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Urinary Hepcidin Levels in Iron Deficiency Anaemia and Correlation with Severity in Children up to 12 Years of Age: Report from a Tertiary Care Centre

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Introduction

Iron Deficiency Anaemia (IDA) remains the commonest nutritional deficiency in developing countries with the highest prevalence in preschool children[1]. IDA in infants and toddlers may significantly contribute to diminished mental, motor, and behavioural effects[2]. Iron is an essential element for every living organism, as it has a vital role in oxygen transport in the form of haemoglobin, myoglobin and catalysis of oxidation-reduction reactions, involved in energy kinetics[3].

IDA is diagnosed by reduction in levels of haemoglobin, ferritin, transferrin saturation, and mean corpuscular volume (MCV) along with other iron parameters[4]. The three stages in IDA as per increasing severity are described by Al-Mazahi *et al* as stage 1 (deficiency depicted by iron store depletion; decreased serum ferritin levels), stage 2 (impaired erythropoiesis, low transferrin saturation) and stage 3 (impaired red blood cell synthesis and Hb concentration. microcytic anaemia)[5]. Serum ferritin is commonly deployed as an indicator of iron reserves besides acute phase reactant in response to inflammation. The standard clinical and laboratory methods for diagnosis of IDA include clinical symptoms such as pallor, breathlessness, fatigability and laboratory evidences from complete blood count (CBC), iron profile, and serum ferritin test[6].

Hepcidin, a 25 amino acid peptide hormone, synthesized in hepatocytes and excreted through kidney. It facilitates control of iron uptake and movement in the form of ferritin, inhibition of absorption of dietary iron in the duodenum and blocks the release of iron from macrophages and controls the movement of iron stored in enterocytes, hepatocytes and macrophages. Studies suggest the role of hepcidin levels in serum and urine in iron pathophysiology[1]. More advanced understanding of the hepcidin kinetics and iron regulation, its role in conditions of disturbed iron metabolism, refractory and chronic anaemias, haemoglobinopathies and hemolytic anaemia[10].

Hepcidin levels can be measured in plasma, serum and urine. Urinary hepcidin seems more useful than serum hepcidin as three variants (hepcidin 20, 22 and 25) are found in urine in comparison to only 2 in serum (while only Hepcidin 25 and 20) and free of diurnal variation[11,8]. Urinary hepcidin assay provides an indirect measure of the circulating hepcidin level and potential non-invasive means for diagnosing ID, which can be particularly useful in children[11,16]. In absence of sufficient data in Indian children, we planned to evaluate urinary hepcidin and its correlation with serum ferritin and serum iron parameters in diagnosis of IDA in children upto 12 years of age.

Methods

This study was a case-control study conducted in the Departments of Paediatrics and Biochemistry, in a tertiary care centre in northern India over a period of 18 months (Nov. 2018 - March 2020). Informed consent from parents/ guardians and institutional ethical clearance was obtained.

Inclusion criterion: All patients of IDA from age 6 months to 12 years.

Exclusion criterion: Age < 6 month or > 12 years, anaemia due to chronic disorders (tuberculosis, rheumatological disorders, kidney disease), hemolytic anaemias, malignancies, infections (Bacterial, viral, parasitic, fungal), megaloblastic anaemia, iron therapy in previous 3 months, or deranged liver or kidney function tests.

Sample size: Sample size in a previous study on urinary hepcidin was taken as reference. Results of this study concluded AUC of urinary hepcidin - 25 level for predicting stage 1, stage 2, and stage 3 were 0.84, 0.95 and 0.99. Taking these values as reference, ä as 0.06 and 5% level of significance, sample size was calculated as 87. We enrolled total 100 children with 1:1:1:1 ratio in 4 groups as described

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below we took 25 samples per group[23].

Informed consent/assent was obtained from parent or guardian of all children enrolled in the study and assent were taken wherever necessary. Approval from Institutional Ethics Committee was obtained. All enrolled children were divided in 4 groups of 25 children each including three groups of IDA with increasing severity:-

Group 1 (IDA stage 1): Low serum ferritin level (≥ 20 ng/ml) and normal serum transferrin and normal haemoglobin, Mean corpuscular volume (MCV) and Mean corpuscular haemoglobin concentration (MCHC values).

Group 2 (IDA stage 2): Low serum ferritin (<12 ng/ml) and low serum transferrin saturation <16% and normal haemoglobin, MCV and MCHC values.

Group 3 (IDA stage 3): Low serum ferritin (<12 ng/ml) and low transferrin saturation (<16%) and microcytic hypochromic anaemia.

Group 4 (Control group): Healthy children, normal serum ferritin level >20 ng/ml and transferrin saturation >35% and normal haemoglobin, MCV and MCHC values.

After enrollment, clinical and demographic were noted. Samples were collected for estimation of:-

1. Haematological parameters: Around 7 - 10 ml of venous blood sample was collected using aseptic techniques for estimation of haemoglobin with RBC indices and peripheral smear, c-reactive protein (CRP), erythrocyte sedimentation rate (ESR), kidney and lung function tests, serum ferritin, serum iron, transferrin levels, iron saturation, and total iron binding capacity (TIBC) and unsaturated iron-binding capacity (UIBC), serum Vitamin B_{12} and folate levels.
2. Urine analysis: A total of 10 ml of urine sample was collected with standard methods for routine microscopy, urinary creatinine, urinary hepcidin. For urine hepcidin measurement, spot urine samples were taken and centrifuged at 3,000 rpm for 10 min. Supernatant was stored at -20° C till the batch was analysed by ELISA.

Statistical analysis was done using SPSS version 21.0. Categorical variables were presented in number and percentage (%) and continuous variables were presented as mean ± SD and median. Quantitative variables were compared using ANOVA/ Kruskal Wallis Test (when the data sets were not normally distributed) between the four groups (control, stage I, stage II, and stage III). Qualitative variables were compared using Chi-Square test. Receiver operating characteristic curve was used to find out cut-off point and sensitivity, specificity, NPV and PPV of urinary hepcidin-2 for predicting stage 1, stage 2, and stage 3. Pearson correlation co-efficient/Spearman rank correlation co-efficient was used to correlate urinary hepcidin with various other quantitative parameters. A p value of < 0.05 is considered statistically significant.

Results

A total of one hundred children (6 months to 12 years) were enrolled to include 75 children with IDA and 25 healthy children as a control group. All children suspected clinically of iron deficiency anaemia were investigated. A total 89 sequential blood samples were sent to include consecutive 25 children in 3 groups that is total of 75 children. For the fourth group of 25 controls, a total of 30 samples were sent. The 100 children who were included in 4 groups were further investigated with urine and blood samples.

Baseline clinical and demographic parameters were comparable in all 4 groups. In the study most of the subjects in group 1 and group 4 were 5 - 10 years of age; while group 2 and group 3 were 1 - 5 years of age. The age-wise distribution of children showed 10 infants [group 1 (1), group 2 (3), group 3 (6), group 4 (0)] and most toddlers (n = 37) [(group 1 (5), group 2 (12), group 3 (15), group 4 (5)]. Thirty three children 5 years - 10 years [group 1 (11), group 2 (5), group 3 (4), group 4 (13)] and twenty children > 10 years [group 1 (8), group 2 (5), group 4 (7)]. Mean age of controls was 8.40 ± 2.97 years while in stage 1, stage 2 and stage 3 ID subjects mean age was 8.04 ± 3.65 years, 5.31 ± 4.00 years and 2.84 ± 2.82 years respectively. No statistically significant difference was observed in gender distribution in different groups (males 52% in group 1, 60% in group 2, 84% in group 3 and 48% in group 4; $p > 0.001$). Baseline laboratory parameters and for anaemia including mean haemoglobin, ferritin, transferrin, etc., are shown in different groups in Table I. Serum ferritin level in normal healthy control group was 115.80 ± 63.88 ng/ml and in stage 1, stage 2, and stage 3 ID subjects was 17.86 ± 1.68 ng/ml, 10.72 ± 1.34 ng/ml and 10.02 ± 1.51 ng/ml respectively. Serum ferritin, total iron and transferrin saturation levels were significantly lower in all stages of ID as compare to control group. More significant reduction in their level was observed with progression of severity of ID ($P < 0.01$). No correlation was seen with CRP, serum folate, and serum vitaminB12. While TIBC and UIBC were significantly higher in all stage of ID compare to controls and also with progress of severity of ID, TIBC and UIBC increased significantly.

Table I: Laboratory parameters and urinary hepcidin in iron deficient patients enrolled in study.

Parameter	Group 4 (n = 25)	Group 1 (n = 25)	Group 2 (n = 25)	Group 3 (n = 25)	P value
Mean Age (±SD)	8.40 ± 2.97	8.04 ± 3.65	5.31 ± 4.00	2.84 ± 2.82	< 0.001
Haemoglobin	12.44 ± 0.47	12.01 ± 0.55	11.32 ± 0.84	8.34 ± 0.92	< 0.001
Total leucocyte count (mm^3)	8446.40 ± 2254.48	8825.20 ± 2407.57	8407.6 ± 2650.63	9935.6 ± 2201.37	0.12
Polymorphs (10^3/uL)	6.15 ± 8.44	5.97 ± 6.56	6.22 ± 9.92	6.12 ± 7.29	0.57
Lymphocytes (10 3/uL)	3.36 ± 10.33	3.62 ± 6.77	3.26 ± 8.66	3.29 ± 8.24	0.26
Eosinophils (10 3/uL)	1.84 ± 1.43	2.12 ± 1.2	3.24 ± 3.56	2.16 ± 0.94	0.22
ESR	9.56 ± 5.40	8.48 ± 5.90	8.48 ± 6.03	10.04 ± 5.60	0.61
MCV (fl)	85.59 ± 6.21	79.74 ± 3.88	75.32 ± 4.20	64.44 ± 9.90	< 0.001
MCH (pg)	29.50 ± 2.35	27.57 ± 2.14	25.52 ± 2.06	20.12 ± 4.87	< 0.001
MCHC	33.78 ± 1.49	32.92 ± 1.41	31.56 ± 1.57	29.31 ± 2.50	< 0.001
PCV (%)	36.94 ± 2.56	36.42 ± 2.00	34.29 ± 2.73	27.27 ± 3.31	< 0.001
RDW (%)	12.28 ± 0.95	12.86 ± 1.02	14.38 ± 1.46	16.77 ± 1.82	< 0.001
Platelet (10^6/mm3)	3.02 ± 0.66	2.88 ± 0.67	3.89 ± 1.82	5.59 ± 1.69	< 0.001
Reticulocyte count (10^9/l)	1.99 ± 0.55	1.71 ± 0.64	2.06 ± 0.90	1.77 ± 0.65	0.32
LDH	219.96 ± 67.91	216.04 ± 59.28	227.12 ± 85.24	235.68 ± 50.88	0.39
S. Ferritin (ng/ml)	115.80 ± 63.88	17.86 ± 1.68	10.72 ± 1.34	10.02 ± 1.51	< 0.001
Total iron (ug/dl)	109.40 ± 43.16	72.40 ± 16.40	43.60 ± 13.33	27.76 ± 8.53	< 0.001
TIBC (µmol/l)	263.68 ± 40.75	287.76 ± 40.42	376.96 ± 90.75	455.12 ± 115.92	< 0.001
UIBC (ug/dl)	163.64 ± 67.39	205.96 ± 66.64	280.32 ± 89.12	359.80 ± 97.81	< 0.001
Transferrin Saturation (%)	50.08 ± 16.05	35.28 ± 12.34	11.98 ± 3.05	8.91 ± 3.77	< 0.001
CRP (mg/dl)	0.44 ± 0.27	0.42 ± 0.28	0.40 ± 0.23	0.54 ± 0.30	0.30
FA (ug/l)	14.36 ± 4.77	16.02 ± 9.39	13.46 ± 6.20	11.58 ± 9.54	0.05
Vitamin B12 (ng/l)	458.28 ±118.48	430.72 ± 158.48	399.84 ± 139.66	353.68 ± 115.88	0.02
Mean Urinary Hepcidin (ng/ml)	114.28 ± 88.94	99.86 ± 145.47	18.31 ± 27.66	16.03 ± 48.78	< 0.001
Median (range) urinary hepcidin (ng/ml)	111.77 (1.26 - 326.18)	34.47 (0 - 449.3)	32.03 (0 - 97.44)	0.06 (0 - 241.48)	< 0.001

Urinary hepcidin level in control group was 114.28 ± 88.94 ng/ml and in stage 1, stage 2 and stage 3 ID subjects was 99.86 ± 145.47 ng/ml, 18.31 ± 27.66 ml and 16.03 ± 48.78 ng/ml respectively. Urinary hepcidin levels were significantly reduced with increasing severity of IDA ($P < 0.01$). The median (range) urinary hepcidin values (ng/ml) also showed similar declining trends among control group: 111.77 (1.26 - 326.18); and stage 1 IDA: 34.47 (0 - 449.3), stage 2 IDA: 32.03 (0 - 97.44) and stage 3 IDA: 0.06 (0 - 241.48) ($P < 0.001$). From above, it is observed that urinary hepcidin levels were significantly lower in all stages of ID and from stage one to stage 3 ($P < 0.05$). Table II shows urinary hepcidin levels showed significant positive correlation with age, weight, haematological parameters (Hb, MCV, MCH, MCHC, PCV, total iron, ferritin level and transferrin saturation ($P < 0.01$). On the contrary, urinary levels of hepcidin showed significant negative correlation with platelets (r value = -0.32), TIBC (r value = -0.63) and UIBC (r value = -0.57).

Receiver operating characteristics (ROC) curve was used to detect three cut-off points for urinary hepcidin level to differentiate IDA and its different stages, from healthy children (≥ 69.41 ng/ml, ≥ 16.63 ng/ml and ≥ 13.04 ng/ml). The area under curve (AUC) was 0.66 ($p < 0.01$), 0.89 ($p < 0.001$) and 0.91 ($p < 0.001$) at 3 cut-off points respectively with 95% CI as (0.49 - 0.81), (0.81 - 0.98) and (0.83 - 0.99) respectively. Sensitivity (72%, 72% and 84%), specificity (68%, 96% and 96%), positive predictive value (69.3%, 94.8% and 95.5%) and negative predictive value (70.0%, 84% and 90%) were highest for urine hepcidin value ≥ 13.04 ng/ml.

Table II: Correlation between urinary hepcidin and clinical laboratory parameters (n = 100).

	Urinary Hepcidin (ng/ml)	
	R value	P value
Age (years)	0.28	0.005
Weight (kg)	0.31	0.002
Haemoglobin (g/dl)	0.55	0.000
MCV (fl)	0.62	0.000
MCH (pg)	0.64	0.000
MCHC (g/dl)	0.49	0.000
PCV (%)	0.46	0.000
Platelet ($\times 10^3/mm^3$)	- 0.33	0.001
Reticulocye count (%)	- 0.04	0.678
Lactate dehydrogenase (U/L)	-0.043	0.668
S. Ferritin (ng/ml)	0.63	0.000
Total iron (µ/dl)	0.66	0.000
TIBC (µ/dl)	-0.64	0.000
UIBC (µ/dl)	-0.57	0.000
Transferrin saturation (%)	0.71	0.000
C-reactive protein (mg/dl)	0.01	0.960
Urinary creatinine (mg/dl)	-0.07	0.451

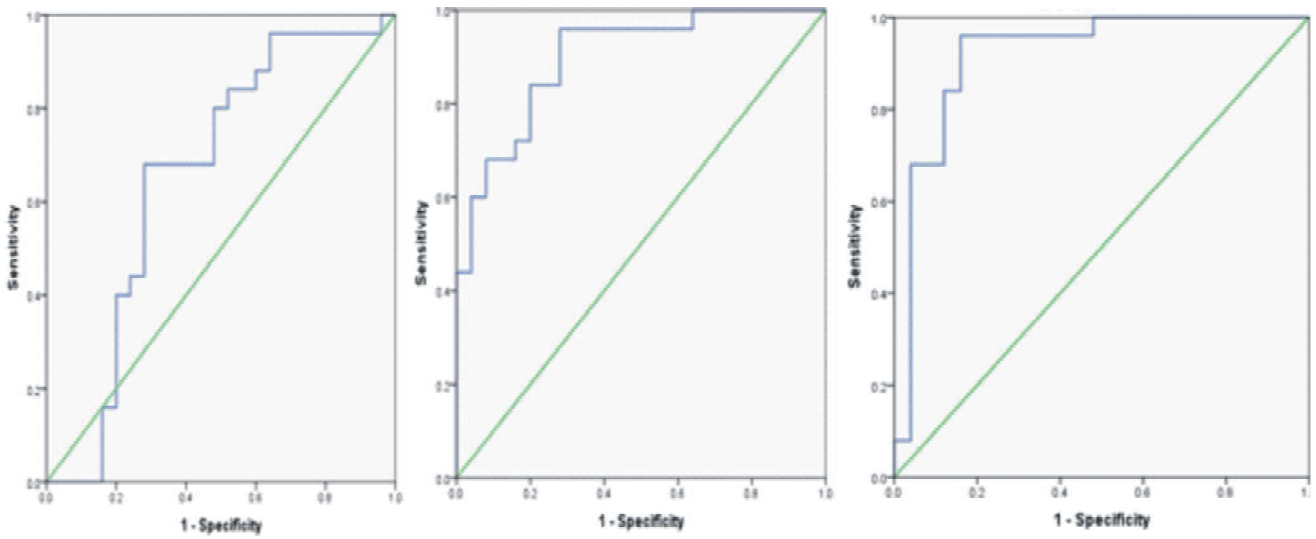
Discussion

Due to rapid growth and development in young infants and children, the demand of iron exceeds the availability of iron rendering them more vulnerable to the effect of iron deficiency anaemia. For diagnosis of IDA, an ideal screening test should be non-invasive, low cost and with high sensitivity to identify IDA before the onset of clinical symptoms for early intervention and adverse motor, behavioural, and mental complications. Currently, conventional blood haemoglobin and serum ferritin levels are the available invasive tests used worldwide popularly[12]. Serum hepcidin has shown high sensitivity[14] in previous studies in adults and children with iron deficiency anaemia with a positive correlation between serum iron, serum ferritin, MCV and transferrin saturation suggesting it as a useful indicator of iron stores. The potential limitation of the use of serum hepcidin levels for diagnostic tests is diurnal variation[19]. Serum iron levels are significantly high at noon and 8 pm in comparison to that at 8 am. Serum hepcidin levels also show similar diurnal variation[18]. In absence of sufficient data, we planned to investigate urinary hepcidin level as a marker for iron deficiency anaemia in children which will have the advantage of being non-invasive and simple screening method.

The results in our study on urinary hepcidin showed low values in all 3 groups of IDA compared to control group (114.28 ± 88.94 ng/ml). The mean urinary hepcidin level further declines with increasing stages of iron deficiency (stage 1:99.86 ± 145.47 ng/ml, stage 2: 18.31 ± 27.66 ng/ml, stage 3: 16.03 ± 48.78 ng/ml) significantly ($P < 0.01$). Similar results were observed in previous studies by Sonia *et al*[13] (urinary hepcidin level: control group (443.92 ± 91.84 ng/ml, stage 1: 362.6 ± 31.36 ng/ml, stage 2: 273.16 ± 33.48 ng/ml and stage 3: 189.12 ± 21.14 ng/ml) and Sanad *et al*[11], (urinary hepcidin (nmol/mmol Cr): healthy: 2.8 ± 1.3 stage 1: 0.7 ± 0.22, stage 2: 0.3 ± 0.009, stage 3: 0.079 ± 0.009) respectively. Mouhamed *et al*[15] also showed similar significant decline in urinary hepcidin in IDA (stage I: 0.69 ± 0.16, stage II: 0.29 ± 0.05 and stage III: 0.08 ± 0.00) in comparison to control group (2.88 ± 0.82 nmol/mmol creatinine) ($P < 0.001$).

With a cut-off point of urinary hepcidin in different stages of IDA (Stage 1: ≥ 69.41 ng/ml, stage 2: ≥ 16.63 ng/ml, stage 3: ≥ 13.04 ng/ml). Area under curve (AUC) was significantly highest in stage 3 (0.91 vs 0.89 vs 0.66; $p < 0.001$). Sensitivity of these three cut-off points was 72%, 72% and 84% respectively while specificity was 68%, 96% and 96% respectively. Positive predictive value (PPV) of these three cut-off points was 69.3%, 94.8% and 95.5% respectively while negative predictive value (NPV) was 70.0%, 84%, and 90% respectively. Our results were similar to Sonia *et al*[13] who showed cut-off points for ID stage 1, stage 2, and stage 3 were ≥ 369 ng/ml, ≥ 315 ng/ml, and ≥ 293 ng/ml, with PPV of 80, 96 and 92%, respectively, and NPV of 85, 95, and 95% respectively. The sensitivity (84, 96, and 96%) and specificity (80, 96, and 92%) of these cut-off values were also reported to be high, but was showing trend of reducing levels of hepcidin. Similar results by Sanad *et al*[11] who reported the cut-off level for stage 1, 2 and 3 ID as ≥ 0.94, ≥ 0.42 and ≥ 0.08 nmol/mmol Cr respectively while the AUC was 0.838, 0.944 and 0.999 respectively ($p < 0.001$). At these cut-off sensitivity of urinary hepcidin to diagnose ID stage 1, 2, and 3 was 88, 96 and 96% respectively while specificity was 88, 92 and 100% respectively. Mouhamed *et al*[15] also used receiver operator curve (ROC) to know the best cut-off level of urinary hepcidin to detect stage 1, stage 2 and stage 3 iron deficiency in healthy children and got the cut-off level as ≥ 0.95, ≥ 0.38, ≥ 0.089 respectively. At these cut-off points, the sensitivity, specificity, PPV and NPV was 100% to detect stage 1, stage 2 and stage 3 ID respectively.

Correlation of urinary hepcidin with iron parameters



	Stage 1	Stage 2	Stage 3
Area under curve	0.66	0.89	0.91
Standard Error	0.08	0.04	0.04
95% CI	0.49-0.81	0.81-0.98	0.83-0.99
Cut off value of hepcidin (ng/ml)	69.41	16.63	13.04
Sensitivity	72.0%	72.0%	84.0%
Specificity	68.0%	96.0%	96.0%
PPV	69.3%	94.8%	95.5%
NPV	70.8%	77.4%	85.7%
Accuracy	70.0%	84.0%	90.0%

Fig. 1: *Predictive values of urinary hepcidin level in detection of iron deficiency in three different stages.*

showed significant positive correlation with Hb (r value = 0.55), MCV (r value = 0.610, MCH (r value = 0.64), MCHC (r value = 0.49), PCV (r value = 0.45), serum iron level (r value = 0.65), ferritin level (r value = 0.62) and Tsat (r value = 0.71) (P < 0.01) and significant negatively correlated with platelet count (r value = -0.32), UIBC (r value = -0.57) and total iron binding capacity (r value = -0.63). Similar observations were reported by Sanad *et al*[11] who reported significant positive correlation of urinary levels of hepcidin with Hb, MCV, MCHC, hematocrit value, serum iron level, ferritin level and Tsat (P < 0.01) and significant negative correlation with serum transferrin and TIBC (P < 0.01). Cherian *et al*[16] they also reported positive association of hepcidin with haemoglobin, MCV, serum iron, serum ferritin and transferrin saturation levels and negative correlation between hepcidin and transferrin. Sonia *et al*[13] also observed that the levels of urinary hepcidin were positively associated with MCV, serum iron, haemoglobin, MCV, serum ferritin level and transferrin saturation while urinary hepcidin was negatively correlated with total iron binding capacity.

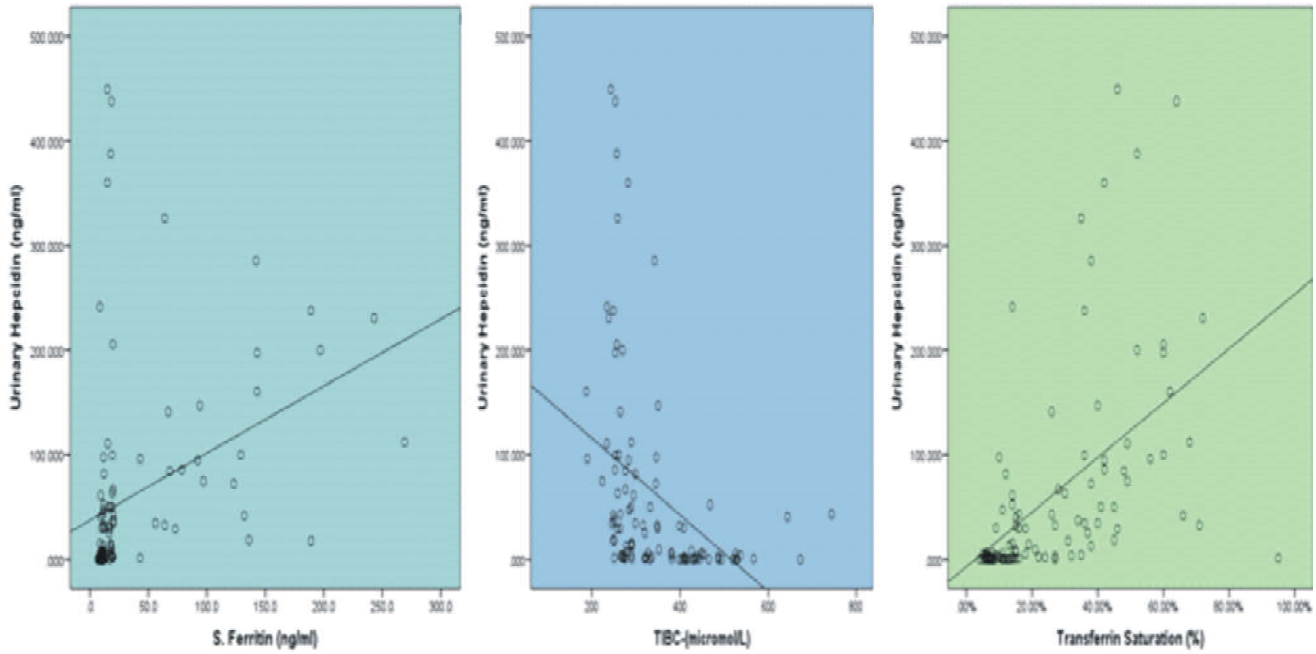


Fig. 2: *Scatterplot showing correlation of urinary hepcidin with serum ferritin, TIBC, and Transferrin saturation.*

Findings of our study was in concordance with Al-Mazahi *et al*[6] and Sonia *et al*[13]. Apart from that, study by Bregman *et al*[17] found positive correlation of hepcidin with ferritin levels. Mouhamed *et al*[15] also found statistically significant correlation between urinary hepcidin level with ferritin level and T. sat ($P < 0.01$). In contrast, urinary levels of hepcidin showed significant negative correlation with TIBC ($P < 0.01$).

The strength of our study was a strong study design. Urine specimen is associated with higher pre-analytical variability compared to serum which is a potential limitation of urinary hepcidin assay[19]. Therefore, further evaluation of urine hepcidin as non-invasive monitoring tool of iron status in children is necessary. The small sample size of this study prevents generalisability of results and warrants future studies with larger sample sizes are required to know the cut-off values of urinary hepcidin for diagnosing and differentiating different stages of ID for confirmation.

We conclude that with increasing severity of IDA, along with routine parameters (Hb, MCV, MCH, MCHC, transferrin saturation, total iron) levels of urinary hepcidin also decreased. Urinary hepcidin may be a useful non-invasive, easy, quick, and a low cost, test for mass screening of IDA.

References

1. Sharma A, Giri A, Pudasaini S. Prevalence of anaemia in children aged 6 months to 15 years: a hospital based study. *J Pathol Nepal* 2017; 7 (2): 1168-71.
2. Radhika K. Magnitude of anaemia and strategies for prevention and control in South East Asia countries. *IJCH* 2018; 30: 119-23.
3. Anderson GJ, Darshan D, Wilkins SJ. Regulation of systemic iron homeostasis: how the body responds to changes in iron demand. *Biometals* 2007; 20 (3-4): 665.
4. Expert Scientific Working Group. Summary of a report on assessment of the iron nutritional status of the United States population. *Am J Clin Nutr* 1985; 42 (6): 1318-30.
5. Cook JD. Diagnosis and management of iron-deficiency anaemia. *Best Prac Res Clin Haemato* 2005; 18 (2): 319-32.
6. Al-Mazahi MM, Mekky AA, Salam MA. Urinary hepcidin in diagnosis of iron deficiency anaemia. *Asian J Clin Nutrition* 2015; 7 (1): 9.
7. Nemeth E, Ganz T. Anaemia of inflammation. *Hematol Oncol Clin North Am* 2014; 28 (4): 671-81.
8. Fleming MD. The regulation of hepcidin and its effects on systemic and cellular iron metabolism. *Hematology* 2008; 1: 151-8.
9. Ganz T. Hepcidin and iron regulation, 10 years later. *Blood* 2011; 117 (17): 4425-33.
10. Reichert CO, Marafon F, Levy D *et al*. Influence of Hepcidin in the Development of Anaemia. *In Current Topics in Anaemia* 2017; 20: 2017-231.
11. Sanad M, Gharib AF. Urinary hepcidin level as an early predictor of iron deficiency in children: A case control study. *Ital J Pediatr* 2011; 37 (1): 37.
12. Kazal LA Jr. Prevention of iron deficiency in infants and toddlers. *Am Fam Physician* 2002; 66 (7): 1217.
13. Al Sharkawy SG, El-Kelany AM, Anani MM *et al*. Urinary hepcidin concentration in assessment of iron homeostasis in pediatrics. *Egyptian J Haematolo* 2019; 44 (2): 105.
14. Ælulafiæ J, Kolaroviæ J, Pezo L *et al*. Serum concentration of hepcidin as an indicator of iron reserves in children. *J Med Biochemistry* 2018; 37 (4): 456-64.
15. Mouhamed HI, El Sheimy A, Omran A *et al*. The role of urinary

hepcidin in early detection of iron deficiency anaemia. *J Med in Scientific Res* 2018; 1 (3): 173.

16. Cherian S, Forbes DA, Cook AG *et al*. An insight into the relationships between hepcidin, anaemia, infections and inflammatory cytokines in paediatric refugees: a cross-sectional study. *PloS One* 2008; 3 (12): e4030.
17. Ganz T. Hepcidin and iron regulation, 10 years later. *Blood* 2011; 117 (17): 4425-33.
18. Ganz T, Olbina G, Girelli D *et al*. Immunoassay for human serum hepcidin. *Blood* 2008; 112 (10): 4292-7.
19. Kroot JJ, Hendriks JC, Laarakkers CM *et al*. (Pre) analytical imprecision, between-subject variability, and daily variations in serum and urine hepcidin: implications for clinical studies. *Analytical Biochemistry* 2009; 389 (2): 124-9.

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