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Hepcidin Status among Iron Deficient Anemic Pregnant Women in Gaza strip: A Case Control Study

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Abstract:

Background: Hepcidin, a peptide hormone composed of 25 amino acids. Hepcidin is synthesized mainly in the liver. Iron deficiency anemia (IDA) is common during pregnancy and is associated with higher maternal morbidity and mortality in Gaza strip. Understanding of hepcidin hormone and its role in iron metabolism could lead to a new sensitive indicator for earlier detection of cases with IDA. **Objective:** To assess hepcidin status among IDA pregnant women and its relationship with some biochemical variables in Gaza strip. **Materials and methods**: A case control study comprised 45 IDA pregnant women and 45 apparently healthy pregnant women. Questionnaire interviews were applied among the study population. Serum hepcidin and ferritin were measured by Enzyme-linked immunosorbent assay (ELISA), iron and total iron binding capacity (TIBC) were determined photometrically. Complete blood count (CBC) was also performed. Transferrin and transferrin saturation were calculated. An approval was obtained from Helsinki committee and ministry of health to conduct this study. Overall data were computer analyzed using SPSS (Ver. 18). **Results:** The mean levels of serum hepcidin, iron, transferrin saturation, and ferritin in cases were significantly lower compared to those of controls (2.6±4 ng/ml, 63.2±25.3 µg/dl, 15.6±8.0% and 8.0±9.7 ng/ml versus 7.5±7.3 ng/ml, 77.7±22.9 µg/dl, 23.5±8.0% and 15.4±14.3 ng/ml respectively with P=0.000). The Pearson correlation test showed that positive significant correlations between hepcidin levels and serum iron, ferritin, and transferrin saturation (P<0.001). On the other hand, negative significant correlations were showed with TIBC and transferrin (P<0.001). **Conclusions:** Serum hepcidin level has a relationship with anemia among pregnant women. Therefore, monitoring of hepcidin levels can play an important role in management of anemia among pregnant women.

Keywords:
Hepcidin, Serum Iron, Ferritin, Iron deficiency anemia, Gaza strip

1. Introduction:

Iron is considered as an essential element for virtually all living organisms. There are two types of iron in foods 1) Heme iron is the type that the body absorbs best. It is found in beef, chicken, turkey, and other types of meat (Lopez, Cacoub, Macdougall, & Peyrin-Biroulet, 2016). 2) Non heme iron is the other type, which is found in plant sources that is absorbed partially (Lopez, et al., 2016). Iron participates in a wide variety of metabolic processes, including oxygen transport from the lungs to the tissues by hemoglobin in red blood cell and electron transport (Abbaspour, Hurrell, & Kelishadi, 2014). In pregnant women significant changes can be observed in iron metabolism. Pregnant women need about twice the amount of iron of non-pregnant women. This causes an increase in the demand for iron during pregnancy, therefore serum iron levels decrease, but the total iron binding capacity increases (Bothwell, 2000). Approximately 50% of pregnant women don't get enough of this important mineral (Ervasti, Kotisaari, Heinonen, & Punnonen, 2007). Therefore, iron deficiency is a common cause of anemia in pregnancy which is associated with maternal and fetal problems such as preterm labor and maternal infections (Goepel, Ulmer, & Neth, 1988). Iron supplementation during pregnancy is usually based on hemoglobin values, although physiological hemodilution in pregnancy often leads to reduced hemoglobin values. Thus, new indicators are necessary for earlier detection of IDA in pregnancy (Goepel, et al., 1988).

Recent studies have evaluated the use of hepcidin as a biomarker for the regulation of iron metabolism (Hare, 2017; Manolov et al., 2015). Human Hepcidin is a 25-amino acid peptide that is secreted by the liver and excreted by the kidneys, and is considered to be a major regulator of iron metabolism and the anemia that is associated with chronic inflammation (Park, Valore, Waring, & Ganz, 2001). Hepcidin was first discovered in human urine and serum in the year 2000 (Krause et al., 2000). The peptide was initially reported as Liver-Expressed Antimicrobial Protein-1 (LEAP-1), and in 2001 became known as hepcidin (Park, et al., 2001). Hepcidin inhibits iron transport by binding to the iron channel ferroportin, which is located on the surface of gut enterocytes and the plasma membrane of reticuloendothelial cells (Rossi, 2005). Hepcidin activity is also partially responsible for iron sequestration seen in anemia in chronic disease (Weiss & Goodnough, 2005). In addition, this hormone regulates the transfer of iron through the placental syncytiotrophoblast during pregnancy (Bastin, Drakesmith, Rees, Sargent, & Townsend, 2006). Despite the central role of hepcidin in the metabolism of iron, limited data are available that associate hepcidin levels with measures of iron status, inflammation, and anemia among pregnant women Hepcidin is a pivotal regulator of iron metabolism because it controls the efflux of iron from enterocytes, hepatocytes, and macrophages by internalization and degradation of the iron exporter (ferroportin), and also regulates the plasma iron level. Hepcidin is up regulated in response to an increase of body iron stores or the onset of infection and is down regulated by anemia or hypoxia, while it is also an acute phase reactant induced by inflammation that shows antimicrobial activity. The discovery of hepcidin and further understanding of how it inhibits the movement of iron and its regulation may eventually help clinicians better evaluate a patient's iron status and may assist in more effective treatment for anemia of chronic diseases (Knutson, 2010). The aim of the

present study was to investigate the hepcidin levels among IDA pregnant women in the third trimester in Gaza strip.

2. Methodology

This case control study comprised 45 IDA pregnant women (cases) and 45 apparently healthy pregnant women (controls). The pregnant women aged (17-38 years old) were selected from Health centers in Gaza Strip (Alaqsa hospital in Deir al-Balah city, Health center of Deir al-Balah and AL-Remal Health center). Control apparently healthy women with no history of IDA were selected from the general population that matches the case group in sex, age and residence. The following exclusion criteria was applied which includes pregnant women: taking iron therapy, hemoglobinopathy, history of smoking, liver and renal disease and obesity.

An ethical approval to conduct the study was obtained from Helsinki committee in the Gaza Strip. All participants were given a full explanation about the purpose of the study and agreed to participate. The participants were interviewed face to face by the researcher. Most questions were dichotomous questions. The questionnaire included questions on personal information (age, height and weight), socioeconomic information (pregnant education, and family income/month) and medical history data.

Venous blood samples (5ml) were drawn from the participants and dispensed into two tubes (EDTA and plain tubes). Then serum sample was obtained by centrifugation (3000 rpm/10 minutes) at room temperature. For all study population different commercial kits were used for analysis of the different analytes and the manufacturers procedures were followed. Hepcidin hormone was determined by ELISA kit (DRG diagnostics – Germany), serum ferritin was determined by ELISA kit (Accubind – USA), serum iron was determined by colorimetric method using Chromazurol B (Elitech Diagnostic system - France), TIBC method was Saturation/precipitation method (Elitech Diagnostic system - France). CBC was analyzed by an auto analyzer (cell Dyn 1800, USA). Transferrin and transferrin saturation were calculated.

Statistical Package for the Social Science (SPSS) ver. 18 was used for data processing and analysis. Different statistical tests were used including Chi-square, unpaired t-test, Pearson's correlation test, and ROC curve was plotted to measure the usefulness of the test in general. The results were considered as statistically significant when the P-value < 0.05.

Results

General characteristics of study population

As shown in Table 1, there was no significant difference between the age of controls (27.4±4.2) and that of the cases (27.3±4.8) (P = 0.944). On the other hand, the income of controls (1817.8±423.9) was higher than that of cases (656.7±177.6) (P < 0.001). The results also showed that there is no significant difference in height, weight or BMI between controls and cases (P = 0.219). Regarding the question if pregnant women were taking meals regularly, the number of cases was (6.7%) which is lower compared to controls (96.6%) (P < 0.001). On the other hand, smokers among family were 24.4% among controls and 88.9% among cases and the difference was statistically significant (P < 0.001). In contrast, recurrent abortions in cases was higher compared to controls and

the difference was found to be statistically significant (P < 0.001). Moreover, cases with gravida ≤ 3 were 46.7% and with gravida > 3 were 53.3% compared to 75.6% and 24.4% for controls respectively, and the difference was also statistically significant (P = 0.023).

Table 1 ***General Characteristics of the study population***

Characteristic		Controls (n=45)		Cases (n=45)	Statistical test	P-value
Age	Mean ± SD (min-max)	27.4±4.2 (18-35)		27.3±4.8 (17-38)	t = 0.070	0.944
Income (NIS)		1817.8±423.9 (1500-3000)		656.7±177.6 (400-1000)	t = 14.517	< 0.001
Height (cm)		162.1±5.3 (153-173)		160.8±4.8 (150-173)	t = 1.237	0.219
Weight (kg)		72.7±10.8 (55-106)		73.6±11 (52-116)	t = -0.396	0.693
BMI (kg/m²)		27.6±3.4 (21.9-37.1)		28.5±4.0 (21.6-42.6)	t = -1.104	0.273
Taking meals regularly	No. (%)	**Yes** **No**	43 (96.6) 2 (4.4)	3 (6.7) 42 (93.3)	$\chi2$ = 71.146	< 0.001
Smokers among family		**Yes** **No**	11 (24.4) 34 (75.6)	40 (88.9) 5 (11.1)	$\chi2$ = 38.054	< 0.001
Recurrent abortion		**No** **1** **≥2**	34 (75.6) 8 (17.8) 3 (6.7)	6 (13.3) 21 (46.7) 18 (40.0)	$\chi2$ = 36.142	< 0.001
Gravida		**≤3** **>3**	34 (75.6) 11 (24.4)	24 (53.3) 21 (46.7)	$\chi2$ = 4.849	0.023

BMI: Body mass index; NIS: New Israeli Shekel

Hepcidin hormone and other iron indicators among the study population

Table 2 shows that the mean level of hepcidin is lower in cases (2.6±4 ng/ml) compared to controls (7.5±7.3 ng/ml) and the difference was statistically significant (P < 0.001). The mean levels of serum iron and serum ferritin were also lower in cases (63.2±25.3 µg/dl & 8.0±9.7 ng/ml) compared to controls (77.7±22.9 µg/dl & 15.4±14.3 ng/ml) respectively and the difference was also statistically significant (P = 0.005). In contrast, TIBC and transferrin were higher in cases (432.7±72 µg/dl, 308.1±52.6 mg/dl) compared to controls (342.3±59.1 µg/dl & 244.5±42.5 mg/dl) respectively, and the difference was statistically significant (P = < 0.001).

Table 2 ***Hepcidin and iron parameters among the study population***

Parameter	Controls (n=45) Mean ± SD	Cases (n=45) Mean ± SD	t	P-value
Hepcidin (ng/ml) (min-max)	7.5 ± 7.3 (1.1-41.8)	2.6 ± 4 (0.1-19.8)	3.975	< 0.001

Serum iron (µg/dl) (min-max)	77.7 ± 22.9 (42.7-174)	63.2 ± 25.3 (33-124.8)	2.864	0.005
TIBC (µg/dL) (min-max)	342.3 ± 59.1 (251-480)	432.7 ± 72.0 (279-621)	-6.512	< 0.001
Serum ferritin (ng/ml) (min-max)	15.4 ± 14.3 (2.8-80.3)	8.0 ± 9.7 (1.4-64)	2.852	0.005
Transferrin saturation (%) (min-max)	23.5 ± 8.0 (9.5-43.7)	15.6 ± 8.0 (6.2-33)	4.703	< 0.001
Transferrin (mg/dl) (min-max)	244.5 ± 42.5 (179.3-345)	308.1 ± 52.6 (199.5-442.6)	-6.311	< 0.001

TIBC: Total iron binding capacity

Complete blood count (CBC) indices among the study population

Table 3 shows the comparison between cases and controls regarding to CBC indices. The difference in all blood indices values between cases and controls was significant. The mean values of RBCs (4.0±0.3, 3.3±0.2 10^6/µl), HB (11.8±0.6, 9.7±0.8 g/dl), HCT (34.7±2.0, 29.4±2.3 %), MCV (86.3±3.3, 76.6±4.8 fl), MCH (29.4±1.3, 25.6±2.2 pg) and MCHC (34±0.9, 33.2±1.5 g/dl) were higher in controls compared to cases, respectively. In contrast, the mean difference of RDW was higher in cases compared to controls (16.6±2.4, 13.7±0.6 fl) respectively.

Table 3 ***Complete blood count (CBC) indices among cases and controls***

CBC indices	Controls (n=45) Mean ± SD	Cases (n=45) Mean ± SD	T	P-value
Hb (g/dl) (min-max)	11.8 ± 0.6 (11-14.2)	9.7 ± 0.8 (7-10.9)	13.82	< 0.001
RBC (10^6/µl) (min-max)	4.0 ± 0.3 (3.5-5.2)	3.3 ± 0.2 (3-3.8)	11.138	< 0.001
HCT (%) (min-max)	34.7 ± 2.0 (33.0-41.7)	29.4 ± 2.3 (22.7-32.8)	11.847	< 0.001
MCV (fl) (min-max)	86.3 ± 3.3 (80-95)	76.6 ± 4.8 (63.3-85)	11.123	< 0.001
MCH (pg) (min-max	29.4 ± 1.3 (27.4-33.1)	25.6 ± 2.2 (20.0-29.4)	9.864	< 0.001
MCHC (g/dl) (min-max)	34 ± 0.9 (31.2-37)	33.2 ± 1.5 (29.1-36.7)	2.829	0.006
RDW (fl) (min-max)	13.7 ± 0.6 (33.0-41.7)	16.6 ± 2.4 (22.7-32.8)	-7.7	< 0.001

Hb: Hemoglobin**; HCT:** Hematocrit; **MCH:** Mean cell hemoglobin; **MCHC:** Mean cell hemoglobin concentration; **MCV:** Mean cell volume; **RBCs:** Red blood corpuscles; **RDW:** Red cell distribution width.

Distribution of hepcidin according to general characteristics of study population

Table 4 shows the levels of hepcidin hormone according to general characteristics of the study population. The mean concentration of hepcidin was higher in participants that take meals regularly and those that experienced recurrent abortion (P = 0.023 & P = 0.020), respectively. Smoking or negative smoking causes a decrease in the mean concentration of hepcidin (P = 0.008). On the other hand, although the mean concentration of hepcidin was higher in women with Gravida ≤ 3 compared to those with Gravida >3, the difference was not statistically significant (P = 0.129).

Table 4 ***Distribution of hepcidin according to general characteristics of study population***

Items	Hepcidin (ng/ml) Mean ± SD (min-max)	t	P-value
Taking meals regularly			
Yes	7.4 ± 7.3 (0.3-41.8)	3.8	0.023
No	2.6 ± 4.1 (0.1-19.8)		
Smoker among family			
Yes	3.5 ± 4.3 (0.1-19.8)	2.735	0.008
No	7.1 ± 7.9 (0.2-41.8)		
Recurrent abortion			
Yes	6.7 ± 7.7 (0.1-41.8)	2.639	0.020
No	3.7 ± 4.7 (0.1-19.8)		
Gravida			
≤3	5.8 ± 7.0 (0.1-41.8)	1.532	0.129
>3	3.6 ± 5.0 (0.1-19.8)		

Correlation between hepcidin and other iron indicators among the study population

Table 5 presents the correlation between hepcidin hormone levels and iron indicators. Hepcidin showed a significant positive correlation with serum iron (r = 0.547, P < 0.001), serum ferritin (r = 0.558, P < 0.001), and transferrin saturation (r = 0.577, P < 0.001). In contrast, hepcidin showed a significant negative correlation with TIBC (r = -0.551, P < 0.001) and transferrin (r = -0.526, P < 0.001).

Table 5 ***Correlation between hepcidin and other iron indicators among the study population.***

Parameters	Serum hepcidin	
	Pearson correlation (r)	P-value
Serum iron (ug/dL)	0.547	< 0.001
TIBC (ug/dL)	-0.551	< 0.001
Serum ferritin (ng/ml)	0.558	< 0.001
Transferrin saturation (%)	0.577	< 0.001
Transferrin (mg/dL)	-0.526	< 0.001

TIBC: Total iron binding capacity

Hepcidin levels correlated with CBC indices among the study population

Table 6 presents the correlation between hepcidin CBC indices. Hepcidin showed a significant positive correlation with Hb (r = 0.524, P < 0.001), RBCs (r = 0.402, P < 0.001), HCT (r = 0.489, P < 0.000), MCV (r = 0.433, P < 0.000), MCH (r = 0.455, P < 0.001) and MCHC (r = 0.249, P = 0.018). In contrast, hepcidin showed a significant negative correlation with RDW (r = -0.490, P < 0.001).

Table 6 ***Correlation between hepcidin and CBC indices among study population.***

Parameters	Hepcidin	
	Pearson correlation (r)	**P-value**
Hb (g/dl)	0.524	< 0.001
RBC (10^6/µL)	0.402	< 0.001
HCT (%)	0.489	< 0.001
MCV (fl)	0.433	< 0.001
MCH (pg)	0.455	< 0.001
MCHC (g/dl)	0.249	0.018
RDW (fl)	-0.490	< 0.001

Hb: Hemoglobin**; HCT:** Hematocrit; **MCH:** Mean cell hemoglobin; **MCHC:** Mean cell hemoglobin concentration; **MCV:** Mean cell volume; **RBCs:** Red blood corpuscles; **RDW:** Red cell distribution width.

Youden index cut-off points for prediction of anemic pregnant women

In the present study, Table 7 illustrates a summary of the receiver operating characteristic curve (ROC curve) (Figure 1) and the cut off value of serum hepcidin level for diagnosis of anemia among pregnant women. Serum hepcidin level was statistically significant to diagnose anemia among pregnant women and the cut-off value for hepcidin was 1.3 pg/ml, the area under the curve (AUC) was 0.826 (P < 0.001), sensitivity and specificity were 91.1 % & 64.4 % respectively. Positive predictive value (PPV) was 77.8 %, negative predictive value (NPV) was 71.9 %, and accuracy was 78.8% for diagnosis of anemia among pregnant women.

Table 7 ***Youden index cut-off points for prediction of anemic pregnant women***

Biomarker	Cases (n=45)	Controls (n=45)	Cut-off point (pg/ml)	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Accuracy (%)	AUC (95% CI)	P value
Hepcidin hormone	29	4	≤1.3	91.1	64.4	77.8	71.9	78.8	0.826 (0.739–0.914)	< 0.001
	16	41	>1.3							

AUC: area under the curve; **n:** number of subjects; **NPV:** Negative predictive value; **PPV:** Positive predictive value; **95% CI**: 95% confidence interval.

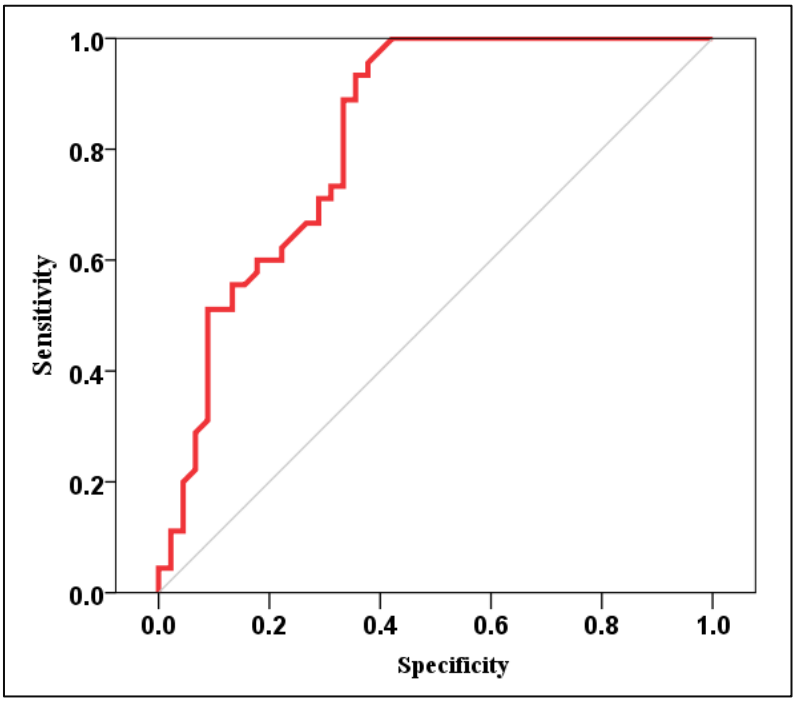

Figure 1 ***Receiver operating characteristic curve (ROC) to prediction anemic pregnant women.***

Discussion

Iron deficiency anemia is a global health problem and a common medical condition seen in every day clinical practice. IDA is the most common type of anemia among pregnant women. The diagnosis and treatment of this condition can clearly be improved. The available biochemical tests for IDA are limited to routine traditional anemia factors including biochemical parameters such as iron, TIBC, transferrin, transferrin saturation, serum ferritin and CBC indices tests. On the other hand, other biochemical features in blood such as hepcidin hormone was recently linked to IDA (Hare, 2017). This discovery has revolutionized our understanding of IDA, and its measurement should advance diagnosis/treatment of this condition.

Our results show that there is a significant association between pregnant women with IDA and the baby's birth weight ($P < 0.001$). Therefore, pregnant women with IDA have an increased risk for delivering a low – birth weight baby. Our results agree with those of (CDC, 1998) which showed that there is a significant difference between pregnant women with IDA and low-birth weight baby**.** There was a significant difference between controls and cases in terms of family income ($P < 0.001$), where controls have higher family income compared to cases. The increase in family income is reflected on the pregnant women's diet and may save them from becoming anemic.

The results also show that there is a statistically significance difference between cases and controls regarding the number of pregnancies ($P = 0.023$). Women with higher pregnancy number are more prone to have iron deficiency anemia. The short interval between different pregnancies (less than one year) creates a large demand of iron, which is needed for the development of the fetus and the loss of iron during delivery increases further the iron requirement. This result is in agreement with a study performed by (Hadipour et al., 2010) who found that the parity is one of the factors that could have influence of IDA. The results of the present study also show that there is a statistically significant difference between cases and controls with regard to pregnancy loss ($P = 0.020$). IDA is one of the factors that cause fetal death and pregnancy loss (Abu-Ouf & Jan, 2015)**.**

Our results show that the mean levels of hepcidin hormone is lower in IDA pregnant women compared to controls ($P < 0.001$). This finding is in agreement with the results of (Koenig, Tussing-Humphreys, Day, Cadwell, & Nemeth, 2014). Their results showed that serum hepcidin was significantly lower in IDA pregnant women and hepcidin levels decrease as pregnancy progress compared to the control group with the lowest hepcidin levels observed in the third trimester. This is due to the increase in fetal iron needs in the third trimester (Koenig, et al., 2014). Another study results also agree with our finding, which showed a statistically significant difference in serum hepcidin levels between pregnant women with IDA and pregnant women without IDA (Manolov, et al., 2015).

Serum hepcidin level was a valid test for anemic pregnant women screening and the ability of the test to discriminate between those with anemia and those without anemia were excellent for pregnant women because the test have high value of sensitivity (91.1 %), specificity (64.4 %), PPV (77.8 %), NPV (71.9 %) and accuracy (78.8%) to diagnostic anemia among pregnant women. The present study agrees with the results of other studies, (Pasricha et al., 2011) concluded that serum hepcidin concentration may be a useful indicator of deficient iron stores; (Lasocki, Longrois, Montravers, & Beaumont, 2011) and (Shu et al., 2015) showed that hepcidin levels may be suppressed by iron deficiency anemia even in inflammation cases and they concluded serum hepcidin was the most accurate threshold for iron deficiency anemia diagnosis in critically ill patients with anemia.

The results of the present study show a significant increase in TIBC concentration in IDA pregnant women compared to controls which was statistically significant ($P < 0.001$). Furthermore, Hepcidin showed a significant negative correlation with TIBC ($r = -0.551$, $P < 0.001$). Our results are comparable with those of (Kwapisz, Zekanowska, & Jasiniewska, 2009) who showed that serum hepcidin levels were significantly negatively correlated with TIBC. All patients with IDA showed significantly lower levels of hepcidin and higher levels of TIBC compared to the control group. On the other hand, our results show that hepcidin has a significant positive correlation with

serum iron (r = 0.547, P < 0.001). The results are in agreement with those of (Koenig, et al., 2014) where there results showed that hepcidin was reduced in IDA pregnant women with low circulating iron. In contrast, our results show that hepcidin has a significant positive correlation with ferritin (r = 0.558, P < 0.001) which are in compatible with those of (Scholl, 2005) who showed that serum hepcidin levels were significantly positively correlated with ferritin.

In the present study, the mean difference of transferrin in cases was significantly higher than that in controls (308.1±52.6, 244.5±42.5 mg/dl respectively, P < 0.001). While the mean difference of transferrin saturation in cases was significantly lower than that in controls (15.6±8.0, 23.5±8.0 mg/dl respectively, P < 0.001). In the present study, the Pearson correlation test showed a significant negative correlation of hepcidin with transferrin (r = -0.526, P < 0.001). On the other hand, hepcidin results showed a significant positive correlation with transferrin saturation (r = 0.577, P < 0.001). These results are in agreement with the results of (Koenig, et al., 2014) who showed that hepcidin is negatively correlated with transferrin and positively correlated with transferrin saturation.

In the present study, the results show a significant decrease in RBCs, Hb, HCT, MCV, MCH and MCHC levels of cases compared to controls. On the other hand, RDW levels were significantly increased in cases, that means the width of the RBC was higher than those in controls. Pearson correlation test showed a positive significant correlation between hepcidin, RBC and Hb levels which are in agreement with (Azab & Esh, 2013). Therefore, in the present study decreased levels of hepcidin was associated with an increase in RDW in IDA patients. Another study is also in agreement with this result which found that the levels of Hb, serum iron, %saturation, ferritin and hepcidin were significantly low in IDA pregnant women than controls group (Singla, Tyagi, Shankar, Dash, & Kumar, 1996).

In conclusion, the mean level of hepcidin was significantly lower in cases compared to controls. There were positive significant associations between hepcidin levels with Iron, Ferritin, and Transferrin saturation. In addition, Serum hepcidin level has a relationship with anemia among pregnant women. Therefore, monitoring of hepcidin levels may play an important role in management anemia among pregnant women.

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