EFFECTIVENESS OF ZINC PROTOPORPHYRIN/HEME RATIO IN IDENTIFICATION OF IRON DEFICIENCY IN A GROUP OF PRESCHOOL AGED CHILDREN

By

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ABSTRACT

Background and aim: *Iron deficiency anemia (IDA) is the ultimate result of untreated iron deficiency. Zinc protoporphyrin (ZPP)/heme ratio levels are a direct marker of iron status in the bone marrow during erythropoiesis. We studied the value of this index in screening for iron depletion or for deficiency in children seen for routine examination in a community pediatric practice.*

Subjects and methods: *The present cross sectional study consisting of 95 child aged 3-6 years old was recruited from outpatient pediatric clinic in Alzahraa University Hospital, Faculty of Medicine for Girls, Al-Azhar University during the period from (April 2020 - April 2021). All participants were subjected to full medical history taking, thorough clinical examination and standard laboratory assessment including complete blood count, serum iron, serum ferritin, TIBC and ZPP/H was assessed.*

Results: *The present study included 95 preschool children. They included 25 anemic children (26.3 %) and 70 non-anemic children (73.4 %). Comparison between anemic and non-anemic children revealed that anemic children had significantly higher ZPP/H [median (IQR): 97.8 (91.13–100.2) versus 51.05 (43.21–75), p□0.001]. Correlation analysis identified significant inverse correlation between ZPP/H and Hb (r=-0.573, p<0.001), and S.ferritin (r=-0.267, p=0.009) and positive correlation with RDW (r=0.439, p<0.001) and TIBC (r=0.484, p =0.000). ROC curve analysis recognized ZPP/H as a reliable diagnostic marker for anemia (AUC=0.819) with sensitivity and specificity of 92.00 and 78.57 respectively.*

Conclusions: *ZPP/H ratio can be considered as a reliable marker for diagnosis of iron deficiency with or without anemia in preschool children. It's well-correlated with other markers of anemia.*

Keywords: *Anemia, Zinc protoporphyrin, Preschool children.*

INTRODUCTION

Iron is one of the essential elements required for human health. Symptoms suggesting iron deficiency are generally vague and can be associated with a number of clinical conditions **(Auerbach and Adamson, 2016)**. Iron deficiency may result from insufficient iron intake, decreased absorption, or blood loss **(Matthew, et al., 2020)**.

Iron deficiency anemia (IDA) is the ultimate result of untreated iron deficiency. Regardless of the presence of symptoms, patients with IDA should be treated as early as possible because they are at risk for organ ischemia and further worsening of the anemia unless the underlying cause is relieved, and the bone marrow iron stores refilled. Likewise, children with IDA should be treated because sideropenia is associated with long-lasting neurocognitive impairments, decreased learning ability, and altered motor function **(Mantadakis et al., 2020)**.

When iron supply for erythropoiesis falls to a suboptimal level, zinc, instead of iron, is incorporated into protoporphyrin IX, and thus zinc protoporphyrin (ZPP) is produced instead of heme. Thus, ZPP levels are a direct marker of iron status in the bone marrow during erythropoiesis **(Eleni et al., 2021)**.

Using the simple technique to measure the ZPP/H ratio in whole blood, we studied the value of this index in screening for iron depletion or for deficiency in children seen for routine examination in a community pediatric practice.

SUBJECTS AND METHODS

The present cross-sectional study was conducted at Al-Zahraa University Hospital, Cairo, Egypt during the period from (April 2020-April 2021).

Ethical consideration:

- Approval by the ethical committee of Al-Azhar Faculty of Medicine was obtained before the study.
- An informed written consent was obtained from all parents' patients and control groups before getting them involved in the study.
- The steps of the study, the aim, the potential benefits and hazards, all were discussed with the parents of the studied groups.
- Confidentially of all data were ensured.
- The patients and the control groups had the right to withdraw from the study at any

time without giving any reasons.

- No conflict of interest regarding the study or publication.

Disclosure of finance: No financial support for the study and puplication.

Sample size calculation:

The study included 95 consecutive children attending the pediatric outpatient clinic. Their age ranged from 3 to 6 years selected by sample random method. The sample size were calculated on MedCalc program version 11.3.0.0 and according to a previous study done by **Zimmermann et al. (2022)** who stated zinc protoporphyrin detecting iron deficiency anemia in 5–15-y-old children with area under curve of 0.718; adjusting the power of the test to 80.0%; confidence interval to 95% and margin of error accepted to 5%; the minimum sample needed for this study was found to be 54 patients.

Inclusion Criteria:

- Age 3-6 years.
- Both Male and female.

Exclusion Criteria:

- Patients with chronic illness as liver cell failure or renal failure.
- Patients with acute or chronic infectious or immunological diseases.
- Patients with cancer.
- Conditions that might affect serum ferritin (e.g. acute inflammation).
- Patients with other hematological diseases.

Study plan:

All participants were subjected to:

I. Full medical history taking with stress on present history, evident pallor, infection, bleeding disorder, mental status assessment, developmental and nutritional history.

II. Clinical examination including:

- General examination insisting on (pallor, any brittle nails or spooning of nails or a sore tongue).
- Vital signs (HR, R.R, tempreture, BL/ P).
- Anthropometric measurements plotted on Egyptian centile including weight and height measurements.
- Systemic examination, with a focus on detecting any organomegaly.

III. Standard laboratory assessment including:

- Complete blood count: done automatically by sysmex (kx-21N) automated hematological counter.
- Serum iron: done by Cobas c 311 auto analyzer using Roch reagent kits.
- TIBC: done by Cobas c 311 auto analyzer using Roch reagent kits.
- ZPP /H and serum ferritin: done by ELISA.

Statistical analysis:

Data obtained from the present study were statistically analyzed using SPSS (IBM. USA). Numerical data were expressed as mean and standard deviation or median and interquartile range (IQR) and compared using t test or Mann-Whitney U test as appropriate. Categorical data were presented as number and percent and compared using Fisher's exact test or chi-square test as appropriate. Binary logistic regression analysis was used to determine predictors of iron deficiency anemia. Receiver operator characteristic (ROC) curve analysis was used to identify diagnostic value of the investigated marker. P value less than 0.05 was considered statistically significant.

Lastly, our studied children were classified into two groups according to CBC finding and definition of anemia according to WHO.

Group A: Anemic, according to this criteria: Children aged 6 months to 6 years are considered anemic at Hb levels less than 11 g/dL, and children aged 6-14 years are considered anemic when Hb levels are less than 12 g/dL **(WHO, 2021)**.

Group B: Non-anemic.

RESULTS

Our results will be demonstrated in the following tables and figure:

Table (1): Comparison between anemic and non-anemic group regarding demographic data

demographics	Anemic n=25	Non-anemic n=70	p value	Sig.
Age (years)	5.05 ± 0.72	4.63 ± 0.92	0.062	NS
Female/male	13/12	36/34	0.961	NS
Weight (kg)	17.26 ± 2.49	18.43 ± 2.16	0.028	S
Height (cm)	100.84 ± 9.36	106.11 ± 8.34	0.010	S

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

The previous table shows that there was statistically significant difference between the anemic and non-anemic groups regarding weight with (P-value = 0.028), height with (P – value = 0.010).

Table (2): Comparison between anemic and non-anemic group regarding Laboratory investigation

Lab. investigations	**Anemic n=25**	**Non-anemic n=70**	**p-value**	**Sig.**
CBC				
MCV(Fl)	72.06 ± 6.27	76.74 ± 4.72	<0.001	HS
MCH(p.g)	22.69 ± 2.67	25.57 ± 1.84	<0.001	HS
RBCs($10^{6/}$µl)	3.73 ± 0.17	4.36 ± 0.39	<0.001	HS
MCHC(g/dl)	31.54 ± 1.10	32.09 ± 1.09	0.032	S
PCV%	31.56 ± 0.99	37.83 ± 3.29	<0.001	HS
RDW%	14.83 ± 0.95	13.32 ± 0.80	<0.001	HS
TLC ($10^{3/}$µl)	9.68 ± 1.42	9.98 ± 1.50	0.386	NS
PLTs ($10^{3/}$µl)	324.76 ± 66.64	322.07 ± 50.73	0.835	NS
S.Iron (mcg/dl)	41 (27 – 52)	57.5 (47 – 73)	<0.001	HS
TIBC (µg/dl)	318.48 ± 55.89	282.44 ± 69.56	0.022	S
S.Ferritin (ng/ml)	10.9 (9 − 15.1)	29.8 (11.7 − 48.4)	<0.000	HS
ZPP/H (µmol/mol)	97.8 (91.13 – 100.2)	51.05 (43.21 – 75)	<0.001	HS

P-value > 0.05: Non significant; P-value < 0.05: Significant; P-value < 0.01: Highly significant
•: Independent t-test; ≠: Mann-Whitney test.

This table shows that there were highly statistically significant difference between the anemic and non-anemic groups regarding MCV, MCH, RBCs, PCV, and S.iron with (P – value<0.001) decreasing in anemic group.

And shows highly statistically significant difference between the anemic and non-anemic groups regarding RDW and ZPP/ heme with (P – value< 0.001) and (P – value< 0.001) respectively increasing in anemic group.

And shows statistically significant difference between the anemic and non-anemic groups regarding MCHC and S.ferritin with (P- value = 0.032 and <0.0٠٠) respectively decreasing in anemic group and TIBC with (P-value = 0.022) increasing in anemic group.

Table (3): Correlation for ZPP/H with clinical and laboratory data in studied children

Demographics & lab. investigations	ZPP/heme	
	r	**P-value**
Age(year)	0.063	0.543
Weight (Kg)	0.014	0.892
Height (Cm)	0.018	0.861
CBC / Hb (gm/dl)	**-0.573****	**<0.00١**
MCV (Fl)	**-0.295****	**0.004**
MCH (p.g)	**-0.399****	**<0.00١**
MCHC (g/dl)	-0.197	0.055
RBCs (10^6/µl)	**-0.483****	**<0.00١**
PCV %	**-0.512****	**<0.00١**
RDW %	**0.439****	**<0.00١**
TLC (10^3/µl)	0.066	0.526
Platlets (10^3/µl)	0.118	0.257
S.Iron (mcg /dl)	**-0.288****	**0.005**
S.Ferritin (ng/ml)	**-0.٢٦٧***	**0.0٠٩**
TIBC (µg /dl)	**0.484****	**0.000**

P-value > 0.05: Non-significant; P-value < 0.05: Significant; P-value < 0.01: Highly significant

Spearman correlation coefficient

This table shows correlation between ZPP/H levels with different parameters in the studied children, there were negative correlation of ZPP/H with Hb, MCV, MCH, PCV, RBCs, S.iron and S.Ferritin.

And Positive significant correlation between ZPP/H and TIBC and RDW.

Table (4): Predictors of iron deficiency anemia in the studied children

Demographics & lab. investigations	Univariate				Multivariate			
	P-value	Odds ratio (OR)	95% C.I. for OR		P-value	Odds ratio (OR)	95% C.I. for OR	
			Lower	Upper			Lower	Upper
Age >4.9	0.006	4.095	1.511	11.098	–	–	–	–
Weight <= 15.7	0.002	6.000	1.962	18.353	–	–	–	–
Height <= 102	0.001	5.700	2.015	16.125	–	–	–	–
Low Socio-economic sat	0.003	4.444	1.689	11.694	–	–	–	–
RBCs ≤ 3.8	0.000	89.333	19.617	406.812	–	–	–	–
MCV ≤ 73.4	0.001	5.542	2.075	14.805	–	–	–	–
MCH≤ 23.3	0.000	13.500	4.413	41.294	–	–	–	–
MCHC≤ 31.7	0.010	3.596	1.363	9.488	–	–	–	–
PCV≤ 33.1	0.000	396.000	42.134	3721.825	0.000	352.000	37.216	33320.387
RDW>14.5	0.000	107.667	20.083	577.218	–	–	–	–
S.Iron≤ 53	0.001	7.422	2.303	23.921	–	–	–	–
S.Ferritin<30.9	0.004	4.472	1.612	12.407	_	_	_	_
TIBC>309	0.000	6.625	2.432	18.050	–	–	–	–
ZPP/H>78.17	0.000	42.167	8.918	199.385	–	–	–	–

This table shows univariate logistic regression analysis for factors associated with anemia showed significant association between anemia and age, weight, height, low socio-economic status, CBC parameters, S.iron, S.ferritin. TIBC and ZPP/H.

Table (5): Validity of CBC indices and iron profile in diagnosis of iron deficiency anemia:

Variable	Cut off point	AUC	Sensitivity	Specificity	+PV	-PV
RBCs(10^6/µL)	≤ 3.8	0.952	80.00	95.71	87.0	93.1
MCV(FL)	≤ 73.4	0.709	64.00	75.71	48.5	85.5
MCH(p.g)	≤ 23.3	0.802	60.00	90.00	68.2	86.3
MCHC(g/dl)	≤ 31.7	0.660	68.00	62.86	39.5	84.6
PCV%	≤ 33.1	0.975	96.00	94.29	85.7	98.5
RDW%	>14.5	0.900	76.00	97.14	90.5	91.9
S.Iron(mcg/dl)	≤ 53	0.773	84.00	58.57	42.0	91.1
S.Ferritin(ng/ml)	≤ 29	0.783	100.00	52.86	43.1	100.0
TIBC(µg/dl)	>309	0.702	68.00	75.71	50.0	86.9
ZPP/H(µmol/mol)	>78.17	0.819	92.00	78.57	60.5	96.5

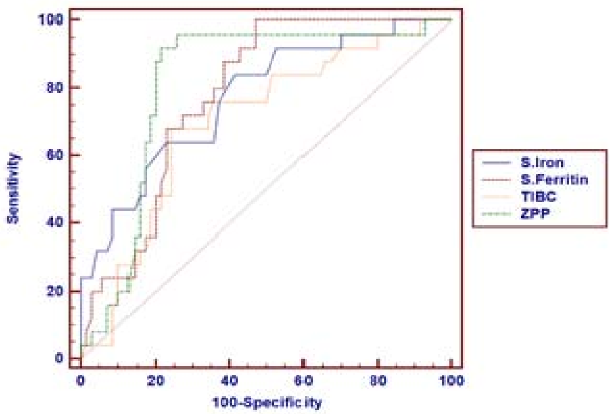
ROC curves analysis and **table (5)** show that the most sensitive test with high specificity to diagnose IDA were ZPP/H, serum ferritin and some parameters of CBC.

Cut off point of ZPP/H ratio to detect IDA (>78.17), with sensitivity of (92%) and Specificity of (78.57%).

PPV of 60.5%, NPV of 96.5% and total accuracy of 81.9%.

Cut off point of S.ferritin to detect IDA (<29), with sensitivity of (100%) and Specificity of (52.86%).

PPV of 43.1%, NPV of 100% and total accuracy of 0.783%.



Figure(1): ROC Curve of iron profile and ZPP/H

ROC curve analysis shows that:

Cut off point of ZPP/H ratio to detect IDA (>78.17), with sensitivity of (92%) and Specificity of (78.57%).

PPV of 60.5%, NPV of 96.5% and total accuracy of 81.9%.

DISCUSSION

Iron deficiency anemia (IDA) is the leading cause of anemia worldwide **(WHO, 2018)** with well established guidelines for diagnosis and treatment **(WHO, 2011**; **McLean at al., 2009)**.

Typically, the diagnosis of IDA is made when the plasma hemoglobin (Hb) falls below normal (<11.0g/dL in children) and the serum ferritin is <12 µg/L **(McLean et al., 1993)**. Unfortunately, the frequent coexistence of inflammation/infection confounds serum ferritin, which is an acute phase protein, mandating the performance of additional tests e.g., C-reactive protein (CRP) and serum transferrin receptor (sTfR). As a result, the diagnosis of IDA often requires a battery of diagnostic tests, trained technicians, and the use of expensive laboratory equipment, which increases costs and delays results. Clearly, developing biomarkers that quickly, easily and reliably detect IDA would be beneficial **(Kanuri et al., 2018)**.

The main aim of this study was to assess the role of ZPP/H in early diagnosis of IDA in a group of Egyptian preschool children. The present study showed that ZPP/H levels were significantly higher in anemic group compared to non-anemic group. These results are supported by Kanuri et al., 2018. In their work, children with IDA had significantly higher ZPP/H when compared with their non-anemic counterparts.

In the present study, there was a negative correlation between ZPP/H and Hb also with MCV, MCH, S.Iron and S.Ferritin and there was a positive correlation with TIBC. In line with these findings, the study by Yu et al., 2011 supported our results as they revealed that ZPP/H was significantly negatively correlated with hemoglobin and positively correlated with TIBC. However, the study by **Yu et al., 2011** reported that ZPP/H ratio was significantly negatively correlated with age and Hemoglobin, but non-significantly correlated with Transferrin saturation and Serum ferritin. In addition, **Magge et al., 2013** reported that iron prescription was significantly associated with ZPP/H reduction. Furthermore, the study by **Teshome et al., 2017** reported that erythrocyte ZPP was reduced in children aged 24– 36 months and, seemed higher in boys than in girls, Whole blood ZPP was independently associated with decreased concentrations of haemoglobin and ferritin and with increased plasma concentrations of transferrin receptor and C-reactive protein.

In our work, ROC curve analysis recognized ZPP/H as a reliable diagnostic marker for anemia (AUC=0.819) with sensitivity and specificity of 92.00 and 78.57 respectively. Similar conclusions were reported by the studies of **Kanuri et al., 2018**, **Teshome et al., 2017** and **Homann et al., 2019**.

In conclusion, ZPP/H can be considered as a reliable marker for diagnosis of anemia in preschool children. It's well-correlated with other markers of anemia.

CONCLUSION

- Our study revealed that (26.3%) of studied children were anemic and (70.3%) were non-anemic based on Hb levels>11(g/dl) and serum ferritin >12 (ng/ml).
- ZPP/H significantly increased in anemic group (p value = 0.001), with a cut of point >78.1.
- ZPP/H has a positive correlation with TIBC and RDW increasing in anemic children, and a negative correlation with Hb, MCV, MCH, RBCs, PCV, serum iron and serum ferritin as they are decreasing in anemic children.
- ZPP/H is good discriminator of anemia in terms of area under ROC curve analysis.

RECOMMENDATIONS

In light of the previous results, the current research recommends the following:

- Further studies on large geographical scale and on larger sample size to emphasize our conclusion.
- National health education program to encourage healthy feeding habits and awareness of iron rich food, iron enhancers and iron inhibitors.
- Iron supply of infants 6-23 months (10-12.5) mg /day for three successive months in areas where IDA is highly prevalent **(WHO, 2016)**.
- Early diagnosis and treatment of iron deficiency anemia is essential to prevent the squeal of iron deficiency anemia.

LIMITATION

Limitations of this study include the following:

- Our study was done on a small sample size and small geographical scale.
- Expensive zinc protoporphyrin kits as total price was 5800E.P for 96 cases so each case costed 61 E.P.

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