Section: Pathology

Original Research Article

SERUM HEPCIDIN LEVELS IN FEMALE BREAST CARCINOMA PATIENTS AND ITS RELATIONSHIP WITH ANEMIA: A CROSS-SECTIONAL STUDY

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ABSTRACT

Background: Breast cancer, comprising 24% of female cancers, is the leading cause of female cancer death globally and the second most common cancer overall. Anemia is prevalent but often undiagnosed in breast cancer patients. Hepcidin, a hepatic peptide hormone, plays a key role in breast carcinoma-related anemia. Considering its pivotal role in iron metabolism, hepcidin-targeting drugs under research may offer novel treatments, potentially improving the quality of life for those with breast carcinoma and anemia. The objectives of this study are to compare serum hepcidin levels in breast carcinoma patients with those having benign breast diseases and to assess serum hepcidin levels in breast carcinoma patients with anemia and those without anemia.

Materials and Methods: The present study is a comparative cross-sectional investigation conducted at the Department of Pathology and Biochemistry, Government Medical College, Thrissur, spanning from January 1, 2020, to December 31, 2021. Two groups were selected for the study population: the first group comprised 30 cases of breast carcinoma patients, while the second group comprised 30 cases of benign breast diseases.

Results: The mean age for breast carcinoma patients was 51.1 years, compared to 35.8 years for those with benign breast disease. In Group 1, serum hepcidin levels were 45.05 ng/ml, significantly higher than 22.08 ng/ml in Group 2 ($p < 0.001$). Group 1 further divided into anemic (mean hepcidin: 53.28 ng/ml) and non-anemic (mean hepcidin: 28.6 ng/ml) subgroups, showing significant differences ($p < 0.001$). Serum hepcidin correlated negatively with RBC indices in both carcinoma (hemoglobin r= -0.830, hematocrit r= -0.813, RBC count r= -0.833) and benign (hemoglobin r= -0.825, hematocrit r= -0.735, RBC count r= -0.633) groups. In the benign group, a weak negative correlation was observed with MCHC (r= -0.385, p= 0.036), and a positive moderate correlation with RDW in the carcinoma group (r= 0.407, p= 0.026).

Conclusion: The study identified elevated serum hepcidin levels in breast carcinoma patients, particularly in those with anemia, as opposed to benign cases.

Keywords: Serum hepcidin, Breast carcinoma, Anemia.

INTRODUCTION

Breast cancer in females has taken the forefront as the most commonly diagnosed cancer, surpassing lung cancer and has secured the fifth position among the leading causes of cancer death worldwide.[1] Breast cancer has held the top position among Indian females, with an age-adjusted rate as

high as 25.8 per 100,000 women and a mortality rate of 12.7 per 100,000 women.[2]

Anemia is frequent in breast cancer patients but often remains undiagnosed and untreated.[3]Hepcidin, a hepatic peptide hormone, plays a pivotal role in the development of anemia in breast carcinoma by closely regulating iron metabolism. Its increased levels have been noted in neoplastic diseases, inflammation, and sepsis, emphasizing its significance.[4,5]Hepcidin, a key regulator of iron metabolism, influences iron release from cells such as enterocytes, macrophages, and hepatocytes. Its interaction with ferroportin inhibits iron release by inducing ferroportin internalization and degradation. Hepcidin expression, responsive to iron, erythropoietic demand, hypoxia, and inflammation, plays a pivotal role in maintaining iron homeostasis but can lead to iron deficiency in chronic inflammatory states, contributing to conditions like the anemia of chronic disease observed in malignancies.[6,7]

The objectives of this study are to compare serum hepcidin levels in breast carcinoma patients with benign breast diseases and to assess serum hepcidin levels in breast carcinoma patients with anemia compared to those without anemia.

MATERIAL AND METHODS

This study was conducted at the Department of Pathology and Biochemistry, Government Medical College, Thrissur, a tertiary care institution in Kerala. The study utilized a comparative cross-sectional design involving two distinct groups of participants. Group 1 was comprised of female breast carcinoma patients undergoing mastectomy in the General Surgery department of Government Medical College, Thrissur. Group 2 included female patients undergoing excision biopsy for benign breast swellings in the same department. The study spanned from January 1, 2020, to December 31, 2021, lasting for 24 months. Inclusion criteria for both groups encompassed females above 18 years, while exclusion criteria involved patients diagnosed with other malignancies, breast carcinoma patients with chronic liver disease, those with bone metastasis and individuals unwilling to provide consent for the study. The targeted sample size for each group was 30 cases: carcinoma breast (Group 1) and benign disease of the breast (Group 2).

Overnight fasting blood samples were collected from patients using EDTA vials for complete blood count and peripheral smear and clot activator vials for hepcidin estimation. Measurements of hemoglobin, hematocrit, MCV, MCH, MCHC, RDW, and RBC count were conducted from EDTA tube blood using a Sysmex XT-1800i 5-part auto analyzer. A manual peripheral smear was prepared from EDTA tube blood stained with Leishman's stain. The serum was separated and stored at -20 °C, and hepcidin values were determined using a 96-well Human Hepc25 (Hepcidin 25) ELISA Kit from Elabscience Biotechnology Inc.

The collected data were coded and entered into an Excel sheet, then analyzed using IBM SPSS Statistics, Version 20.0, a statistical software package. Continuous measurement results are presented as Mean ± SD, while categorical measurements are expressed in numbers and percentages. Significance was assessed at a 5% level. Independent T-tests were conducted to identify statistically significant differences in serum hepcidin levels (ng/ml) between breast carcinoma and benign breast disease groups. Additionally, the tests were performed to compare serum hepcidin levels between anemic and non-anemic groups of breast carcinoma patients, with a significance threshold set at $p < 0.05$. Bivariate Pearson correlations were conducted to identify statistically significant correlations between serum hepcidin levels and hemoglobin, hematocrit, MCV, MCH, MCHC, RBC count, and RDW among patients with breast carcinoma and benign breast diseases. For determining the optimal cutoff of serum hepcidin levels in benign and breast carcinoma patients, receiver operating characteristic (ROC) curve analysis was conducted. The obtained cutoff value categorized serum hepcidin levels into two groups. A Chi-square test was then performed to identify statistically significant differences between benign breast disease and breast carcinoma groups using the optimal cutoff level of serum hepcidin.

RESULTS

In the current study, the first group comprised 30 patients with breast carcinoma, while the second group consisted of 30 patients with benign breast diseases. Among the 30 cases of benign breast diseases in the second group, 21 were diagnosed as fibroadenoma, 4 as fibrocystic disease of the breast, 2 as benign adenomyoepithelioma, 2 as benign phyllodestumor, and a breast lipoma. Group 1 was subdivided into two subgroups. The first subgroup comprised patients with anemia and breast carcinoma(n=20), while the second subgroup included patients without anemia(n=10).

The age of patients in the breast carcinoma group ranged from 30 to 67 years, while the age of patients in the benign breast disease group ranged from 18 to 64 years. The mean age of patients in the breast carcinoma group is 55.1 years, while the mean age of patients with benign breast disease is 35.8 years. A comparison of various parameters between the two study groups is shown in Table 1. The serum hepcidin level in the breast carcinoma group is 45.05ng/ml (SD-14.61), whereas in the benign breast disease group, it is 22.08ng/ml (SD-3.51). Among breast cancer patients, the mean serum hepcidin levels are 53.28 ng/ml (SD=8.82) for anemia patients and 28.6 ng/ml (SD=8.5) for non-anemia patients. [Table 2]

ROC curve analysis was conducted to determine serum hepcidin's sensitivity, specificity, and optimal cut off value. The ROC curve yielded an area under the curve (AUC) of 0.903, with a 95% confidence interval of 0.817-0.990 and a p-value less than 0.001. The serum hepcidin value of 24.5ng/ml was identified as the most accurate cut off point, above which a patient can be categorized as belonging to the breast carcinoma group. This cut off demonstrated a sensitivity of 83.3% and a specificity of 76.7%. The positive likelihood ratio was also calculated to be 3.58, while the negative likelihood ratio was determined to be 0.22. The comparison between breast carcinoma and benign breast disease groups is presented in Table 3, categorized according to serum hepcidin cut off values. The distribution of peripheral smear pictures among the two groups is illustrated in Figure 1. The various presentations included are dimorphic anemia, microcytic hypochromic anemia, normocytic normochromic anemia, normocytic normochromic anemia with macrocytes and normocytic normochromic blood picture. The association between serum hepcidin and age was assessed using linear regression for both the breast carcinoma group (Figure 2) and the benign breast disease group. [Figure 3]

Strong negative correlations were observed between serum hepcidin and hemoglobin in both the carcinoma group ($r = -0.830$, $p < 0.001$) and the benign group ($r = -0.825$, $p < 0.001$). Similarly, strong negative correlations were found between serum hepcidin and hematocrit in the carcinoma group ($r = -0.813$, $p < 0.001$) and the benign group ($r = -0.735$, $p < 0.001$). The benign group had a weak negative correlation with MCHC ($r = -0.385$, $p = 0.036$). Additionally, strong negative correlations were observed between serum hepcidin and RBC count in both the carcinoma group ($r = -0.833$, $p < 0.001$) and the benign group ($r = -0.633$, $p < 0.001$). Furthermore, a positive moderate correlation was noted between serum hepcidin and RDW in the carcinoma group ($r = 0.407$, $p = 0.026$). [Table 4]

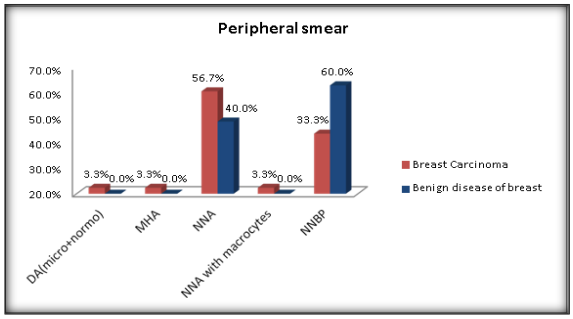

Figure 1: Peripheral smear distribution among two groups

[DA- Dimorphic anemia, MHA- Microcytic hypochromic anemia, NNA- Normocytic normochromic anemia, NNBP- Normocytic normochromic blood picture]

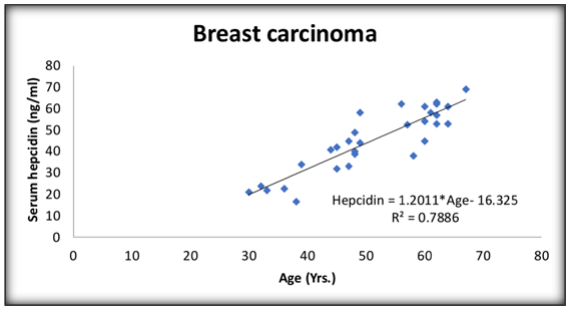

Figure 2: Linear regression to measure the association of serum hepcidin with age among breast carcinoma group

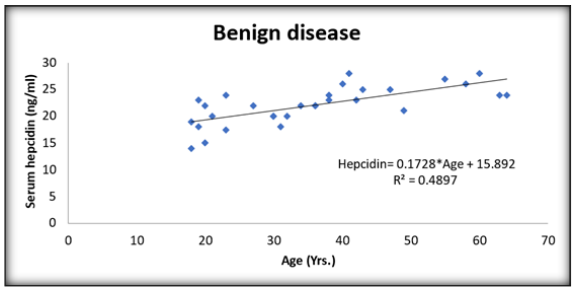

Figure 3: Linear regression to measure the association of serum hepcidin with age among benign disease of breast group

Table 1: Comparison of various parameters between the two study groups

	Breast Carcinoma (N=30)		Benign disease of breast (N=30)		
Parameters	**Mean**	**Std. Deviation**	**Mean**	**Std. Deviation**	**p-value**
Hemoglobin (g/dl)	11.16	1.49	12.25	1.06	0.010
Hematocrit %	33.49	4.32	35.76	3.01	0.022
MCV (fl)	86.38	3.69	85.97	3.93	0.679
MCH (pg)	28.75	1.36	29.04	1.82	0.486
MCHC (g/dl)	33.31	1.25	33.78	1.22	0.151
RBC count(x10^6/mm3)	3.89	0.56	4.17	0.4	0.036
RDW (fl)	46.08	4.34	44.81	3.73	0.23
Serum hepcidin (ng/ml)	45.05	14.61	22.08	3.51	<0.001

Table 2: Mean serum hepcidin levels among anemia and non- anemia patients in breast carcinoma group

Parameter	Anaemia(N=20) (Mean±SD)	Non-Anaemia(N=10) (Mean±SD)	Pvalue
Serum hepcidin level(ng/ml)	53.28±8.82	28.6±8.5	<0.001

Table 3: Comparison of breast carcinoma and benign disease of breast groups based on serum hepcidin cut off values

Groups	Serum hepcidin(ng/ml)		Total	p value
	>=24.5	<24.5		
Breastcarcinoma	25 (78.1%)	5 (17.9%)	30	<0.001
Benigndiseaseof breast	7 (21.9%)	23 (82.1%)	30 (50%)	

Table 4: The correlation between serum hepcidin levels and RBC indices in the two study groups

Variables	Breast Carcinoma(N=30)	Benign diseaseof breast(N=30)
	r value,p value	r value,p value
Hemoglobin(g/dl)	-0.830, p<0.001	-0.825,p<0.001
Hematocrit%	-0.813, p<0.001	-0.735,p<0.001
MCV (fl)	0.319, p=0.086	-0.026,p=890
MCH(pg)	0.192, p=0.310	-0.250,p=0.184
MCHC(g/dl)	-0.119,p=0.531	-0.385,p=0.036
RBCcount(x10^6/mm3)	-0.833, p<0.001	-0.633,p<0.001
RDW(fl)	0.407, p=0.026	0.281, p=0.133

DISCUSSION

Globally, breast cancer is the most frequently diagnosed cancer among women. In India, the incidence of breast cancer is rapidly increasing, and it has become the most common cancer affecting Indian women. There is compelling evidence linking anemia to unfavourable outcomes in individuals diagnosed with breast cancer. Unfortunately, anemia is frequently underestimated, adversely impacting both the quality of life for patients and diminishing their overall survival. Hepcidin, a peptide hormone primarily synthesized by the liver and released into the bloodstream, is crucial in regulating iron absorption and its distribution within tissues.[7,8]

In a study by Shao X et al., the incidence of anemia significantly differed among breast cancer patients with bone metastasis, breast cancer patients without bone metastasis, and those with breast hyperplasia.[9]The hepcidin levels followed this order: metastatic breast cancer (86.37±16.30) > non-metastatic breast cancer (47.21±18.48) > breast hyperplasia (34.31±16.79) - which is comparable to our study. In a study conducted by Pan X et al, the mean serum hepcidin was 8.18 ± 3.75µg/L in breast carcinoma patients with anemia and 4.53 ± 2.07µg/L in those without anemia, indicating a statistically significant difference (t=3.7090, p< 0.01). Moreover, there was a negative correlation between serum hepcidin and hemoglobin levels ($r = -0.502$, $p < 0.01$).[10] These results align with the observations made in the current study. In a study conducted by Ciniselli et al., elevated hepcidin levels were observed in malignant breast tumours as opposed to benign ones.[11]

In the current study, it is evident that hepcidin levels are increased in breast cancer and in association with anemia. Reduction of Serum hepcidin may prove beneficial in restraining growth of tumour cells. Besides, correcting anemia in a patient with breast carcinoma through iron therapy alone may prove inadequate, given the persistently elevated serum hepcidin levels. Consequently, iron-restrictive anemia in these cases might respond well to therapy involving hepcidin antagonists.

CONCLUSION

Our study revealed increased serum hepcidin levels in patients with breast carcinoma, as compared to cases of benign breast disease. We analyzed serum hepcidin levels in both anemic and non-anemic patients within the breast carcinoma group, finding higher levels in breast carcinoma patients with anemia. The correlation between elevated hepcidin levels and lower hemoglobin, hematocrit, and RBC count was observed in both groups. These findings provide valuable insight into the relationship between serum hepcidin and anemia across different breast disease categories.

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