

Original Research Article

Study the role of hepcidin in diagnosis of iron deficiency anemia in young females of northern India

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ABSTRACT

Background: Iron deficiency is one of the most common nutritional disorder. Maintenance of body iron status is an integral part of healthcare in young female of reproductive age group. Thereby early detection could lead to early intervention and reduce its comorbidity. Indeed, an ideal screening test should be capable of identifying iron deficiency long before developing anemia. Henceforth, the present study was aimed to determine utility of serum hepcidin in iron deficiency and to access the baseline value of hepcidin in young female.

Methods: This sectional study was conducted in the Department of biochemistry SGT Medical College Hospital and Research Institute, Budhera, Gurugram. It included non-pregnant female students of age 18-25 years with normal RBC indices and hemoglobin >12 gm%. Estimation of serum hepcidin-25 was by ELISA.

Results: The reference range of hepcidin established in this study was 12.14-139.89 ng/ml for females with the mean being 42.4±29.13 ng/ml. It showed higher discriminating power in evaluating iron status in young healthy women (AUC 0.984) with best combination of diagnostic sensitivity (95.7%) and specificity (93.2%) at a cut off of >15.7 ng/ml. Serum hepcidin identified 17% of young healthy females with normal hemoglobin to have functional or storage iron deficiency.

Conclusions: The prevention of iron deficiency anemia remains insufficient worldwide especially among underprivileged women and children. Therefore, estimation of serum hepcidin may be considered as a valuable tool in assessing iron status in young healthy female population who are the prime target group for iron supplements to reduce comorbidity associated with iron deficiency and anemia.

Keywords: Hemoglobin, Iron deficiency, Serum hepcidin, Young female

INTRODUCTION

Hepcidin, a 25 amino acid peptide largely produced in the liver, plays a predominant role in iron regulation. It is a principal coordinator of systemic iron homeostasis harmonizing the use and storage of iron with iron acquisition. Mostly, it functions via a single biochemical mechanism called hepcidin-ferroportin interaction. Ferroportin acts as an iron transporter present on the intestinal duodenum cells, macrophages, and placenta. Hepcidin binds to this iron transporter, ferroportin and induces its internalization and degradation. Therefore,

loss of ferroportin from the cell surface subsequently prevents iron entry into plasma, leading to low transferrin saturation and less iron supply to the developing erythroblast.¹⁻³ On the other way decreased expression of hepcidin leads to increased cell surface ferroportin thereby increasing iron absorption.

Therefore, screening of hepcidin may be used as an indicator of iron bioavailability. Currently hepcidin testing is not being practiced in routine diagnostic laboratories. Pasricha et al established reference range values for hepcidin as 5.4 ng/ml to 174.6 ng/ml in healthy

premenopausal women in Australia.⁴ Galesloot et al also established normal ranges for hepcidin in the Netherlands.⁵ The more recent work on hepcidin reference ranges was performed in Greece by Sdogou et al.⁶

The present study was aimed to establish reference range of hepcidin in non-anemic young female students. The established reference values in north India could potentially form the baseline and reference point for studying quantitative characteristics of hepcidin. Further, it may provide an important prospective to compare them with already established data on reference values elsewhere.

METHODS

This sectional study was conducted in the Department of biochemistry SGT Medical College Hospital and Research Institute, Budhera, Gurugram. The study included 200 Non-Pregnant Female students of age 18-25 years with normal RBC indices and hemoglobin >12 gm% volunteering from SGT. Whereas, female students on iron therapy and oral contraceptive pills, with chronic liver disease, any systemic illness, acute or chronic infection were excluded from the study.

Sample collection and processing

Under strict aseptic precautions 3 ml of venous blood was collected in EDTA vacutainers which was used for estimation of (WBC, RBC, Hb, HCT, MCV, MCHC, PLT count, MCH) via the SYSMEX KX 21 Autoanalyser and 5 ml was drawn in plain vacutainers which was centrifuged to separate serum. The serum was stored at -20°C which was used to measure serum hepcidin, iron, TIBC, ferritin, transferrin level. Estimation of serum Iron and TIBC was done using semi-autoanalyser (ERBA CHEM 7). Spinreact, SA, Spain diagnostic kit. Reference value for serum iron was 40-150 µg/dl or 7.16-26.85 µmol/l and for TIBC was 200-400 µg/dl for females. Serum ferritin and transferrin were estimated by quantitative turbidimetric method using semi-autoanalyser (ERBA CHEM 7). Spinreact, SA, Spain diagnostic kit. The reference value for serum ferritin was 10-110 µg/l and for serum transferrin was 200-360 mg/dl for females

Hepcidin estimation was done by Enzyme-linked Immunosorbent Assay (Human Hepcidin (Hepc) ELISA Kit, Sincere Biotech Co., Ltd, Beijing, China). Purified human hepcidin antibody was used by kit to coat micro titer plate wells to make solid-phase antibody followed by addition of samples. Human hepcidin antigen combined with antibody followed by addition of HRP labeled antibody to form antibody-antigen-enzyme-antibody complex. After washing, TMB substrate solution was added which changed to blue color. HRP enzyme-catalyzed, reaction was terminated by the addition of a sulphuric acid solution and the color change was

measured spectrophotometrically at a wavelength of 450 nm. Thereafter, concentration of Human hepcidin in the serum samples was determined by comparing the O. D. of the samples to the standard curve.

Statistical analysis

The result was analyzed statistically using suitable software (SPSS) v.20.0. Mean and standard deviation of the study parameters was calculated. Optimal cut off value of hepcidin was determined by ROC curve. P<0.05 was considered as statistically significant.

RESULTS

A total of 262 students were screened, of which 62 were excluded because of low hemoglobin <12 gm/dl, seven had BMI ≥30. Thus 200 healthy non-pregnant female university students were taken for the study.

Study population comprised of young healthy female with the mean age of 19.4 year, and mean BMI 25.8 (Table 1).

Table 1: Demographic characteristics of study population.

Parameter	Mean	SD	Range
Age(years)	19.4	1.96	18-25
Body weight (Ibs)	153.2	6.87	141-175
Height (cm)	162.8	6.15	150-173
BMI (kg/m ²)	25.8	2.14	23-29

The mean hemoglobin (13.1 gm/dl), hematocrit and red cell indices of all the subjects were within the normal reference indicating no evidence of anemia. The total leucocytes count was also within the normal range ruling out any inflammation (Table 2).

Table 2: Hematologic parameters of study group.

Parameter	Mean	SD	Range	Normal reference range
Hemoglobin (gm/dl)	13.1	0.69	12-15	12-15
Hematocrit (%)	40.1	2.11	39.7-44.7	40-50
RBC Count (millions/mm ³)	4.14	0.261	3.8-4.99	3.5-5.2
MCV (fL)	96.7	1.34	91.58-95.25	80-100
MCH (pg)	31.6	0.42	30-31.5	27-32
MCHC (gm/dL)	32.7	0.03	32.7-33.8	30-35
TLC (thousand/mm ³)	8028.5	648.34	5700-9100	4000-10000

Serum iron was within the normal reference range in all the subjects, whereas TIBC, serum transferrin, TSAT and serum ferritin were below the normal reference range in

some of the subjects; indicating an iron deficient status (Table 3).

Table 3: Iron status of the study group based on traditional parameters.

Parameter (normal reference range)	Mean	SD	Range
Serum iron (40-150 µg/dl)	87.4	23.59	50.25-149.92
TIBC (200-400 gm/dl)	425.1	16.38	386.6-460.9
Serum transferrin (200-360 mg/dl)	297.3	25.62	184.1-326.8
TSAT (>16%)	20.7	6.26	12.03-38.78
Serum ferritin (10-110 µgm/l)	33.8	21.1	9.8-97.7

Serum hepcidin range was distributed from 12.14-139.89 ng/ml (Table 4). A peak was observed above 18.7 ng/ml. Frequency distribution bar graph for serum hepcidin is shown in Figure 1.

Table 4: Iron status of the study group based on serum hepcidin.

Parameter	Mean	SD	Range
Serum hepcidin (ng/ml)	42.4	29.13	12.14-139.89

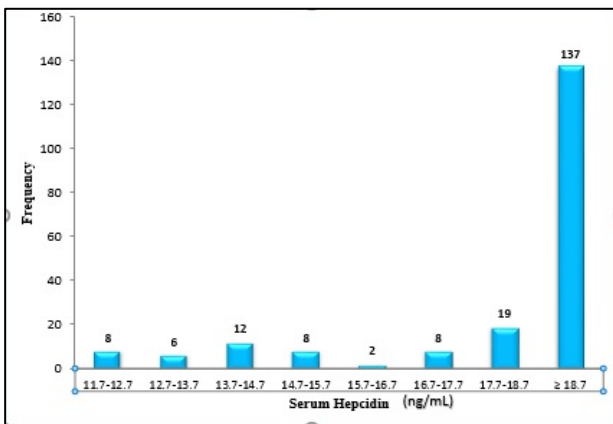


Figure 1: Frequency distribution bar diagram of serum hepcidin.

Table 5: ROC analysis of serum hepcidin in evaluation of iron status (n=200).

Serum hepcidin (ng/ml)	
Optimal cutoff	≤15.7
Sensitivity	95.7
Specificity	93.2
AUC	0.984
Standard error	0.0075
P value	<0.001

The serum hepcidin showed higher discriminating power in evaluating iron status in young healthy women in the

age group 18-25 years (AUC 0.984) with best combination of diagnostic sensitivity (95.7%) and specificity (93.2%) at a cut off of >15.7 ng/ml (Table 5, Figure 2).

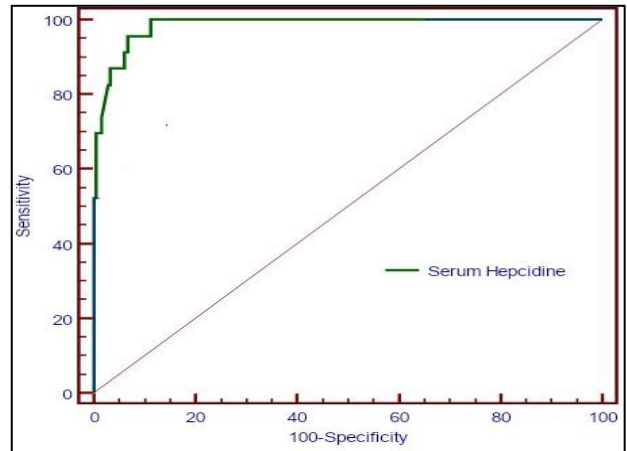


Figure 2: Receptor operating characteristic (ROC) curve of serum hepcidin.

Table 6: Discrimination between ID and NID population based on serum hepcidin.

Parameter	Iron deficient (ID)/non-iron deficient (NID)	N (total 200)	Percentage
serum hepcidin ≤15.7 ng/ml	ID	34	17
serum hepcidin ≥15.7 ng/ml	NID	166	83

On classification of serum hepcidin based on cut off with sensitivity of 95.7% and specificity of 93.2% derived from ROC 17% (34/200) were included in the ID group and the rest 83% were NID. Hence hepcidin in this cut off value was able to screen 17% of women with normal RBC indices (Table 6) compared to serum ferritin where 11.5% were classified as iron deficient (Table 7).

Table 7: Discrimination between ID and NID based on serum ferritin.

Parameter	Iron deficient (ID)/non-iron deficient (NID)	Total n=200	
		N	Percentage
Serum ferritin ≤12 µg/l	ID	23	11.5
Serum ferritin 12-20 µg/l	Equivocal	50	25
Serum ferritin >20 µg/l	NID	127	63.5

Further, a significant positive correlation was seen between serum ferritin and serum hepcidin (Table 8).

Table 8: Correlation of serum hepcidin with traditional iron parameters.

Variable	Pearsons correlation coefficient (r)	P value
Serum hepcidin versus serum ferritin	0.994	<0.0001

DISCUSSION

The study comprised of young university students with a mean age of 19.4 year and BMI of 25 (Table 1). The mean Hemoglobin being 13.1 gm/dl and all the hematologic parameters like HCT, MCV, MCH, MCHC, TLC were well within the reference range indicating no evidence of anemia or inflammation in these women whereas TIBC, serum transferrin, TSAT and serum ferritin were below the normal reference range indicating an iron deficient status in some.

The mean serum hepcidin value and reference range was estimated to be 42.4±29.13 ng/ml, 12.14-139.89 ng/ml respectively. Pasricha et al estimated a mean of 28.5 ng/ml among 261 healthy premenopausal females.⁴ Ganz et al observed reference range 17.186-91.237 ng/ml for female blood donor population. Ganz et al also observed a reference range in women 29-254 ng/mL.⁷

Similarly, different studies Swinkles et al using time of flight mass spectrometry, Grebenchtchikov et al using high-sensitive radioimmunoassay have assigned different values.^{8,9} The difference in absolute hepcidin concentrations between studies could be accounted by the type of different hepcidin assays which warrants harmonization.⁸ Moreover Serum hepcidin is influenced by circadian rhythm, age, sex, transcription factors reflecting the heterogeneity in values.¹⁰

Hepcidin reference range values are useful as baseline information for the explication of the properties of this molecule in iron deficient states. Establishment of these reference range values may enlighten the importance and usefulness of hepcidin values in iron deficiency anemia.

Next, on receptor operative curve (ROC) analysis, the hepcidin showed higher discriminating power in evaluating iron status in young healthy women in the age group 18-25 years (AUC 0.984) with best combination of diagnostic sensitivity (95.7%) and specificity (93.2%) at a cut off of >15.7 ng/ml (Table 4). On basis of serum hepcidin derived cutoff value of 15.7 ng/ml 17% of young healthy females with normal hemoglobin were identified to be functional and/or storage iron deficiency (Table 5).

The present study included young non-pregnant females who had Hb >12 gm/dl and went beyond the routine investigations to estimate other conventional iron parameters like serum ferritin, transferrin, iron, TIBC and TSAT. We evaluated the sensitivity and specificity of

serum hepcidin over the standard cut offs and found that it could pick up 17% of females as iron deficient as compared to 11% by serum ferritin. We also found a significant correlation between serum ferritin and hepcidin.

Hepcidin concentration is a useful index of iron status. Hepatic hepcidin is suppressed in iron deficiency, facilitating increased intestinal iron absorption and release from macrophage stores through ferroportin interaction as a physiological response to replenish iron stores.^{5,12-15} This novel peptide hepcidin could be used as an indicator of iron deficiency and in targeting patients who would be optimally benefitted from oral iron therapy. Hepcidin utility is been widely studied in various population and anemia.¹⁶⁻¹⁹

Nevertheless, the diurnal variations, age, gender, harmonization of assays should be considered. It is important to conduct more studies involving a larger population to come up with reference values that can be generalized and reflect the iron status of the young Indian female population.

CONCLUSION

Hepcidin the principal regulator of iron homeostasis, is a sensitive marker of iron deficiency. It can be used as potential marker for early detection of body iron status and seek timely intervention in terms of supplementation to build up optimal iron stores. Therefore, estimation of serum hepcidin in young females may help in early detection of anemia, thereby reduce its comorbidity, ultimately leading to improvement of health status of next generation child.

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