Evaluation of Urine Ferritin Concentration as a Non-Invasive Test for Iron Status of Preterm Neonates

Review Article

Rabab Gameel Allam[1], Nehal Mohamed El-Raggal[1], Rasha Ahmed Ghorab[2], Ahmed Nashat Shawky Fahmy[3] and Tayseer Moustafa Mahmoud Mohamed[1]

[1]Department of Pediatrics, [2]Department of Clinical Pathology and [3]Faculty of Medicine, Ain Shams University

ABSTRACT

Background: Preterm neonates are at high risk of iron deficiency due to low iron store at birth. Recent research has shown a link between low iron stores in neonates and poor neurocognitive outcome. Serum ferritin is often used as a measure of iron stores in neonates that require phlebotomy.

Objective: To investigate the potential use of urinary ferritin as non-invasive screening method for evaluating iron reserve in preterm neonates without the need for phlebotomy.

Patients and Methods: Paired blood and urine samples were collected from 35 preterm neonates enrolled in this cross-sectional study on first day of life.

Measurement of urine ferritin was done using ELISA while, serum ferritin and urine creatinine were assessed by routine laboratory assay. The included neonates were consequently identified as either normal or deficient iron storage groups according to a predefined minimal serum ferritin value of 35 ng/mL approved for preterm and low birth weight neonates

Results: The median concentrations of serum and urinary ferritin were 230ng/mL and 154ng/mL, respectively. urinary ferritin concentrations correlated positively with gestational age, birth weight and serum ferritin concentrations. Urinary ferritin concentrations and urine ferritin/creatinine ratio were found significantly lower in the deficient iron storage group as compared to the normal iron storage group. A cut-off value for urinary ferritin of ≤50 ng/mL had a sensitivity83.3 %and a specificity96.5% for indication of iron storage deficiency with a PPV83.3%.

Conclusion: Urinary ferritin levels in preterm neonates could be a feasible non-invasive screen for iron deficiency in preterm neonates since low values could identify iron store depletion.

Key Words: Neonatal intensive care unit, preterm neonates, urinary ferritin.

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Corresponding Author: Rabab Gameel Allam, Lecturer of Pediatrics, Faculty of Medicine, Ain Shams University, Cairo, Egypt. **Tel.:** +20109069425, **E-mail**: rabab_allam@med.asu.edu.eg

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INTRODUCTION

Cognitive and behavioral issues may result from iron deficit during the newborn period, and these difficulties may continue long after the iron deficiency has been cured[1]. Neonatal iron insufficiency is likely to be prevalent among NICU patients due to the shared risk factors with iron deficiency, such as preterm delivery, maternal diabetes/obesity and small for gestational age (SGA)[2]. The volume of blood lost due to phlebotomy is another danger. Existing iron deficiency screening approaches primarily prioritize the identification of anemia. However, anemia is a consequence that occurs later in the course of iron shortage and is likely to happen when the infant's brain has a neurological injury due to the lack of iron[3].

AIM OF THE WORK

To study the possibility of measuring urinary ferritin level as a non-invasive screening tool to assess iron stores in preterm neonates.

PATIENTS AND METHODS

Study Population:

This cross sectional research has been conducted at the Post Resuscitation Care, Maternity Hospital, Ain

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Shams University, Cairo, Egypt., from September 2023 till February 2024. This study included preterm neonates with a gestational age less than 37 weeks, who did not receive red packed cells transfusion before enrollment.

A total number of 35 preterm neonates were randomly allocated by simple method,by choosing randomly from pieces of paper with the preterm neonate's names on it in the delivery room then ferritin was measured in paired serum and urine samples collected at the same time on their first day of life, then according to the results of the serum ferritin, the neonates were classified into deficient iron storage and normal iron storage groups according to a predefined minimal serum ferritin value of 35 ng/mL approved for preterm and low birth weight neonates[4].

Sample Size calculation: (at least 35 subjects)

After examining previous research results, the sample size was calculated using power analysis and Sample Size Software (PASS 15) (Version 15.0.10), with a confidence level of eighty percent and a margin of error of +/– 0.2.[5] showing that the spearman correlation between log10 transformed serum and log10 transformed urine ferritin values among NICU patients was (r=0.35); based on that a sample size of at least 35 preterm neonates will be sufficient to achieve research aim.

Ethical Considerations:

This study was conducted after approval of "Research Ethical Committee" of Ain-Shams University Faculty of Medicine. An informed consent was obtained from the parents or the legal guardians of the patient before enrollment. Full explanation of the nature of the study was done and parents were enlightened that they have the right to withdraw from the study at any time. Ethical Committee Approval Number: FMASU MS 560/2023.

Study Tools:

All of the studied neonates were subjected to the following: Gestational age was estimated by using the date of the last menstrual period and was confirmed by new Ballard score[6], Birth weight was measured and gender was determined. then Complete clinical examination including cardiac, chest, abdominal and neurological examinations were done. Every newborn got standard neonatal care in accordance with our Neonatal Intensive Care Unit protocol.

Laboratory analysis:

Laboratory investigations were done for serum ferritin and urinary creatinine by electro-chemiluminescence immunoassay and urinary ferritin by enzyme linked immunosorbent assay (ELISA) and calculation of urine ferritin /creatinine ratio by dividing urinary ferritin by urine creatinine.

Sampling Method:

Blood and urine samples have been obtained within the first day of life.

Quantitative measurement of serum ferritin by electro-chemiluminescence immunoassay:

The sample of blood was collected into a gel vacutainer tube and centrifugated at 4000 rpm for 20 minutes to acquire serum. Serum sample has been stored at -20^{o}C till analysis. Measurement of serum ferritin levels were done by electro-chemiluminescence immunoassay on Cobas e 411 (Roche Diagnostics, CH-6343 Rotkreuz, Switzerland). The findings had been demonstrated in ng/mL.

Urine Sampling and quantitative measurement of urinary ferritin by Enzyme Linked Immunosorbent Assay (ELISA):

A sample of urine was obtained from each preterm neonate into urinary bag at same time of collection of serum sample. Urine samples were centrifugated to remove any precipitate and stored at -80^{o}C until analysis. Urine supernatant was used for measurement of urinary ferritin and urine creatinine. The urinary ferritin was quantitatively measured using a commercially available ELISA Kit provided by Bioassay technology laboratory (Cat No: E1702Hu), following the instructions provided by the manufacturer. The data were quantified in ng/mL. The absorbance of both the standards and samples was measured at a wavelength of 450 nanometers using a microtiter plate ELISA reader manufactured by Biotek in the United States. The range of detection for the kit was one to four hundred ng/mL. Urine creatinine was measured using rate blanked jaffe method on Cobas c501 (Roche Diagnostics, CH-6343 Rotkreuz, Switzerland) for correction of urinary ferritin by dividing urinary ferritin by urine creatinine to obtain urine ferritin/ creatinine ratio (UFCR).

Statistical Analysis:

The quantitative variables of serum ferritin level, gestational age, and urinary ferritin amount were characterized using statistical indices of mean and standard deviation. Qualitative information was described using tables and percentages. The data were gathered, reviewed, coded, and put into the Statistical Package for Social Science (IBM SPSS) version 27. The quantitative data have been reported using descriptive statistics. For data with a parametric distribution, the mean, standard deviation, and range were provided. For data with a non-parametric distribution, the median and inter-quartile range (IQR) have been reported. In addition, the qualitative information have been represented using numerical values and percentages. The comparison between groups containing qualitative information was conducted using the Chi-square test. The comparison of quantitative data between two independent groups with a parametric distribution has been conducted using an independent t-test. The Spearman correlation coefficients have been used to evaluate the correlation between two quantitative parameters within the same group. The r-value is used to ascertain the correlation coefficient, which ranges from -1 to +1. A value of 0 indicates no association, whereas a value of 1 indicates a perfect and full correlation. The r-value indicates the direction of the correlation (direct or inversed).

The receiver operating characteristics (ROC) curve has been utilized to define the area under the curve to determine cutoff point of urinary ferritin to predict iron storage deficiency. The confidence interval has been established at a level of 95% and the acceptable margin of error has been set at five percent. The *p-value* was taken as significant if it was less than 0.05, and highly significant if it was less than 0.01.

RESULTS

Our study included 35 preterm neonates,with gestational age ranging from 28 to 36 weeks, 27 (77.1%) were males. Birth weight range was from 850 to 2490 g.

Table 1: Demographic data of all the studied preterm neonates according to serum ferritin level as a reflection on iron storage.

		Normal Iron Storage group *n*=29	Deficient Iron storage group *n*=6	Test-value	*P*
Gestational age (weeks)	Mean±SD	34.03 ± 1.4	30.33 ± 2.88	4.83 •	0.00
	Range	30 – 36	28 – 34		
Gender, (n %)	Male	24 (82.8%)	3 (50.0%)	3.02 *	0.08
Birth weight (g)	Mean±SD	1850±310	1340±300	-4.832•	0.000
	Range	1600 – 2490	850 – 1750		

*: Chi-square test; • : Independent t-test; *P-value* > 0.05: Non significant; *P-value* < 0.05: Significant; *P-value* < 0.01: Highly significant.

As demonstrated in previous table, deficient iron storage group had considerably lower gestational ages as well as birth weight.

Table 2: Laboratory parameters of all the studied preterm neonates.

		Total no =35
Serum ferritin (ng/mL)	Median(IQR)	230 (103 – 289)
	Range	6 – 632
Urinary ferritin (ng/mL)	Median(IQR)	154.9 (81.66 – 197.1)
	Range	25 – 480
Urinary creat (mg/dL)	Median(IQR)	13.3 (9 – 25)
	Range	6.1 – 87
Urine ferritin/creat ratio	Median(IQR)	0.00092 (0.00043 – 0.00111)
	Range	0.000032 – 0.00186
Urinary Ferritin as a percent of Serum Ferritin, %		67.35%

(Table 2) displays the results of laboratory tests for all the studied preterm neonates. It shows The urinary ferritin as a percent of serum ferritin was 67.35%.

Table 3: Laboratory parameters of all the studied preterm neonates according to serum ferritin as a reflection on iron storage.

		Normal Iron Storage group No=29	Deficient Iron storage group No=6	Test-value•	*P*
Urinary ferritin, (ng/ml)	Median (IQR)	157.9 (114 – 197)	8.7 (6.5 – 10.3)	-2.539	**0.008**
	Range	25 – 480	2 – 209.1		
Urinary creat, (mg/dl)	Median (IQR)	15.5 (10.1 - 25)	9.45 (6.8 - 18.6)	-0.876	0.381
	Range	6.1 – 87	6.2 – 57.6		
Urine ferritin/ creat ratio	Median (IQR)	0.00097 (0.00706 – 0.001219)	0.0000122 (0.00007 – 0.00029)	-3.064	**0.002**
	Range	0.000403 – 0.00186	0.000032 – 0.000363		
Urinary Ferritin as a percent of Serum Ferritin		**62.1%**	**60%**		

In (Table 3) as regards the laboratory results of both groups. A clear difference has been detected between the two groups, urinary ferritin level and urine ferritin/creatinine ratio were significantly lower in deficient iron storage group compared to other group.

Table 4: Correlations of serum ferritin with urinary ferritin and other studied parameters.

	Serum ferritin ng/mL		Urinary ferritin ng/mL	
	r	*P*	r	*P*
Serum ferritin, (ng/mL)	-	-	0.68*	0.000
Urinary ferritin,(ng/mL)	0.68*	0.000	-	-
Urine ferritin/ creat ratio	0.44*	0.007	0.62*	0.000
Gestational age (Weeks)	0.70*	0.001	0.73*	0.000
Birth weight (Kg)	0.81*	0.000	0.73*	0.000

(Tables 4) shows a direct significant correlation between serum and urinary ferritin levels with correlation coefficient 0.68 and *p value* = 0.000 Also, a statistically significant relationship has been detected between serum and urinary ferritin levels on one hand and gestational age and birth weight on the other hand.

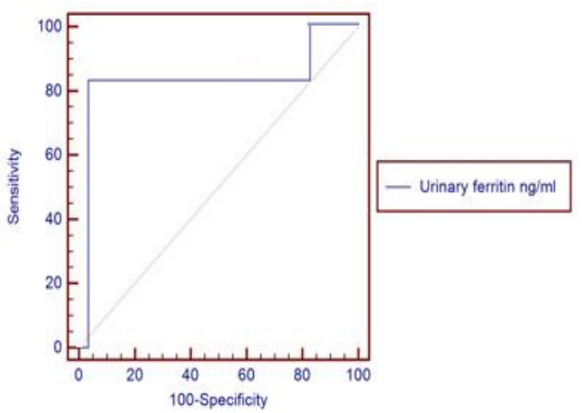

Fig. 1: ROC curve of urinary ferritin to detect iron storage deficiency.

Table 5: Cutoff value of urinary ferritin level.

Parameter	AUC	Cut off Point	Sensitivity	Specificity	PPV	NPV
Urinary ferritin	0.833	≤50	83.33	96.55	83.3	96.6

AUC: Area Under ROC Curve; PPV: Positive Predictive Value; NPV: Negative Predictive Value.

(Table 5) displays that the cut-off value of urinary ferritin level to detect iron storage deficiency was ≤50 ng/ml, positive and negative predictive values were 83.3% and 96.6 % respectively.

DISCUSSION

One of the most crucial micronutrients for the proper operation of all body systems, particularly the brain, is iron. If iron shortage develops during pregnancy or the early stages of infancy, it may result in long term neurological and behavioral problems[1]. Infants with low and extremely

low birth weights, infants whose mothers smoke and those whose mothers have diabetes are among high risk groups to have iron insufficiency at delivery[2].

Urinary ferritin, a non-invasive test for iron status, has inherent benefits compared to serum-based testing[7]. However, it is possible that the accuracy of the results may be compromised due to the artifactually increased level of urinary ferritin caused by inflammation.

Unfortunately, the literature review yielded a scarcity of comparable studies in this field, specifically examining the correlation between serum and urinary ferritin levels in this particular age range[8].

Correlations between paired random urine and serum results may be enhanced by corrected urinary ferritin creatinine ratio[5].

In our study, we used correction of urinary ferritin by dividing urine ferritin by urine creatinine to obtain urine ferritin/ creatinine ratio (UFCR) as a more accurate measurement of urinary ferritin concentration. This method of correction was also used by[5] and is more accurate than using specific gravity as it has a very small effect. However[8], did not make this correction in studying 61 premature neonates to establish an association between serum and urinary ferritin levels.

In our results, deficient iron storage group - based on serum ferritin level less than 35 ng/ml according to previous studies[4] -had a lower birth weight and gestational age compared to normal group with $p< 0.001$ for both.

Furthermore we have found a direct correlation between serum and urinary ferritin on one side and both gestational age and birth weight on the other side.

This was in accordance with the study of[9], they found that serum ferritin concentration in preterm infants at 24–48 hours of age had a significantly lower value in infants <34 weeks gestation, compared to infants >34 weeks gestation.

Similarly[10], reported that iron reserves were significantly lower in very preterm compared to moderate and late preterm neonates depending on serum ferritin measurements.

Although low iron stores at premature birth results from the mother's interruption of the majority of fetal iron transfer during the third trimester of gestation, the growth velocity of premature birth reaches its maximum at post menstrual age of 24 – 38 weeks indicating a particularly high iron needs[11].

Infants with lower birth weight are more susceptible to iron deficiency because they have lower iron reserves at birth. as evidenced by the study conducted by[8]. This study found a significant correlation between gestational age, birth weight, and levels of serum and urinary ferritin, which aligns with our own findings.

Our results were similar to the study of[8] which stated that both urinary and serum ferritin levels were significantly correlated.

A research done by[12] in Japan found a statistically significant correlation between the levels of ferritin in the blood and urine of healthy adults.

As well as, the results of[5] showed that urinary ferritin correlated with serum ferritin (correlation coefficient formed value 0.44) and in iron limited erythropoiesis which is the stage before developing anemia, there was a lower urinary ferritin value which was coincident with the results of our study. However, the sole distinction in the iron storage deficiency diagnosis which was made by the low RET-Hb. The range of the urinary and serum ferritin levels were 36- 536 ng/mL and 1- 1442 mL respectively.

Moreover, a positive correlation between serum and urinary ferritin levels was observed in the study of[7]. The median urinary and serum ferritin levels were 128 and 5.1 ng/mL respectively but the urinary ferritin as a percent of serum ferritin was only 4 % in 8 preterm neonates and 20 % in 5 healthy full term neonates. Moreover, in the research of[13], urinary ferritin was about 3% only of serum ferritin.

We found that the cutoff value of urinary ferritin level to predict the iron status of our preterm neonates was ≤ 50 ng/mL with sensitivity 83.33 %, specificity 96.55% and positive predictive value of 83.3%, but the study of[5] showed a corrected urine ferritin level < 12 ng/mL in 30 patients with iron limited erythropoiesis depending on RET- Hb cutoff value < 28 pg for diagnosis of them with sensitivity 82 %, specificity100% and positive predictive value 100%. While the negative predictive value was 56%.

The difference between our estimated cutoff value and that of[5] may be due to difference in sample size as they studied 30 neonates with iron limited erythropoiesis, while in our study only 6 neonates had low iron storage.

Negative predictive value of urinary ferritin is rather poor due to elevation of ferritin during inflammation state, Therefore, a decreased ferritin level seems to be a reliable indicator of iron storage deficit, whereas an elevated level does not guarantee that iron levels are indeed normal.

CONCLUSION

In conclusion, ferritin level in urine could be utilized as a noninvasive marker to assess neonatal iron stores without phlebotomy.

Limitations

1. The measured serum and urinary ferritin is likely quite heterogeneous and may not always be intact ferritin but pieces of ferritin shell. This is particularly true in urine where a molecule as large as ferritin is not expected to normally be filtered by the glomerulus.

2. Presence of different reference ranges for serum and urinary ferritin in the previous studies.

CONFLICT OF INTEREST

1. No competing interests of financial or personal nature.

2. The manuscript is not under consideration elsewhere.

3. Funding not received.

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تركيز الفريتين فى البول كإختبار غير غازى لتقييم حالة الحديد فى حديثى الولادة الخدج

رباب جميل علام[١]، نهال محمد الرجال[١]، رشا أحمد غراب[٢]، أحمد نشأت شوقى فهمى[٣] و تيسير مصطفى محمود محمد[١]

[١]قسم طب الأطفال، [٢]قسم الباثولوجيا الإكلينيكية، [٣]بكالوريوس الطب والجراحة، كليه الطب، جامعه عين شمس، القاهرة، مصر

الخلفية: يعد الحديد عنصرا حيويا للعديد من وظائف الخلايا فى جسم الإنسان، كما إنه عنصر غذائى اساسى. يترتب على نقص الحديد تغيرات مرضية بخلايا و انسجة المخ و الجهاز العصبى مما يؤدى إلى إضطرابات فى التطور العصبى غير قابلة للشفاء حتى بعد إعطاء الحديد للطفل.

يعكس مستوى الفريتين فى الدم إجمالى مخزون الحديد فى الجسم و بالتالى فإن انخفاض نسبته يعد دلالة على نقص الحديد و يمكن إستخدام مستوى الفريتين فى البول كوسيلة غير غائرة لتحرى مخزون الحديد فى الجسم و خاصة فى حديثى الولادة و الأطفال لتجنب عملية سحب الدم.

الهدف: تهدف هذه الدراسة إلى تقييم فاعلية تحليل نسبة الفريتين فى البول كوسيلة بسيطة لحساب مخزون الحديد فى الجسم بدلا من الفريتين فى الدم عند الخدج.

تم عمل هذه الدراسة على عدد ٣٥ من الخدج بمحضن مستشفى الأطفال بجامعة عين شمس بالقاهرة فى الفترة من سبتمبر ٢٠٢٣ حتى فبراير ٢٠٢٤.

تم إدراج الخدج ذوى الأعمار الجنينية الأقل من ٣٧ اسبوع و الذين لم يسبق لهم نقل الدم .

تم قياس نسبة الفريتين فى الدم و البول معا فى اليوم الأول بعد الولادة مع تقسيم الخدج إلى مجموعتين طبقا لنسبة الفريتين فى الدم:

مجموعة نقص الحديد فى الجسم حيث نسبة الفريتين بالدم اقل من ٣٥ نانوجرام لكل ملليلتر و مجموعة طبيعية حيث نسبة الفريتين بالدم تعادل أو أكثر من ٣٥ نانوجرام لكل ميلليلتر.

النتائج: و قد أظهرت نتائج الدراسة وجود علاقة طردية ما بين العمر الرحمى و الوزن عند الولادة مع مستويات الفريتين فى الدم و البول. كما أظهرت نتائج الدراسة إنخفاض مستوى الفريتين فى الدم و البول فى مجموعة نقص الحديد فى الجسم عن المجموعة الطبيعية مع وجود علاقة طردية ذات دلالة إحصائية بين مستوى الفريتين فى الدم و البول .

كما وجد أن مستوى الفريتين فى البول يساوى أو أقل من ٥٠ نانوجرام لكل ميلليتر يمكن استخدامه كدليل على نقص مستوى مخزون الحديد فى الجسم لدى الخدج.

النتيجه: و من ثم نستخلص من تلك الدراسة أن قياس مستوى الفريتين فى البول يمكن استخدامه لمعرفة مستوى مخزون الحديد فى الجسم للعلاج فى الوقت المناسب قبل حدوث مضاعفات.