Evaluation of Some Inflammtory Proteins and Immunological Parameters in Breast Cancer Patients Attending Oncology Clinic at American Cancer Hospital, Iho, Imo State

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Abstract

Breast cancer is associated with complex alterations in inflammatory and immunological parameters,. This study aimed to evaluate inflammatory proteins, immunological parameters in breast cancer patients attending the Oncology Clinic at American Cancer Clinic, Iho. The study population comprised seventy-five (75) histologically confirmed breast cancer patients and seventy-five (75) apparently healthy individuals without any known malignancy who served as the control group. Informed and written consent was sought and obtained from prospective study participants who are eligible for the study before collecting their samples. The blood samples were collected in plain tubes and serum obtained from the clotted blood was analyzed for CRP, IL-6, IgM, and IgG using appropriate immunoassay techniques. Data obtained in this study was analyzed using Statistical Package for Social Sciences (SPSS) version 21. The student independent t-test and one-way ANOVA were used to compare means across groups. Results were expressed as Mean ± standard deviation. Pearson's correlation coefficient was employed to assess associations among parameters. Values with $p<0.05$ were considered statistically significant. The results showed that the mean values of CRP, IL-6, IgM and IgG were significantly higher ($p=0.001$) in newly diagnosed breast cancer patients (28.37 ± 9.57 mg/L, 35.69 ± 13.89 pg/mL, 1.84 ± 0.40 g/L, 14.89 ± 1.13 g/L) when compared to those on chemotherapy (20.97 ± 6.93 mg/L, 27.59 ± 7.64 pg/mL, 1.14 ± 0.22 g/L, 12.38 ± 0.93 g/L), post-surgery breast cancer patients on chemo (15.28 ± 4.76 mg/L, 15.00 ± 4.63 pg/mL, 1.12 ± 0.32 g/L, 11.04 ± 1.35 g/L) and controls (9.27 ± 8.16 mg/L, 8.51 ± 11.38 pg/mL, 1.61 ± 0.46 g/L, 13.19 ± 1.41 g/L). In conclusion, breast cancer is associated with significant alterations in inflammatory and immunological profiles, especially in newly diagnosed patients due to immune deregulations likely linked to tumor progression and treatment response but improved with chemotherapy and surgery.

Keywords

Breast Cancer, Inflammorty Proteins, Immunological Parameters, Oncology Clinic

1. Introduction

A major contributor to the worldwide cancer burden, breast cancer remains the most often diagnosed cancer and one of the leading causes of cancer-related mortality for women worldwide. This complicated disease has significant biochemical, genetic, and clinical heterogeneity, which leads to variations in prognosis and responsiveness to treatment. Breast cancer originates from the epithelial cells lining the breast tissue's ducts (ductal carcinoma) or lobules (lobular carcinoma). The normal regulatory mechanisms that govern cell division, proliferation, and death are disrupted by the genetic and epigenetic alterations in these cells. As a result, these mutated cells proliferate unrestrained, penetrate nearby tissues, and often move to distant organs such as the liver, lungs, brain, and bones. Breast cancer can develop and spread due to a variety of reasons, including lifestyle decisions, environmental exposures, hormonal imbalances, and genetic predispositions (such BRCA1 and BRCA2 mutations). Thanks to developments in molecular profiling, breast cancer is now divided into several subtypes, such as hormone receptor-positive, HER2-positive, and triple-negative breast cancers, each having unique therapy implications and clinical outcomes. Despite improvements in early detection, targeted therapy, and personalised medicine, breast cancer still poses serious public health challenges, particularly in low- and middle-income countries where access to screening and care is still limited [1].

With an anticipated 2.3 million new cases and 685,000 deaths in 2020 alone, breast cancer is the most common disease diagnosed and the leading cause of cancer-related mortality among women globally [2]. Recent epidemiological trends indicate that breast cancer is becoming prevalent in low- and middle-income regions, especially sub-Saharan Africa, despite the long-held belief that the disease mostly affected women in wealthier countries. This phenomenon is attributed to lifestyle changes, urbanisation, higher life spans, and changing reproductive patterns. In Nigeria, breast cancer is currently the most prevalent cancer among women and a major contributor to cancer-related sickness and mortality. The 5-year survival rate for Nigerian women with breast cancer is still shockingly lower than that reported in affluent nations, largely due to late-stage presentation, limited access to screening programs, delayed diagnosis, and inadequate treatment infrastructure [3].

The multifactorial aetiology of breast cancer is characterised by a complex interplay among genetic predispositions, hormonal influences, environmental exposures, and epigenetic changes. Traditional risk factors include obesity, hormone replacement treatment use, nulliparity, early menarche, late menopause, and a family history of breast cancer. In recent years, there has been increased interest in the roles that chronic inflammation and the immune system play in carcinogenesis. Chronic inflammation is now widely recognised as a feature of cancer, contributing to a pro-tumorigenic environment through immune evasion, DNA damage, genomic instability, and angiogenesis. [4] Immuno-inflammatory responses in breast cancer are coordinated by a complex network of immune cells, including T lymphocytes, neutrophils, dendritic cells, and macrophages, as well as soluble mediators, such as prostaglandins, cytokines, chemokines, and acute-phase reactants. By constantly changing the tumour microenvironment, these factors influence the initiation, growth, and metastasis of tumours. Tumor-associated macrophages (TAMs) and regulatory T cells (Tregs) in particular have been shown to impair anti-tumor immune responses and encourage tumour immune evasion, resulting in worse than ideal clinical results.

Our increasing knowledge of the molecular relationships between the immune system and cancer cells has enabled new approaches to targeted therapies, such as immunotherapy and anti-inflammatory strategies. Determining immunological biomarkers and describing the inflammatory milieu in breast tissue.

Hepatocytes mainly produce C-reactive protein (CRP), a well-known classical acute-phase reactant, in response to pro-inflammatory cytokines, especially interleukin-6 (IL-6), interleukin-1β (IL-1β), and tumour necrosis factor-alpha (TNF-α) [5]. Acute phase reactants are proteins whose plasma concentrations increase (positive acute-phase proteins) or decrease (negative acute-phase proteins) in response to inflammation. The innate immune response depends on CRP, a sensitive measure of systemic inflammation, which stimulates opsonisation, complement activation, and phagocytosis. In the context of cancer, elevated circulating CRP levels are increasingly recognised as indicators of tumor-associated inflammation and have been linked to worse clinical outcomes.

Elevated CRP levels in breast cancer have been frequently associated with more aggressive disease phenotypes, advanced tumour stages, and poorer overall survival, making them a possible prognostic biomarker [6]. The increased CRP in breast cancer patients is thought to be a reflection of both the tumour load and the systemic inflammatory state that the tumour microenvironment orchestrates. By promoting angiogenesis, cancer cell proliferation, immune evasion, and metastasis, this pro-inflammatory condition makes the disease worse. Moreover, elevated CRP may indicate underlying host-tumor interactions, including immune cell activation and recruitment as well as tumor-associated macrophage recruitment, which promote cancer progression.

Because of its clinical importance, CRP is being researched as a potential therapeutic target in oncology as well as a prognostic tool. Monitoring CRP levels may aid in the evaluation of medication response, disease progression, and patient stratification based on inflammatory status for tailored treatment plans. Further study is required to elucidate the precise molecular relationships among CRP, tumour biology, and immune modulation in breast cancer [7].

A multipurpose cytokine, interleukin-6 (IL-6) is essential for haematopoiesis, immunological control, and inflammation. By triggering downstream signalling pathways like STAT3 and NF-κB, IL-6 stimulates tumour development, angiogenesis, and resistance to apoptosis in breast cancer. Aggression and metastasis of the disease have been associated with elevated levels of circulating IL-6 [8].

The humoral immune response depends heavily on immunoglobulins, especially immunoglobulin G (IgG) and immunoglobulin M (IgM). Complement activation and pathogen neutralisation are two processes that are aided by IgM, the first antibody generated in response to antigen exposure. The most prevalent immunoglobulin, IgG, promotes opsonisation and offers long-term immunity. Immunoglobulin level changes in cancer may be a sign of tumor-induced immune modulation or immunological failure. In individuals with breast cancer, elevated or decreased IgG and IgM levels may indicate an imbalance in immune surveillance and tumour growth [9].

Evaluating these biomarkers may help with diagnosis, prognosis, and therapy monitoring while providing information on the immunological and inflammatory environment of breast cancer. In order to ascertain their potential usefulness in clinical therapy, particularly in areas with limited resources, this study aims to assess CRP, IL-6, IgM, IgG, and haematological markers in patients with breast cancer.

With disproportionately high death rates in low- and middle-income nations, breast cancer remains a major global public health concern. The late discovery and diagnosis of the disease, which frequently results from insufficient screening programs, low awareness, and restricted access to diagnostic facilities, is a significant factor to this mortality. The majority of breast cancer patients in Nigeria are discovered at an advanced stage, with few available treatments and poor prognoses [10].

In many countries with limited resources, the incorporation of immunological and haematological indicators in routine breast cancer evaluation is still poor, despite advancements in oncological therapy. The potential of inflammation-related biomarkers including CRP, IL-6, and immunoglobulins to enhance early detection, disease monitoring, and prognosis in patients with breast cancer is not fully realised, and their therapeutic value is understudied. Despite their predictive importance, haematological markers are frequently disregarded in cancer evaluation, even though they are usually available.

Information about the immuno-hematological characteristics of patients with breast cancer is scarce in the setting of the American Cancer Clinic, Iho. This disparity emphasises how important it is to thoroughly assess these biomarkers in order to improve patient care and results [11].

Finding trustworthy, easily available, and reasonably priced biomarkers for breast cancer detection and tracking is crucial, especially in environments with limited resources. Given that they represent tumour biology and the host immune response, inflammation-immune indicators including CRP, IL-6, and immunoglobulins present a viable path. Numerous studies have shown how useful these markers are for diagnosis and prognosis. For example, higher CRP levels have been linked to a higher death rate among Danish patients with breast cancer. Likewise, worse clinical outcomes for Chinese patients with breast cancer have been associated with increased IL-6 levels [12].

Studies conducted in different contexts have also brought attention to changes in immunoglobulin profiles in patients with breast cancer, suggesting a possible role for these changes in immune surveillance and disease progression.

However, there is a lack of localised research, especially in Imo State, Nigeria, that thoroughly assesses these parameters in patients with breast cancer. There are many breast cancer patients treated at the American Cancer Clinic in Iho, but nothing is known about their immunological status. Therefore, the purpose of this study is to assess the levels of CRP, IL-6, immunoglobulin M, and G, parameters in breast cancer patients attending the oncology clinic at American Cancer Clinic, Iho, in order to close this knowledge gap and provide baseline data that may guide clinical practice and future research.

2. Materials and Methods

Study Area

The study was conducted at the American Cancer Clinic, Iho, located in Ikeduru Local Government Area of Imo State, Nigeria. The clinic is managed by the International Health Care Consulting in the United States of America (USA). It operates as a public-private partnership (PPP) venture with the Imo International Health System (IIHS). The American Cancer Clinic offers comprehensive cancer treatment and care services, including detoxification and rejuvenation, radiation therapy, chemotherapy, surgery, and palliative care. The facility serves as a referral center for cancer patients within and outside the state.

2.1 Ethics Advocacy and Pre-Survey Contact

The Ethical clearance was obtained from American Cancer Clinic, Iho. Informed consent was obtained from all study participants after explaining the study's objectives, procedures, risks, and benefits.

2.2 Study Population

The sample size for the study will be calculated using the formular below and this is according to [13].

$$n = \frac{z^2 pq}{d^2}$$

n =desired sample size

z =the standard deviation usually set at 95%=1.96

p =the prevalence of patients with breast cancer 5.0

$q = 1 - p$

d =degree of accuracy set at 0.05

According to Anele *et al*. (2014), the prevalence of breast cancer in Imo State is 5.0%.

$$n = \frac{1.96^2 \times 0.05 \times (1 - 0.05)}{0.05^2}$$

$$n = \frac{3.814 \times 0.05 \times 0.95}{0.0025} = \frac{0.18248}{0.0025} = 72.99 \approx 73$$

$n = 73$

The minimum sample size would be 73.

A total of one hundred and fifty (150) individuals between the ages of twenty-five (25) and seventy (70) years were recruited for this study. Seventy-five (75) of the participants were histologically confirmed breast cancer patients attending the Oncology Clinic at the American Cancer Clinic, Iho, while the remaining seventy-five (75) were apparently healthy individuals without any known malignancy, and they served as the control group.

3. Selection Criteria

Inclusion Criteria

Participants included in the study met the following criteria:

i. Female subjects within the ages of 25 and 70 years.

ii. Breast cancer patients confirmed by histopathological diagnosis.

iii. Individuals who voluntarily provided informed consent for participation in the study.

iv. Apparently healthy female individuals of the same age range with no history of malignancy served as control.

Exclusion Criteria

i. Subjects below 25 years or above 70 years.

ii. Subjects diagnosed with other chronic illnesses such as autoimmune, infectious, or hematological disorders, HIV/AIDS, or other forms of carcinoma.

iii. Pregnant women or women with recent blood transfusion.

iv. Subjects who declined to give informed consent.

3.1 Study Design

This was a hospital-based cross-sectional study carried out between April and June 2025 and all eligible individuals who filled the questionnaire and gave a written informed consent for the study period were sampled. Participants were recruited consecutively from the clinic during scheduled outpatient visits. The study consisted of two groups. Group 1 comprised seventy-five (75) histologically confirmed breast cancer patients attending the Oncology Clinic at the American Cancer Clinic, Iho, while Group 2 comprised seventy-five (75) apparently healthy individuals without any known malignancy, and they served as the control group. The breast cancer group was further categorized into: Group A: 25 newly diagnosed breast cancer patients not yet initiated on any treatment; Group B: 25 breast cancer patients currently undergoing chemotherapy; and Group C: 25 breast cancer patients who had undergone surgical resection and were being maintained on chemotherapy. All participants were sampled only once and blood samples were collected under standard aseptic conditions to evaluate the levels of C-reactive Protein, immunoglobulin M, immunoglobulin G, and interleukin-6.

3.2 Sample Collection

Five milliliters (5 mL) of venous blood was aseptically collected from the ante-cubital vein of each participant using a sterile disposable 10 mL syringe and needle through venipuncture. The blood was dispensed into a plain tube. Each tube was labeled with the participant's name, sample number, and date of collection. The blood in the plain tube was allowed to clot and then centrifuged at 3000 rpm for 5 minutes to separate the serum, which was stored at -20°C until analyzed for CRP, IgM, IgG, and IL-6 levels. All assays were completed within 48 hours of sample collection to ensure reliable and accurate results.

3.3 Laboratory Procedures

All reagents and kits used were commercially purchased and the manufacturer’s standard operating procedures were strictly followed.

A. Determination of C-reactive protein (CRP)

The test was done by latex-enhanced Immunoturbidimetric method as modified by Agape diagnostics limited, India (Lot number: 32308474).

Principle

This is a latex-enhanced turbidimetric immunoassay. CRP in the sample binds to specific anti-CRP antibodies that have been adsorbed onto latex particles, leading to agglutination. The extent of agglutination is directly proportional to the concentration of CRP in the sample. The actual CRP concentration is determined by interpolation from a calibration curve prepared using calibrators of known concentrations.

Procedure

The determination of C-reactive protein (CRP) using a semi-auto analyzer involved the addition of 450 µL of CRP R1 reagent into two cuvettes designated for the calibrator and the test sample or control, respectively. Into the calibrator cuvette, 5 µL of diluted calibrator was added, while 5 µL of the test sample or control was added into the corresponding cuvette. Subsequently, 150 µL of CRP R2 reagent was added to both cuvettes. The mixtures were thoroughly mixed, and absorbance readings were taken immediately (A1) and again after 2 minutes (A2) at 578 nm.

Calculation

The change in absorbance (A2 - A1) was calculated for each calibrator point. These values were used to construct a standard curve by plotting delta absorbance against known CRP concentrations. The concentrations of the test samples and controls were determined by interpolating their delta absorbance values from the standard curve.

Reference Range

CRP: < 10.0 mg/L

B. Determination of Immunoglobulin M (IgM)

The test was done by Immunoturbidimetric method as modified by Biosino Bio-technology and Science Inc., China (Catalogue number: 000109015).

Principle

Immunoglobulin M (IgM) in the test sample reacts with special antibody in the assay reagents, forming undissolved compounds and turbidity in the reaction solution. When the quantity of antibody is fixed, the resulting turbidity is determined by the concentration of IgM in the sample at the test wavelength.

Procedure

The assay was carried out at a wavelength of 340 nm, a temperature of 37°C, and using a 1 cm cuvette. Three tubes were prepared: blank, calibrator, and sample. To the blank tube, 0.05 ml of distilled water was added; to the calibrator tube, 0.05 ml of calibrator; and to the sample tube, 0.05 ml of the test sample. Then, 2.5 ml of reagent R1 was added to each of the tubes. The contents were mixed thoroughly and incubated at 37°C for 5 minutes. After incubation, the spectrophotometer was set to zero using the blank tube, and absorbance readings of the calibrator and sample tubes (A1) were taken. Next, 0.85 ml of reagent R2 was added to all tubes, mixed thoroughly, and incubated again at 37°C for 5 minutes. Final absorbance readings (A2) were taken, setting the blank as zero. The IgM concentration in the sample was calculated using the absorbance values.

Calculation

The absorbance difference (A2 - A1) was calculated for each calibrator dilution point. These values were then plotted against the corresponding IgM concentrations of the calibrators to generate a calibration curve. The IgM concentration in the test sample was determined by calculating its absorbance difference (A2 - A1) and interpolating this value on the calibration curve.

C. Determination of Immunoglobulin G (IgG)

The test was done by Immunoturbidimetric method as modified by Biosino Bio-technology and Science Inc., China (Catalogue number: 000109005).

Principle

Immunoglobulin G (IgG) in the test sample reacts with special antibody in the assay reagents, forming undissolved compounds and turbidity in the reaction solution. When the quantity of antibody is fixed, the resulting turbidity is determined by the concentration of IgG in the sample at the test wavelength.

Procedure

The assay was carried out at a wavelength of 700 nm, a temperature of 37°C, and using a 1 cm cuvette. Three tubes were prepared: blank, calibrator, and sample. To the blank tube, 0.02 ml of distilled water was added; to the calibrator tube, 0.02 ml of calibrator; and to the sample tube, 0.02 ml of the test sample. Then, 2.5 ml of reagent R1 was added to each of the tubes. The contents were mixed thoroughly and incubated at 37°C for 5 minutes. After incubation, the spectrophotometer was set to zero using the blank tube, and absorbance readings of the calibrator and sample tubes (A1) were taken. Next, 0.85 ml of reagent R2 was added to all tubes, mixed thoroughly, and incubated again at 37°C for 5 minutes. Final absorbance readings (A2) were taken, setting the blank as zero. The IgG concentration in the sample was calculated using the absorbance values.

Calculation

The absorbance difference (A2 - A1) was calculated for each calibrator dilution point. These values were then plotted against the corresponding IgG concentrations of the calibrators to generate a calibration curve. The IgG concentration in the test sample was determined by calculating its absorbance difference (A2 - A1) and interpolating this value on the calibration curve.

D. Determination of Interleukin-6 (IL-6)

The test was done by Sandwich Enzyme-Linked Immunosorbent Assay (ELISA) method as modified by MyBioSource, Inc., USA (Catalogue number: MBS8506129096).

Principle

The assay for IL-6 was based on the quantitative sandwich enzyme immunoassay technique. In this method, a monoclonal antibody specific to IL-6 had been precoated onto a microplate. Standards and samples were pipetted into the wells, where any IL-6 present in the samples bound to the immobilized capture antibodies. After an incubation period, unbound substances were removed through a series of washing steps. A biotin-conjugated detection antibody specific for IL-6 was then added, which bound to the captured IL-6. Following another wash, a streptavidin-horseradish peroxidase (HRP) conjugate was introduced to bind the biotin-labeled detection antibody. After incubation and washing to remove any unbound enzyme conjugate, a substrate solution was added. The HRP catalyzed a color change in the substrate in proportion to the amount of IL-6 present in the sample. The reaction was stopped with the addition of an acidic stop solution, and the absorbance was measured at 450 nm using a microplate reader. A standard curve was generated from the known concentrations of IL-6 standards, and the concentration of IL-6 in each sample was extrapolated from this curve.

Procedure

All reagents and standards were prepared according to the manufacturer's instructions. The required number of microwell strips was determined based on the number of test samples and the standards. One hundred microliters (100 µL) of standards, controls, and serum samples were added into appropriate wells of the microplate. The plate was covered with an adhesive strip and incubated for 1.5 hours at 37°C. After incubation, the contents of each well were aspirated, and the wells were washed four times using 350 µL of wash buffer per wash. Complete removal of wash buffer was ensured by inverting the plate and blotting on absorbent paper.

Following the washes, 100 µL of the biotin-conjugate working solution was added to each well. The plate was covered again and incubated for 1 hour at 37°C. Another washing step was performed as before. Subsequently, 100 µL of streptavidin-HRP working solution was dispensed into each well, and the plate was incubated for 30 minutes at 37°C, avoiding direct light exposure. The wells were then washed again four times to remove unbound enzyme conjugate. Thereafter, 100 µL of substrate solution was added to each well and incubated for 10–20 minutes at 37°C, again protected from light. A yellow-colored product developed in proportion to the IL-6 concentration. Finally, 100 µL of stop solution was added to each well, and the plate was gently tapped to mix thoroughly. The optical density (OD) of each well was measured immediately at 450 nm using a microplate reader.

Calculation

The mean absorbance readings of duplicate standards, controls, and test samples were calculated. The average optical density value of the zero standard was subtracted from all other readings. A standard curve was constructed by plotting the mean absorbance values of the IL-6 standards on the y-axis against their corresponding concentrations on the x-axis. A four-parameter logistic (4PL) curve fitting method was used to generate the standard curve. For samples that were diluted, the calculated IL-6 concentrations were multiplied by the dilution factor. The IL-6 concentrations in the test samples were then extrapolated from the curve and expressed in picograms per milliliter (pg/mL).

Statistical Analysis

Data obtained in this study were analyzed using Statistical Package for Social Sciences (SPSS) version 21.0. The student independent T-test and one-way ANOVA were used to determine mean differences across groups. Results were expressed as Mean ± standard deviation. Pearson's correlation coefficient was employed for correlation analyses among parameters. Test with probability of $p<0.05$ was considered statistically significant.

4. Results

Table 1 presents the mean ± standard deviation, ANOVA, and post-hoc comparison values of inflammatory markers (CRP and IL-6) among newly diagnosed breast cancer patients, patients currently on chemotherapy, post-surgery breast cancer patients receiving chemotherapy, and apparently healthy individuals.

There were significant differences in the mean values of CRP and IL-6 across the four groups ($p = 0.001$). Post-hoc analysis revealed that the mean ± SD value of CRP in newly diagnosed breast cancer patients (28.37 ± 9.57 mg/L) was significantly higher ($p = 0.006$) than those on chemotherapy (20.97 ± 6.93 mg/L), and also significantly higher ($p = 0.000$) when compared to post-surgery patients on chemotherapy (15.28 ± 4.76 mg/L) and healthy controls (9.27 ± 8.16 mg/L). Likewise, CRP levels in patients on chemotherapy (20.97 ± 6.93 mg/L) were significantly higher ($p = 0.000$) than those of healthy controls (9.27 ± 8.16 mg/L), though the difference between patients on chemotherapy (20.97 ± 6.93 mg/L) and post-surgery patients on chemotherapy (15.28 ± 4.76 mg/L) was not statistically significant ($p = 0.064$). However, CRP in post-surgery patients on chemotherapy (15.28 ± 4.76 mg/L) remained significantly elevated ($p = 0.006$) compared to the healthy control group (9.27 ± 8.16 mg/L).

Similarly, IL-6 levels followed a comparable trend. The mean ± SD value of IL-6 in newly diagnosed patients (35.69 ± 13.89 pg/mL) was significantly higher ($p = 0.043$) than in those on chemotherapy (27.59 ± 7.64 pg/mL), and significantly higher ($p = 0.000$) than in post-surgery chemotherapy patients (15.00 ± 4.63 pg/mL) and healthy individuals (8.51 ± 11.38 pg/mL). IL-6 was also significantly increased ($p = 0.000$) in chemotherapy patients (27.59 ± 7.64 pg/mL) compared to healthy controls (8.51 ± 11.38 pg/mL), and in patients on chemotherapy

(27.59 ± 7.64 pg/mL) when compared with post-surgery chemotherapy patients (15.00 ± 4.63 pg/mL) (p = 0.000). However, IL-6 levels between post-surgery patients on chemotherapy (15.00 ± 4.63 pg/mL) and healthy controls (8.51 ± 11.38 pg/mL) showed borderline significance (p = 0.050).

Table 1. Mean ± Standard Deviation, ANOVA and Post-Hoc Values of Inflammatory Markers in Newly Diagnosed Breast Cancer Patients, Patients on Chemotherapy, Post-Surgery Patients on Chemotherapy and Apparently Healthy Individuals.

Group	Variables (Units)	
	CRP (mg/L)	IL-6 (pg/mL)
Newly Diagnosed (A) (n=25)	28.37 ± 9.57	35.69 ± 13.89
On Chemotherapy (B) (n=25)	20.97 ± 6.93	27.59 ± 7.64
Post-Surgery + Chemo (C) (n=25)	15.28 ± 4.76	15.00 ± 4.63
Healthy Control (D) (n=75)	9.27 ± 8.16	8.51 ± 11.38
F-value	43.015	51.381
P-value	*0.001	*0.001
A Vs B	*0.006	*0.043
A Vs C	*0.000	*0.000
A Vs D	*0.000	*0.000
B Vs C	0.064	*0.000
B Vs D	*0.000	*0.000
C Vs D	*0.006	0.050

Key: CRP=C-reactive protein, IL-6=Interleukin-6, Vs=Versus, n=Sample size, mg/L=milligrams per liter, pg/mL=picograms per milliliter, *=Statistically significant at P≤0.05.

Table 2 shows the mean ± standard deviation, ANOVA and Post-hoc values of immunological markers (IgM and IgG) in newly diagnosed breast cancer patients, patients on chemotherapy, post-surgery patients on chemotherapy, and apparently healthy individuals.

There were significant differences in the mean values of IgM and IgG among the various groups (p = 0.001 for both). Specifically, the mean ± SD value of IgM (1.84 ± 0.40 g/L) in newly diagnosed breast cancer patients was significantly higher (p = 0.000) than in patients on chemotherapy (1.14 ± 0.22 g/L) and post-surgery patients on chemotherapy (1.12 ± 0.32 g/L), but showed no significant difference (p = 0.082) when compared with the healthy control group (1.61 ± 0.46 g/L). The mean IgM level in patients on chemotherapy (1.14 ± 0.22 g/L) was significantly lower (p = 0.000) than that of the healthy control group (1.61 ± 0.46 g/L) but not significantly different (p = 1.000) from post-surgery chemotherapy patients (1.12 ± 0.32 g/L). Similarly, post-surgery chemotherapy patients had significantly lower IgM levels (1.12 ± 0.32 g/L) compared to the healthy control group (p = 0.000).

For IgG, the mean ± SD value in newly diagnosed patients (14.89 ± 1.13 g/L) was significantly higher (p = 0.000) than in those on chemotherapy (12.38 ± 0.93 g/L), post-surgery chemotherapy patients (11.04 ± 1.35 g/L), and the healthy control group (13.19 ± 1.41 g/L). The mean IgG level in patients on chemotherapy (12.38 ± 0.93 g/L) was also significantly higher than in post-surgery chemotherapy patients (11.04 ± 1.35 g/L; p = 0.002), and significantly lower (p = 0.044) than in the healthy control group (13.19 ± 1.41 g/L). Additionally, post-surgery chemotherapy patients had significantly lower (p = 0.000) IgG levels (11.04 ± 1.35 g/L) than the healthy control group (13.19 ± 1.41 g/L).

Table 2. Mean ± Standard Deviation, ANOVA and Post-Hoc Values of Immunological Markers in Newly Diagnosed Breast Cancer Patients, Patients on Chemotherapy, Post-Surgery Patients on Chemotherapy and Apparently Healthy Individuals

Group	Variables (Units)	
	IgM (g/L)	IgG (g/L)
Newly Diagnosed (A) (n=25)	1.84 ± 0.40	14.89 ± 1.13
On Chemotherapy (B) (n=25)	1.14 ± 0.22	12.38 ± 0.93
Post-Surgery + Chemo (C) (n=25)	1.12 ± 0.32	11.04 ± 1.35
Healthy Control (D) (n=75)	1.61 ± 0.46	13.19 ± 1.41
F-value	22.272	39.746
P-value	*0.001	*0.001
A Vs B	*0.000	*0.000
A Vs C	*0.000	*0.000
A Vs D	0.082	*0.000
B Vs C	1.000	*0.002
B Vs D	*0.000	*0.044
C Vs D	*0.000	*0.000

Key: IgM = Immunoglobulin M, IgG = Immunoglobulin G, Vs = Versus, n = Sample size, g/L = grams per liter, *= Statistically significant at P ≤ 0.05.

5. Discussion

Breast cancer is a diverse disease that is marked by substantial immunological and haematological abnormalities that indicate the severity of the disease and the effectiveness of treatment, in addition to the malignant transformation of breast epithelial cells. Immunological measures and inflammatory indicators have become important diagnostic and prognostic tools for cancer [14]. The purpose of this study was to assess serum levels of immunological parameters and inflammatory proteins in patients with breast cancer at various stages of disease management, including those who had just received a diagnosis, were receiving chemotherapy, and were post-surgery patients still receiving chemotherapy at the American Cancer Clinic in Iho.

According to the study's results (Table 4.1), newly diagnosed breast cancer patients have noticeably higher levels of C-reactive protein (CRP) and interleukin-6 (IL-6) than do patients receiving chemotherapy, patients who have had surgery and are receiving chemotherapy, and people who appear to be in good health. It has long been known that CRP, an acute-phase protein produced by hepatocytes in reaction to pro-inflammatory cytokines like IL-6, is a biomarker of systemic inflammation. The host's reaction to tumour invasion, necrosis, and progression is usually reflected in elevated CRP levels in cancer patients. The noticeably elevated CRP levels in recently diagnosed patients are in line with previous findings by [15], who highlighted the crucial part that CRP plays in inflammation linked to cancer. In a similar vein, elevated CRP levels have been reported in a number of cancers, with a correlation to tumour aggressiveness and a poor prognosis. This provided more evidence that CRP is a useful indicator of disease activity, especially in breast cancer [16].

Following chemotherapy and surgery, a decrease in tumour burden and inflammatory drive may be the cause of the observed drop in CRP levels among patients receiving treatment. This pattern is consistent with research by [17], who showed that therapeutic tumour load decrease frequently results in CRP normalisation, demonstrating the usefulness of the biomarker in tracking treatment response.

In addition to CRP, IL-6 levels were significantly higher in patients with newly diagnosed breast cancer, and they steadily decreased in patients undergoing chemotherapy and those recovering after surgery. This trend implies that IL-6 expression and tumour activity are closely related. Because it promotes angiogenesis, tumour cell survival, and immune evasion, the pleiotropic cytokine IL-6 is recognised to be important in the development and spread of cancer. Additionally, it promotes the development of cancerous cells and increases resistance to apoptosis [18]. High levels of IL-6 in patients with breast cancer have been shown to be closely associated with the severity of the illness and poor clinical outcomes.

The study's findings, which show that both CRP and IL-6 peak in individuals who are not receiving treatment and decrease with treatment, lend credence to the idea that systemic inflammation and breast cancer activity are tightly related. It also emphasises the possibility of these markers as surrogate indications of therapy response in addition to their use as diagnostic or prognostic tools. as highlighted in [19]. Given its crucial involvement in tumor-associated inflammation and treatment resistance, IL-6 is a prospective target in the management of breast cancer.

Additionally, as Table 4.2 illustrates, the immunological markers showed a clear pattern in both the controls and the breast cancer subgroups, providing information about the humoral immune dynamics in cancer. In contrast to other breast cancer subgroups and healthy controls, this study discovered that newly diagnosed patients had much higher IgM concentrations. This could indicate that before tumor-induced immunosuppression sets in, tumor-associated antigens first stimulated an increase of the humoral immune response. As part of the immune system's effort at surveillance, tumour cells, particularly in the early stages of the disease, might cause increased B-cell activation that results in the release of polyclonal immunoglobulins [20]. Due to either treatment-related cytotoxicity or a decrease in antigenic stimulation, these immune responses may wane when the disease worsens and therapeutic measures like chemotherapy or surgery are implemented. This result seems to deviate with the findings of [21], who discovered significantly reduced IgM levels in patients with treated breast cancer, and [22], who highlighted a widespread decrease of humoral immunity in cancer. However, the present finding lends credence to the idea that IgM might be momentarily raised in the early stages of cancer, before the tumour microenvironment modulates the immune system.

Similar trends were seen in IgG levels, which peaked in initially diagnosed patients and then significantly decreased in chemotherapy and post-operative patients. IgG's rise in early-stage disease may indicate prolonged immunological activation or dysregulation in response to tumour progression, even though it normally represents long-term antibody-mediated immunity [23]. Significantly, untreated breast cancer patients have been found to have higher serum immunoglobulin levels in the past, suggesting that these antibodies may play a part in early tumor-host immunological interactions [24]. The observed decrease in IgG as treatment goes on may be due to a general inhibition of B-cell function or therapeutic attenuation of this immune response [25-27].

6. Conclusion

Patients with breast cancer have elevated levels of CRP, IL-6, IgM, and IgG, which are indicative of anaemia, immunological dysregulation, and inflammation brought on by tumours. Chemotherapy and surgery tend to partially normalise or improve these anomalies, which are particularly noticeable in newly diagnosed patients. All things

considered, the results imply that immunological indicators can help evaluate the state of the disease and the effectiveness of treatment for breast cancer.

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