and cytokines have been suggested to play a key role in breast cancer (9). Previous studies have shown that IL-8 ribonucleic acid (RNA) transcription is higher in breast cancer cells, however levels of IL-6, IL-1 α, IL-4 and IL-1β were not significantly different from those of healthy volunteers (20). In another study, IL-10, IL-8 and IL-6 concentrations

were higher in breast cancer patients than in healthy women (21). Chavey et al. reported that the serum concentrations of TNF-α, MIP-1β, IL-6, IFN-γ, IL-8, MCP-1, IL-13 and IL-12 were significantly elevated in breast cancer patients compared to healthy individuals (10). We found that serum concentrations of IL-23, IL-10, TNF-α, IL-18, IL-6, IL-12, and IL-8 were significantly higher in breast cancer patients compared to healthy volunteers. The cytokines that enhance the immune response against breast cancer are specifically linked to Th1 and Th17 cells. These results suggest that these cells have a significant role in the immune response to breast cancer.

Several publications have demonstrated that well-known proinflammatory cytokines, such as TNF-α, IL-8, IL-6, and IL-1, are higher in patients with breast cancer and contribute to increased tumor invasion (22, 23). Elevated levels of TNF-α in the bloodstream are linked to invasion and poorer prognosis. However, it is still uncertain whether there is a direct connection between these proinflammatory cytokines and tumor stage or lymph node metastasis (24). Ma et al. reported that patients with invasive ductal carcinoma had higher serum levels of IL-8 and IL-6 compared to healthy women. They observed that levels of TNF-α, IL-6 and IL-8 were particularly increased in patients with stage II and III cancers, as well as in those with lymph node involvement. TNF-α levels were only elevated in patients with stage III carcinoma and lymph node metastases. The same study reported that patients with ER-positive tumors had higher levels of blood IL-6, and lower levels of IL-8 compared to patients with ER-negative tumors. No correlation was found between TNF-α levels and ER, PR, or HER2 status (25). Autenshlyus et al. demonstrated that molecular subtypes are linked to cytokines, particularly IL-6 and IL-8. They also found that the triple-negative subtype exhibited the lowest potential for cytokine production compared to other subtypes (26). Some studies have reported that the expression of estrogen and progesterone receptors, as well as the activity of steroidogenesis enzymes have been influenced by IL-8, and IL-6 (27, 28). In our study, we found that breast cancer patients had increased levels of TNF-α, IL-8 and IL-6, especially in the ER-positive Luminal A and Luminal B groups, compared to the ER-negative triple-negative patients. While IL-1β levels did not differ significantly among the groups, the lowest levels were noted in triple-negative breast cancer. This suggests that the immune response against may be irregular. This observation raises the possibility that, instead of blocking IL-6 and IL-8 receptors, increasing these cytokine levels or enhancing receptor activation might improve treatment response, particularly in triple-negative breast cancers.

The immunosuppressive and tumor-promoting effects of IL-10 are well established, but its role in breast cancer development remains uncertain (29). In a study by Li et al., the link between IL-10 expression and clinicopathological characteristics in cancer cells was examined. The study found that low IL-10 expression was associated with a higher risk of recurrence and metastasis (30). In line with this, Kozłowski et al. observed significantly higher levels of IL-10 in breast cancer tissues compared to normal breast tissues (21). However, conflicting findings were reported in another study, which found no significant difference in IL-10 expression between breast cancer and normal tissue (31). Another study reported that breast cancer patients had higher serum IL-10 levels than healthy volunteers, and ER and PR-negative tumors had even higher levels (32). In our study the IL-10 level is significantly higher in patients with breast cancer. This level is highest in luminal A patients with the lowest Ki-67 score. However, it is lower than the normal population in triple-negative patients with the highest Ki-67 score. The inverse connection between Ki-67 scores and IL-10 suggests that IL-10 may be an important parameter thatmodulates the balance between immune suppression and tumor proliferation.

In less aggressive tumors such as Luminal A, high levels of IL-10 may help maintain a controlled tumor microenvironment with lower levels of proliferation. In contrast, the low levels of IL-10 in TNBC could indicate a lack of this regulatory mechanism, potentially contributing to the unchecked proliferation and aggressiveness of these tumors.

IL-17 is the primary cytokine produced by Th17 cells. The development of these cells is known to depend on a combination of IL-21, TGF-β, IL-23 and IL-6 (33). Th17 is important in carcinogenesis as studies have shown that STAT3 plays a significant role in cancer-associated inflammation and that IL-23 promotes tumor growth (34). Kaur et al. reported that patients with breast cancer had significantly higher levels of IL-17A compared to individuals with normal breast tissue. They also stated that high levels of IL-17A negatively impact disease-free survival (11). Gangemi et al. reported that patients with breast cancer had increased levels of IL-23, but found no correlation with molecular subtypes (24). Yang et al. observed an increase in the number of Th17 cells in breast cancer patients compared to healthy individuals (34). In our study, although IL-17 levels did not show a significant difference in breast cancer cases, IL-23 levels were significantly elevated. This suggests a potential key role for Th17 cells in breast cancer. The observation of the lowest levels in triple-negative breast cancer patients raises the question of whether Th17 cells contribute to cancer development or affect the body's response to the disease.

IL-33, a member of the IL-1 family, is known for promoting Th2 immune responses and playing a key role in mucosal healing and epithelial repair (35). ST2, another member of the IL-1 superfamily, has IL-33 as its sole identified ligand. Research has demonstrated that soluble ST2 levels are higher in patients with metastatic breast cancer compared to those with primary breast cancer (12). Liu et al. found that IL-33 levels were higher in breast cancer patients, especially in the ER-positive group, compared to individuals with benign breast diseases and healthy controls (36). We found that breast cancer patients have higher IL-33 levels than healthy controls, although these differences were not significant. The ER-positive group had higher levels than the ER-negative patients but the difference was not significant. IL33 might contribute to a pro-tumor microenvironment by promoting inflammation. Luminal A and B tumors tend to be hormone receptor-positive, and these cytokines might be involved in pathways related to hormone receptor signaling or the immune response specific to these subtypes.

IL-12 is a pro-inflammatory cytokine that play role in activating the cell-mediated immune responses. It is involved in the proliferation of natural killer cells and T cells (37). Derin et al. reported that there were no significant differences in serum levels of IL-12 and IL-8 between breast cancer patients and healthy volunteers (38). Youssef et al. reported that IL-12 was observed at lower levels in cancer patients compared to healthy individuals. When breast cancer patients were evaluated internally, the levels were significantly higher in hormone receptor-negative and lymph node positive patients compared to other breast cancer patients (39). On the contrary, we noted a significant increase in the level of IL-12 serum cytokines in breast cancer patients. A significant decrease was observed in the triple-negative group compared to other breast cancer subtypes. This finding supports our conclusion that triple-negative tumors are less immunogenic and cause irregular immune system responses.

IL-18 is involved in the Th1 response synergistically with IL-12 and through the activation of natural killer cells producing IFN-γ (40). A cohort study found that serum IL-18 levels were significantly higher in patients with metastatic breast cancer compared to those without metastases or healthy volunteers (14). Park et al. evaluated the correlation between subgroups of breast cancer patients and IL-18 levels. The group reported that the highest IL18 serum levels

were found in the triple-negative subgroup, while the lowest IL-18 serum levels were found in the luminal subgroup (41). In this study, we observed a significant increase in serum cytokine levels of IL-18 in breast cancer patients, consistent with previous literature. However but no significant difference was observed between the subtypes.

MCP-1 is secreted from adipocytes and may be involved in macrophage-related migration. Recent studies have shown that macrophages assist tumor cells in moving out of blood vessels and causing metastases. Elevated levels of MCP-1 in the tumor microenvironment may contribute to increased cancer cell invasiveness by stimulating both autocrine and paracrine signaling pathways (42). Lebrecht et al. reported that MCP-1 levels were higher in patients with breast cancer, although they were not statistically significant (43). Another study by Dutta et al. reported that triple-negative breast cancer cells showed higher MCP-1 expression than luminal breast cancer cells (13). Here, no statistically significant differences were found although the highest serum MCP-1 levels were observed in breast cancer patients,especially in the triple-negative subgroup. MCP-1 which recruits monocytes to the tumor site, contributes to the tumor-associated macrophage population, often associated with tumor progression and metastasis. The elevated levels in breast cancer patients, particularly in triple-negative subtypes, suggest a more aggressive tumor phenotype and an increase in tumor-associated macrophages in the tumor microenvironment of these patients.

IFN-γ is the key cytokine responsible for activating macrophages. Studies have shown that at high doses, IFN-γ can induce apoptosis through the Jak-STAT-1-caspase signaling pathway. Additionally, research indicates that tumors exposed to low doses of IFN-γ are more likely to develop metastatic traits, whereas high-dose treatment can lead to tumor regression (44). Singh et al. found that the simultaneous loss of the transcription factor Elf5 and the ubiquitin ligase FBXW7 activates intrinsic IFN-γ signaling, which drives tumor progression and metastasis in triple-negative breast cancer cells (45). Another study found a significant correlation between higher expression of an IFN-related metagene and a poorer prognosis in ER-positive, HER2-negative breast cancer (46). In our study, we observed higher levels of IFN-γ in breast cancer patients, although it was not statistically significant. Subgroup analysis revealed significantly elevated IFN-γ levels in the luminal B subtype compared to all other groups. The higher level of IFN-γ, known for its antitumor effects, in breast cancer patients is an expected outcome.

The limitations of our study include the small number of patients. In addition, the immune system cells that are effective in combination with cytokines could not be examined through immunophenotyping. Furthermore, the values of the cytokines had upper and lower detection limits due to the measurement technique, and there was unknown status of cytokines and immune system cells in the tumor microenvironment.

These results did not reveal whether cytokines are involved in the development of breast cancer or if they are part of the immune system's response to breast cancer. The increase in IL-10 suppresses the immune system and boosts cytokines like IL-12, which possesses antitumor properties. There is also an increase in IL-23, which has been reported to play a role in tumor development in breast cancer patients. This suggests that the immune system and cytokines play an important role in both tumor development and the immune response against the tumor simultaneously. Our study shows that Th1- and Th17-related cytokines play a crucial role in breast cancer progression and the immune system's response to cancer. In addition, the low detection of all other cytokines except MCP-1 in triple-negative breast cancer suggests that these tumors may have lower immunogenicity. Consequently, these tumors progress more aggressively due to the inadequate immune system response.

## ACKNOWLEDGMENTS

This research was funded by Saglik Bilimleri University (Grant Number: 2020/098).

## AUTHORS' CONTRIBUTION

AD conceptualized the study, conducted the literature review, collected the data, performed the statistical analysis, and drafted the manuscript. UOI contributed to the study design, participated in the literature review, analyzed the data, and wrote and revised the manuscript. HS, MG, FT, CC, and MMS conducted the clinical studies and contributed to the literature review. EK and UOI performed the critical review. All authors reviewed and approved the final manuscript.

## CONFLICTS OF INTEREST

The authors declare no conflict of interest.

## REFERENCES

1. Siegel RL, Miller KD, Fuchs HE, Jemal A. Cancer statics, 2021. *CA: A Cancer Journal for Clinicians* 2021;71:7–33.
2. Pilipow K, Darwich A, Losurdo A. T-cell-based breast cancer immunotherapy. Semin Cancer Biol. 2021;72:90-101.
3. Liu C, Chu D, Kalantar-Zadeh K, George J, Young HA, Liu G. Cytokines: From Clinical Significance to Quantification. Adv Sci (Weinh). 2021;8(15):e2004433.
4. Yi M, Li T, Niu M, Zhang H, Wu Y, Wu K, Dai Z. Targeting cytokine and chemokine signaling pathways for cancer therapy. Signal Transduct Target Ther. 2024;9(1):176.
5. Wang Y, Zhang H, Liu C, Wang Z, Wu W, Zhang N, et al. Immune checkpoint modulators in cancer immunotherapy: recent advances and emerging concepts. J Hematol Oncol. 2022;15(1):111.
6. Atallah-Yunes SA, Robertson MJ. Cytokine Based Immunotherapy for Cancer and Lymphoma: Biology, Challenges and Future Perspectives. Front Immunol. 2022;13:872010.
7. Briukhovetska D, Dörr J, Endres S, Libby P, Dinarello CA, Kobold S. Interleukins in cancer: from biology to therapy. Nat Rev Cancer. 2021;21(8):481-499.
8. Briukhovetska D, Dörr J, Endres S, Libby P, Dinarello CA, Kobold S. Interleukins in cancer: from biology to therapy. *Nature reviews. Cancer* 2021;21:481–499.
9. DeNardo DG, Coussens LM. Inflammation and breast cancer. Balancing immune response: crosstalk between adaptive and innate immune cells during breast cancer progression. *Breast cancer research*. 2007;9(4):212.
10. Chavey C, Bibeau F, Gourgou-Bourgade S, Burlinchon S, Boissière F, Laune D, et al. Oestrogen receptor negative breast cancers exhibit high cytokine content. *Breast cancer research*. 2007;9:R15.
11. Kortylewski M, Xin H, Kujawski M, Lee H, Liu Y, Harris T, et al. Regulation of the IL-23 and IL-12 balance by Stat3 signaling in the tumor microenvironment. *Cancer cell*, 2009;15:114–123.
12. Gillibert-Duplantier J, Duthey B, Sisirak V, Salaün, D, Gargi T, Trédan O, et al. Gene expression profiling identifies sST2 as an effector of ErbB2-driven breast carcinoma cell motility, associated with metastasis. *Oncogene,* 2012;31:3516–3524.
13. Dutta P, Sarkissyan M, Paico K, Wu Y, Vadgama JV. MCP-1 is overexpressed in triple-negative breast cancers and drives cancer invasiveness and metastasis. *Breast cancer research and treatment*, 2018;170:477–486.
14. Günel N, Coşkun U, Sancak B, Günel U, Hasdemir O, Bozkurt S. Clinical importance of serum interleukin-18 and nitric oxide activities in breast carcinoma patients. *Cancer,* 2002;95:663–667.
15. Hamidullah Changkija B, Konwar R. Role of interleukin-10 in breast cancer. *Breast cancer research and treatment,* 2012;133:11–21.
16. Hariharan N, Rao TS, Naidu CK, Raju KVVN, Rajappa S, Ayyagari S, et al. The Impact of Stage and Molecular Subtypes on Survival Outcomes in Young Women with Breast Cancer. *Journal of adolescent and young adult oncology,* 2019;8:628–634.
17. Kulkarni A, Stroup AM, Paddock LE, Hill SM, Plascak JJ, Llanos AAM. Breast Cancer Incidence and Mortality by Molecular Subtype: Statewide Age and Racial/Ethnic Disparities in New Jersey. *Cancer health disparities*, 2019;3:e1–e17.
18. Copeland J, Oyedeji A, Powell N, Cherian CJ, Tokumaru Y, Murthy V, et al. Breast Cancer in Jamaica: Stage, Grade and Molecular Subtype Distributions Across Age Blocks, the Implications for Screening and Treatment. *World journal of oncology*, 2021;12:93-103.
19. Li CI, Uribe DJ, Daling JR. Clinical characteristics of different histologic types of breast cancer.

*British journal of cancer,* 2005;93:1046–1052.
20. Green AR, Green VL, White MC, Speirs V. Expression of cytokine messenger RNA in normal and neoplastic human breast tissue: identification of interleukin-8 as a potential regulatory factor in breast tumours. *International journal of cancer*, 1997;72:937–941.
21. Kozłowski L, Zakrzewska I, Tokajuk P, Wojtukiewicz MZ. Concentration of interleukin-6 (IL-6), interleukin-8 (IL-8) and interleukin-10 (IL-10) in blood serum of breast cancer patients. *Roczniki Akademii Medycznej w Bialymstoku* 2003;48:82–84.
22. Gangemi S, Minciullo P, Adamo B, Franchina T, Ricciardi GR, Ferraro M, et al. Clinical significance of circulating interleukin-23 as a prognostic factor in breast cancer patients. *Journal of cellular biochemistry*, 2012;113:2122–2125.
23. Soria G, Ofri-Shahak M, Haas I, Yaal-Hahoshen N, Leider-Trejo L, Leibovich-Rivkin T, et al. Inflammatory mediators in breast cancer: coordinated expression of TNFα & IL-1β with CCL2 & CCL5 and effects on epithelial-to-mesenchymal transition. *BMC cancer*, 2011;11:130.
24. Chin AR, Wang SE. Cytokines driving breast cancer stemness. *Molecular and cellular endocrinology*, 2014;382:598–602.
25. Ma Y, Ren Y, Dai ZJ, Wu CJ, Ji YH, Xu J. IL-6, IL-8 and TNF-α levels correlate with disease stage in breast cancer patients. *Advances in clinical and experimental medicine:official organ Wroclaw Medical University*, 2017;26:421–426.
26. Autenshlyus A, Davletova K, Varaksin N, Marinkin I, Lyakhovich V. Cytokines in various molecular subtypes of breast cancer. *International journal of immunopathology and pharmacology*, 2021;35:1-13.
27. Uciechowski P, Dempke WCM. Interleukin-6: A Masterplayer in the Cytokine Network. *Oncology*, 2020;98:131–137.
28. Hilborn E, Stål O, Jansson A. Estrogen and androgen-converting enzymes 17β-hydroxysteroid dehydrogenase and their involvement in cancer: with a special focus on 17β-hydroxysteroid dehydrogenase type 1, 2, and breast cancer. *Oncotarget*, 2017;8:30552–30562.
29. Chang CM, Lam HYP, Hsu HJ, Jiang SJ. Interleukin-10: A double-edged sword in breast cancer. *Tzu chi medical journal*, 2021;33:203–211.
30. Li Y, Gao P, Yang J, Yu H, Zhu Y, Si W. Relationship between IL-10 expression and prognosis in patients with primary breast cancer. *Tumour biology: the journal of the International Society for Oncodevelopmental Biology and Medicine*, 2014;35:11533–11540.
31. Tanikawa T, Wilke CM, Kryczek I, Chen GY, Kao J, Núñez G, et al. Interleukin-10 ablation promotes tumor development growth and metastasis. *Cancer research*, 2012;72:420–429.
32. Lv Z, Liu M, Shen J, Xiang D, Ma Y, Ji Y. Association of serum interleukin-10, interleukin-17A and transforming growth factor-α levels with human benign and malignant breast diseases. *Experimental and therapeutic medicine*, 2018;15:5475–5480.
33. Bettelli E, Carrier Y, Gao W, Korn T, Strom TB, Oukka M, et al. Reciprocal developmental pathways for the generation of pathogenic effector TH17 and regulatory T cells. *Nature*, 2006;441:235–238.
34. Yang L, Qi Y, Hu J, Tang L, Zhao S, Shan B. Expression of Th17 cells in breast cancer tissue and its association with clinical parameters. *Cell biochemistry and biophysics*, 2012;62:153–159.
35. Lopetuso LR, Scaldaferri F, Pizarro TT. Emerging role of the interleukin (IL)-33/ST2 axis in gut mucosal wound healing and fibrosis. *Fibrogenesis & tissue repair*, 2012;5:18.
36. Liu J, Shen JX, Hu JL, Huang WH, Zhang GJ. Significance of interleukin-33 and its related cytokines in patients with breast cancers. *Frontiers in immunology*, 2014;5:141.
37. Lissoni P, Mengo S, Mandalà M, Mauri E, Brivio F, Rovelli F, et al. Physiopathology of IL-12 in human solid neoplasms: blood levels of IL-12 in early or advanced cancer patients, and their variations with surgery and immunotherapy. *Journal of biological regulators and homeostatic agents,* 1998;12:38–41.
38. Derin D, Soydinc HO, Guney N, Tas F, Camlica H, Duranyildiz D, et al. Serum IL-8 and IL-12 levels in breast cancer. *Medical oncology* 2007;24:163–168.
39. Youssef SS, Mohammad MM, Ezz-El-Arab LR. Clinical Significance of Serum IL-12 Level in Patients with Early Breast Carcinoma and Its Correlation with Other Tumor Markers. *Open access Macedonian journal of medical sciences*, 2015;3:640–644.
40. Akdis M, Burgler S, Crameri R, Eiwegger T, Fujita H, Gomez E, et al. Interleukins, from 1 to 37, and interferon-γ: receptors, functions, and roles in diseases. *The Journal of allergy and clinical immunology*, 2011;127:701–721.
41. Park IH, Yang HN, Lee KJ, Kim TS, Lee ES, Jung S, et al. Tumor-derived IL-18 induces PD-1 expression on immunosuppressive NK cells in triple-negative breast cancer. *Oncotarget,* 2017;8:32722–32730.
42. Santander AM, Lopez-Ocejo O, Casas O, Agostini T, Sanchez L, Lamas-Basulto E, et al. Paracrine Interactions between Adipocytes and Tumor Cells Recruit and Modify Macrophages to the Mammary Tumor Microenvironment: The Role

of Obesity and Inflammation in Breast Adipose Tissue. *Cancers,* 2015;7:143–178.
43. Lebrecht A, Grimm C, Lantzsch T, Ludwig E, Hefler L, Ulbrich E, et al. Monocyte chemoattractant protein-1 serum levels in patients with breast cancer. *Tumour biology: the journal of the International Society for Oncodevelopmental Biology and Medicine*, 2004;25:14–17.
44. Song M, Ping Y, Zhang K, Yang L, Li F, Zhang C, et al. Low-Dose IFNγ Induces Tumor Cell Stemness in Tumor Microenvironment of Non-Small Cell Lung Cancer. *Cancer research*, 2019;79:3737–3748.
45. Singh S, Kumar S, Srivastava RK, Nandi A, Thacker G, Murali H, et al. Loss of ELF5-FBXW7 stabilizes IFNGR1 to promote the growth and metastasis of triple-negative breast cancer through interferon-γ signalling. *Nature cell biology*, 2020;22:591–602.
46. Callari M, Musella V, Di Buduo E, Sensi M, Miodini P, Dugo M, et al. Subtype-dependent prognostic relevance of an interferon-induced pathway metagene in node-negative breast cancer. *Molecular oncology*, 2014;8:1278–1289.