Madonna Journal of Medicine and Health Sciences
Volume 2 issue 1 (2021), Pp. 20 – 32

http://madonnauniversity.edu.ng/journals/index.php/medicine

ASSOCIATION OF SERUM HAPTOGLOBLIN WITH SOME MARKERS OF INFLAMMATION IN POST DIAGNOSTIC BREAST CANCER PATIENTS

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ABSTRACT

Changes in serum Haptoglobulin is associated with poor prognosis in many malignant diseases. This study aimed at evaluating the serum haptoglobin and markers of inflammation in post diagnostic breast cancer patients. A total of 50 subjects were recruited for the study. Twenty were post diagnostic breast cancer patients attending cancer clinic at Federal Medical Centre Owerri, Nigeria while 30 were apparently healthy subjects age matched with breast cancer and served as the control. The 50 subjects were within the age of 25-60 years. Serum samples obtained from the participants were used for the analysis of serum haptoglobin using immune-assay technique. C-reactive protein was determined using latex-enhanced nephelometry method, while ESR was determined using westergren method**.** Data obtained was analyzed using SPSS version 21 and results were expressed as mean and standard deviation (mean ± SD). Difference in mean values between groups were assessed by student t-test. Tests with a probability value of

P< 0.05 were considered statistically significant. The mean value of haptoglobulin was significantly increased (p=0.000) in post diagnostic breast cancer patients (1224.02±109.18 µg/ml) when compared to controls (693.59±165.11 µg/ml). The mean value of CRP was significantly increased (p=0.000) in post diagnostic breast cancer patients (5.25±0.61 mg/L) when compared to controls (1.98±0.23 mg/L). The mean value of ESR was significantly increased (p=0.000) in post diagnostic breast cancer patients (89.00±16.08 mm/hr) when compared to controls (7.47±2.12 mm/hr). Results show Breast cancer is associated with significant elevated levels of haptogloblin, C-reactive protein and ESR. This indicates a positive association between Breast cancer and haptogloblin, which is predictive of poor disease outcome and survival in breast cancer.

Keywords*: haptogloblin, markers, inflammation, diagnostic, breast cancer*

INTRODUCTION

Breast cancer is cancer that develops in breast cells. Typically, the cancer forms in either the lobules or the ducts of the breast. However cancer of the breast is responsible for the death of millions of women worldwide. Malignancy of the breast is one of the commonest causes of death in women aged between 40-44 years (Berry *et al*., 2015). Cancer of the breast is so widespread that it has become a genuine problem for public health, with about one woman in ten developing it in her lifetime throughout the world. Its incidence increases with age, being uncommon below the age of 30; and its behavior varies from a slowly progressive to a rapidly progressive disease despite all forms of treatment. Breast cancer primarily affects women; however, it occasionally affects men. The female to male ratio of breast cancer prevalence is 100:1 (Wernberg *et al.*, 2017). Breast cancer accounts for 0.2% of all cancer cases in men. The aetiology of the disease is unknown, although both low radiation and oncogenic viruses may play a role. A variety of interrelated genetic, hormonal, environmental, sociobiological and physiological factors exert an influence on the development of this disease. Despite the identification of high risk factors, only

35% of breast cancer may be explained by known or suspected risk factors, including modifiable behaviours involving diet, overweight, exercise and alcohol use (Polednak, 2015).

Breast cancer incidence, mortality and survival varies widely among women of different racial or ethnic background (Miller, 2013). There is a high mortality and poor survival among Africans both in the diaspora and on the mother continent. This has been attributed at least partially to low utilization of breast cancer screening measures to detect tumours at a more treatable stage (Gordon *et al.,* 2012).

The incidence of breast cancer has been much lower in other parts of the world as compared to the United Kingdom and North America where it accounts for the largest number of deaths of approximately 34,000 per annum (Sainsbury, 2015). However, the incidence of this disease is rising in many countries such as Japan and developing nations such as Ghana. Wernberg *et al.*, (2017) reported that breast cancer formed 11% of all cancers histologically diagnosed at the Pathology Department of Korle Bu Teaching Hospital (K'BTH). During a free physical examination of breast exercise carried out in Armstrong and Doll, (2013) detected 13 breast cancer cases out of 712 women aged 20-80 years (mean 39.9 years). Of the 13 breast malignancy 3 out of 412 cases were found in southern and 10 out of 300 in Northern Ghana, (Sainsbury, 2015).

Diet may also be a factor in the variation of the incidence of breast cancer among women from different racial or ethnic communities (Armstrong and Doll, 2013).

Haptoglobin (Hp) is a serum a2-sialoglycoprotein that functions as an acute-phase protein, binds with free hemoglobin and exhibits anti-oxidative activity (Beckman *et al*., 2006). Formation of Hp-hemoglobin complex in the circulation prevents the loss of iron although the kidneys and prevents the formation and accumulation of the Fenton reaction-derived free radicals (Nnatuanyai *et al.,* 2017; Nnatuanya *et al.,* 2018; Obeagu and Obeagu, 2018).

Some human breast carcinomas express the haptoglobin related protein (Hpr) or proteins that share epitopes with it. In a recent retrospective immunohistochemical study of 70 patients with early breast carcinoma (stages 1 and 11), Kuhajda *et al*., (2009) showed that Hpr epitope

expression by mammary tumours was associated with a significantly increased risk of recurrence. These studies suggested that a link exists between Hpr-epitope expression and the development of aggressive malignant potential. Therefore, if this phenomenon represents a biologic event linked to the heightened expression of malignancy, it is expected to occur in metastasis as the disease progresses. Moreover, metastases from initially Hpr-negative tumors would be expected to acquire Hpr epitopes, (Kuhajda *et al.*, 2009).

Erythrocyte sedimentation rate (ESR) is a simple inexpensive index of measurement of chronic inflammation frequently ordered in clinical medicine (Saadeh, 2018) .In cancer management, a high ESR has been found to correlate with the prognosis of breast cancer and other types of cancer such as Hodgkin's disease, gastric carcinoma, renal cell carcinoma, chronic lymphocytic leukemia, colorectal cancer, and prostate cancer (Johansson *et al*., 2012). The diagnostic use of ESR has been generally replaced by the measurement of C-reactive protein (CRP). CRP is a classical positive acute-phase protein displaying rapid and pronounced rise of its plasma concentration in response to acute inflammation, infection, and tissue damage (Johnson, 2016). CRP is produced by the liver, predominantly under transcriptional control by the cytokine interleukin-6 originating from the site of pathology. Serum CRP has been shown to parallel carcinogenesis possibly as an expression of the host defense reaction or as paraneoplastic syndrome (Deodhar, 2009). Previous epidemiologic studies have reported that elevated CRP levels may be associated with a poor prognosis of several types of solid cancers, including endometrial, cervical, colorectal, pancreatic, hepatocellular, esophageal, renal cell, bladder, prostate, ovarian, and non-small cell lung cancer. Breast cancers are characterized by significant histological inflammation; emerging evidence suggests that inflammatory pathways also play an important role in breast cancer progression (Das *et al*., 2009)**.**

The study was done to evaluate the serum haptoglobin and markers of inflammation in post diagnostic breast cancer patients.

MATERIALS AND METHODS

Study Area

The study was conducted at the Federal Medical Centre Owerri, Imo state. **Ethics, Advocacy and Pre-Survey Contacts**

A letter of introduction from the Head of Department of Medical Laboratory Science, Imo State University was collected. I met with the Chief Medical Director and Head of Clinical Services and also Chairman Ethics Committee of Federal Medical Centre, Owerri. On request, an approval was obtained. Modalities for the survey were reached and dates were fixed for blood sample collection at appropriate clinic days. Informed consent was sought and obtained before commencement of collection of samples.

Study Population

Total of 50 subjects attending oncology/surgery unit at Federal Medical Centre, Owerri were used for the study. The 50 subjects were within the age of 25-50 years. The 50 subjects were divided into two groups:

Group 1 (Test) consists of 20 women with breast cancer (0-3 month post diagnostic) and not yet on chemotherapy.

Group 2 (Control) consist of 30 apparently healthy women without any cancer

Selection Criteria

Inclusion

i. Women with breast cancer were between 0-3months post diagnostic
ii. Women with breast cancer who are not on chemotherapy.
iii. Breast cancer patients not diagnosed of liver disease, kidney disease, HIV or any malignancies.
iv. Breast cancer and healthy women whose informed consent was obtained.

Exclusion

i. Women with benign or malignant tumor

ii. Breast cancer patients with a history of diabetes, renal diseases, other cardiovascular diseases and other metabolic derangement.

iii. Breast cancer patients whose informed consent could not be obtained because they were skeptical about the research work.

Study Design

A cross-sectional study was conducted in the month of November 2019 and all eligible subjects who filled the questionnaire and gave a written informed consent for the study period were sampled.

The 80 subjects were divided into two groups. Group 1 consists of 40 women with breast cancer while group 2 consists of 20 apparently healthy women.

Sample Collection

With a sterile syringe, 5ml of blood was collected from each subject from the antecubital vein, using the standard venipuncture technique. The blood sample was dispensed into plain tubes. The sample in the tube was centrifuged at 3000rpm for 5 minutes to separate the serum. The serum samples obtained were taken to the research laboratory of the Department of Medical Laboratory Science, Imo State University for analysis.

Laboratory Methods and Procedures

All reagents were commercially procured and the manufacturer's standard operational procedures were strictly followed.

A. Determination of Serum Haptoglobin using Immuno-assay technique (Bergstrom and Lefvert, 2010)

Procedure

The serum haptoglobin levels were measured in the hospital laboratory using a Beckmen Coulter Unicel DXC by combining an antihaptoglobin antibody with the haptoglobin molecule, forming

an insoluble complex. This antigen-antibody complex was measured by optical absorbance at 340 nanometers using turbidimetry principle. The serum haptoglobin was reported in milligrams per deciliter. The normal range of serum haptoglobin in our hospital laboratory is 36–195 mg/dl.

B. Determination of C-reactive Protein Using ELISA Method (Samira *et al.*, 2012)

Procedure

This assay employs an antibody specific for Human CRP coated on a 96-well plate. Standards and samples were pipetted into the wells and CRP present in a sample was bound to the wells by the immobilized antibody. The wells were washed and biotinylated anti-Human CRP antibody was added. After washing away unbound biotinylated antibody, HRP-conjugated streptavidin was pipetted to the wells. The wells were again washed, a TMB substrate solution was added to the wells and color develops in proportion to the amount of CRP bound. The Stop Solution changes the color from blue to yellow, and the intensity of the color was measured at 450 nm wavelength.

Calculation:

$$\text{Conc. of serum CRP} = \frac{\text{Absorbance of test}}{\text{Absorbance of standard}} \times \text{Concentration of Std}$$

The standard curve was constructed as follows: The absorbance for the standards (vertical axis) versus the standard concentrations (horizontal axis) was plotted on a linear graph paper.

C. Determinatiion of Erythrocyte Sedimentation Rate (Westergren Method) (Ochei and Kolhatkar, 2000)

Procedure

0.5ml of 3.8% of sodium citrate was added in a small test tube. 2ml of blood was added into the test tube. The blood was added into the westergren tube to the top mark 0. The tube was then stood vertically in a stop rack and allowed to stand for 1 hour.

Statistical Analysis

Data was analysed using software statistical package for social sciences (SPSS) version 20.0 the results were expressed as mean and standard deviation (mean ± SD). Differences in mean values between groups were assessed by student t-test. Tests with a probability value of $P< 0.05$ were considered statistically significant.

RESULTS

Table 1: Mean Value of Haptogloblin, C-reactive protein and ESR in Post Diagnostic Breast Cancer Patients

Parameter	Test	Control	t-value	p-value
Haptogloblin (µg/ml)	1224.02±109.18	693.59±165.11	6.56	0.000
CRP (mg/L)	5.25±0.61	1.98±0.23	12.30	0.000
ESR (mm/hr)	89.00±16.08	7.47±2.12	12.31	0.000

Table 1 shows that the mean value of haptogloblin was significantly increased (p=0.000) in post diagnostic breast cancer patients (1224.02±109.18 µg/ml) when compared to controls (693.59±165.11 µg/ml).

The mean value of CRP was significantly increased (p=0.000) in post diagnostic breast cancer patients (5.25±0.61 mg/L) when compared to controls (1.98±0.23 mg/L).

The mean value of ESR was significantly increased (p=0.000) in post diagnostic breast cancer patients (89.00±16.08 mm/hr) when compared to controls (7.47±2.12 mm/hr).

Table 2: Correlation of Haptogloblin with C-reactive protein and ESR in Post Diagnostic Breast Cancer Patients

Dependent Variable	n	r	p-value
CRP	20	0.61	0.201
ESR	20	0.89	0.015

Table 2 shows that there was a non-significant positive correlation of haptogloblin with C-reactive protein (r=0.61 and p=0.201) in post diagnostic breast cancer patients.

There was a significant positive correlation of haptogloblin with ESR (r=0.89 and p=0.015) in post diagnostic breast cancer patients.

Discussion

The high mortality rate and poor survival association in breast cancer patients in African has been attributed to utilize members that detects tumour at the early and treatable stage.

In the present study, results show that the mean values of haptogloblin was significantly increased ($p<0.05$) in post diagnostic breast cancer patients when compared to controls. However the mechanism is not yet confirmed, but previous literature have indicated that breast iron levels are increased in breast cancer and have been directly linked to breast cancer development through the production of oxidants, the increase in level of iron will stimulate the production of haptoglobulin, hence it increase in breast cancer patient (Kumar *et al*., 2010). This result is in agreement with the report by Beckman *et al*., (2006) who stated that haptoglobin expression is characteristic of many types of malignant tumors, including breast cancer and is significantly increased in women with breast cancer.

In this study, the mean value of C-reactive protein (CRP) was significantly increased ($p<0.05$) in post diagnostic breast cancer patients, when compared to controls. Rapid tumor growth may cause an immune response, and many inflammatory factors such as C-reactive protein are released, this is probably due to the rise in the plasma concentration of interleukin-6 which is produced predominantly by macrophages during inflammation (Al Murri *et al*., 2007). CRP is a representative marker for inflammatory status. It is a classical acute-phase protein displaying a rapid and pronounced rise of its plasma concentration in response to acute inflammation,

infection, and tissue damage (Tas *et al*., 2013). The result of this study is similar to the findings of Eboreime *et al*. (2015) who in his study reported an elevated level of serum C-reactive protein level in female patients with breast cancer. Studies by Strojnik *et al*. (2014) also stated that C-reactive protein is a classical predictor of breast cancer, because of it raised level and have therefore been identified and applied for predicting BC outcomes.

The current study revealed that the mean value of ESR was significantly increased ($p<0.05$) in post diagnostic breast cancer patients when compared to controls. Elevated ESR is frequently encountered in patients with inflammatory diseases such as cancer. In inflammatory conditions, fibrinogen, other clotting proteins, and alpha globulin are positively charged, thus increasing the ESR (Bray *et al*., 2016). ESR begins to rise at 24 to 48 hours after the onset of acute self-limited inflammation, decreases slowly as inflammation resolves, and can take weeks to months to return to normal levels. The result of this study is in agreement with report from Brigden, (2008) they reported that an elevated ESR level has also been identified as a prognostic factor adversely affecting survival in cancer patients.

There was a non-significant positive correlation of haptogloblin with C-reactive protein and a significant positive correlation of haptogloblin with ESR in post diagnostic breast cancer patients. The positive correlation indicates a direct relation between haptogloblin and ESR.

Conclusion

This shows a positive association between haptogloblin and markers of inflammation (CRP and ESR) which may be predictive of poor disease outcome and survival in post diagnostic breast cancer patients.

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