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Hepcidin Status Correlated with Biochemical Parameters and Hematological Indices among Iron Deficient Anemic Children aged (6 – 12) Years in Gaza City: A Case Control Study

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Abstract

Background: Hepcidin is a small cysteine-rich peptide hormone produced in the liver. This molecule regulates the absorption of iron in the body. Recent studies demonstrated that hepcidin is a master iron regulator. Therefore, assessment of the status of hepcidin and clarifying its association in iron deficiency anemia (IDA) could constitute a promising therapy of the disease.

Objective: To correlate hepcidin hormone status with some biochemical parameters among IDA children aged 6 – 12 years in Gaza City.

Methods: This case-control study comprised 80 IDA children patients and 80 healthy non IDA children controls. Questionnaire interview was applied. Serum hepcidin, serum ferritin were measured by ELISA. Serum iron and total iron binding capacity (TIBC) were determined photometrically. Complete blood count (CBC) was also performed by [Cell-Dyn-1800] autoanalyser. Transferrin and transferrin saturation were calculated by different equations. Ethical considerations were observed. Data was analyzed using SPSS package version 20.0.

Results: The mean level of serum hepcidin was significantly lower in IDA children patients compared to healthy non IDA children controls (P=0.001). The mean serum iron, transferrin saturation and serum ferritin in cases were significantly lower than that in controls (P=0.000). The Pearson correlation test showed negative significant correlations between hepcidin levels and serum iron (r = -0.232, P= 0.003), and negative non-significant correlation with transferrin saturation (r = -0.028, p=0.722), and positive significant correlations with serum ferritin (r=0.320 P=0.000). The mean TIBC and transferrin in cases were significantly higher than that in controls (P=0.000).

Conclusions: Hepcidin is strongly correlated with serum iron, transferrin, TIBC and serum ferritin. Thus it is considered as a good marker and promising therapeutic agent of IDA.

Keywords Hepcidin, Serum iron, Ferritin, Iron deficiency anemia, Gaza.

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حالة الهيبسيدين وعلاقته ببعض المعايير الكيميائية والدموية لدى الأطفال المرضى بأنيميا نقص الحديد من عمر 6 – 12 سنة في مدينة غزة: دراسة مجموعة مرضية وأخرى مجموعة ضابطة

ملخص الدراسة

المقدمة: يعتبر الهيبسيدين هرموناً صغيراً وهو غني بالحامض ألأميني السيستيين، ويصنع هذا الهرمون في الكبد. ويحتوي الهيبسيدين على 25 حامض أميني. ينظم الهيبسيدين امتصاص الحديد في الجسم. وقد أكتشف عام 2000م. أثبتت الدراسات الحديثة أن الهيبسيدين هو المنظم الرئيسي للحديد. لذلك فإن تقييم ذلك الوضع للهيبسيدين وتوضيح مثل هذا الارتباط مع أنيميا نقص الحديد ربما يؤسس لعلاجات واعدة لهذا المرض.

الهدف: تهدف هذه الدراسة لتقييم علاقة الهيبسيدين مع بعض المعايير الكيميائية والدموية لدى الأطفال المرضى بأنيميا نقص الحديد من عمر 6 – 12 سنة في مدينة غزة.

الطرق والأدوات: اشتملت هذه الدراسة على مجموعة مرضية وأخرى مجموعة ضابطة، تحتوي المجموعة المرضية على 80 طفلاً مريضاً بأنيميا نقص الحديد و تحتوي المجموعة الضابطة على 80 طفلاً من غير المرضى بأنيميا نقص الحديد. وقد تم الحصول على البيانات المستخدمة في الدراسة من خلال المقابلة المباشرة مع المرضى وذويهم. وتم قياس مستوى الهيبسيدين ومخزون الحديد بواسطة تقنية ELISA. كما تم تحديد مستوى الحديد في الدم و الحديد المرتبط بواسطة تقنية القياسات الضوئية. وكذلك كل مكونات الدم بواسطة جهاز Cell–Dyn–1800. كما تم حساب نواقل الحديد والنواقل المشبعة باستخدام معادلات مختلفة. وقد أخذت المعايير الأخلاقية لإجراء هذا البحث بعين الاعتبار وتم تحليل النتائج بواسطة البرنامج الإحصائي المحوسب SPSS package version 20.0.

النتائج: أظهرت النتائج أن متوسط مستوى هرمون الهيبسيدين كان أقل في المجموعة المرضية مقارنة بالمجموعة الضابطة و كانت النتائج ذات دلالة إحصائية. كانت نتيجة مستوى الحديد في الدم و نواقل الحديد المشبعة ومخزون الحديد أقل وذات دلالة إحصائية في المجموعة المرضية منها في المجموعة الضابطة. وقد أظهرت النتائج وجود علاقة عكسية ذات دلالة إحصائية بين مستوى هرمون الهيبسيدين والحديد في الدم، وعلاقة عكسية بدون دلالة إحصائية مع نواقل الحديد المشبعة. وكانت العلاقة طردية ذات دلالة إحصائية مع مخزون الحديد. من ناحية أخرى كانت نتيجة مستوى الحديد المرتبط و نواقل الحديد أعلى بدلالة إحصائية في المجموعة المرضية منها في المجموعة الضابطة. وقد أظهرت النتائج وجود علاقة عكسية ذات دلالة إحصائية بين مستوى هرمون الهيبسيدين والحديد المرتبط و نواقل الحديد.

الاستنتاجات: تبين أن هرمون الهيبسيدين مرتبط بشدة مع مستوى الحديد في الدم ونواقل الحديد والحديد المرتبط ومخزون الحديد. ولذلك فإنه يعتبر كاشفاً جيداً وعاملاً علاجياً واعداً لأنيميا نقص الحديد.

الكلمات المفتاحية: الهيبسيدين، الحديد في الدم، مخزون الحديد، أنيميا نقص الحديد، غزة.

1. Background

Iron is an essential element for nearly all living organisms. It is a key component of oxygen storage and transporting proteins, such as hemoglobin and myoglobin, and of many enzymes that catalyze oxidation-reduction reactions necessary to generate energy and produce various metabolic intermediates for host defense [1,2]. Thus, maintenance of body iron stores is essential, because many human diets contain iron sufficient only to replace the small iron losses. When iron intake is more abundant, apparently iron absorption is appropriately controlled [3]. Iron deficiency may occur by inadequate dietary intake, by increased physiological needs of the nutrient, and/or by increased losses, which may lead to anemia. Anemia is the most widespread nutritional disorder in the world, affecting mainly women of childbearing age and children under two years [4]. Serum iron is a test which measures the amount of circulating iron that is bound to transferrin. Clinicians order this laboratory test when they are concerned about iron deficiency, which can cause anemia and other problems [5]. Total iron-binding capacity (TIBC) is a test that measures the blood's capacity to bind iron with transferrin [6]. Ferritin is a ubiquitous intracellular protein that stores iron and releases it in a controlled fashion. The amount of ferritin stored reflects the amount of iron stored [7]. Transferrin is an iron-binding blood plasma glycoprotein that controls the level of free iron in biological fluids [8]. Human transferrin is encoded by the TF gene [9]. Transferrin glycoprotein binds iron very tightly, but reversibly [10]. Transferrin saturation, abbreviated as TSAT and measured as a percentage, is a medical laboratory value. It is the ratio of serum iron and total iron-binding capacity, multiplied by 100. Recent studies have evaluated the use of hepcidin as a biomarker for the regulation of iron metabolism. Hepcidin has evolved as the primary regulator of iron homeostasis and a probable mediator of chronic anemia disease and inflammation. This role has been widely demonstrated in a number of recent studies [11]. Initially, the peptide was reported as LEAP-1, for Liver-Expressed Antimicrobial Protein and later became known as hepcidin [12]. Independently, in a search for antimicrobial peptides, researchers working in the lab of Tomas Ganz discovered a peptide associated with inflammation, and named it "hepcidin" after observing that it was produced in the liver [13]. Hepcidin is a small cysteine-rich peptide hormone. This molecule regulates the absorption of iron in the body and it appears to be the master regulator of iron homeostasis in humans and mammals, In humans, HAMP (hepcidin antimicrobial peptide) is the gene that encodes for hepcidin. Hepcidin is also a tightly folded polypeptide containing 25 residues in length and is 32% beta sheets. The 25 amino acids have a hairpin structure and are stabilized by 4 disulfide bonds which has been shown to act as the principal regulator of iron homeostasis in vertebrates [14]. Hepcidin binds the iron channel ferroportin, which is located on the basolateral surface of gut enterocytes and the plasma membrane of reticuloendothelial cells, and the degrading ferroportin shuts off the iron transport out of these cells that store it [15]. Ferroportin is also present on enterocytes and macrophages. By inhibiting ferroportin, hepcidin prevents enterocytes of the intestines from secreting iron into the hepatic portal system, thereby functionally reducing iron absorption. The iron release from macrophages is also prevented by ferroportin inhibition; therefore the hepcidin maintains iron homeostasis. Hepcidin has shown fairly consistent antifungal activity. Hepcidin antibacterial activity currently seems to be inconsistent [16]. There are many diseases where failure to adequately absorb iron contributes to iron deficiency and iron deficiency anemia. The treatment will depend on the hepcidin levels that are present, as oral treatment will be unlikely to be effective if hepcidin is blocking internal absorption, in which cases parenteral iron treatment would be appropriate. Studies have found that measuring hepcidin would be of benefit to establish optimal treatment [17]. There are some factors regulating hepcidin levels such Iron loading,

Inflammation and Erythropoeisis activity [18,19]. The aim of this study is to correlate hepcidin hormone status with some biochemical parameters and hematological indices among IDA children patients aged (6 – 12) years in Gaza City. In addition to this. IDA is very prevalent in Gaza City. Also children are most affected by this disease. Beside that the findings of the present study should draw the attention of physicians to consider the analysis of hepcidin hormone level for anemic children. Understanding of hepcidin hormone and its role in iron metabolism could lead to new therapies for hemochromatosis and anemia of inflammation.

Related studies

A study conducted by a group of researchers at Pediatric Department, Faculty of Medicine, Zagazig University, Egypt; concluded that obesity modulates serum hepcidin and treatment outcome of iron deficiency anemia in children [20].

Another study which was carried out for anemic Egyptian children who were infected with H. pylori showed that this bacteria causes up regulation of serum hepcidin levels and they were not responsive to oral iron therapy [21].

A study conducted by German researchers revealed that hepcidin levels could be used to differentiate between IDA and chronic anemia [22].

A group of researchers found that serum hepcidin levels were significantly associated with iron status and could be a useful indicator of IDA [23].

2. Materials and Methods

i. Subjects

This case-control study which comprised 80 IDA children patients and 80 healthy non IDA children controls was conducted during the period from march 2012 to october 2013. Informed parental consent was obtained for enrollment into the study. Questionnaire interview was applied. Serum hepcidin and ferritin were measured by ELISA. Serum iron and TIBC were determined Photometrically. Complete Blood Count (CBC) was performed by [Cell-Dyn-1800] autoanalyser. Transferrin and transferrin saturation were calculated. Patients were selected from Al-Dorra Pediatric hospital in Gaza City, Ard Al-Insan clinic, World Council of churches clinics and Al Remal Health Center. Controls were apparently healthy individuals and matched cases with age, gender and the socioeconomic conditions with the exception of family income which was hard to match, they were chosen from non IDA children in local community.

ii. Iron Parameters

Serum iron and total iron binding capacity (TIBC) were analyzed photometrically. Transferrin saturation was calculated by using this equation: [TS= (Iron/TIBC)*100%] [24]. Transferrin was calculated by using this equation: **[**Transferrin= (Iron/Transferrin saturation)*71.24] [25]. Ferritin level was measured by a competitive ELISA (C-ELISA), (DRG Kit, Germany).

iii. Serum Hepcidin Measurement

Blood samples were collected in plain tubes without anticoagulant, sera were separated and stored at -30°C until assay. Hepcidin levels were measured by a C-ELISA using a commercial kit from (DRG-Kit, Germany). Patients' samples were assayed in duplicate. The results from the C-ELISA were compared with those of the standard curves developed from calibrators run simultaneously with study samples. According to the manufacturer's protocol; 96-well plates were coated with anti-Hepcidin antibody (monoclonal). Ten µL of *Sample Buffer were added* to each of these wells. Twenty µL of each *Standard, Control* and samples with new disposable tips were disposed into appropriate wells. Reaction mixture was incubated for 30 minutes at room temperature on a plate shaker

at ≈ 500 rpm. A volume of 150 µL *Assay Buffer* and 100 µL *Enzyme Conjugate were added* to each of these wells. Reaction mixture was incubated for 180 minutes at room temperature on a plate shaker at ≈ 500 rpm, and briskly shaked. The wells were rinsed 5 times with *distilled water* (400 µL per well). The wells were Stroked sharply on absorbent paper to remove residual droplets. A volume 100 µL of *Enzyme Complex was* dispensed into each well. Reaction mixture was incubated for 45 minutes at room temperature. The contents of the wells were briskly shaked out. The wells were rinsed 5 times with distilled water (400 µL per well). The wells were Stroked sharply on absorbent paper to remove residual droplets. A volume 100 µL of Substrate Solution was added to each well. Reaction mixture was incubated for 30 minu**tes** at room temperature. The enzymatic reaction was stopped by adding 100 µL of Stop Solution to each well. The absorbance (OD) of each well was determined at 450 ± 10 nm with a microtiter plate reader.

iv. Statistics and Data Analysis

Data were computer analyzed using SPSS/ PC (Statistical package for the Social Science Inc. Chicago, Illinois USA version 20.0).
The statistical tests of significance were applied as follows:
- Simple distribution of the study variables and the cross tabulation were applied.
- Chi-square (X^2) was used to identify the significance of the relations, associations, and interactions among various variables, such as child education between cases and controls.
- The independent sample t-test procedure was used to compare means of quantitative variables.
- Pearson's correlation test was applied.
- The results in all the above mentioned procedures were accepted as statistically significant when the p-value was less than 5% ($p<0.05$).

v. Ethical Considerations

The necessary approval to conduct the study was obtained from Helsinki committee in the Gaza Strip. Helsinki committee is an authorized professional body for giving permission to researchers to conduct their studies with ethical concern in the area.

3. Results

Sociodemographic data of the study population

The present study was a case control and comprised 80 IDA children patients aged (6 – 12) years cases and 80 apparently healthy control children in the same ages.

Gaza City Regions distribution classification showed that 11 (13.8%) controls and 10 (12.5%) cases were from West of Gaza. North of Gaza comprised 22 (27.5%) controls and 21 (26.2%) cases. South of Gaza comprised 17 (21.2%) controls and 19 (23.8%) cases. Controls and cases in east of Gaza were 30 (37.5%) and 30 (37.55%) respectively. The difference between controls and cases in term of Gaza City regions distribution was not significant (x^2= 0.182, P=0.980).

Age distribution showed that 59 (73.75%) controls and 59 (73.75%) cases were 6 – 12 years old. Age group 9 – 10 comprised 17 (21.25%) controls and 17 (21.25%) cases. Controls and cases aged 11 – 12 years old were 4 (5.0%) and 4 (5.0%), respectively. The difference between controls and cases in term of age distribution was not significant (x^2= 0.000, P=1.000).

Gender distribution showed that 54 (67.5%) controls and 54 (67.5%) cases were male. Female were 26 (32.5%) controls and 26 (32.5%) cases. The difference between controls and cases in term of gender distribution was not significant (x^2= 0.000, P=1.000).

Table 1.0 summarizes the Sociodemographic data of the study population. Analysis of family income classification showed that 7 (8.7%) controls and 19 (12.5%) cases were less than 1000 NIS. 1001 – 2000 NIS comprised 34 (42.5%) controls and 44 (55.0%) cases. 2001 – 3000 NIS comprised 24 (30.0%)

controls and 11 (13.8%) cases. Controls and cases in more than 3000 NIS were 15 (18.8%) and 6 (7.5%), respectively. The difference between controls and cases in term of family income was significant (x^2= 15.699, P=0.003).

Physical activity data among the study population

As shown in table 2.0 there's a highly significant difference in comparison between cases and controls according to physical activity. The number of cases and controls on high physical activity were 34 (42.5%) and 56 (70.0%) respectively, whereas those who were practicing moderate physical activity were 24 (30.0%) 18 (22.5%), and low physical activity were 22 (27.5%) and 6 (7.5%). The difference between the three groups was strongly significant (x^2=15.378, p=0.000).

Biochemical tests and hematological indices among the study population

Table 3.0 shows the comparison between cases and controls according to biochemical parameters and hematological Indices. There is a statistically significant difference between the values of cases and those of controls for hepcidin, serum iron, transferrin, transferrin saturation, TIBC and ferritin. All blood indices values were significant as well. The mean difference of TIBC, transferrin and RDW were higher in cases compared to controls (367.9±41.4, 315.6±38.9), (262.1±29.5, 224.9±27.7) and (14.8±2.6, 13.7±0.8) respectively. The independent sample t-test showed statistically significant difference between mean difference of cases and controls [(t-test=8.24, p=0.000), (t-test=8.240, p=0.000) and (t-test=3.712, p=0.000)]. On the other hand, the mean difference of serum iron, transferrin saturation, ferritin, hepcidin hormone, R.B.Cs, Hb, HCT, MCV, MCH and MCHC were higher in controls compared to cases [(80.7±21.3, 48.4±16.3), (25.9±7.4, 13.8±6.3), (20.6±12.7, 13.4±7.2), (8.0±6.7, 4.8±5.7), (4.8±0.4, 4.6±0.7), (12.0±0.7, 10.3±0.5), (35.8±2.1, 32.2±2.5), (75.6±4.7, 71.1±8.8), (25.7±2.0, 22.9±3.4), (33.4±1.6, 32.0±1.7)], respectively.

Serum hepcidin levels among the study population

As shown in table 4.0, there is a high significant difference in the level of hepcidin between cases and control groups according to age category, 59 (73.75%) controls and 59 (73.75%) cases were 6 – 12 years old. Age group 9 – 10 comprised 17 (21.25%) controls and 17 (21.25%) cases. Controls and cases aged 11 – 12 years old were 4 (5.0%) and 4 (5.0%) respectively. The independent sample t-test showed significant difference of hepcidin between cases and control groups in relation to age ranges 6 – 8 years and 9 – 10 years (t= 5.905, P-value= 0.017) and (t= 8.223, P-value= 0.007) respectively. And showed no significant difference between mean ages of controls and cases in term of age 11 – 12 years (t= 0.413, P=0.544).

As shown in table 5.0 there is a high significant difference between male and female in relation to hepcidin levels. The number of male=54 cases and number of female= 26 cases. The mean difference of male was higher than female (5.7±6.3, 2.8±3.2 ng/ml). The independent sample t-test showed statistically significant difference between mean difference of male and female subjects (t-test=-2.697, p=0.009).

Hepcidin levels in relation to BMI among the study population

The Pearson correlation test showed that the higher the BMI, the higher the level of hepcidin. However, this negative correlation was not statistically significant (r=0.009, p=0.912).

Hepcidin levels in relation to biochemical parameters among the study population

Table 6.0 presents the relationship between hepcidin level with TIBC, serum iron, transferrin, transferrin saturation and ferritin of the study population. Hepcidin showed significant negative correlation with TIBC, serum iron and transferrin (r=-0.172, p= 0.030, r=-0.232, p=0.003 and r=-0.168, p=0.033,

respectively). On the other hand, hepcidin showed significant positive correlation with ferritin. (r=0.320, p= 0.000). in contrast, it showed negative correlation with transferrin saturation and this correlation was not significant (r=-0.028, p=0.722).

Hepcidin levels in relation to hematological indices among the study population

Table 7.0 presents the relationship between hepcidin level with R.B.Cs, HB, HCT, MCV, MCH, MCHC and RDW. Hepcidin showed significant positive correlation with R.B.Cs and Hb (r=0.160, p=0.043 and r=0.165, p=0.037, respectively). In contrast it showed negative correlation with MCV and RDW and this correlation was not significant (r=-0.030, p= 0.706, and r=-0.106, p=0.184, respectively). On the other hand, hepcidin showed significant positive correlation with HCT, MCH and MCHC and this correlation was also not significant (r=0.143, p= 0.072, r=0.037, p=0.645 and r=0.0.10, p=0.899, respectively).

Table 1.0 *Comparison between cases and controls according to family income*

Family income	Cases (n=80)	Controls (n=80)	X^2	P -value
Less than 1000 NIS	19 23.8%	7 8.7%	15.699	0.003
1001-2000 NIS	44 55.0%	34 42.5%		
2001-3000 NIS	11 13.8%	24 30.0%		
More than 3001 NIS	6 7.5%	15 18.8%		

* the relation is significant at the p-value < 0.05 level**.** NIS: New Israeli Shekel.

Table 2.0 *Comparison between cases and control group according to physical activity*

Physical activity	CASES (n=80)	CONTROLS (n=80)	X^2	P -value
HIGH	34 42.5%	56 70.0%	15.378	0.000*
MODERATE	24 30.0%	18 22.5%		
Low	22 27.5%	6 7.5%		

* the relation is significant at the p-value < 0.05 level.

Table 3.0 *Comparison between cases and controls according to biochemical parameters and hematological indices*

Items	Controls (n=80) Mean ±SD	Cases (n=80) Mean ±SD	t-test	p-value
TIBC (µg/dL)	315.6±38.9	367.9±41.4	8.240	0.000*
Serum iron (µg/dL)	80.7±21.3	48.4±16.3	-10.774	0.000*
Transferrin (mg/dL)	224.9±27.7	262.1±29.5	8.240	0.000*
Transferrin s. (%)	25.9±7.4	13.8±6.3	11.123	0.000*
Ferritin (ng/ml)	20.6±12.7	13.4±7.2	-4.471	0.000*
Hepcidin h. (ng/ml)	8.0±6.7	4.8±5.7	-3.259	0.001*
R.B.Cs (M/UL)	4.8±0.4	4.6±0.7	-2.419	0.000*
Hb (g/dl)	12.0±0.7	10.3±0.5	-18.443	0.000*
HCT (%)	35.8±2.1	32.2±2.5	-9.906	0.000*
MCV (fl)	75.6±4.7	71.1±8.8	-3.949	0.000*
MCH (pg)	25.7±2.0	22.9±3.4	-6.380	0.000*
MCHC (g/dl)	33.4±1.6	32.0±1.7	-5.146	0.000*
RDW (fl)	13.7±0.8	14.8±2.6	3.712	0.000*

* the relation is significant at the p-value < 0.05 level.

(TIBC: Total iron binding capacity, R.B.Cs: Red blood corpuscles, HB: Hemoglobin, HCT: Hematocrit, MCV: Mean cell volume, MCH: Mean cell hemoglobin, MCHC: Mean cell hemoglobin concentration, RDW: Red cell distribution width).

Table 4.0 *Distribution of hepcidin between cases and control group according to age category*

Items	Controls (n=80) Mean ±SD (ng/ml)	Cases (n=80) Mean ±SD (ng/ml)	t-test	p-value
6-8y (n=59)	7.9±7.2	4.9±6.4	5.905	0.017*
9-10y (n=17)	7.8±5.4	3.9±1.6	8.223	0.007*
11-12y (n=4)	10.2±6.1	7.4±6.1	0.413	0.544

* the relation is significant at the p-value < 0.05 level.

Table 5.0 *Distribution of hepcidin according to gender in cases*

Items	Male (n=54) Mean ±SD (ng/ml)	Female (n=26) Mean ±SD (ng/ml)	t-test	p-value
Hepcidin h.	5.7±6.3	2.8±3.2	2.697	0.009*

* the relation is significant at the p-value < 0.05 level.

Table 6.0 *Correlation between hepcidin and biochemical parameters*

Items	Person correlation (r)	P-value
TIBC	-0.172	0.030*
Serum iron	-0.232	0.003*
Transferrin	-0.168	0.033*
Transferrin saturation	-0.028	0.722
Ferritin	0.320	0.000*

* Correlation is significant at the p-value < 0.05 level. ***(TIBC: Total iron binding capacity).***

Table 7.0 *Correlation between hepcidin and hematological indices*

Items	Person correlation (r)	P-value
R.B.Cs	0.160	0.043*
Hb	0.165	0.037*
HCT	0.143	0.072
MCV	-0.030	0.706
MCH	0.037	0.645
MCHC	0.010	0.899
RDW	-0.106	0.184

* Correlation is significant at the p-value < 0.05 level.

(R.B.Cs: Red Blood Corpuscles, Hb: Hemoglobin, HCT: Hematocrit, MCV: Mean Cell Volume, MCH: Mean Cell Hemoglobin, MCHC: Mean Cell Hemoglobin Concentration, RDW: Red cell Distribution Width).

4. Discussion

Iron deficiency anemia (IDA) is prevalent worldwide as well as in Palestine. There are many published data about IDA in Gaza Strip. Despite that the published data about hepcidin hormone are few in Gaza Strip. The available biochemical tests of IDA were limited to routine traditional anemia factors including biochemical parameters such as serum iron, TIBC, transferrin, transferrin saturation, serum ferritin and complete blood count (CBC) indices tests when the patient visits the clinic. This necessitated further assessment of other biochemical features in blood such as hepcidin hormone which was linked to IDA. The present work is the first to assess hepcidin hormone status among IDA children in Gaza City.

The independent sample t-test showed that there was significant difference between cases and control groups in relation to age ranges 6 – 8 and 9 – 10 years. On the other hand there is no significant difference regarding the age group 11 – 12 years. This finding may be explained on the basis that this group of children receive more care from their families, so they take healthy food which contains all necessary nutrients, this practice probably is the cause of normal values of Hb, serum iron, and hepcidin hormone in their blood.

The mean difference of three groups showed that there is a significant difference between cases and control group in relation to physical activity. It appears that the more active the child is the more normal its biochemical and hematological parameters are.

The individuals with family history of IDA did not have a significant knowledge about the disease and its previous history. All of the children did not have a renal disease, cardio vascular disease (CVD) and other diseases. It seems that these individuals did not take iron therapy.

Body mass index provides a reliable indicator of body fatness for most people and it is used as screening for weight categories that may lead to health problems according to (CDC) [26]. The results presented in this study revealed that the mean level of hepcidin decreased in IDA children patients compared to healthy non IDA controls (P=0.001). This finding is in agreement with that observed in a study conducted by a group of researchers who showed that; serum hepcidin was significantly lower in non-obese children with IDA ($p< 0.01$) and significantly higher in obese children with IDA ($p< 0.01$) [20]. Another study conducted by a group of researchers who showed that serum hepcidin was significantly lower in *H. pylori*-non infected children with IDA ($P < 0.01$) and significantly higher in *H. pylori*-infected children with IDA ($P < 0.01$) [21]. A study conducted by a group of researchers showed that there is a statistic significant difference of hepcidin levels among all groups ($p= 0.034$) [22]. Also a study conducted by a group of researchers showed that serum hepcidin levels differed significantly between groups ($P<0.0001$) [23].

On the other hand, as in a study conducted by a group of researchers who reported that hepcidin predicts non responsiveness to oral iron in patients with IDA. Nonresponse to oral iron therapy does not rule out IDA, since two-thirds of patients subsequently responded to intravenous iron [27]. The difference among various studies and ours may be explained on the basis of different sample size, gender, age, BMI and duration of anemia. Also as in a study conducted by a group of researchers who showed that the increase seen in serum hepcidin levels after the iron treatment was statistically significant. Hepcidin levels were significantly higher in children with IDA who received iron treatment compared to healthy children [28]. We did not follow our subjects if they receive iron therapy or not.

Data provided in the present study showed significant increase in TIBC concentration in IDA children's compared to controls with significant mean difference. Hepcidin showed significant negative correlation with TIBC. Similar results were documented in a study conducted by another group of researchers who showed that serum hepcidin levels were significantly negatively correlated with total iron binding capacity [23]. In this study, hepcidin showed significant negative correlation with serum iron. Similar results were documented by a study conducted by a group of researchers who showed that; although hepcidin showed significant positive correlations with serum iron in non-obese children with IDA, it showed significant negative correlations with serum iron in obese children with IDA [20]. Another study conducted by Choi HS et.al, who showed that serum hepcidin levels were significantly associated with iron status and can be a useful indicator of ID [23]. On the other hand hepcidin showed significant positive correlation with

serum ferritin. Similar results were documented by another group who showed that serum hepcidin levels were significantly correlated with ferritin [23].

As indicated in the present results, the mean difference of transferrin in cases was significantly higher than that in controls with significant mean difference, while the mean difference of transferrin saturation in cases was significantly lower than that in controls with significant mean difference.

Pearson correlation test showed significant negative correlation with transferrin. More over hepcidin showed negative correlation with transferrin saturation and this correlation was not significant. Opposite result was documented by another study which showed that serum hepcidin levels were significantly correlated with transferrin saturation [23]. This negative relationship reflects the change of the IDA parameters with hepcidin and suggests an involvement of hepcidin signaling in iron homeostasis and iron deficiency anemia development.

The present data demonstrate a significant decrease in R.B.Cs, Hb, HCT, MCV, MCH also MCHC levels of cases compared to controls. On the other hand, RDW level was significantly increased in cases, that means the width of the R.B.Cs was higher than the normal R.B.Cs, because the iron did not reach to the bone marrow and R.B.Cs waited iron until this size is elevated. In IDA disease iron does not present in the enterocytes and macrophages because iron passes from enterocytes across ferroportin channel into plasma, as there's no hepcidin hormone enough to prevent iron releasing into the plasma.

Pearson correlation test showed a positive significant correlation between serum hepcidin and R.B.Cs and Hb levels. These findings in agreement with others [21]. Similar findings were documented in a study which showed that serum hepcidin levels were significantly correlated with hemoglobin levels [23]. Therefore, decreased level of hepcidin hormone observed in the present study is expected to increase RDW in IDA patients. However, more research studies are needed and in particular the genetic defects related to hepcidin and how to correct its concentration in the body. Follow up of children who showed low levels of hepcidin in this study should also be emphasized. Our findings have brought into light the importance of hepcidin as a biomarker for IDA patients. Physicians should consider hepcidin level when they are dealing with IDA.

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