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Assessment of Serum Hepcidin Levels and Iron Status in Anemic Patients Admitted to Medical Intensive Care Unit

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ABSTRACT

Background: Anemia is very common during critical illness and results from two main factors: inflammation and iron deficiency. Hepcidin, a peptide hormone made in the liver, is the principal regulator of systemic iron homeostasis.

Objective: We aimed in this study to improve the prognosis of anemia in critically ill patients through assessment of serum hepcidin and iron levels in critically ill anemic patients admitted to the medical ICU of Zagazig university hospital and to find out the relationship between levels of serum hepcidin and outcome of critically ill anemic patients.

Patients and Methods: This study was a prospective study that was conducted on 80 patients who fulfilled the inclusion and exclusion criteria were diagnosed, assessed, received medical support, and followed during their stay in MICU.

Results: The mean Hb and hematocrit levels were 9.7±1.3 g/dl and 28.9±4% respectively. Serum hepcidin and serum ferritin levels are markedly elevated among critically ill anemic patients in MICU compared to what is reported in the literature for the general population which reflects the relationship with inflammation. Serum Hepcidin was not correlated with any serum Hb, hematocrit, APACHE II score, or ICU length of stay. Serum hepcidin level had no impact on mortality of the study population in MICU.

Conclusion: Serum hepcidin and ferritin levels are significantly elevated in critically ill anemic patients who are characterized by the inflammatory stress of their illnesses. Serum hepcidin level measured on admission to medical ICU has no impact on patients' short-term ICU outcome in terms of mortality or length of hospital stay.

Keywords: Serum Hepcidin, Iron status, Anemic Patients, Medical Intensive Care Unit.

INTRODUCTION

Anemia is a decrease in the total amount of red blood cells or hemoglobin in blood or lowered ability of blood to carry oxygen [(1)].

There are several types and classifications of anemia. The occurrence of anemia is due to the various red cell defects such as production defect (aplastic anemia), maturation defect (megaloblastic anemia), defects in hemoglobin synthesis (iron deficiency anemia), genetic defects of hemoglobin maturation (thalassemia), or due to the synthesis of abnormal hemoglobin (hemoglobinopathies, sickle cell anemia, and thalassemia) and physical loss of red cells (hemolytic anemia) [(2)].

Anemia is very common during critical illness and results from two main factors: inflammation and iron deficiency [(3)].

Inflammation-induced anemia is frequent among critically ill patients and can be aggravated by true iron deficiency (ID) resulting from blood losses. The serum hepcidin level controls the availability of iron for erythropoiesis, and its determination offers new perspectives for the diagnosis of ID in the presence of inflammation [(4)].

Hepcidin, a peptide hormone made in the liver, is the principal regulator of systemic iron homeostasis. Hepcidin controls plasma iron concentration and tissue distribution of iron by inhibiting intestinal iron absorption, iron recycling by macrophages, and iron mobilization from hepatic stores. Hepcidin acts by inhibiting cellular iron efflux through binding to and inducing the degradation of ferroportin, the sole known cellular iron exporter [(5)].

Understanding the role of hepcidin shows why previous studies using either iron or erythropoietin failed to boost red blood cell synthesis in trauma or critically ill patients and may show the way for decreasing blood transfusions in the future [(6)].

This study aimed to improve the prognosis of anemia in critically ill patients through assessment of serum hepcidin and iron level in critically ill anemic patients admitted to the medical ICU of Zagazig University Hospital and to find out the relationship between levels of serum hepcidin and the outcome of critically ill anemic patients.

PATIENTS AND METHODS

This study was a prospective study that was conducted on 80 patients who fulfilled the inclusion and exclusion criteria. They were diagnosed, assessed, received medical support, and followed during their stay in the Medical Intensive Care Unit (MICU) of the Internal Medicine Department with anemia at the time of admission during the study period of six months.

Critically ill patients admitted to different units of MICU of Zagazig University Hospitals starting from August 2020 to January 2021 whose CBC showed anemia on admission with hemoglobin < 12 g/dl.

The study was conducted at "The Medical Intensive Care Unit of Zagazig University Hospitals" and the laboratory work was done at "The Clinical Pathology Department".

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Inclusion Criteria: Aged 18 years or over, both sexes, and admission hemoglobin level < 12 g/dl.

Exclusion Criteria: History of iron intake or erythropoietin injection in the last month before admission, subjects with blood transfusion within the preceding 3 months, likely to be unavailable for follow-up, and refusal to give consent to participate in the study by the patient's relatives.

Ethical consent:

The study protocol was approved by The Institution Review Board (IRB) of the Faculty of Medicine of Zagazig University and informed written consent was obtained from the patient's relatives. This work was carried out following The Code of Ethics of the World Medical Association (Declaration of Helsinki) for studies involving humans.

Methodology:

During the study period, 96 patients who fulfilled the inclusion and exclusion criteria were enrolled in the present work. Patients were diagnosed, assessed, received medical support, and followed during their stay in MICU. After analysis of patients' data, only 80 of them completed assessment and follow-up to be eligible for statistical analysis.

Baseline data for patients were collected immediately after admission and blood samples were taken for laboratory investigations (visit 1). Subsequent visits were conducted daily with a record of progress notes until the time of discharge from MICU.

All patients were subjected to the following:

1. **Full history taking and thorough physical examination** with emphasis on demographic and clinical data as well as identifying the cause of admission to MICU.
2. Blood samples were extracted immediately and sent to the laboratory for **routine and specific investigations** with the assessment of complete blood count CBC) before any interventional measures and fluid administration.
3. Patients who proved to have anemia with a hemoglobin level below 12 g/dl were enrolled in the study.
4. After the initial assessment, patients were stratified to different units of the MICU according to their provisional diagnosis and immediate resuscitation and medical support were given to them according to our protocols.
5. **Routine Laboratory Investigations included:** CBC with a calculation of blood indices (Hemoglobin, Hematocrit, MCV, MCH, MCHC), and other investigations according to the clinical situation.
6. Specific Investigations to assess iron status: Serum iron, serum ferritin, total iron-binding capacity (TIBC), transferrin Saturation.
7. **Lab devices used in Zagazig University Hospital laboratory as fellow:** CBC analysis was performed by the device (Sysmex-x5 500i). Serum iron & TIBC by the device (Cobas 6000), and serum ferritin performed by the device (Vidas biomerinex)
8. **Measurement of serum Hepcidin level:** Serum Hepcidin was estimated following the standardized technique of the Human Hepcidin (Hepc) ELISA Kit. The kit uses a double-antibody sandwich enzyme-linked immunosorbent assay (ELISA) to determine the level of Human Hepc in samples.
9. Hepc was added to the monoclonal antibody-enzyme well which is pre-coated with Human Hepc monoclonal antibody, incubated; then, labeled Hepc antibodies with biotin were added and combined with treptavidin-HRP to form an immune complex, then incubated and washed again to remove the uncombined enzyme. Then Chromogen Solution A, B, was added and the color of the liquid was changed into blue, where changed finally into yellow. The chroma of color and the concentration of the Human Substance Hepc of the sample were positively correlated.
10. **The outcome in MICU was identified with a record of the following data:** Length of stay in ICU and Mortality in ICU.

Statistical Analysis:

Data collected throughout history, basic clinical examination, laboratory investigations, and outcome measures were coded, entered, and analyzed using Microsoft Excel software. Data were then imported into Statistical Package for the Social Sciences (SPSS version 20.0) (Statistical Package for the Social Sciences) software for analysis. According to the type of data qualitative represent as number and percentage, quantitative continues group represented by mean ± SD and median (range), the following tests were used to test differences for significance; difference and association of qualitative variable by Chi-square test (X^2). Differences between quantitative independent groups by t-test, correlation by Pearson's correlation. P-value < 0.05 was considered significant.

RESULTS

Table (1) showed that age was distributed as 52.06 ±17.95 with a minimum18 and maximum of 88 years. As regards, sex distribution females were 52.5% and males were 47.5%, 33.8% were diabetic and 32.5% were hypertensive.

Table (1): Basic demographic and clinical data distribution of studied group (N=80)

		Age	
Mean± SD		52.06±17.95	
Median (Range)		57.0 (18-88)	
		N	**%**
Sex	**Female**	42	52.5
	Male	38	47.5
	Total	80	100.0
DM	**-VE**	53	66.3
	+VE	27	33.8
HTN	**-VE**	54	67.5
	+VE	26	32.5
	Total	80	100.0

Table (2) showed that HB, WBCs, Hematocrit, MCV, MCH, MCHC, and PLT were distributed as 9.72±1.27, 12.27±6.26, 29.38±6.19, 80.20±8.86, 27.41±3.40, 33.66±1.57 and 200.64±64.36 respectively.

Table (2): CBC distribution of studied group (N=80)

HB (g/dL)	Mean± SD	9.72±1.27
WBCs (× 10^3/µL)	Mean± SD	12.27±2.26
Hematocrit (%)	Mean± SD	28.87±4
MCV (fL)	Mean± SD	80.20±8.86
MCH (pg)	Mean± SD	27.41±3.40
MCHC (g/dL)	Mean± SD	33.66±1.57
PLT (× 10^3/µL)	Mean± SD	200.64±6.36

Table (3) showed that S. Iron, S. Ferritin, TIBC, Transferrin saturation were distributed as 48.07±13.2, 814.26±751.9, 304.97±38.08, and 15.53±4.28 respectively. Serum Hepcidin was distributed as 310.49±135.9 with a minimum of 114.6 and a maximum of 796.1%.

Table (3): Iron profile and Serum Hepcidin distribution of studied group (N=80)

S Iron (mcg/dL)	Mean± SD	48.07±3.2
S Ferritin (ng/mL)	Mean± SD	814.26±51.9
TIBC (mcg/dL)	Mean± SD	304.97±38.08
Transferrin saturation (%)	Mean± SD	15.53±3.28
Serum Hepcidin (ng/mL)	Mean± SD	310.49±35.9

Table (4) showed that the APACHE II score was distributed as 12.62±4.33 and ICU stay was distributed as 9.12±4.58 with a minimum of 2 days and a maximum of 30 days.

Table (4): APACHE II score and ICU stay distribution of study group (N=80)

	APACHE II score	**ICU stay (days)**
Mean± SD	12.62±4.33	9.12±4.58
Median (Range)	12.0 (1-27)	7.0 (2-30)

Table (5) showed that 43.8% of the studied cases died while 56.2% survived.

Table (5): Outcome distribution of studied group (N=80)

		N	**%**
Outcome	**Survived**	45	56.2
	Deceased	35	43.8
	Total	80	100.0

Table (6) showed a significant positive correlation with Cr but a significant negative correlation with ferritin.

Table (6): Correlation of serum hepcidin with other parameters

	r	**p**
APACHE II Score	0.026	.818
ICU stay	-0121	.289
WBCs	0.029	.801
hematocrit	-0.167	.140
MCV	0.63	.579
MCH	0.038	.736
MCHC	0.021	.857
PLT	-0.196	.081
SGPT	0.024	.845
PT	-0.077	.539
INR	0.073	.559
S._creat	0.289	**.010**
Na	-0.067	.556
K	0.104	.356
RBS	0.229	.113
PH	-0.146	.196
PaO2	0.147	.193
PaCO2	-0.144	.201
S. iron	0.068	.550
S. ferritin	-0.241	**.045**
TIBC	-0111	.326
Transferrin saturation	0.090	.426

In table (7) there was non statistically significance between mortality and (iron, and hepcidin).

Table (7): Correlation between mortality and (iron and hepcidin)

	Survived	Deceased	t/ X^2	P
S. iron(mcg/dL)	48.83±3.11	47.10±3.55	0.579	0.565
S. hepcidin(ng/mL)	328.76±13.6	287.02±9.69	1.671	0.124

DISCUSSION

The obtained results showed that the mean hemoglobin concentration was 9.72±1.27g/dl; 40 patients (50%) were moderate, 39 patients (48.8%) were mild and only one patient (1.2%) was severe anemia. The variation in hemoglobin concentration may be related to the patient's age, sex, or medical cause of admission, and the study excludes patients who received blood in the previous 3 months. These results were found in partial agreement with those of **Thomas *et al.*** [7] who stated that the mean admission hemoglobin level was 10.8±0.22 g/dL, with 71% having an admission hemoglobin level <12 mg/dL, and 39% having an admission hemoglobin level<10 mg/dL.

Regarding each means of serum Iron, 48.07±13.2 µg/dl, serum ferritin, 814.26±751.9 ng/ml, TIBC, 814.26±751.9 µg/dl, and Transferrin saturation 15.53±4.28 % were reported for tested patients. The high value of serum ferritin is, mostly because of the inflammatory state which is common in most critically ill patients. According to **Greenwood *et al.*** [8]**,** the hyperferritinemia correlates with the laboratory parameters indicative of severe hyper inflammation and organ dysfunction in critically ill ICU patients, indicating its value in identifying hyperinflammatory critically ill patients for early intervention.

As per our study results, hepcidin in patient serum was distributed as 310.49 ± 135.9 ng/ml with a minimum of 114.6 ng/dl and a maximum of 796.1 ng/dl, which cleared a relative increase in hepcidin in the study population compared with the reported standard reference range of serum hepcidin levels in normal controls: median 19.5 ng/mL (*n* = 2,998 general population cohort) [9]. **Gutschow *et al.*** [10] defined a healthy, normal iron status, the reference range for hepcidin to be from 4.0 to 129.9 ng/mL and that serum hepcidin in healthy female donors was significantly lower than in healthy male donors, with the median hepcidin for the groups with low, normal, and high iron status were 6.7 ng/ mL, 21.4 ng/ml, and 44.9 ng/mL, respectively.

The current study found serum hepcidin level positively correlated with serum creatinine level, r=0.289 ($p \leq 0.010$) suggesting that hepcidin levels increase as renal function deteriorates, possibly due to decreased hepcidin renal clearance which agrees with the results of **Troutt *et al.*** [11] who found that Hepcidin-25 concentrations in CKD patients were significantly increased compared to healthy subjects with Hepcidin-25 concentrations were directly correlated with creatinine. **Basseri *et al.*** [12] found that there was an excellent correlation between urine (expressed as ng/mg of creatinine) and serum hepcidin levels expressed as ng/ml; the same result was reported by **Malyszko *et al.*** [13]**.**

Serum hepcidin was not correlated (negative insignificant correlation) with any of serum Hb r=-0.185 (p=.100), hematocrit r= - 0.167 (p=.140), as hepcidin was measured at admission and these results agree with those of **Détivaud *et al.*** [14] where they did not find a significant correlation between erythroid parameters and urinary hepcidin concentration, suggesting again additional regulatory mechanisms. On the other hand, the result was found to disagree with those of [15] who reported a marked inverse correlation between hemoglobin and urinary hepcidin level (P = 0.014) strongly supporting a causal relationship between up-regulated hepcidin expression and anemia. The urinary hepcidin also significantly (P < 0.05) correlated with serum ferritin and C-reactive protein.

In the same trend, our study cleared that hepcidin was not correlated with APACHE II score r=0.026 (p=.818) or ICU length of stay r=-0121 (p=.289). These results are consistent with findings by **Cherry-Bukowiec *et al.*** [16] who said that serum hepcidin levels on the day of admission were not correlated with ICU LOS.

CONCLUSION

Serum hepcidin and ferritin levels are significantly elevated in critically ill anemic patients who are characterized by the inflammatory stress of their illnesses. Serum hepcidin level measured on admission to medical ICU has no impact on patients' short-term ICU outcome in terms of mortality or length of hospital stay.

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