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Serum High Sensitivity C - reactive protein At Different Stages of Breast Cancer

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Abstract **Original Research Article**

***Background*:** Breast cancer continues to be a leading cause of morbidity and mortality worldwide. In recent years, there has been a growing interest in identifying reliable biomarkers that can aid in the early detection, prognosis, and monitoring of breast cancer. High sensitivity C-reactive protein (hs-CRP), a sensitive marker of systemic inflammation, has gained attention for its potential association with various malignancies, including breast cancer. ***Objective*:** This study was designed to estimate the serum level of hs-CRP in different stages of breast cancer patients and determine its relation with the disease progression. ***Methods*:** This cross-sectional analytical study included a total of 60 women ranging in age from 30 to 65, and divided into two groups. Group A includes 30 diagnosed breast cancer patients, and group B includes 30 age-matched apparently healthy women according to the selection criteria. Serum hs-CRP (mg/L) was estimated for both groups. The mean and median values of the variable were compared between the groups by unpaired students't-tests and Mann-Whitney U tests. The level of significance was defined as p-value <0.05 at 95% confidence interval. ***Results*:** There was no significant difference in age and BMI between study groups. The median value of serum hs-CRP (mg/L) level was significantly (p<0.009) higher in breast cancer patients (3.57 mg/L) when compared to age-matched healthy women (1.56 mg/L). Serum hs-CRP concentrations of cases were increased significantly with the staging of breast cancer. ***Conclusion*:** This study revealed a significant relationship between hs-CRP levels with different stages of breast cancer. Estimating hs-CRP might be a useful tool for early detection, prognosis, and monitoring of breast cancer.

Keywords: Breast cancer; High Sensitivity C - reactive protein; hs-CRP; hs-CRP in breast cancer; Stages of Breast Cancer.

1. INTRODUCTION

Breast cancer is the most widespread and primary contributor to female mortality on a global scale [1]. It ranks as the second most prevalent form of cancer, following lung cancer, accounting for 10.4% of all cancer cases when considering both genders. Additionally, it stands as the fifth most frequent cause of cancer-related deaths [2].

Evidence from clinical and epidemiological information indicates that hormones, genetic factors, environmental elements, and obesity play a role in causing breast cancer. The initiation and advancement of malignancies have been linked to chronic inflammation. Rudoff Virchow, in 1863, observed infiltration of leucocytes in the malignant tissues and proposed the site of chronic inflammation as the origin of cancer. For the first time, he linked inflammation with carcinogenesis [3]. Subsequently, numerous researchers have investigated the influence of inflammation on various aspects of cancer. Certain solid tumors originate in regions of chronic inflammation, and some prompt inflammatory microenvironments in the tumor [4]. Histologically, distinct signs of inflammation are infrequently observed in breast cancer. Nevertheless, the inflammatory element exists in the tumor cell microenvironment, where white blood cells, along with cytokines and chemokines, serve as the primary

mediators of inflammation [5]. It has been demonstrated that inflammatory pathways play a role in the development, invasion, and distant metastasis of tumors [6].

C-reactive protein (CRP), a plasma protein that rises during systemic inflammation as part of the acute-phase response, serves as an indicator of inflammation. Its primary production occurs in the liver and is controlled by pro-inflammatory cytokines, particularly interleukin-6 [7]. In healthy individuals, detecting CRP levels in the blood is typically challenging due to their naturally low concentrations. However, its levels are elevated in response to acute infections, inflammatory conditions, and trauma [8].

Utilizing a high-sensitivity assay enables the measurement of subtle levels of systemic inflammation in the absence of apparent systemic inflammatory or immunologic disorders. So, the hs-CRP test can more accurately detect lower concentrations of the protein than the standard CRP test [9]. Elevated concentrations of high-sensitivity C-reactive protein (hs-CRP) have been documented in various conditions, including infectious diseases, cardiovascular diseases, diabetes, autoimmune disorders, inflammatory bowel diseases, arthritis, and numerous types of cancers. [10].

Increased hsCRP assessed after diagnosis has been associated with worse breast cancer prognosis, aggressiveness of the tumor, poor survival rate, and increased recurrence [11]. This study was designed to estimate the serum level of hs-CRP in breast cancer patients and to find out its differences with the staging of the disease.

2. MATERIALS AND METHODS

The present case-control study enrolled 30 new histologically diagnosed cases of breast cancer admitted in the Department of Surgery of Dhaka Medical College Hospital, Bangladesh, and 30 healthy age-matched controls were selected after a thorough breast examination spanning from July 2018 to June 2019. Ethical permission was taken from the ethical review committee of Dhaka Medical College. A thorough explanation was provided regarding the goals, characteristics, intent, and possible risks associated with all procedures employed in the study. Informed written consent was obtained from both the patients and healthy individuals.

2.1. Objective

2.1.1. General Objective: To estimate serum high sensitivity C- reactive protein at different stages of breast cancer.

2.1.2. Specific Objectives

1. To measure serum hsCRP at different stages of breast cancer.
2. To measure and compare serum hs-CRP in breast cancer patients and healthy women.

2.2. Inclusion Criteria

1. Age: 30-65 years
2. Gender: Female

For study group: Diagnosed cases of breast cancer patients.

For the control group: Age-matched, apparently healthy women.

2.3. Exclusion Criteria

Individuals with a previous medical history of hypertension, type 2 diabetes mellitus, acute or chronic inflammatory conditions, cardiovascular diseases, and other malignancies were excluded from participation in the study. Additionally, women who were taking anti-inflammatory medications were not included in the study.

Demographic data related to age, history of smoking, alcoholism, family history of breast cancer, and use of hormone therapy were recorded through a standard questionnaire. Body weight (kg) and height (cm) were recorded for anthropometric measurements. For all the subjects, body mass index was calculated.

Patients were staged according to the TNM (tumor size, lymph nodes, and metastasis) system by the doctors of the surgery department of Dhaka Medical College.

Then, with all aseptic precautions, blood samples were collected by sterile syringe and transferred into a dry, clean, and deionized test tube kept in a slanting position till the formation of a clot, then centrifuged at 3000 rpm for 5 minutes and the separated serum was kept in labeled Eppendorf after proper labeling to estimate serum hs-CRP in both groups. Serum hs-CRP was assayed at the Microbiology department of BSMMU using the immuno-nephelometric method.

All data were recorded in a predesigned data collection sheet. Continuous variables with normal distribution were expressed as mean ± SD, and without normal distribution were expressed as median and compared between groups by unpaired student's t-test and Mann-Whitney U test, respectively. ANOVA and Bonferroni test were done to compare the variables among the stages of breast cancer. Categorical variables were compared using the chi-square test. The level of significance was defined as p-value <0.05 at 95% confidence interval. All analyses were done using the SPSS version 22 package for Windows.

3. RESULTS

This study is designed to estimate the serum level of hs-CRP of breast cancer patients at different stages of the disease. A purposive type of sampling technique was applied to collect samples from the study population. Thirty diagnosed breast cancer patients were included in group A, and thirty age-matched apparently healthy women were included in group B.

Table 1: Comparison of age and BMI between study subjects (N=60)

	Group A (n=30) Mean±SD	**Group B (n=30) Mean±SD**	**p-value**
Age (years) (Range)	50.07 ± 9.84 (30 - 65)	48.33 ± 10.51 (30 - 65)	0.512
Body Mass Index (kg/m^2) (Range)	23.52 ± 2.94 (18.5 – 31.1)	23.98 ± 3.16 (18.5 – 31.1)	0.566

An unpaired t-test was done to measure the level of significance**.** [Table 1] shows that the age and BMI (Mean±SD) of both groups were matched in study subjects as there was no statistically significant difference.

Table 2: Comparison of serum hs-CRP between study subjects (N=60)

	Group A (n=30) Median (Min-Max)	**Group B (n=30) Median (Min-Max)**	**p-value**
hs-CRP (mg/L) (Range)	3.57 (0.42-37.10)	1.56 (0.58-5.78)	0.009

Mann-Whitney U test was done to measure the level of significance. [Table 2] [Figure 1] shows the serum hsCRP concentration (Median) of both groups in study subjects. The median value of serum hs-CRP (mg/L) was significantly higher in breast cancer patients.

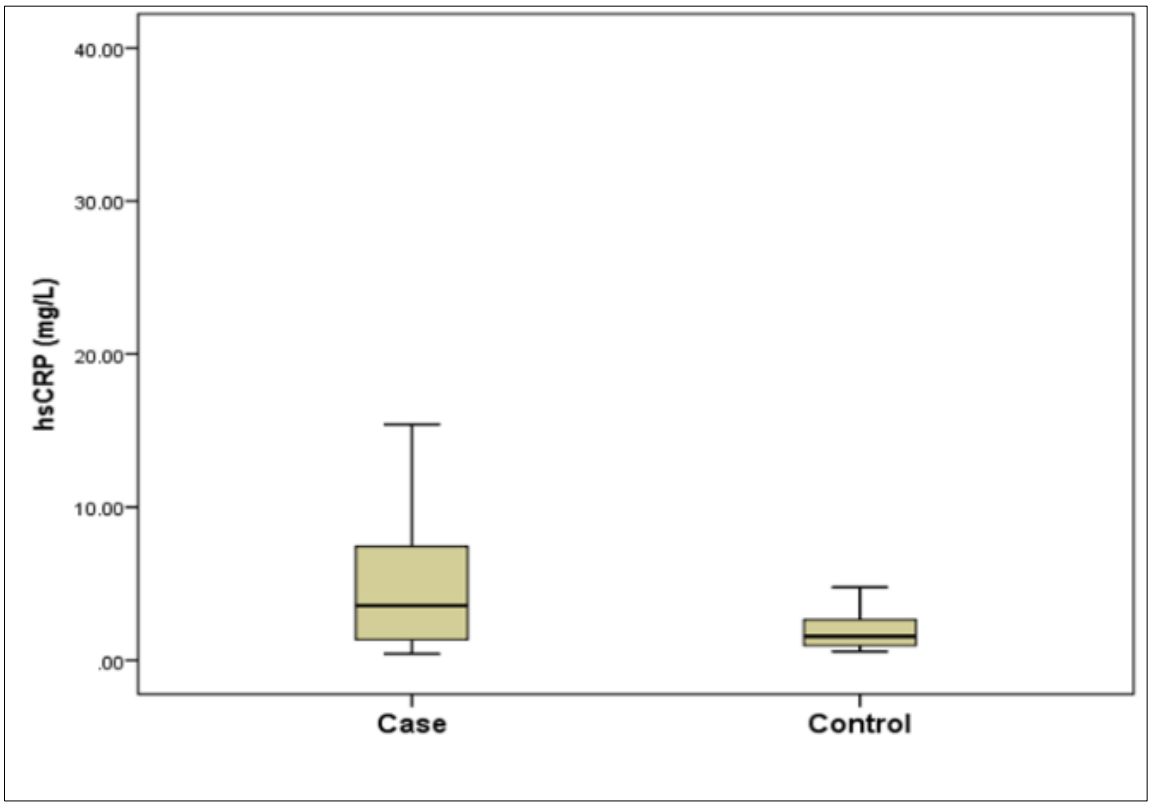

Figure 1: Box plot of serum hs-CRP of study subjects in both groups.

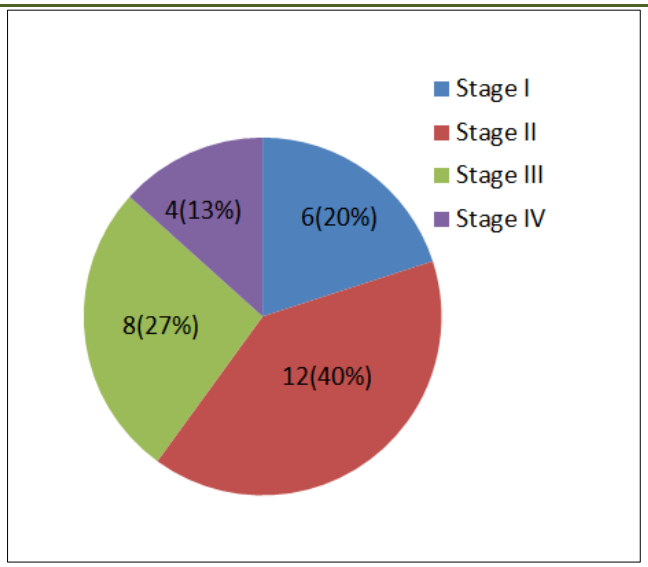

Figure 2: Pie chart showing distribution of the patients according to the staging of breast cancer

In [Figure 2], patient distribution according to the staging shows that 20% of patients were in stage I, 40% of patients were in stage II, 27% of patients were in stage III, and 13% of patients were in stage IV. A large number of participants were in stage II breast carcinoma.

Table 3: Comparison of serum hs-CRP concentration at different stages of breast cancer (n=30)

Staging of breast cancer	hs-CRP Mean±SD	p-value
Stage I (Range)	0.82 ± 0.33 (0.42 - 1.34)	
Stage II (Range)	2.98 ± 1.32 (0.96 - 5.63)	<0.001
Stage III (Range)	8.22 ± 6.05 (1.99 - 21.7)	
Stage IV (Range)	33.06 ± 13.74 (14.15 - 37.1)	

ANOVA test was done to measure the level of significance
Statistical analysis done by the Bonferroni test

Stages	hs-CRP
I vs II	1.000
I vs III	0.007
I vs IV	<0.001
II vs III	0.038
II vs IV	<0.001
III vs IV	<0.001
Control vs I	1.000
Control vs II	1.000
Control vs III	<0.001
Control vs IV	<0.001

[Table 3] shows serum hsCRP concentration (mean±SD) at different stages of breast cancer. As there was a statistically highly significant difference in hs-CRP concentration, the Bonferroni test was done between the stages. The increase of hs-CRP level in stage IV and stage III was more statistically significant than all other stages.

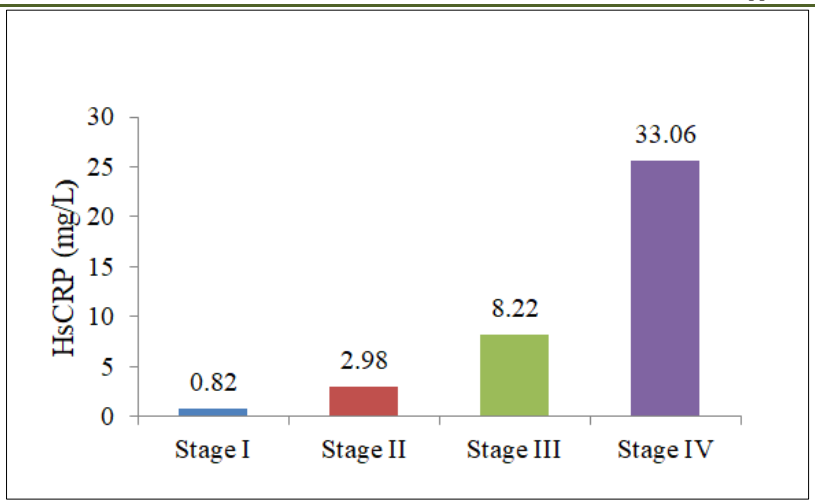

Figure 3: Bar diagram showing mean hs-CRP level in different stages of breast cancer.

Bar diagram in [Figure 3] showing mean hs-CRP level is increasing with progression of breast cancer stages.

4. DISCUSSION

Globally, breast cancer is the most commonly diagnosed cancer among women and the most common cancer-related death worldwide [12]. The association between breast cancer and inflammation has been suggested as the seventh hallmark of cancer [5]. There is a bidirectional link between chronic inflammation and carcinogenesis [3]. The improved sensitivity of hsCRP is used to detect low-grade inflammation. The present study aimed to estimate serum hs-CRP levels in diagnosed breast cancer patients at different stages of the disease.

In this present study, it was observed that the mean±SD value of age was found 50.07 ± 9.84 years, ranging from 30 to 65 years in group A, and 48.33 ± 10.51 years, ranging from 30 to 65 years in group B. The difference was almost identical between the two groups. In this study, the difference in age is not statistically significant ($p>0.05$) in breast cancer patients than healthy women.

BMI in group A was 23.52 ± 2.94 (kg/m^2), and in group B was 23.98 ± 3.16 (kg/m^2). In this study, both baseline parameters (age and BMI) are not statistically significant ($p>0.05$) in breast cancer patients than age-matched healthy women. Similarly, Hong *et al*., (2013) did not differ significantly by age and BMI. On the contrary, Asegaonkar *et al*., undertook a case-control study in 2014 and observed a significant difference in BMI between breast cancer patients and healthy women [13]. Pacholczak, Piotrowska, and Kuszmiersz undertook a detailed anthropometric assessment in 2016, which was conducted on 487 women, of whom 193 had been diagnosed with breast cancer. There was a significant difference in BMI between study subjects [14]. The difference in the mean±SD value of BMI in current and previous studies may be due to geographical variations, racial and ethnic differences between the countries.

In this study, the Median value of serum hs-CRP (mg/L) level was significantly ($p<0.009$) higher in breast cancer patients (3.57 mg/L) than in age-matched healthy women (1.56 mg/L). BMI and menopausal status act as confounding variables and affect both the risk of breast cancer and serum hs-CRP level. Equality of the menopausal status was not maintained. Similarly, a significant increase in hs-CRP is also observed in a case-control, cross-sectional study conducted in 2014 by Asegaonkar *et al*., [13]. In a study conducted in 2015 by Abdollahi, Bakhshi, and Farahani, there is also a significant elevation in hs-CRP in patients with breast cancer than in benign breast tumors [15]. Shahi, Dey, and Chowdhury observed in 2018 a statistically significant increase in serum level of hsCRP among the samples of breast cancer patients compared to healthy control ($p < 0.05$). Higher values of hs-CRP also showed a strong correlation with larger tumor size, LN invasion, and metastasis [16]. However, Zhang *et al*., found no association of hs-CRP with breast cancer in their study conducted in 2007. They added that in the case of breast cancers, inflammation is rare evidence on histopathological examination [17].

In this study, the mean±SD value of hs-CRP (mg/L) was measured at different stages of breast cancer. The level increases significantly with every stage of breast cancer. Serum hs-CRP values were significantly raised in stage IV patients with distant metastasis compared to all other stages. ANOVA and the Bonferroni test were done to compare the variables among the stages. Asegaonkar *et al*., observed serum hsCRP levels were associated with the advanced stage. In stage I, an association of hs-CRP was not significant with disease, while there was a significant association in stage II and III [13]. In a study from Tamil Nadu by Ravishankaran and Karunanithi conducted in 2011, positive correlations were found among CRP and IL-6

levels with lymph node and distant metastasis and TNM stage of breast cancer [18]. In another study conducted by Allin *et al*., in 2011, preoperative hsCRP level may provide short-term prognostic information as a subclinical marker of tumor stage, size, and presence of metastatic disease [19]. All these studies support the findings of our study. An increase of hs-CRP with every stage suggests a significant association of the state of inflammation with the stage of breast cancer. However, Al Murri *et al*., reported negative findings of the association of CRP as a prognostic marker of breast cancer [20].

5. CONCLUSION

This study revealed significantly higher hs-CRP levels in breast cancer patients than in healthy women and also higher hs-CRP levels with disease progression. Therefore, assessing hs-CRP levels could serve as a valuable tool for risk assessment, screening individuals at high risk, and predicting outcomes in diagnosed cases.

6. Compliance with ethical standards

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Disclosure of Conflict of Interest: All authors declared no conflicts of interest.

Statement of Ethical Approval: Proper ethical approval was taken.

Statement of Informed Consent: Written consent was obtained from the parents.

Author Contributions

Farzana Binte Abedin Leera: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Resources, Software, Writing – original draft, Writing – review & editing.

Md. Masum Billah: Data curation, Formal analysis, Investigation, Writing – review & editing

Other Author Contributions: Data curation, Formal analysis.

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