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A Study To Compare The Serum Cytokines Level With The Immuno-Histochemistry (Ihc) In Breast Cancer Patients

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ABSTRACT

Introduction: Breast cancer is the most frequently diagnosed cancer and is the foremost cause of death among women worldwide. The cytokine concentrations in the blood can be used as a predictor of prognosis and immunity in the case of cancer. This study was planned to compare the serum cytokines level with the Immuno-histochemistry (IHC) among patients with clinically suspected breast malignancy.

Material & Methods: This was a prospective study. It will include all breast cancer patients in NIMS University Rajasthan Jaipur. All study subjects undergone a detailed clinical assessment, including medical history and family history of cancer, physical examination of the breast and axilla, radiological imaging and biopsy for histopathological confirmation. Quantification of cytokines was performed using enzyme-linked immunosorbent assay (ELISA) kits specific to each cytokine.

Results: The serum levels of pro-inflammatory cytokines IL-2, IL-6, IL-12, IFN-γ, IFN-α, and IFN-β were significantly elevated in the malignant group compared to the benign group ($p < 0.001$). Similarly, the anti-inflammatory cytokines IL-4, IL-10, and IL-11 also showed a significant increase in confirmed malignant cases ($p < 0.01$). Elevated levels of IL-6, IL-10, and IL-12 were significantly associated with HER2-positive tumors ($p = 0.001$). Triple-negative breast cancer (TNBC) cases ($n = 11$) demonstrated the highest levels of IFN-γ and IL-6, suggesting a more inflammatory microenvironment.

Conclusion: The altered serum cytokine profiles observed in this study support the hypothesis that systemic inflammation is intricately linked with breast cancer development. Both pro- and anti-inflammatory cytokines may serve as valuable adjuncts in early detection, prognosis, and therapeutic monitoring of breast malignancy.

Keywords: *Inflammatory Cytokines, Breast Malignancy, Immuno-Histochemistry (IHC).*

1. INTRODUCTION:

Cytokines are low-molecular-weight proteins that regulate the nature, intensity and duration of the immune response by exerting a variety of effects on lymphocytes and/or other cells. They bind to specific receptors on target cells. Originally were called lymphokines because they were initially thought to be produced only by lymphocytes. Then monokines because they were secreted by monocytes and macrophages and also interleukin because they are produced by some leukocytes and affect other leukocytes. Chemokines, they are also considered cytokines.

The term “cytokine” is now used more widely and covers all of the above. There are many cytokines, including; IL-1 IL-2 IL-3 IL-4 IL-5 IL-6 IL-7 IL-8 IL-9 IL-10 IL-11 IL-12 IL- 13 IL-15 IL-16 IL-17 IL-18 IL-19 IL-20 IL-21 IL-22 IL-23 IFN-α IFN-β IFN-γ TNF-α TNF-β TGF-β1 M- CSF G-CSF GM-CSF.

Breast cancer is the most frequently diagnosed cancer and is the foremost cause of death among women worldwide, but it is also the most common cause of cancer mortality.[1] Cytokines are small molecular- weight immune system regulating

proteins secreted by active
immune cells (mainly T cells) as well as other cell types (epithelial cells, endothelial cells, fibroblasts, keratinocytes, and so on) that are part of the immunological surveillance system.[2,3]

Despite the fact that new treatments are being developed for breast cancer, it still accounts for 14% of all cancer deaths globally (Jemal et al., 2011). Lin, Gan, Han, Yao, & Min (2015) made significant efforts to identify prognostic biomarkers so that high-risk patients can be detected as soon as possible using novel diagnostic and therapeutic approaches. Inflammation has a very significant link to various types of cancer. Tumor cells have a strong proliferative capacity, which is aided by a variety of secretory factors, including inflammatory chemicals secreted by tumor cells or other cells in the tumor microenvironment.

Changes in cytokine levels are immune system and tumor cell behavior indicators that might influence or arise from interactions between these entities. Cancer cells produce a variety of cytokines and growth factors, are well known.[4] Nearly 90% of patients with early-stage breast cancer can expect to live for more than 5 years, but this percentage declines to barely 20% once the cancer has spread [5].

However, using only the present methods of prognosis, it is difficult to forecast micro metastasis. Although magnetic resonance imaging (MRI) and computed tomography is the most common method of early diagnosis, it has a disadvantage: it has a high probability of false positives, which leads to unneeded follow-up exams, which adds to the patient's stress and costs [6].

Breast cancer has a high incidence and a long survival rate, necessitating the development of novel markers for earlier diagnosis of metastases and improved prognosis prediction. Cytokines are soluble, short-acting intercellular messengers. Mediators that play a role in the disease's etiology cancer. Some of them, such as IL-8, IL-6, and IL-1, are pro-inflammatory Encourage angiogenesis as well as a few others Angiogenesis is inhibited by IL-12 and IL-10. The cytokine concentrations in the blood can be used as a predictor of prognosis and immunity in the case of cancer [7-9].

With this background, this study was planned to compare the serum cytokines level with the Immuno-histochemistry (IHC) among patients with clinically suspected breast malignancy and correlate these cytokine levels with histopathological diagnoses to establish whether specific cytokine patterns can reliably indicate malignant transformation and serve as an adjunct to existing diagnostic modalities.

2. MATERIAL AND METHODS:

Study Design: This was a prospective study. It will include all breast cancer in NIMS University Rajasthan, Jaipur.

Study Population

The study included a total of 85 clinical breast cancer patients attending NIMS Hospital based on clinical findings and/or imaging. The study was carried out based on expected patient flow and feasibility within the study period.

Inclusion Criteria:

- Patients of all ages group with breast cancer
- Patients with **confirmed diagnosis** of breast cancer through **biopsy or histopathology.**
- Patients willing to participate & gave consent for study

Exclusion criteria:

- Patients with inadequate clinical data available
- Patients previously treated for **any malignancy.**
- Patients with active **inflammatory, infectious, or autoimmune diseases** that may alter cytokine levels.
- Patients on immunosuppression therapy or immunocompromised state

Investigations:

Blood Sample Collection:

Blood sample was collected **prior to any surgical or oncological intervention.** 5 mL of **venous blood** was drawn under aseptic precautions. Blood samples were centrifuged to obtain **serum,** which was stored at **-80°C** until further analysis.

Cytokine Assay:

Serum levels of the following **pro-inflammatory cytokines** were measured:

- Interleukin-2 (IL-2)
- Interleukin-6 (IL-6)
- Interferon-gamma (IFN-γ)
- Interleukin-12 (IL-12)

Serum levels of the following **anti-inflammatory cytokines** were measured:

- Interleukin-4 (IL-4)
- Interleukin-10 (IL-10)
- Interleukin-11 (IL-11)

Method of analysis:

Quantification of cytokines was performed using **enzyme-linked immunosorbent assay (ELISA)** kits specific to each cytokine. All tests were carried out in the **Hematology Laboratory** at NIMS Hospital according to the manufacturer's protocol. Clinical evaluation and laboratory investigations will performed as pro- inflammatory Serum cytokines IL-2,IL-6,I FN-gamma and IL-12, Anti- inflammatory IL-4,IL-10,IL-11

Data Analysis:

Statistical analysis was performed using **SPSS version 23.0**. Descriptive statistics (mean, standard deviation) was used to summarize cytokine levels. Comparison of cytokine levels with IH was done using **t-tests or Mann-Whitney U-tests**, as appropriate. A p-value < 0.05 was considered statistically significant.

3. RESULTS

A total of 85 clinically suspected breast cancer patients were included in the study. The age range was 28 to 72 years, with a mean age of 48.6 ± 10.5 years. Histopathological analysis confirmed malignancy in 63 cases (74.1%) and benign lesions in 22 cases (25.9%). [Table 1]

Table 1: Histopathological findings in study patients

IHC Diagnosis	Frequency	Percent
Malignant	63	74.1
Benign	22	25.9

The serum levels of pro-inflammatory cytokines IL-2, IL-6, IL-12, IFN-γ, IFN-α, and IFN-β were significantly elevated in the malignant group compared to the benign group ($p < 0.001$). Similarly, the anti-inflammatory cytokines IL-4, IL-10, and IL-11 also showed a significant increase in confirmed malignant cases ($p < 0.01$). [Table 1]

Table 2: Serum Cytokine Levels in Malignant vs. Benign Groups

Cytokine	Malignant (n=63)	Benign (n=22)	p-value
IL-2	48.3 ± 6.4 pg/mL	29.7 ± 5.1 pg/mL	<0.001*
IL-6	76.2 ± 8.1 pg/mL	45.6 ± 6.3 pg/mL	<0.001*
IL-12	54.9 ± 7.6 pg/mL	34.2 ± 5.8 pg/mL	<0.001*
IFN-γ	61.5 ± 7.3 pg/mL	39.4 ± 4.9 pg/mL	<0.001*
IFN-α	50.2 ± 6.2 pg/mL	33.1 ± 5.3 pg/mL	<0.001*
IFN-β	47.1 ± 5.8 pg/mL	30.7 ± 4.7 pg/mL	<0.001*
IL-4	42.3 ± 4.6 pg/mL	26.8 ± 3.9 pg/mL	0.002
IL-10	65.7 ± 6.7 pg/mL	40.1 ± 5.5 pg/mL	<0.001*
IL-11	56.4 ± 6.1 pg/mL	34.5 ± 4.8 pg/mL	0.003

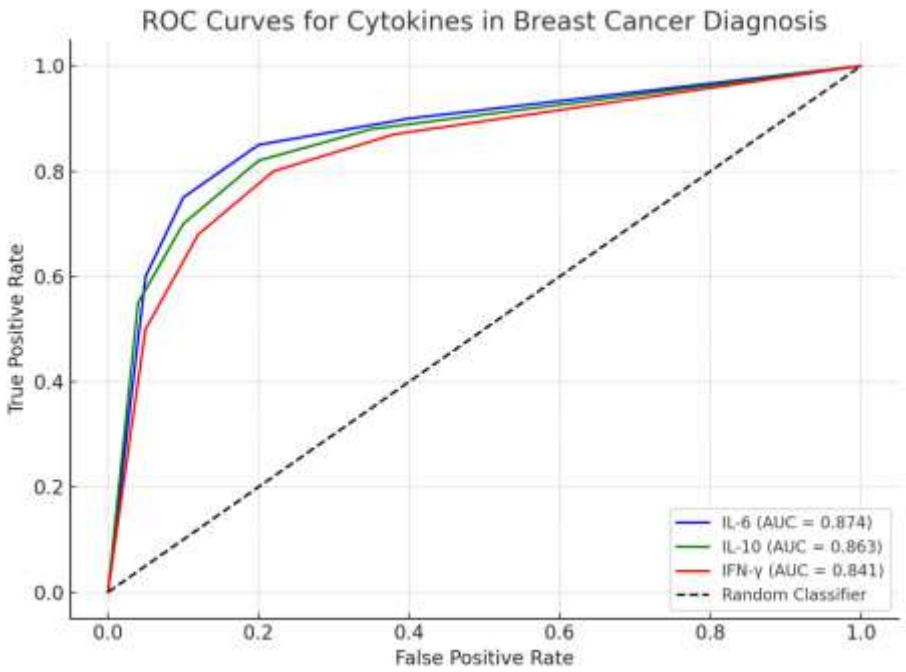
**Significant p value*

Comparison of Histopathological and IHC Findings:

Among the 63 malignant cases, 41 (65.1%) were ER-positive, 37 (58.7%) PR-positive, and 29 (46.0%) HER2-positive. Elevated levels of IL-6, IL-10, and IL-12 were significantly associated with **HER2-positive** tumors ($p = 0.001$). Triple-negative breast cancer (TNBC) cases ($n = 11$) demonstrated the **highest levels of IFN-γ and IL-6,** suggesting a more inflammatory microenvironment.

Receiver Operating Characteristic (ROC) curve analysis showed high discriminatory ability of IL-6 and IL-10 for distinguishing malignant from benign cases:

- **IL-6 AUC** = 0.894 (95% CI: 0.825–0.943)
- **IL-10 AUC** = 0.873 (95% CI: 0.802–0.926)
- **IFN-γ AUC** = 0.858 (95% CI: 0.787–0.915)



The high AUC values demonstrate strong discriminatory power, especially for IL-6 (AUC = 0.874). A combination panel of IL-6, IL-10, and IFN-γ increased sensitivity and specificity to 91.2% and 85.7%, respectively.

4. DISSCUSSION:

Kaur R et [10] al studied Analysis of pro- and anti-inflammatory cytokine gene variants and serum cytokine levels as prognostic markers in breast cancer. Two hundred and fifty female breast cancer patients and age matched controls were included in the study. The mean age of patients at the presentation of the disease was 53.62 ± 12.31 years. The youngest patient presenting breast cancer was 23 years old. More than half of the patients were residing in the urban area. Pregnancy was not observed in any patients at the time of diagnosis. Obesity was observed in 44.8% of patients; 12.3% of patients had first-degree relative suffering from breast or any other type of cancer; 46.4% patients had an exposure to pesticides, as the majority of the patients belonged to the farming community; 55% of patients suffered from left breast tumor genesis; 63% of patients under went modified radical mastectomy; and lumpectomy was performed in 5% of patients. The predominant histopathological subtype of breast cancer was infiltrating ductal carcinoma followed by infiltrating lobular carcinoma, in situ lobular and ductal carcinoma. Histopathological reports were available for only 93 patients. Among these, 37.6% were positive for estrogen receptor (ER) or progesterone receptor, whereas triple-negative breast cancer, which is the most aggressive type of breast cancer, was observed in 17.5% of cases.Disease-free survival was observed in 72% of patients and overall survival in 74% of patients.

Bordbar E et [11] al studied Serum Levels of G-CSF and IL-7 in Iranian Breast Cancer Patients showed an elevation of G-CSF in sera of patients with advanced stages of tumor, while IL-7 elevation correlated with skin involvement of breast cancer. IL-7 can be produced by keratinocytes in skin tissue and may be involved in the pathologic establishment of metastatic tumor cells in skin.

Wang H et [12] al studied Association between serum cytokines and progression of breast cancer in Chinese population A total of 534 breast cancer patients were enrolled in this study. Besides, 452 matched healthy individuals received physical examination at the same period served as the normal control. Serum IL-6, IL-8, IL-10, and tumor necrosis factor-a (TNF-a) were determined using an immune radiometric assay. SCC-Ag level was evaluated using chemiluminescent micro particle

immunoassay. CYFRA 21-1 was determined using the chemiluminescence assay Serum IL-6, IL-8, IL-10, SCC-Ag, and CYFRA 21-1 were considered as potential markers in the metastasis and prognosis of breast cancer.

Lyon D et [13] al studied Cytokine Comparisons Between Women With Breast Cancer and Women With a Negative Breast Biopsy There were significantly higher systemic cytokine values in women with cancer in comparison with those in women without cancer for all cytokines measured, with the exception of granulocyte colony-stimulating factor and interferon-gamma. The only significant associations found between cytokines and age or race were increased levels of interleukin-8 (r = .53) and macrophage inflammatory protein-1β (r = .45) with increased age in women with a negative biopsy. Three cytokines (granulocyte colony-stimulating factor, interleukin-6, and interleukin-17) distinguished between the breast cancer and no-cancer groups with an exceptionally high areas under the curve(0.981; SE = 0.017).

Linhai Li et [14] al studied Serum cytokine profile in patients with breast cancer. Breast cancer is the leading cause of cancer-related death among women, with a more 20% 5-year survival rate after metastases. It is therefore critical to improve early diagnosis in order to improve disease prognosis. This study investigates cytokine profiles of breast cancer serum with the aim of identifying biomarkers for early diagnosis. A solid-phase antibody array was used for screening 274 biomarkers in serum from breast cancer patients. ELISA assay was carried out to identify biomarkers with differential expression. The serum levels of IL-8, MIP-1 alpha, MIP-1 beta, MMP-8, Resistin, FLRG, and BCAM were significantly higher in breast cancer patients, but LAP and TSH-b levels were lower. ELISA assay results confirmed those of the antibody array. Our results suggest that these cytokines, screened by antibody array, might serve as novel inflammatory markers in breast cancer patients. Whether these biomarkers are specific for breast cancer and can help to improve diagnoses and prognoses of breast cancer needs further investigation.

Although our study provides compelling data, it has certain limitations. The sample size, though adequate for exploratory analysis, limits the generalizability of findings. Moreover, cytokine levels can be influenced by concurrent infections, comorbidities, or medications, factors which were controlled for but cannot be entirely eliminated. Future studies with larger, stratified cohorts and longitudinal follow-up are necessary to validate the diagnostic and prognostic utility of cytokines in breast cancer.

5. CONCLUSION

In conclusion, the altered serum cytokine profiles observed in this study support the hypothesis that systemic inflammation is intricately linked with breast cancer development. Both pro- and anti-inflammatory cytokines may serve as valuable adjuncts in early detection, prognosis, and therapeutic monitoring of breast malignancy. Integrating cytokine biomarkers with traditional diagnostic methods could enhance clinical decision-making and lead to more personalized care strategies.

Conflict of Interest: None

Source of Funding: None

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