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Original Research Article

Immunological Profile Assessment of Breast Cancer Patients in Wasit Province

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Abstract: One of the more prevalent diseases in women is breast cancer, however males might also get it sometimes. A tumor is often formed when certain cells in the breast tissue start to grow quickly and multiply uncontrollably, leading to breast cancer. These cancerous cells continue to invade the nearby healthy cells in the breast if not treated and eradicated, and may spread to the nearby lymph nodes. The cancer may also penetrate breast tissues and spread to nearby organs, and in some cases, it can reach distant parts of the body. The current study include a group of (80) females. 50 patients with breast cancer and 30 healthy, Blood samples were taken at Al-Karama Teaching Hospital based on 50 breast cancer, their age range from (30-30),(40-49),50 years and over from the period (2024/10/1-2025/2/1.The study aim to Evaluate immune levels in women diagnosed with breast cancer, focusing on key immune markers by using ELISA technique.Interleukin 8 (IL-8) levels in miserable and well individuals changed considerably according to the study's findings (P = 0.001). The average level in patients was 120 ± 15 ng/L, which was greater than the average level in healthy people (40 ± 10 ng/L). Interleukin 12 (IL-12) levels were also found to differ significantly between the two groups (P = 0.001), with the average level in patients being 25 ± 5 ng/L, lower than that of healthy people (60 ± 10 ng/L). Based to an immunological the investigation, women diagnosed breast cancer have markedly lower levels of cytokines 12 (IL-12), which is diagnostic of an impaired immune response targeting the tumor. Because IL-12 is essential for T lymphocyte and natural killer (NK) cell activation, low levels of this cytokine make it easier for cancer cells to evade cellular defense. Conversely, the findings found that, in comparison with people in good health, cancerous tissue from breast tumor patients of any age had greater quantities of factor 8 (IL-8). This increase is in keeping with the established function of IL-8 in fostering tumor metastasis and angiogenesis, all of which are directly linked to the advancement of illness. Thus, two similar mechanisms that inhibit the immune response and increase the development and spread of tumors are reduced IL-12 and higher IL-8. For the purpose of to evaluate the immune-mediated features of patients with breast cancer, this investigation looked at two crucial cytokines, interleukin-8 (IL-8) and interleukin-12 (IL-12). In order to confirm the possibility of association between these cytokines and the severity of the disease or its development of metastases, it also aimed to examine the levels of these cytokines in various age groups and disease stages. The study is to help build a scientific basis that can support the creation of tailored immunotherapeutic approaches for the treatment of breast cancer by exposing these immunological alterations.

Keywords: Cancer immunity, inflammation, tumor development, cytokines, immunological response, ELISA, breast cancer, interleukin-8, interleukin-12, and suppression of immunity.

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INTRODUCTION

Millions of women become impacted by breast cancer every year, so it is one of the most common illnesses in the world. The World Health Organization (WHO) reports that breast cancer is the most common cause of death due to cancer for women and makes for approximately eleven percent of all cancer cases world (Tao *et al.,* 2015)

While every individual develops breast cancer in distinct ways, there are likely identical signs that may identify the illness. A painless lump or expansion in a breast or armpit might represent an early indication of abnormal modification (Shetty, 2021). Furthermore, the breast's shape or create may alter, giving the appearance of asymmetry or swelling. Another major sign of infection is irregular nipple fluid, which sometimes includes blood. Indicating cellular changes, the breast skin's texture may also change, becoming red or wrinkled. discover blister growth and development, blistering, or nibble abduction. For a prompt diagnosis and suitable therapy, these are symptoms that require to be examined quickly by a breast surgery or oncologist (Pei *et al.,* 2019).

A variety of basic techniques are utilized to effectively identify and monitor breast tumors. A clinical examination is typically the first step in the evaluation procedure, where a physician looks for lumps or unusual changes in the breast tissue While trying to establish the existence of the disease or rule out other disorders, this physical checkup aids to recognize any signs that may qualify for more tests or analyses. (McDonald *et al.,* 2004).

The determination of cancer of the breast depends on a variety of core methods and techniques targeted to provide detailed tumor evaluation and early recognition. A clinically breast examination, in which the physician looks for lumps or unusual changes in the breast tissue, is typically an initial step in the diagnosis method. This investigation help in finding any signals that need more research. In order to identify worrisome tumors that might not be seen during a physical examination, mammography is an essential diagnostic technique for the early diagnosis of malignancies. When more precise imaging is required, magnetic resonance imaging (MRI) and ultrasound are used to obtain detailed information about the tumor, including its nature (solid or liquid) and other characteristics. To definitively confirm the presence of cancer, a biopsy is performed. A portion of the affected tissue is removed and analyzed microscopically, allowing doctors to evaluate the cells and determine whether they are malignant. This integrated approach plays a critical role in early detection and optimal treatment planning for breast cancer patients (Tang *et al.,* 2009). In the immune response associated with cancer, interleukin eight (IL-8) and interleukin-12 (IL-12) have incompatible function. While IL-8 promotes in angiogenesis and encourages the proliferation of cancerous cells, IL-12 is an immunostimulant that induces T cell lymphocytes and cell killer cells to destroy the tumor. As consequently, low IL-12 together with elevated IL-8 levels are important variables in the development of illness (Zielonka *et al.,* 2007).

The progression of tumors and metastasis are greatly stimulated by the inflammatory cytokine interleukin-8 (IL-8). Promoting angiogenesis, or the formation of new blood vessels, is one of its main roles. This ensures the tumor has enough blood to thrive and supply the oxygen and nutrients required for its continuous growth. Additionally, by increasing in the migration and invasion of cancer cells into tissues nearby, IL-8 enhances the growing of tumors (Mir *et al.,* 2023).

Interleukin-12 (IL-12) has an anticancer influence by amplifying the immune system's response to cancer cells. It promotes T lymphocytes and natural killer (NK) cells, two essential cell types which detect and kill cancer cells. Additionally, by preventing angiogenesis, which limits the blood flow to tumors and limits their capacity to proliferate and spread, IL-12 inhibits the formation of tumors (Zundler and Neurath, 2015).

BREAST CANCER

One of the most prevalent tumors in women is breast cancer, however it can also strike males sometimes. The development of breast cancer happens when certain cells in the breast tissue proliferate uncontrollably and increase quickly, usually resulting in a tumor. These cancerous cells continue to invade the nearby healthy cells in the breast if not treated and eradicated, and may spread to the nearby lymph nodes. The cancer may also penetrate breast tissues and spread to nearby organs, and in some cases, it can reach distant parts of the body (Akram *et al.,* 2017) Breast cancer typically originates from the milk-producing glands (lobules) or the ducts that carry milk to the nipple (milk ducts). It can also sometimes arise from the fatty tissue or the fibrous connective tissue within the breast (Franjić, 2023).

BREAST CANCER INDUCED CYTOKINES

Interleukin-8's Function in Breast Cancer

Interleukin-8 (IL-8) is a pro-inflammatory cytokine that plays a multifaceted role in breast cancer progression through its effects on tumor growth, metastasis, and modulation of the immune response. Functioning as a signaling molecule, IL-8

regulates inflammatory and immune processes within the tumor microenvironment (Waugh and Wilson, 2008).

Its main purpose is to promote angiogenesis and growth of tumors. Interleukin-8 (IL-8) supports the formation of new blood vessels, providing constant supply of oxygen and nutrients necessary for tumor development and maintenance (Todorović-Raković and Milovanović, 2013).

Also, interleukin-8 (IL-8) promotes metastasis by facilitating the invasion and migration of cancer cells, and higher levels of IL-8 lead to a higher promotion of tumor cells to other regions of the body (De Larco *et al.,* 2001).

Interleukin-8 (IL-8) also helps forms of cancer avoid the immune system through altering the tumor environment and limiting immune cell activation, and this protects cancer cells via immune destruction (Fousek and Palena, 2021).

Moreover, the disease state created by interleukin-8 (IL-8) stimulates tumor formation and can create resistance to some treatments especially chemotherapy and immunotherapy (Gonzalez-Aparicio and Alfaro, 2020).

While it acts an essential role of different pathways which foster growth of tumors, interleukin-8 (IL-8) is a possible therapeutic target in the treatment of breast cancer. Methods that target IL-8 or its receptors might be helpful by minimizing metastasis, inhibiting tumor progression, and reviving the immune response's anticancer response. (Yi *et al.,* 2024).

INTERLEUKIN-12'S FUNCTION IN BREAST CANCER

T lymphocytes and natural killer cells (NK cells), two critical cell types that recognize and destroy cancer cells, are mostly controlled by the immunostimulating cytokine interleukin-12 (IL-12). A number of studies focused on its therapeutic properties, especially for cancers of the breast, where it enhances the immune system and inhibits the formation of cancers (Mirlekar and Pylayeva-Gupta, 2021).

One of the main roles of interleukin-12 (IL-12) is to trigger the body's defenses. By stimulating the growth of type 1 helper T cells (Th1) and promoting the production of interferon gamma (IFN-γ), it promotes the cytotoxic efficacy of T cells and natural killer (NK) cells against cancer cells (Lu, 2017).

These mechanisms enhance the immediate impact of interleukin-12 (IL-12) in prevent cancer. IL-12 stimulates immune cells to invade the tumor environment, limits tumor formation, and prevent cells from growing (extend to other parts of the body). Also, it has been confirmed that IL-12 improves the effectiveness of existing treatments for cancer, such immunotherapy and chemotherapy (Wang *et al.,* 2019).

The immune system is promoted by interleucina-12 (IL-12), which results in a Th1 infection that makes the tumor eliminative and a difference in a Th2 response that aids in the tumor's growth. (Xu *et al.,* 2010). Although side effects such as cytokine release syndrome caused difficulties which have limited its clinical application, interleukin-12's (IL-12) therapeutic potential has prompted the investigation into its therapeutic uses in clinical trials for the treatment of breast cancer (Li and Liu, 2022).

By encouraging an allergic and immunostimulatory state, interleukin-12 (IL-12) can alter the tumor environment and reverse the immunosuppressive effects of cytokines like TGF-β and IL-10. Although further study is required to enhance treatment protocols and lower toxicity, IL-12 is a viable option for targeted immunotherapy in the treatment of breast cancer because of its capacity to rewire the tumor environment (Haist *et al.,* 2021).

Materials and Techniques

Research Design

From the first of October 2024, to February 1, 2025, this case-control research was carried out at Al-Karama Teaching Hospital in Kut City, Iraq. The study comprised 30 women who seemed to be in good health as controls and 50 women who had been diagnosed with cancer of the breast.

Study Population

- **Inclusion Criteria:** Female patients with histopathologically confirmed breast cancer, aged ≥30 years, prior to receiving chemotherapy or radiotherapy.
- **Exclusion Criteria:** Patients with other malignancies, autoimmune disorders, or active infections.

Three age groups were created from the participants: those aged 30 to 39, 40 to 49, and ≥50.

Ethical Approval

All study participants gave their informed permission, and the local ethics committee accepted the study procedure.

SAMPLE COLLECTION AND PROCESSING

Four milliliters of venous blood were drawn from each participant and placed in gel tubes. Serum was separated from samples by centrifuging them for 10 minutes at 1500 rpm. The separated serum was then kept at -20°C until analysis.

MATERIALS AND EQUIPMENT

- **Consumables:** Eppendorf tubes (1.5 mL, Eppendorf, Germany), gel tubes (Trust Lab, China), sterile surgical gloves (Himedia, China), syringes (BIO ZEK Medical, Netherlands).
- **Instruments:** ELISA reader and washer (Bio-Tek, USA), incubator (Fisher Scientific, Germany).
- **Kits:** Human Interleukin-8 and Interleukin-12 ELISA kits (Shanghai YL Biotech, China), including pre-coated ELISA plates, standards, buffers, and chromogenic reagents.

INTERLEUKINS-8 AND INTERLEUKINS-12 DETERMINATION

As directed by the manufacturer, a sandwich enzyme- linked immunosorbent assay (ELISA), which was utilized to determine the levels of cytokines. In short, biotin-labeled identification markers and streptavidin–HRP conjugate were added to antibody-coated wells after serum specimens as well as standards were added. Following washing, chromogen solutions A and B were added, and acid was utilized to halt the production of color. A microplate reader was applied to measure the optical density at 450 nm. Normal curves were used to calculate values.

STATISTICAL ANALYSIS

SPSS version 26 was utilized for data analysis. The Shapiro-Wilk and Kolmogorov-Smirnov tests were used to determine normality. The Independent-Samples Mann-Whitney U test on continuous factors and the Chi-square or Fisher's exact test for categorical data were utilized to assess group differences. the p-value less than 0.05 were regarded as being statistically significant.

RESULT

Interleukin 8 (IL-8)

Interleukin 8(IL-8) of this study result shows a significant (P-value = 0.001) mean difference between patients and Healthy. patients had a higher mean (120± 15) ng/L than the Healthy (40±10) ng/L, as shown in Table (1).

Table 1: Mean difference of IL-8 between the study sample with breast cancer and the Healthy.

30-39	120± 15	40±10
40-49	135± 18	45±12
50	150±20	50±14

Interleukin-8 (IL-8) is a proinflammatory cytokine secreted primarily by cancer cells and immune cells in the tumor environment. Higher levels of IL-8 are observed in patients with breast cancer compared to individuals without cancer across all age groups, consistent with its role in promoting tumor growth and spread (metastasis). An increase in the mean IL-8 levels with age among patients, which may indicate greater aggressiveness as age progresses (Roumeguere *et al.,*2018).

high IL-8 levels in breast cancer patients which Enhances cancer cell migration and invasion, contributing to metastasis. Promotes the formation of new blood vessels (angiogenesis), supplying oxygen and nutrients for tumor growth (Abou Shousha *et al.,* 2022). Suppresses the immune system's response against the tumor, allowing it to evade natural immunity. May be associated with resistance to chemotherapy, as some studies have shown that high IL-8 levels reduce tumor response to treatment (David et al.,2016).

INTERLEUKIN 12 (IL-12)

Interleukin 12(IL-12) of this study result shows a significant (P-value = 0.001) mean difference between patients and Healthy. patients had a low mean (25± 5) ng/L than the Healthy (60±10) ng/L, as shown in Table (2).

Table 2: Mean difference of IL-12 between the study sample with breast cancer and the Healthy

Age (years)	IL-12 Mean ± SD in patient with breast cancer	IL-12 Mean ± SD in Non- breast cancer
30-39	25± 5	60±10
40-49	20± 4	55±9
50	15±3	50±8

Mechanisms Underlying the Decrease of IL-12 Levels in Breast Cancer A reduction in IL-12 levels among breast cancer patients reflects a compromised anti-tumor immune response, enabling malignant cells to evade immune surveillance. Several mechanisms contribute to this decline.

First, tumor-mediated immune suppression plays a central role. Breast tumors secrete immunosuppressive cytokines such as interleukin-10 (IL-10) and transforming growth factor-beta (TGF-β), which inhibit IL-12 production by macrophages and dendritic cells (Mirlekar and Pylayeva-Gupta, 2021). Antitumor immunity is compromised by the

development of T cells with regulatory functions (called Tregs and suppressor cells made up of myeloid cells (MDSCs), together increase the production of interleukin-12 (IL-12) (Marigo *et al.,* 2008).

Second, immune cell inability lead to low levels of interleukin-12 (IL-12). Tumor-associated macrophages may be more likely to create interleukin-10 (IL-10), that decreases the availability of IL-12, and dendritic cells can become dysfunctional in the tumor environment (Ruffell *et al.,* 2014).

Third, the transcription factor high-stress-inducible factor one alpha (HIF-1α) is stimulated through high blood pressure and cancer inflammation, ultimately resulting in an inhibition in the expression of interleukin-12 (IL-12) and an increased level in interleukin-10 (IL-10). Additionally, free radicals originated via oxygen that the develop during tumor development prevent immune cells from generating IL-12. (Meng *et al.,* 2018).

Fourth, immune cell damage and bone marrow function decreased set on by therapies for cancer including chemotherapy and radiation therapy can generally end in a decrease in the production of cytokines, including interleukin-12 (IL-12) (Xu *et al.,* 2001).

Fifth, genetic modifications in immune signaling pathways (including mutations affecting NF-κB or STAT4) may affect the transcription of the interleukin-12 (IL-12) gene, and modifications in IL-12 receptor components can influence subsequent signaling if the same cytokines are presented. (Trinchieri, 2003).

At last, that absence of inflammatory stimul ation in the tumor environment inhibits the activatio n of interleukin-12 (IL-12) a synthesis process

The sterile and immunosuppressive environ ment of the tumor further affects immune response by blocking inflammation from functioning as an effe ctive trigger of IL-12 production (Ma *et al.,* 2015).

CONCLUSIONS

1. The results demonstrated that women with cancer of the breast had quite higher quantities of interleukin eight (IL-8) than women without the malignancy. These results indicate that IL-8 is possibly responsible for the stimulation of inflammation and vascular development, two biological processes that assist in the formation and supplying of tumors.
2. Interleukin-12 (IL-12) levels generally significantly decreased in patients, showing an impaired immune reaction targeting the cancerous growth. This decreased effectiveness might cause it even harder for the body to fight off cells that develop cancer.
3. Patients experiencing cancer of the breast indicate obvious immunological impairments. Low concentrations of interleukin-12 (IL-12) are related to a decreased immune response, which can affect the evolution of the sickness and the body's reaction to treatments, whereas interleukin-8 (IL-8) aids in the growth and spread of tumors.

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