

## Assessment of Some Hematological Parameters in Iraqi Women with Different Breast Cancer Stages

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### Abstract

Breast cancer (BC) is the most commonly diagnosed cancer in women. The metabolism of iron is closely regulated by hepcidin which exerts its action by interacting with a ferroportin.

The aim of the present study was to assess the alterations in the levels of some serum biomarkers that have a role in iron homeostasis (hepcidin and ferroportin) in addition to hematological parameters (hemoglobin, leukocyte and platelets count) in different stages of BC.

This study included 66 women with BC. The patients were categorized as follows : group 1 includes :22 patients with stage I disease ,group 2 includes: 22 patients with stage II disease ,and group 3 include: 22 patients with stage III disease .Group 4 includes :22 apparently healthy women as control.

Data analysis revealed a significant elevation of serum hepcidin levels of patients groups 1, 2, and 3 ( $437.2 \pm 26.4$ ,  $501.4 \pm 31.8$  and  $558.5 \pm 21.3$  pg/ml respectively) ,vs ( $179.4 \pm 19.8$  pg/ml) of control, with steady elevations from stage I to III . Furthermore, serum ferroportin levels were significantly lowered in groups 1 and 3 compared to control ( $0.589 \pm 0.107$  and  $0.733 \pm 0.1$  vs  $1.37 \pm 0.28$  ng/ml respectively). While blood hemoglobin level of group 3 were lower ( $11.96 \pm 0.18$  vs  $12.7 \pm 0.13$  g/dl ) compared with controls . Blood leukocyte count of patients (all groups) ( $7.39 \pm 0.28$ ,  $8.93 \pm 0.48$ ,  $9.86 \pm 0.52$  ( $\times 10^3/\mu\text{l}$ ) respectively) were markedly increased compared to controls ( $6.06 \pm 0.23$ ), while mean platelet count for patients in group 2&3 were significantly increased compared to controls ( $313.9 \pm 19.3$ ,  $309.2 \pm 25.3$  vs  $233.3 \pm 9.1$  respectively).

In conclusion, hepcidin, ferroportin and hematological markers including hemoglobin, WBC count and platelets count are altered in women with BC compared to healthy control. The changes occur mostly in accordance with disease stages .

**Key words:** Breast cancer, Hpcidin , Ferroportin.

### تقييم بعض المعلمات الدموية لدى المريضات العراقيات المصابات بمراحل مختلفة من سرطان الثدي

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### الخلاصة

سرطان الثدي هو أكثر سرطان مشخص لدى النساء. إن أيض الحديد منظم بدقة بواسطة بروتين الهبسيدين الذي يعمل بالتنسيق مع الفروپورتين.

تهدف هذه الدراسة الى تقييم فائدة قياس بروتينات في مصل الدم التي لها دور في تنظيم معدلات الحديد (الهبسيدين والفروپورتين) بالإضافة الى قياس العوامل الدموية (الهيموغلوبين وكريات الدم البيضاء والصفائح الدموية) كوسائل تساعد في دراسة تقدم سرطان الثدي .

شملت هذه الدراسة ٦٦ انثى مصابة بسرطان الثدي، يتم تقسيم المريضات حسب مراحل المرض: المجموعة الاولى شملت ٢٢ مريضة ( بالمرحلة الاولى من المرض)، المجموعة الثانية شملت ٢٢ مريضة (بالمرحلة الثانية من المرض)، المجموعة الثالثة شملت ٢٢ مريضة (بالمرحلة الثالثة من المرض). المجموعة الرابعة شملت ٢٢ انثى تبدو بصحة جيدة كمجموعة مقارنة .

تحليل النتائج اظهرت ارتفاع مهم في معدلات الهبسيدين لدى كل مجاميع المرضى ١ و٢ و٣ ( $437.2 \pm 26.4$ ،  $501.4 \pm 31.8$  و  $558.5 \pm 21.3$  pg/ml) مقابل ( $179.4 \pm 19.8$  pg/ml) لمجموعة المقارنة وكانت هناك زيادة متناسبة مع تقدم المرض من المرحلة الاولى الى المرحلة الثالثة . بالإضافة الى ذلك كان هناك نقصان مهم في معدلات الفروپورتين لدى مرضى المرحلة الاولى والثالثة بالمقارنة مع معدلات مجموعة الاصحاء ( $0.589 \pm 0.107$  و  $0.733 \pm 0.1$  مقابل  $1.37 \pm 0.28$  ng/ml). بينما اظهرت معدلات الهيموغلوبين للمجموعة الثالثة نقصان مهم ( $11.96 \pm 0.18$  مقابل  $12.7 \pm 0.13$  g/dl)، مع مجموعة الاصحاء. كان متوسط معدلات كريات الدم البيضاء لمجاميع المرضى ١ و٢ و٣ هي ( $7.39 \pm 0.28$ ،  $8.93 \pm 0.48$  و  $9.86 \pm 0.52$  مقابل  $6.06 \pm 0.23$   $\times 10^3/\mu\text{l}$ )، بينما كانت متوسطات معدلات الصفائح الدموية للمجاميع ٢ و٣ مرتفعة ارتفاعاً مهماً مقارنة لمجموعة الاصحاء ( $313.9 \pm 19.3$  و  $309.2 \pm 25.3$  مقابل  $233.3 \pm 9.1$   $\times 10^3/\mu\text{l}$ ) .

وبذلك يمكن ان نستنتج ان الهبسيدين والفروپورتين والعوامل الدموية (الهيموغلوبين واعداد كريات الدم البيضاء واعداد الصفائح الدموية) اظهرت انحرافات في النساء المصابات بسرطان الثدي بالمقارنة مع مجموعة الاصحاء. معظم هذه الانحرافات كانت متناسبة مع مرحلة المرض. الكلمات المفتاحية: سرطان الثدي، الهبسيدين، الفروپورتين .

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## Introduction

Breast cancer (BC) is the most commonly diagnosed cancer in women, it is estimated to affect (24.2%) of women worldwide. In Iraq, the number of new cases in 2018 for both sexes, and for all age groups was 5141 which represent 20.3% of all new cases for all cancer types (25320), while the number of prevalent cases of all cancer types over 5 years for all ages was 54809 cases<sup>(1)</sup>.

Breast carcinomas is the most common type of BC although different types of sarcomas and lymphomas also can be encountered<sup>(2)</sup>.

Chronic inflammation is a key participant in cancer development and progression<sup>(3)</sup>. The mechanism by which chronic inflammation is related to BC prognosis is not fully clear. However, complex processes through which chronic inflammation may promote carcinogenesis such as polarization of M2 tumor-associated macrophages via inflammatory cytokines with the subsequent production of tumor growth factors or promotion of angiogenesis<sup>(4,5)</sup>. In addition, several prognostic factors are correlated with inflammatory status such as body fatness, physical activity, and cardiovascular comorbidity, which may affect tumor prognosis through alternate mechanisms. Meanwhile, the presence of undetected cancer cells may result in inflammation, thus inflammation may not only contributor to tumor promotion, but also result of these cancer cells<sup>(6)</sup>.

The American Society of Clinical Oncology (ASCO) recommends the use of tumor markers in prevention, screening, treatment and surveillance of BC. The most common clinically applied tumor markers for breast cancer are CA 15-3, CA 27.29, Carcinoembryonic antigen (CEA), Estrogen receptor (ER), Progesterone receptor (PR), Human epidermal growth factor receptor 2 (HER2), urokinase plasminogen activator (uPA), plasminogen activator inhibitor 1 (PAI-1) and multiparameter assays for gene expression<sup>(7)</sup>.

The metabolism of iron is closely regulated by hepcidin which is liver-derived peptide. It exerts its action by interacting with a ferroportin (FPN), a transmembrane protein implicated in iron efflux from the body iron stores<sup>(8)</sup>. Binding of hepcidin with FPN exerts a negative effect on erythropoiesis by inducing internalization and then subsequently destruction of FPN<sup>(8,9)</sup>.

Increased erythropoiesis suppresses hepcidin production, while iron loading and inflammation induce its production. Blood loss, anemia, increased erythropoietin or hypoxia stimulate bone marrow erythropoiesis and then reduce hepcidin<sup>(10,11)</sup>. Increase in serum hepcidin has been reported increasingly from diverse clinical states, (neoplastic diseases, inflammation, and sepsis)<sup>(10,12,13)</sup>.

Sideropenic anemia is frequently reported in cancer patients,<sup>(14)</sup> that is mediated by the inflammatory cytokines produced by cancer cells and by macrophages infiltrating the cancer tissue<sup>(15)</sup>. These

cytokines stimulate the hepatic production of hepcidin, which in turn inhibit iron absorption in the duodenum and promote iron sequestration by macrophages limiting its recycling<sup>(16)</sup>. Moreover, the inflammatory cytokines decrease FPN expression in duodenal enterocytes blocking the transport of iron from enterocytes to transferrin<sup>(16)</sup>. IL-10 also induces an increase in serum ferritin and soluble transferrin receptor (sTfR) levels causing anemia<sup>(17)</sup>.

The role of peripheral WBC in predicting incident BC remains to be determined in spite of the established role of peripheral WBCs in BC prognosis<sup>(18-19)</sup>. Many studies showed the lack of association between total white blood cell count (TWBCC) and BC risk<sup>(20-21)</sup>; yet, TWBCC serves as a significant predictor of certain types of BC and for other types of cancer like lung<sup>(20)</sup>, gastric<sup>(22)</sup>, and colorectal cancers<sup>(23)</sup>.

Furthermore, platelets have important roles in various physiological and pathophysiological processes, including inflammation, immunity, angiogenesis, wound healing, and cancer progression<sup>(24-25)</sup>. Platelet dysfunction and thrombotic disorders are important manifestations of cancer progression<sup>(24-26)</sup>. Thrombocytosis is associated with poor cancer prognosis, suggesting a potential role for platelets in the pathogenesis of the disease<sup>(27-28)</sup>.

The aim of the present study was to assess the alterations in the levels of some serum biomarkers that have a role in iron homeostasis (hepcidin and ferroportin) in addition to hematological parameters (hemoglobin, leukocyte and platelets count) in different stages of BC.

## Material and Methods

This study was conducted at The Oncology Teaching Hospital / Baghdad Medical City from October/2018 to February/2019.

Subjects enrolled in the study were 66 women that were recently diagnosed as having BC at different stages of the disease; and 22 apparently healthy women of comparable age to serve as control group. The age range of the subjects was 30-70 years. Specialist oncologist performed diagnosis and staging of BC according to Breast Cancer Staging, NCCN guidelines Version 4.2017<sup>(29)</sup>; a specialized pathologist using the WHO grading system,<sup>(30)</sup> performed the histological confirmation and grading.

BC patients at stage IV of the disease is characterized by metastasis to other organs, and those at stage 0 having either ductal carcinoma in situ (DCIS) or lobular carcinoma in situ (LCIS) of disease, were excluded from the study. Other excluded patients were those whom were already undergoing chemo-, radio-, or hormonal therapy; or those with autoimmune diseases or hemoglobinopathy, except iron deficiency anemia.

Subjects enrolled in the study were grouped as: Group 1 including 22 BC women at stage I of the disease, Group 2 including 22 BC women at stage II of the disease, Group 3 including 22 BC women at stage III of the disease and Group 4 including 22 apparently healthy women serving as control group. Blood specimens were obtained from all participants. Serum levels of hepcidin and FPN were estimated quantitatively by sandwich type of enzyme linked immune-sorbent assay (ELISA),<sup>(31)</sup> using kits purchased by R&D® (USA) for hepcidin and MyBioSource® (USA) for ferroportin. Measurements of total white blood cell count<sup>(32)</sup>, platelet cell count<sup>(33)</sup> were performed utilizing whole blood samples based on DynaHelix Flow technology. Estimation of blood hemoglobin was performed utilizing colorimetric surfactant method<sup>(34)</sup>.

#### Statistical analysis

Analysis of data was carried out using the available statistical package of SPSS-23 (Statistical Packages for Social Sciences- version 23). Data were presented in simple measures of mean, standard error, and range. Student t-test was used for testing the significant difference between two groups, whereas ANOVA among more than two groups by LSD post hoc multiple comparisons of data. Person's correlation was calculated for studied quantitative variables. Statistical significance was considered whenever the P value was less than 0.05.

**Table2.Descriptive data of studied groups.**

group parameter	Group 1	Group 2	Group 3	Group 4
Age (years)	51.5±2.3	46.2±1.66	45.9±1.79	44.3±1.5
Body weight (Kg)	73.3±1.76	72.4±1.96	76.4±2.24	72.04±2.75
Height (cm)	160.1±1.42	157.9±1.46	161.6±1.26	163.04±2.05
BMI(kg/m <sup>2</sup> )	28.4±0.45	28.9±0.41	29.1±0.7	26.9±0.82

Data are expressed as mean± SE , BMI: body mass index

Data of Table-3 describe the differences between patients and control groups regarding family history, a number of smoker subjects, presence of diabetes mellitus , presence of hypertension . Sixteen of BC patients were presented with one or more first degree relative with BC in contrast to two of female control, all healthy control females were selected as none of them having diabetes mellitus nor hypertension and all of them were nonsmoker, while five of BC patients were diabetic and eight of them were hypertensive.

## Results

Age range for group 1 was 32-67 years (51±2.3), for group 2 was 34-68 years, (47.63±1.87), and for group 3 was 32-65 year (45.5±1.75), While age range for control group was 31-60 years (44.3±1.5).

Table-1 summarized the descriptive data of BC patients and healthy control regarding age, body weight, height, and body mass index (BMI). There was no significant difference between total patients group and healthy control group regarding age, body weight, and height (P value 0.064,0.502 ,0.064 respectively) and significant difference regarding BMI (P value 0.041).

**Table1. Descriptive Data of Total Patients and Control Groups**

Parameter	BC Patients (n=66)	Healthy control (n=22)	P value
Age (years)	47.9±1.14	44.3±1.5	0.064
Body weight (Kg)	74.07±1.15	72.04±2.7	0.502
Height (cm)	159.8±0.81	163.04±2.05	0.064
BMI (kg/m <sup>2</sup> )	28.8±0.3	26.96±0.82	0.041*

Data are expressed as mean± SE , BC: breast cancer, BMI: body mass index,\* significant difference from control group (p<0.05)

Table 2 presents the data of studied groups regarding age, body weight, height and body mass index

**Table 3 . Medical history of studied groups**

Parameter	BC patients n=66	Healthy control n=22
Family history	16	2
Smoker **	2	None
DM	5	None
Hypertension***	8	None

BC: breast cancer, n=number, DM: diabetes mellitus,

Table 4 describes the differences among studied groups of patients regarding hormonal receptors state [estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor 2 receptor (Her-2)], and histological classification of BC as

invasive ductal carcinoma (IDC) was the predominant type followed by ductal carcinoma in situ (DCIS) with IDC, then invasive lobular carcinoma (ILC) with IDC and finally ILC.

**Table4. Basic criteria of studied patients in different stages of breast cancer**

Parameter	Group		Stage I (n=22)		Stage II (n=22)		Stage III (n=22)		Total (n=66)	
		%		%		%		%		%
ER positive	10	45.4	16	72.7	17	77.2	43	65.1		
PR positive	12	54.5	16	72.7	16	72.7	44	66.6		
Her-2 positive	9	40.9	10	45.4	9	40.9	28	42.4		
IDC	16	72.7	15	68.1	14	63.6	45	68.1		
DCIS&IDC	5	22.7	6	27.2	4	18.1	15	22.7		
ILC	0	0	1	4.5	0	0	1	1.5		
ILC&IDC	1	4.5	0	0	4	18.1	5	7.2		

ER: estrogen receptor, PR : progesterone receptor, Her-2:human epidermal growth factor receptor, IDC :invasive ductal carcinoma, DCIS: ductal carcinoma in situ, ILC :invasive lobular carcinoma.

As presented in Table-5, there were significant elevations in serum hepcidin level, blood WBC count and blood platelet count of total BC patients as compared to control group, while there was

significant decrease in blood hemoglobin level of total patients as compared to control and no significant difference between these groups regarding a serum ferroportin level.

**Table5. Blood and serum levels of the studied parameters among total patients and control groups**

Parameter	Total patients (n 66)	Control group (n 22)	P value
S. hepcidin pg/ml	499.09±16.4	179.4±19.8	0.000*
S.ferroportin ng/ml	0.794±0.11	1.37±0.28	0.07
B.hemoglobin gm/dl	12.1±0.16	12.7±0.13	0.009*
B.WBC count (^10 <sup>3</sup> /μl)	8.73±0.28	6.06±0.23	0.000*
B. platelet count (^10 <sup>3</sup> /μl)	301.8±12.4	233.3±9.16	0.000*

Data are expressed as mean ±SE, WBC: white blood cell ,B: blood ,S: serum

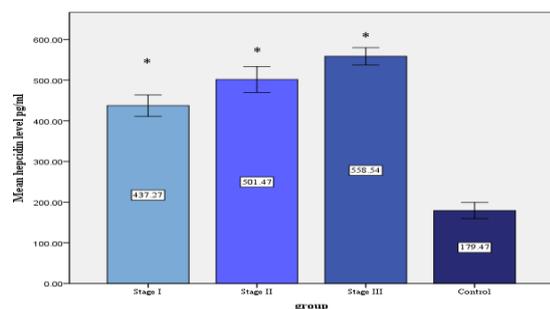
**Table6. Blood and serum levels of the studied parameters among the different study groups**

Parameter	Group 1 (n=22)	Group 2 (n=22)	Group 3 (n=22)	Group 4 (control) (n=22)	P value
S. hepcidin pg/ml	437.2± 26.4	501.4± 31.8	558.5± 21.3	179.4± 19.8	0.000*
S.ferroportin ng/ml	0.589± 0.107	1.06± 0.31	0.733 ± 0.102	1.37± 0.28	0.07
B.hemoglobin gm/dl	12.2± 0.28	12.4± 0.37	11.9± 0.18	12.7± 0.13	0.17
B . WBC count (^10 <sup>3</sup> /μL)	7.39± 0.28	8.93± 0.48	9.86± 0.52	6.06± 0.23	0.000*
B.platelet count(^10 <sup>3</sup> /μL)	282.2± 20.09	313.9± 19.3	309.2 ±25.3	233.3 ±9.16	0.016*

Data are expressed as mean ±SE, WBC: white blood cell , B: blood ,S: serum

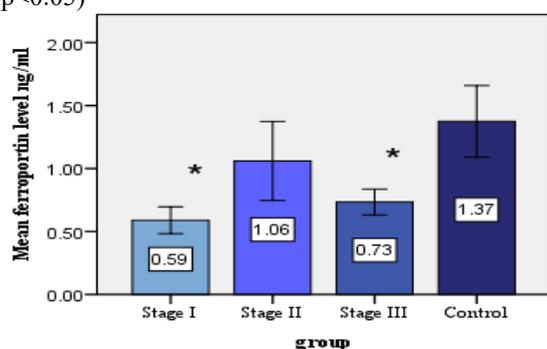
Figure-1 illustrates that serum hepcidin levels of patient groups are significantly higher than that of controls through different stages from I-III. Hecpidin levels tends to increase as the disease progresses; as presented by significantly higher levels in stage III compared to that in stage I.

Serum levels of FPN of stage I and III were significantly lower than that of control group with no significant difference between patient groups (Figure-2). Blood hemoglobin levels of stage III was significantly lower than that of control group with no significant difference between patients groups (Figure-3).



**Figure1. Mean value of serum hepcidin among studied groups**

\*= significantly different from control group (p<0.05)



**Figure2. Mean value of serum ferroportin among studied groups**

\*= significantly different from control group (p<0.05).

**Table 5. Correlation study among studied groups**

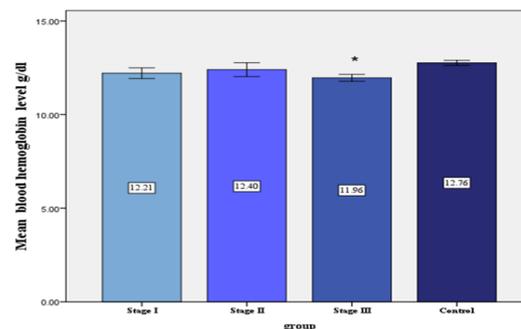
x-axis	y-axis	r-value	P value	Group
S.hepcidin	S.ferroportin	-0.678	0.001	Stage II
S.hepcidin	S.ferroportin	-0.433	0.044	Stage III
S.hepcidin	S.ferroportin	-0.407	0.001	Total patient
S.ferroportin	B.Platelet	-0.438	0.041	Stage III

S: serum, B: blood

**Discussion**

Globally, more than 95% of BCs diagnosed as adenocarcinomas (35), while IDC is the most common form of invasive breast cancer (accounting for 55% of incidence upon diagnosis) (36), and ILC constitutes only 5%–15% of invasive breast carcinoma (37). The results of this study agree with these results, as 68.1% of the patients having IDC, and only 1.5% of patients having ILC as mentioned in Table-2.

The important role of iron in DNA synthesis could lead to cell cycle arrest after iron depletion, (38) whereas chronic sub toxic levels feeding of iron to cancer cells transform them into a the more aggressive phenotype which is prone to metastasis (39). Hecpidin induces FPN degradation, thus, interfering with iron efflux and cause iron



**Figure 3. Mean value of blood hemoglobin among studied groups**

\*= significantly different from control group (p<0.05)

Correlation studies were done between all studied parameters among all groups including total patients, group 1, group 2, group 3, and group 4 and the significant results (that indicated positive or negative correlation) are tabulated in table-5. Serum hepcidin levels were significantly correlated with serum FPN levels in BC patients (total patient r=-0.407, stage II r=-0.678, stage III r=-0.433). More advanced stages of BC (stage III) were presented with more significant correlation between FPN levels and blood platelet count (r=-0.438).

sequestration in tumor cells. Moreover, hepcidin blocks iron export from cells such as macrophages and enterocytes (40). Serum levels of hepcidin are strictly controlled by different stimuli; iron status is the prime controller of hepcidin expression under basal conditions (41); yet, other factors can also control liver hepcidin expression, such as hormones, growth factors and heparins (42-44).

In this study, serum levels of hepcidin in all patients groups are significantly higher than that of the control group and the elevation was coordinated with the stage of the disease (P value 0.000, 0.000, 0.000 respectively) with significant elevation of stage III as compared to stage I (P value 0.001), while there was no significant difference between levels of stage I &II, stage II&III (P value 0.076

,0.115 respectively). While, serum FPN levels are significantly lower in patients with stage I and III than in the control group (P value 0.015 , 0.047 respectively). Serum FPN levels in patients with stage II was also lower than that of the control group but the difference was statistically non-significant (P value 0.325),and there were no significant differences between stages I&II and stages I&III and stages II&III (P value 0.142 ,0.65 ,0.307 respectively).

Similar findings were reported in previous studies. Orlandi et al<sup>(45)</sup> reported that patients with BC and benign breast lesions had significantly higher hepcidin levels than healthy control group. Similarly, Ciniselli et al<sup>(46)</sup> found that when compared with patients with benign breast diseases, patients with BC had significantly higher hepcidin levels, Also BC patients were reported to have higher serum hepcidin that is associated with lower levels of FPN when compared to healthy controls<sup>(45,47)</sup>.

Patients with stage III showed significantly lower blood hemoglobin levels compared to that of the control group (P value 0.032) and non-significant decrease of stage I and stage II as compared to control group (P value 0.138 ,0.325 respectively), and there were non-significant differences among patients groups (stages I&II, stages I&III, and stages II&III) (P value 0.613 ,0.498 ,0.238 respectively). These changes occur in accordance with the high hepcidin and low FPN levels. Twelve out of twenty two of BC patients at stage III have iron deficiency anemia at time of BC diagnosis. The number of anemic BC patients at stage I and stage II was much lower than that of patients at advance stage (stage III) (4/22 and 5/22) respectively; these findings point the important role of hepcidin in iron metabolism.

Hepcidin overexpression is not only associated with various hematological malignancies and some solid organs tumors such as breast, prostate cancers, but it also participate in the development of anemia in those patients<sup>(10,45,48)</sup>.

In this study, there was a steady increase in TWBCC throughout disease stages, and there were significant elevations in all patients groups (stage I, II, and III) as compared to control group (P value 0.022 ,0.000 ,0.000 respectively), even that the increase is still within normal range of blood WBC count [4-10( $\times 10^3/\mu\text{l}$ )]<sup>(49)</sup>. There were significant elevations of stage II and III than that of stage I (P value 0.008 ,0.000 respectively), and no-significant elevation of stage III than that of stage II (P value 0.103). Elevated WBC count has been shown to be associated with increased risks of the lung<sup>(19)</sup>, gastric<sup>(22)</sup>, and colorectal<sup>(23)</sup> cancers.

leukocytosis is associated with thrombocytosis which is negative prognostic indicator in patients with cancer<sup>(50-51)</sup>. Thrombocytosis is associated with a poor cancer prognosis, suggesting a potential role

for platelets in the pathogenesis of the disease<sup>(28)</sup>. In this study, platelet count of all patients group still within normal values.

There were significant negative correlations between serum hepcidin levels and serum ferroportin levels of total patients, stage II patients group and stage III patient group, and these results may be related to negative regulatory role of hepcidin on FPN by induction of the later degradation<sup>(8,9)</sup> as these groups showed significant elevation of serum hepcidin level with decrease in FPN levels as compared to control. Also previous studies reported elevation of serum hepcidin in association with decreased FPN levels as compared to healthy control<sup>(45,52)</sup>.

## Conclusion

Hepcidin, ferroportin and hematological markers including hemoglobin, WBC count and platelets count are altered in women with BC compared to healthy control. The changes occur mostly in accordance with disease stage. Larger scale study testing validity of these markers in diagnosis and staging of BC is recommended.

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