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Original Research Article

Evaluation the Role of Hepcidin and Alkaline Phosphatasein the Blood of Breast Cancer Women

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Abstract: Breast cancer is a common malignancy and a major cause of death in women. In 2015, 570,000 women died from breast cancer that is approximately 15% of all cancer deaths among women. The goal of this study was to examine the changes in hepcidin, and alkaline phosphatase (ALP) in women with breast cancer concerning its validity for the early detection and the severity of the disease. A cross sectional study was carried out in Kirkuk City from November 2022 to March 2023. The number of breast cancer women under study was 60 whose ages were between 30 - 70 years old. These women were consisting Kirkuk Oncology Cancer. The control group included 30 healthy women and their ages were between 30 - 70 years old, women with breast cancer were collected from Kirkuk Oncology Center. The body weight and height were measured for the calculation of the body mass index. This study showed that the peak age of women with breast cancer was between 45 – 56 years and its percentage was 40%, while the least age group 35-44 years, and its percentage which was found to be 27 %. Also this study shows that the mean of BMI was number of breast cancer, were 7(12%) women underweight (<18.5), 10(17%) women normal weight (18.5 – 24.9), 20 (33%) overweight (25 – 29.9), and 23 (38%) with obese (≥ 30). The blood samples were collected from each women included in this study for the estimation ALP and Hepcidin (HP). This study aims to assess the role of Hepcidin in women with breast cancer and to investigate the correlation between ALP. The mean of the serum level of Alkaline Phosphatase (ALP) in breast cancer women comparing with the control group (95.2 ± 59.0 versus 82.4 ±37.4 U/L). The result was no significant (p>0.215).

Keywords: Hepcidin, Alkaline phosphatase, Breast cancer, Lobular epithelial tumors.

INTRODUCTION

Cancer is characterized by loss of control of cellular growth and development leading to excessive proliferation and spread of cells [1, 2]. Breast cancer (BC) is the most common term for a set of breast tumor subtypes with distinct molecular and cellular origins and clinical behavior. Most of them are the origin of ductal or lobular epithelial tumors. Globally, it is the most common malignant diseases of women's diagnosed every year. It accounts for about 30% of the mortality of women ages of 40–49 years, followed by lung cancer, with an estimated 1.67 million cases worldwide each year worldwide. Approximately 12.5% of women in the United States have been affected by invasive breast cancer during their lifetime [3-5]. Hepcidin a peptide hormone, is prominently synthesized by the liver, and secreted into serum followed by tissue localization through circulation. Hepcidin inhibits iron absorption from the duodenum and iron egress from macrophages and hepatocytes through its binding and inducing degradation of iron exporter ferroportin. Hepcidin increases are induced by proinflammatory cytokines, in particular IL-6. IL-6 that plays an important role in the regulation of inflammation, immune responses and erythropoiesis [4]. Deregulated hepcidin–ferroportin signaling is implicated not only in iron diseases, but also in cancers such as increased serum hepcidin increase in patients with malignant breast tumors and patients with benign breast lesions with respect to healthy subjects [1]. Hepcidin increases in 41–82% of breast cancer patients which leads to develop anemia. Unfortunately, this status is often under-recognized, under-treated and affects the quality of life but also is associated with reduced overall survival [4, 6]. Alkaline phosphatase (ALP) is a

nonspecific enzyme that hydrolyzes phosphate in alkaline medium and its total level reflects the combined activity of several isozymes found in a variety of tissues, but it is most abundant in the liver, bone, kidney, and intestinal lining. ALP is a predictors of metastasis and related to prognosis in BC [6]. Bilirubin, a metabolic by-product of hemoglobin breakdown, is one of the most potent endogenous antioxidants of the human body and also has substantial anti-inflammatory properties Therefore; bilirubin may play a preventive role in breast cancer development [7-9]. The present study aimed at analyzing hepcidin and ALP among patients with breast cancer in comparison with healthy controls.

MATERIALS AND METHODS

Subjects

This study has investigated 90 women (60 patients and 30 controls), their ages between (30-70) years. The patients were referred to three main facilities, Kirkuk oncology center, consultation of early detection of breast tumor in Azadi hospital, and Kirkuk general hospital from November 2022 to March 2023. Clinical history data, information on age, weight, height, marital status, menopausal status, family history of breast cancer, chronic diseases, and course of treatment were collected in a short questionnaire form.

Sample Collection

About 5 ml venous blood was collected from each case by using a sterile disposable syringe then unloaded into gel tubes and allowed to clot at room temperature for 20 minutes. All samples were centrifuged at 3000 rpm for 15 minutes; sera removed and divided into four Eppendorf tubes 500 µl for each sample, then stored at - 30 C until used to the time of biochemical assay which included parameters, Hepcidin and ALP.

Hepcidin was analyzed by ELISA Kit from Biomeriuex, France. ALP was measured by spectrophotometric kit, by randox GOD\APA-USA kit.

RESULTS AND DISCUSSION

Distribution of Breast Cancer Women According to Age

The total number of a subject that participate are 90 (60 patient and 30 control)This study showed that the peak age of women with breast cancer was between 45 – 56 years and its percentage was 40%, while the least age group 35-44 years and its percentage which was found to be 27 %, see Table (1).

Table 1: Relation the number of breast cancer women with Age.

Age group(years)	NO.	%
35-44	16	27
45-56	24	40
57-76	20	33
Total	**60**	**100%**

This study reveals that, the highest rate of breast cancer in women was within the age group 45 – 56 years and the least was within the age group of 35-44 years. Numerous results obtained by other studies were focused on the age of women with breast cancer, e.g. the study that carried by Ghanim H *et al.,* [10] who found that the mean age of breast cancer women was (42.2 ± 10.41) years, while the study by Armstrong K [11] Who found that the mean age was (50.4± 12.45) years with range of 22 to 80 years. In United Kingdom, where the age-standardized incidence for the breast cancer among women aged between 50 to 60 years, may be due to familial and the hormonal factors [12]. As shown in figure (1) that on distributing cases according to age it was seen that most malignant cases were in aged group 45 – 56 years of age group (i.e. 40 %), while no cases was observed < 23 years of age group, this results are similar to study in breast cancer by Mac Mahon B *et al.*, [13] were in 40 – 49 years of age (i.e. 52.5%), and there were no cases in < 23 years of age, while the findings were observed by Navneet Kaur A [14] that the maximum number of cases of carcinoma of breast were in the age group of 35 to 44 years. Previous study done in Khalid found similar finding and agree with the present study [15].

Relation of BMI to Breast Cancer

Table 2 explains the number of breast cancer women with BMI, were 7(12%) women underweight (<18.5), 10(17%) women normal weight (18.5 – 24.9), 20 (33%) overweight (25 – 29.9), and 23 (38%) with obese (≥ 30).

Our finding was very similar to the result of a study in Iraq by Al-Saady who reported a highly significant increase in BMI among breast cancer patients [16]. The association between obesity and breast cancer is widely explained by four mechanisms which are sex hormone metabolism; low adiponectin levels circulating in obese patients which act on ERα-positive cells as a growth factor stimulating proliferation [17], deregulated insulin signaling, and chronic low-grade inflammation [18]. Results of data analysis in Table 2 showed that BMI ≥ 30 significantly increases

the risk of breast cancer in Iraqi women. Our results were consistent with the results of other studies Amadou *et al.,* Li *et al.,* and Zahmatkesh *et al.,* [19-21], their results demonstrated the risk of breast cancer in obese women (BMI > 30) was higher than women with normal BMI. The results of Hosseinzadeh *et al.,* demonstrated no association between high BMI and developing breast cancer [23]. Some studies showed that a higher BMI increases the risk of breast cancer after menopause, but it decreases it during a premenopausal period [20].

Table 2: Relation the number of breast cancer women with BMI.

BMI (Kg/m^2)		**Studied group**
		Breast cancer women
Underweight (<18.5)	N	7
	%	12
Normal weight (18.5 – 24.9)	N	10
	%	17
Overweight (25 – 29.9)	N	20
	%	33
Obese (≥ 30)	N	23
	%	38
Total	**N**	**60**
	%	**100**

Serum Level of Alkaline Phosphatase (ALP) in Women with Breast Cancer

As shown in table (3), the mean of the serum level of Alkaline Phosphatase (ALP) in breast cancer women comparing with the control group (95.2 ± 59.0 versus 82.4 ±37.4 U/L). The result was no significant (p>0.215).

Table 3: Serum Level of Alkaline Phosphatase (ALP) in Women with Breast Cancer

Study groups	**No.**	**A L P U/L**	**T. test**	**P. value**
Breast cancer women	60	95.2 ± 59.0	1.25	0.215 NS
Control group	30	82.4 ±37.4		

Serum ALP activity was no significant at P value = 0.215, in breast cancer women as compared with control, as shown in Table 3.

Many studies by Amritpal F *et al.,* [24] and Choudhari A *et al.,* [25] where found levels of serum ALP were significantly higher in patients of carcinoma breast as compared to controls and that there was a progressive increase in serum ALP activities with advance in stage of disease and metastasis. Enzymes were one of the first groups of tumor markers, identified their elevated activities were used to indicate the presence of cancer, the observed increase in alkaline phosphatase activity in sera of patients with malignant breast tumors can be explained by the fact that the changes in the membrane permeability of the tumor cells lead to the release of the enzyme into the circulation [26, 27]. Serum alkaline phosphatase activity elevated in a variety of malignancies, like lung, gastrointestinal and in Hodgkin's disease. Alteration in alkaline phosphatase activity leads to increase inorganic phosphate which is important for the synthesis of adenosine triphosphate that is needed as core of the requirement energy for the tumor cells [24]. Resulting of increase in alkaline phosphatase activity also increase the synthesis of DNA by provide inorganic phosphate which need to nucleotide synthesis. The progressive increase in the serum ALP activity in breast cancer patients is an indication of metastasis [28]. This marker is used to evaluate the patient's response to treatment and to detect the presence of metastasis or recurrence the increase in alkaline phosphatase activity is a constant feature in neoplastic transformation, and this was noted in all cases of breast carcinoma found that alkaline phosphatase levels were higher in sera of the groups with infiltrating carcinoma than in the women with benign disorders of the breast, in addition to that the elevated levels of ALP were seen in primary and secondary liver cancer [22]. Some studies used this non-specific enzyme markers can be routinely used easy to estimate assay, it does not require sophisticated center or any latest technology and can be performed even at the rural centers for diagnosing breast cancer, detecting metastasis and monitoring the cancer progression and treatment. Alkindi and Alhashemi (2022) [29] study, who reported ALP levels are significantly higher in cases of breast cancer patients than healthy controls. ALP is a sensitive liver indicator. High levels imply bone or liver metastases. Hepatic metastases were seen in 55-75% of breast cancer deaths. Hepatic metastases are crucial for patient survival. Early detection of liver metastases improves prognosis. CT, MRI, and PET can identify breast cancer liver metastases. Acute hepatic symptoms such as jaundice, hepatomegaly, and ascites occur late in the disease process [29]. Our study agreement with Anber (2022) [30] study showed that there was a non-significant difference in the serum levels of alkaline phosphatase breast cancer patients and patients treated with chemotherapy. These results were on the same line with a study done by Oluboyo *et al.,* who stated that there was no statistically significant difference in the ALP activity between the breast cancer subjects on chemotherapy and those not on chemotherapy, It has been reported that

chemotherapeutic agents can have both direct and indirect effects on the bone microenvironment ultimately leading to a decrease in the bone mineral density [31]. Also, the present study was in agreement with other studies in which they didn't find any significant difference in ALP levels in non-metastatic breast cancer. On the other hand the present results were in contrary to previous studies in which they stated that the progressive increase in serum ALP was due to metastasis of breast cancer either to bone or liver. Also, inconsistent with previous studies in which they stated that chemotherapy induces bone marrow density loss [32, 33].

Serum Level of Hepcidin in Women with Breast Cancer

As shown in Table (4), the mean of the serum level of Hepcidin in breast cancer women compared with the control group (6.43 ±3.27 versus 4.63 ±2.84 ng/mL). The result was highly significant ($p<0.009$).

Table 4: Comparison between breast cancer and healthy women regarding the mean ± SD of Hepcidin

Study groups	No.	Hepcidin (ng/mL)	T. test	P. value
Breast cancer women	60	6.43 ±3.27	2.70	0.009
Control group	30	4.63 ±2.84		

Our study agreement with Ali *et al.,* (2020) ([33] reported that serum levels of hepcidin in breast cancer patients 437.2± 26.4 compared with the control group 179.4± 19.8. In this study there were significant elevations in serum hepcidin level, the important role of iron in DNA synthesis could lead to cell cycle arrest after iron depletion, whereas chronic sub-toxic levels feeding of iron to cancer cells transform them into a more aggressive phenotype which is prone to metastasis [34]. Hepcidin induces FPN degradation, thus, interfering with iron efflux and cause iron sequestration in tumor cells. Moreover, hepcidin blocks iron export from cells such as macrophages and enterocytes. Serum levels of hepcidin are strictly controlled by different stimuli; iron status is the prime controller of hepcidin expression under basal conditions; yet, other factors can also control liver hepcidin expression, such as hormones, growth factors, and heparins [35].

In this study, serum levels of hepcidin in all patient groups are significantly higher than that of the control group, and the elevation was coordinated with the stage of the disease.

Similar findings were reported in previous studies Orlandi *et al.,* [36] reported that patients with BC lesions had significantly higher hepcidin levels than healthy control group.

Correlation between S. Hepcidin Level and AIP Level in Women with Breast Cancer

This study found that there was (Inverse) a moderate negative correlation between S. Hepcidin Level and AIP Level in Women with Breast Cancer (R: -0.184), Figure (1).

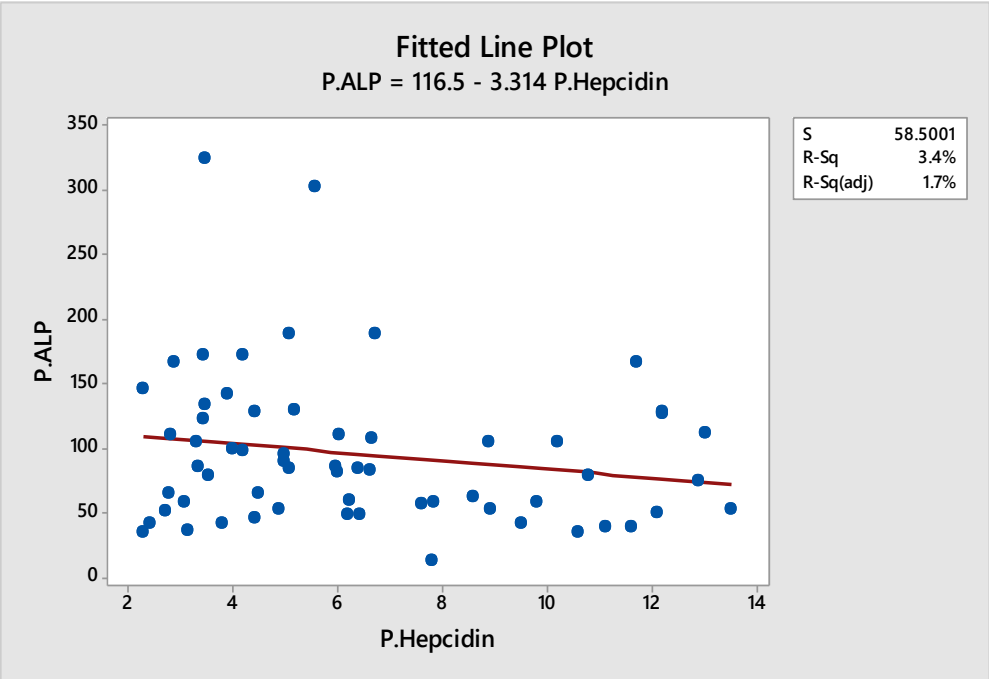

Figure 1: correlation between S. Hepcidin Level and AIP Level in Women with Breast Cancer

CONCLUSIONS

This study showed that the peak age of women with breast cancer was between 45 – 56 years. Serum Level of Alkaline Phosphatase (ALP) in breast cancer women comparing with the control group was no significant difference. This study found that there was (Inverse) a moderate negative correlation between S. Hepcidin Level and AIP Level in Women with Breast Cancer

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