Evaluation of Reticulocyte Hemoglobin Content and Serum Neutrophil Gelatinase-Associated Lipocalin as Predictive Biomarkers of Anemia in Children on Hemodialysis

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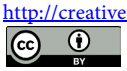

Abstract

Background: Diagnosis of iron deficiency anemia with currently available tests is rendered difficult in hemodialysis patients. **The aim:** To investigate the role of reticulocyte Hemoglobin Content (CHr) in the diagnosis of iron deficiency anemia in hemodialysis children in comparison to the used traditional markers and assess the impressiveness and the utility of Neutrophil Gelatinase Associated Lipocalin (NGAL) as a novel biomarker of iron status in those patients. **Methods:** This study investigated CHr in addition to NGAL serum level in the same line with traditional markers for anemia, including: CBC, serum iron, ferritin, total iron-binding capacity (TIBC), and transferrin saturation (TSAT%). **Results:** It is more significant that CHr content in hemodialysis children is lower than their controls as they are (27.06 ± 2.90) pg and (32.86 ± 3.59) pg, respectively, $p = 0.01$. There is no significant difference regarding NGAL between the study groups. Significant negative correlation between CHr with ferritin, urea, creatinine, and positively correlated with iron and RBCS. CHr showed a sensitivity of 90% and specificity of 86.67% to detect iron-deficiency anemia with a cut-off value of 27 pg. **Conclusion:** CHr is superior to ferritin and TSAT % for the early diagnosis of iron deficiency anemia in hemodialysis children; our results do not support NGAL as a marker of anemia in hemodialysis patients.

Keywords

Children, Hemodialysis, Reticulocyte Hemoglobin Content, Neutrophil Gelatinase-Associated Lipocalin

1. Introduction

Anemia is a common complication of advanced chronic kidney disease (CKD), with a prevalence exceeding 87% in children with CKD stages 4 and 5 (Hayes, 2019) [1].

Young patients with chronic kidney disease CKD, especially those on hemodialysis (HD), experience marked alterations in iron balance and tissue distribution because of reduced iron absorption, increased iron losses, and impaired mobilization of iron from stores (Wish *et al.*, 2018) [2].

Iron deficiency is common in patients on chronic hemodialysis, and most require iron-replacement therapy. In addition to absolute iron deficiency, many patients have functional iron deficiency as shown by a suboptimal response to the use of erythropoietin stimulating agents (ESAs) (Pandey and Daloul, 2016) [3].

The traditional biomarkers used for the diagnosis of iron-deficiency anemia (IDA) in patients with CKD have limitations, leading to persistent challenges in the detection and monitoring of IDA in these patients (Batchelor and Kapitsinou, 2020) [4].

The reticulocytes last only 24 to 48 h in the circulation before developing into mature red blood cells. During the initial stage of iron deficiency, insufficient iron supply would cause a decline of hemoglobin production in reticulocytes in the bone marrow, which can be detected through CHr (Dinh and Cheanh, 2020) [5].

Soluble transferrin receptor, serum iron, serum ferritin, and transferrin saturation are frequently affected by inflammation, chronic diseases, and in the normal aging process (except soluble transferrin receptor) (Gelaw and Melku, 2019) [6].

CHr has a high specificity for not being affected by inflammation, and also exhibits a low coefficient of variation. Compared with the traumatic bone marrow biopsy used for the evaluation of iron status, CHr is relatively cheap, convenient, and less invasive, because only several milliliters of peripheral blood are needed to get CHr data (Cai *et al.*, 2017) [7].

Neutrophil gelatinase-associated lipocalin (NGAL) also known as lipocalin 2, siderocalin or 24p3) is a 25 kDa glycosylated protein from the lipocalin family (Karur and Batra, 2012) [8].

NGAL is produced and secreted by kidney tubule cells at low levels, but the amount produced and secreted into the urine and serum increases dramatically after ischemic, septic, or nephrotoxic injury of the kidneys (Tasanarong and Hutayanon, 2013) [9].

NGAL was recently proposed as a portentous early predictive biomarker for kidney injury (Rysz *et al.*, 2017) [10].

NGAL was initially characterized as an antibacterial immune factor via the pocket's ability to capture siderophores (such as bacterial enterochelin and mammalian endogenous catechols) that bind iron with high affinity, causing

iron depletion and thus the inhibition of bacterial cell growth (Xiao and Yeoh, 2017) [11].

We aimed to investigate the role of CHr in the diagnosis of iron deficiency anemia in hemodialysis children in comparison to the used traditional markers and to assess the impressiveness and the utility of NGAL as a novel biomarker of iron status in children with CKD on regular hemodialysis.

2. Material and Methods

This case-control study was carried out on 60 children, 35 males and 25 females, aged from 6 to 16 years, selected from those attending the nephrology, hemodialysis unit, and the outpatient clinic of Al-Zahraa Hospital, Al-Azhar University. Informed consent was obtained from the participating parents in adherence to the ethical committee guidelines of Alzhraa hospital, AL-Azhar University, Cairo, Egypt. This study was conducted with the (nephrology and hemodialysis) unit, pediatric department, hematology unit, clinical pathology department, Alzhraa hospital, AL-Azhar University.

Children included in the study were divided into the following two groups:

Group I: patients group; 30 children (19 males and 11 females) with end-stage renal disease on regular hemodialysis who fulfill the criteria for definition and classification of CKD (KDIGO, 2020) [12] and attended the pediatric hemodialysis unit during the period of the study; they were on regular hemodialysis for more than three months at the time of the study, for 4 hours/setting, three times weekly, with low flux polysulphone dialyzer by 4008 Fresenius machine. They were subjected to whole history taking, including etiology, the onset of CKD, duration of hemodialysis, and laboratory investigations. The most common cause of chronic kidney disease was congenital (43.3%), followed by acquired (23.3%), hereditary causes (16.7%), and unknown (16.7%).

The patient's group was divided into anemic and nonanemic groups according to the hemoglobin level < 110 g/L (KDIGO, 2012; Cai *et al.*, 2017) [7] [13].

The anemic patient's group was subdivided into two groups: absolute iron deficiency is defined when the transferrin saturation (TSAT) is ≤20% and the serum ferritin concentration is ≤100 ng/mL among predialysis and peritoneal dialysis patients or ≤200 ng/mL among hemodialysis patients. Functional iron deficiency is characterized by TSAT ≤ 20% and elevated ferritin levels ≥ 200 (Gafter-Gvili *et al.*, 2019) [14]. Group II: controls group, 30 healthy children, matched age and sex with patients group. Children with hemoglobinopathies, hemolytic anemia, other acute or chronic diseases, and recent blood transfusion within the last three months were excluded from the study.

2.1. Sample Collection

Five ml of peripheral venous blood were withdrawn under complete aseptic condition from each subject. The first two ml were evacuated in an EDTA-containing tube for C.B.C. and measurement of reticulocyte hemoglobin content. The re-

maining three ml were evacuated in a plain tube and left to clot for 20 minutes at room temperature before centrifugation at 3000 pm. The serum was separated and divided into two parts; the first part was used for measurement of serum urea, creatinine, iron, total iron-binding capacity (TIBC), ferritin level, and C-reactive protein (C.R.P.), while the remaining part of the serum was frozen at -20C until the serum NGAL level analysis.

The complete blood count (C.B.C.) using the Cell dyne Ruby cell counter, Abbott (Germany). The reticulocyte hemoglobin content was measured using Sysmex XN 1000, Kobe, Japan. Serum urea, creatinine, iron, and total iron-binding capacity (TIBC) were measured using Cobas C311 and kits of Roche (Germany), followed by calculation of transferrin saturation as follows: transferrin saturation (%) = (serum iron/serum TIBC) × 100. Serum ferritin level was measured using Cobas E411 and kits of Roche (Germany), C-reactive protein (C.R.P.) level were measured using turbidimetric method (BioSystems, lot 19420). According to the manufacturer's instructions, the concentration of NGAL was measured by ELISA using a quantitative double-antibody sandwich ELISA kit (Bioassay Technology Laboratory, China, Cat. No. E1719Hu). Its level was expressed as ng/ml.

2.2. Statistical Analysis

Data were collected, revised, coded, and entered the Statistical Package for the Social Science version 20 (I.B.M. Corp., Armonk, NY, U.S.A.). Spearman correlation coefficients were used to assess the correlation between two studied parameters in the same group. The Receiver Operating Characteristic (R.O.C.) curve assessed the best cutoff point with sensitivity and specificity. Interpretation of probability values was as follows: $p > 0.05$: non-significant; $p < 0.05$: significant.

3. Results

Table 1 shows a comparison between dialysis children and healthy controls regarding demographic data, anthropometric measurements, blood pressure, and laboratory data; it revealed: a significant decrease in weight and height z score in dialysis children compared to their controls. The patient's group had high blood pressure as expected. There is a significant decrease in WBC, R.B.C.s, Hb, and platelets in dialysis children; meanwhile, there is a significant increase in serum urea, creatinine, and C.R.P. in the dialysis group than in their controls.

Table 2 shows a comparison between dialysis children and healthy controls regarding iron status parameters, CHr and NGAL. It revealed a significant decrease in the serum iron, TIBC, and CHr meanwhile, there is a significant increase in the serum ferritin and TSAT% in the dialysis group compared to their controls; meanwhile there is no significant difference regarding NGAL serum level among the study groups.

Table 3 shows a comparison between functional iron deficiency (FID) and iron deficiency anemia (I.D.A.) regarding laboratory data among dialysis patients. It shows a significant decrease in R.B.C., Hb, Hct%, creat, C.R.P., CHr, iron, and fer-

ritin, while there is a significant increase in TSAT% in the iron-deficiency anemia (I.D.A.) compared to functional iron deficiency (FID) group.

Table 4 shows a correlation between ferritin & TSAT% and CHr with the study clinical and laboratory data shows a significant negative correlation between ferritin

Table 1. Comparison between the patient's group and the controls regarding age, anthropometric measurements, and laboratory data.

Variables	Group I Cases group (No = 30)	Group II Control group (No = 30)	Test value	P-value
Age (yrs)	12.05 ± 3.17	12.05 ± 3.17	0.000[a]	1.000
Z score: Weight (kg)	−0.69 (−1.04 - 0.26)	0.42 (−0.06 - 0.74)	3.013[c]	0.003
Z score: Height (cm)	−0.33 (−0.78 - 0.06)	0.20 (−0.36 - 1.10)	2.813[c]	0.005
Z score: BMI	−0.36 (−0.89 - 0.28)	0.54 (−0.44 - 1.29)	2.063[c]	0.039
SBP (mmHg)	127.0 ± 22.0	109.0 ± 8.03	4.210[b]	**0.001**
DBP (mmHg)	84.67 ± 16.55	73.0 ± 5.35	3.673[b]	**0.001**
WBCs ($\times 10^3/mm^3$)	6.19 ± 1.81	7.65 ± 1.26	−3.645[b]	**0.001**
RBCs ($10^6/mm^3$)	3.76 ± 0.77	4.47 ± 0.26	−4.804[b]	**0.001**
Hb (g/dl)	10.14 ± 2.01	11.98 ± 0.49	−4.843[b]	**0.001**
Hct (%)	32.50 ± 6.76	39.42 ± 1.60	−5.451[b]	**0.001**
MCV (fL)	84.60 ± 11.20	85.90 ± 5.31	−0.576[b]	0.567
Platelets ($\times 10^3/mm^3$)	204.27 ± 62.89	248.17 ± 52.74	−2.930[b]	**0.005**
Urea (mg/dl)	161.00 ± 51.70	25.33 ± 6.47	14.262[b]	**0.001**
Creat (mg/dl)	8.02 ± 2.43	0.44 ± 0.14	17.064[b]	**0.001**
CRP (mg/l)	16 (13 - 19)	3 (2 - 4)	−6.663[c]	**0.001**

P-value < 0.05: significant; [a]Chi-square test; [b]Independent t-test; [c]Mann-Whitney test.

Table 2. Comparison between the patient's group and the controls regarding iron status parameters, CHr levels, and serum NGAL.

Variable	Group I Cases group (No = 30)	Group II Control group (No = 30)	Test value	P-value
Iron (µg/dl)	92 (40 - 160)	105.5 (91 - 113)	−0.769[b]	0.026
Ferritin (µg/l)	1634 (400 - 2000)	145 (139 - 156)	4.411[b]	0.001
TIBC (µg /dl)	207 (171 - 261)	320 (308 - 367)	−4.882[b]	0.001
TSAT %	43.1 (18.81 - 70.36)	30.46(28.81 - 37.58)	−2.099[b]	0.036
CHr (pg)	27.06 ± 2.90	32.86 ± 3.59	−6.890[a]	0.001
NGAL (ng/ml)	2.73 (1.61 - 4.6)	3.22 (1.09 - 18.5)	−0.076[b]	0.829

P-value < 0.05: significant; [a]Independent t-test; [b]Mann-Whitney test.

Table 3. Comparison between functional iron deficiency (FID) and iron deficiency anemia regarding laboratory data.

	FDA (No = 18)	IDA (No = 6)	Test value	P-value
WBCs ($\times 10^3/mm^3$)	6.14 ± 1.76	5.33 ± 1.42	1.009[a]	0.324
RBCs ($10^6/mm^3$)	4.53 ± 0.80	3.40 ± 0.49	−4.178[a]	0.01
Hb (g/dl)	11.50 ± 1.84	9.08 ± 1.22	−3.700[a]	0.001
Hct (%)	36.18 ± 8.15	29.74 ± 4.65	−2.421[a]	0.024
MCV (fL)	90.02 ± 5.48	83.43 ± 5.11	−2.688[a]	0.013
PLAT ($\times 10^3/mm^3$)	225.50 ± 111.21	191.17 ± 31.99	−1.214[a]	0.238
Urea (mg/dl)	178.44 ± 56.01	136.17 ± 36.15	1.719[a]	0.100
Creat (mg/dl)	8.71 ± 2.34	6.03 ± 0.69	2.722[a]	0.012
CRP (mg/l)	17 (16 - 21)	13 (11 - 13)	−2.519[b]	0.012
Iron (µg/dl)	151 (108 - 182)	72 (38 - 132)	−1.901[b]	0.057
Ferritin (µg/l)	2000 (1240.4 - 2000)	108.2 (105 - 109)	−3.848[b]	0.000
TIBC (µg/dl)	227 (180 - 318)	204.5 (138 - 207)	−1.135[b]	0.256
TSAT %	19.17 (16.67 - 51.28)	68.5 (57.64 - 87.92)	−2.733[b]	0.006
CHr (pg)	27.1 (27.0 - 27.2)	25.5 (22.5.1 - 26.6.)	−2.922[b]	0.003
NGAL (ng/ml)	2.65 (1.61 - 4.03)	2.43 (1.52 - 9.60)	0.367[b]	0.714

[a]Independent t-test; [b]Mann-Whitney test.

Table 4. Correlation of ferritin, TSAT%, and CHr with laboratory data.

Variable	Ferritin (ug/l)		TSAT %		CHr (pg)	
	r	P-value	r	P-value	r	P-value
Ferritin (µg/l)	–	–	−0.636**	0.001	−0.603**	0.001
TSAT %	−0.636**	0.001	–	–	−0.621**	0.001
CHr (pg)	−0.603**	0.001	−0.621**	0.001	–	–
NGAL (ng/ml)	0.110	0.564	0.054	0.777	0.031	0.869
WBCs ($\times 10^3/mm^3$)	−0.105	0.582	0.101	0.594	0.266	0.156
RBCs ($10^6/mm^3$)	−0.482**	0.007	0.556**	0.001	0.411*	0.024
Hb (g/dl)	−0.829**	0.001	0.574**	0.001	0.476**	0.008
Hct (%)	−0.629**	0.001	0.634**	0.001	0.514**	0.004
MCV (fL)	−0.507**	0.004	0.425*	0.019	0.388*	0.034
Platelets ($\times 10^3/mm^3$)	−0.026	0.891	0.344	0.063	0.306	0.100
Urea (mg/dl)	0.546**	0.002	−0.608**	0.001	−0.451**	0.012
Creat (mg/dl)	0.706**	0.001	−0.673**	0.001	−0.472**	0.009
Iron (µg/dl)	−0.581**	0.001	0.850**	0.001	0.505**	0.004
TIBC (µg/dl)	0.192	0.309	−0.357	0.053	−0.352	0.056
CRP (mg/l)	0.740**	0.001	−0.638**	0.001	−0.503**	0.005

with TSAT %**,** CHr, RBCS, Hb, and its indices with significant positive correlation to urea, creat, and CRP. Meanwhile, CHr had a significant positive correlation with RBCs, Hb, and indices but negatively correlated with urea, creatinine, and CRP. The same table shows a significant positive correlation between TSAT% with RBCs, Hb, and its indices but shows a significant negative correlation with urea, creatinine, and CRP.

Table 5 and Figure 1 demonstrate the specificity and sensitivity, positive and negative predictive values of the CHr, ferritin, and TSAT% levels to detect iron deficiency anemia in the patient's group. The diagnostic performance of CHr, 90% sensitivity, and specificity 86.67% at cut off < 27.2 pg, meanwhile the diagnostic performance of ferritin and TSAT % was 80.0%, sensitivity,96.67 specificities, 56.6%; sensitivity, and 776.67% specificity, respectively.

Figure 2 demonstrates that children with anemia are 80.00% of the study patients on regular hemodialysis.

Figure 3 demonstrates that children with functional iron deficiency anemia are 75.00%, and iron deficiency anemia is 25.00% of the study patients group.

Figure 4 demonstrates a significant negative correlation between ferritin and Hb level, respectively.

Figure 5 demonstrates a significant negative correlation between ferritin and

Table 5. The sensitivity, specificity positive and negative predictive values of ferritin, CHr and TSAT% for the diagnosis of iron deficiency anemia in hemodialysis children

Variables	Cut off point	AUC	Sensitivity/%	Specificity/%	+PV	−PV
Ferritin (µg/l)	>200	0.829	80.0	96.67	96.6	82.9
CHr (pg)	≤27.2	0.844	90.00	86.67	87.1	89.7
TSAT %	>37.58	0.591	56.67	76.67	70.8	63.9

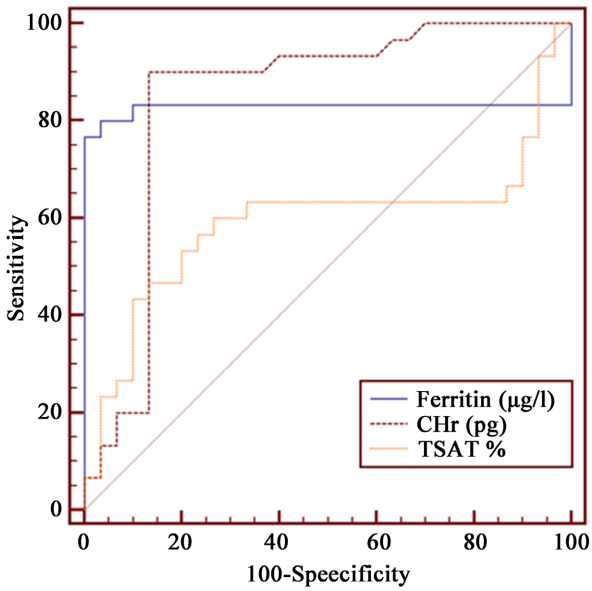

Figure 1. Specificity and sensitivity CHr, ferritin and TSAT%.

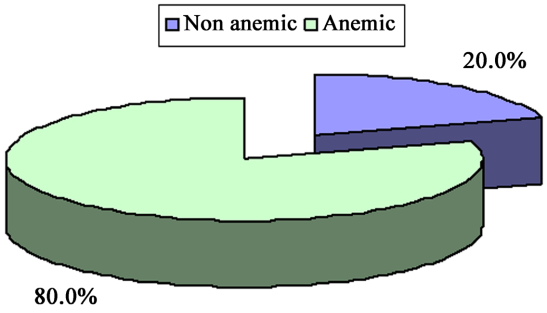

Figure 2. Frequency of anemia in patients group.

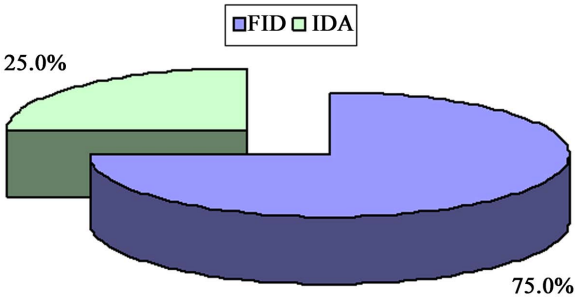

Figure 3. Frequency of FID and IDA in patients group.

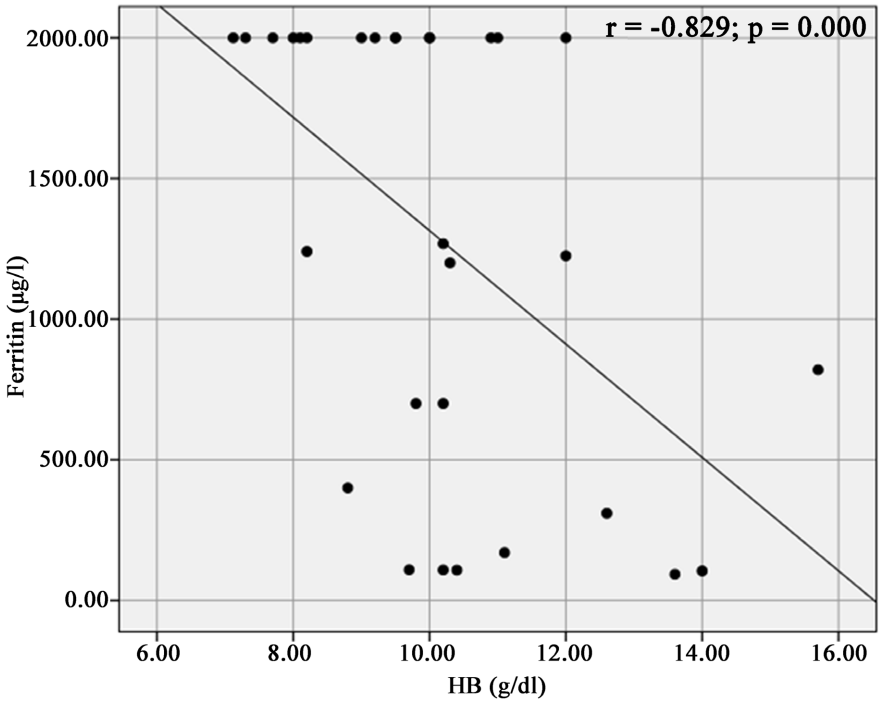

Figure 4. Negative correlation between ferritin and Hb level.

CHr level.

Figure 6 demonstrates a significant negative correlation between ferritin and iron level.

Figure 7 demonstrates a significant positive correlation between ferritin and CRP level.

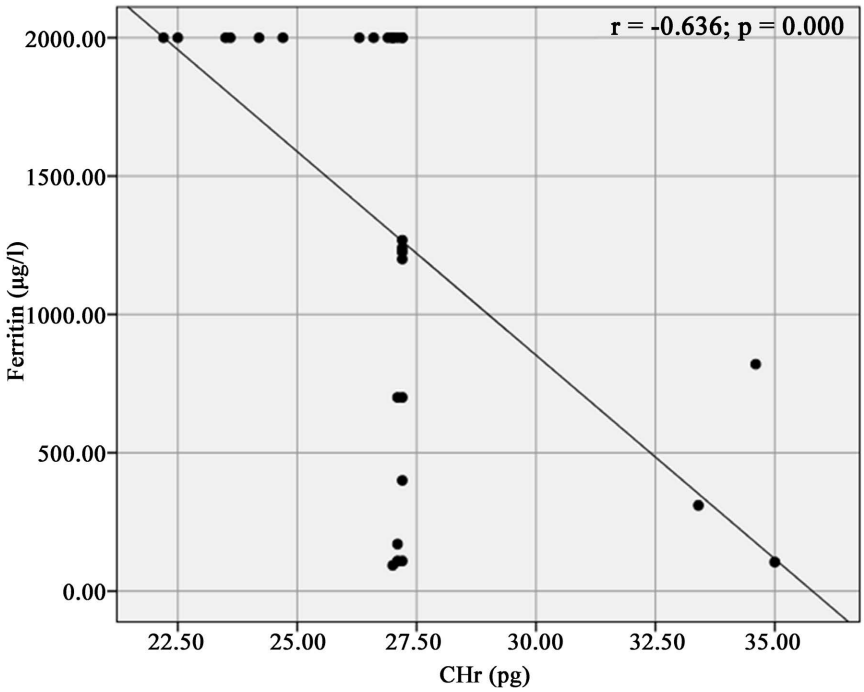

Figure 5. Negative correlation between ferritin and CHr.

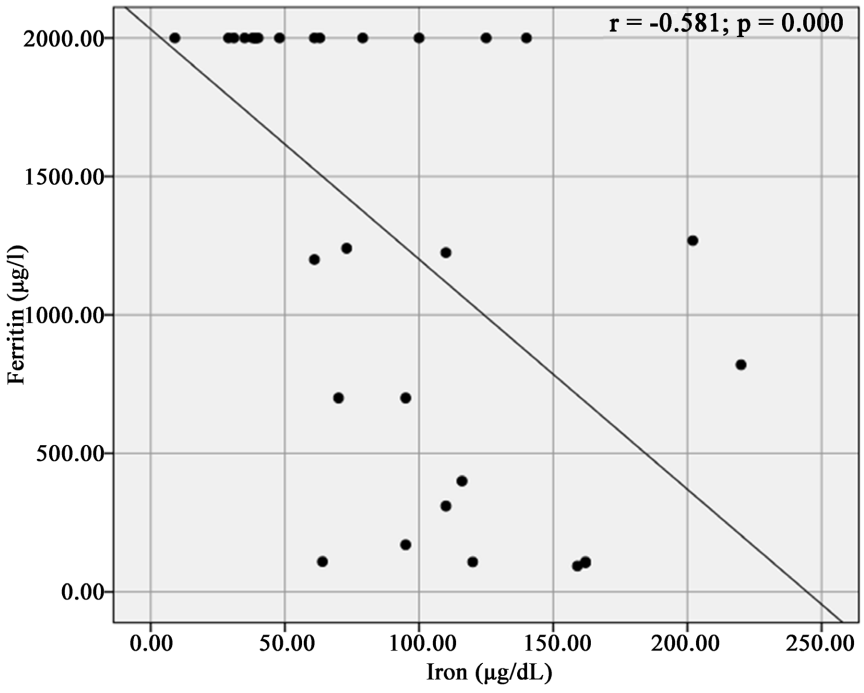

Figure 6. Negative correlation between ferritin and iron.

Figure 8 demonstrates a significant positive correlation between CHr and iron level.

4. Discussion

It is essential to identify the status of anemia and iron deficiency and correct it

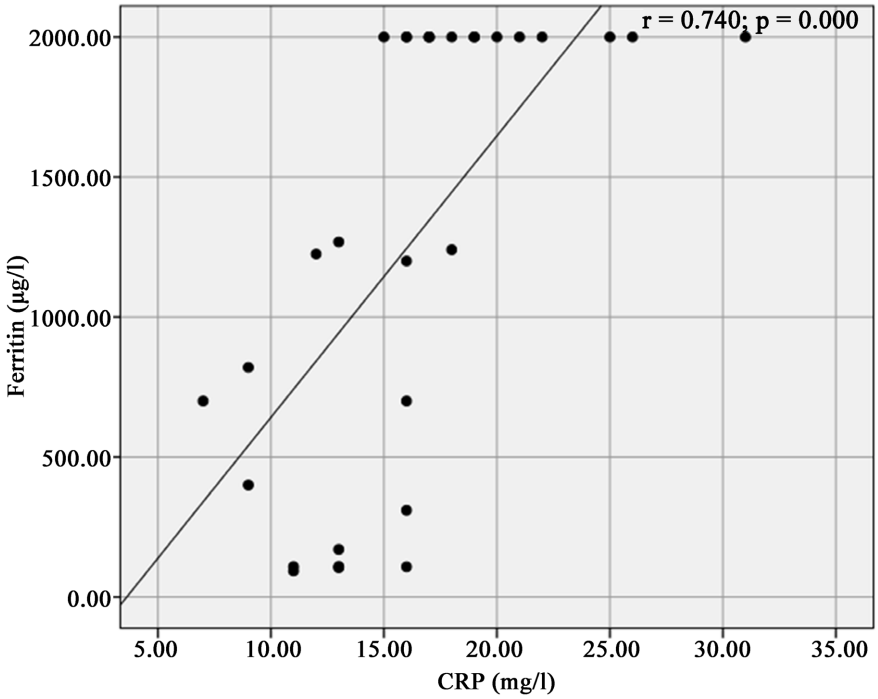

Figure 7. Positive correlation between ferritin and CRP level.

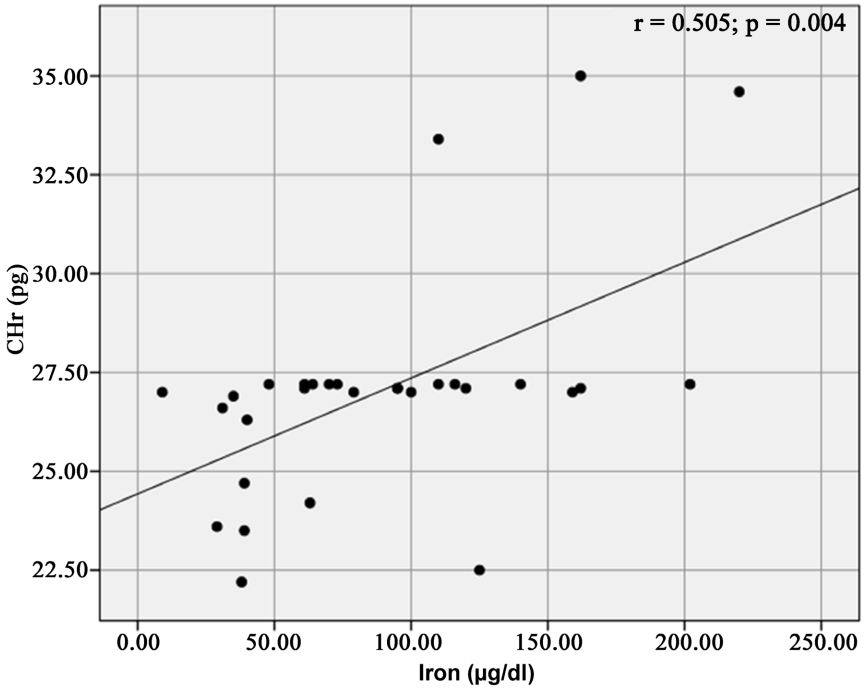

Figure 8. Positive correlation between CHr and iron.

actively before correcting anemia to avoid unnecessary supplementation in hemodialysis children (Davidkova *et al.*, 2016) [15].

The current study aimed to assess the CHr as a predictor of iron deficiency anemia in hemodialysis children and assess NGAL as a predictor of iron status in that group in addition to the traditional makers, iron, ferritin, TSAT% and

TIBC. In the current study, we observed the high prevalence of anemia in the study patients group in 80% of the study cases, and cases with iron and functional anemia were 75% and 25%, respectively. Anemia is common in CKD, particularly in the hemodialysis patients (Dalimunthe *et al.*, 2016) [16], other studies estimation of anemia was 15% in US CKD patients, 45% - 55% in Asian CKD patients, and 50% - 90% in African CKD patients (Stauffer and Fan, 2014; Ryu *et al.*, 2017; Maina *et al.*, 2016; Amoako *et al.*, 2014; Ijoma *et al.*, 2010) [17] [18] [19] [20] [21].

We found high ferritin, TSAT%, and low iron in the hemodialysis group; meanwhile, there is a significant low CHr content in the hemodialysis group despite the high serum ferritin. CHr estimate the Hb content of red blood cells rather than the amount of storage iron, providing a snapshot of recent iron availability for Hb synthesis; in addition, it acts as a sensitive indicator of functional iron deficiency and is possibly better than TSAT% and ferritin. CHr help in predicting whether or not there will be a response to iron administration despite serum ferritin and the TSAT% which are usually used (Yilmaz *et al.*, 2011; Shani and Elise, 2013) [22] [23].

The hemoglobin content of the reticulocytes measures iron supply to erythropoiesis and thus the quality of the cells (Hönemann *et al.*, 2021) [24].

Ferritin can even be increased in the presence of iron deficiency due to the impact of underlying inflammatory conditions, which result from several factors such as uremia, infections (Bahrainwala and Berns, 2016; Buttarello *et al.*, 2016; Mehta *et al.*, 2016) [25] [26] [27], making its interpretation difficult in patients with CKD (Atkinson and Warady, 2018; Davidkova *et al.*, 2016; Gaweda, 2017) [15] [28] [29].

TSAT% are considered good indicators for iron deficiency. On the other hand, it can be influenced by the circadian variability of serum iron. The measurement of soluble transferrin receptor levels is part of a more detailed workup of complicated cases of anemia (Hönemann *et al.*, 2021) [24].

In the current study, findings agree with a study done in Vietnam by Dinh and Cheanh [5] which reported that CHr significantly decreased in children on hemodialysis compared with the control group, CHr significantly decreased in the IDA group and some of the FID group but not significant in all patients of FID. CHr is a good indication of iron availability of iron-deficient erythropoiesis (Gelaw *et al.*, 2019; Gopesh and Modi, 2017) [6] [30]. Also, our result agrees with Suari and Ariawati [31] who reported similar finding.

Also similar finding was reordered by Karagülle and Gündüz (2013); Abdul Gafor and Subramaniam (2018) and Cai *et al.* (2017) [7] [32] [33].

By analyzing the current study results, we found a strong positive correlation between CHr level and RBCs, Hb, Hct%, MCV, and serum iron (Kariyawasan and Samarasekara, 2020) [34] reported similar findings. In addition, a significant negative correlation between CHr level and inflammatory markers, including serum ferritin and CRP, in addition to the uremic toxins, in contrast, serum ferritin had a strong negative correlation with RBCs, Hb, Hct%, MCV, but ferritin

is positively correlated with uremic toxins and CRP that augment the role of CHr as a strong predictor of iron deficiency in hemodialysis patients.

Our study agrees with (Kariyawasan and Samarasekara, 2020) [34], who reported a positive correlation between CHr and serum iron and TSAT% level and hematological test that suggests CHr is the strong predictor in the diagnosis of iron deficiency anemia (IDA). TSAT% is not measured but derived from serum iron and total iron-binding capacity (TIBC) measurements. TIBC is a negative acute-phase reactant; that is, its plasma concentration is suppressed by inflammation. In the context of systemic inflammation, reductions in TIBC lead to higher levels of TSAT% independent of patients' iron status. Therefore, inflammation is implicated in the poor reliability of TSAT% as a measure of iron status in CKD (Hayes, 2019) [1].

Hematocrit, serum ferritin, and unsaturated iron-binding capacity were significantly affected by inflammation, while reticulocyte hemoglobin content and other parameters were not (Cai *et al.*, 2017) [7]. These findings explained that CHr is the strongest predictor for the diagnosis of IDA.

In our study, the diagnostic performance of CHr is 90% sensitivity and specificity 86.67% at cut off < 27.2 pg for diagnosis of IDA of CKD on hemodialysis was comparable to ferritin and TSAT % their diagnostic performance is 80%, 50% sensitivity, and 96%, 70% specificity respectively, so CHr is the most robust marker for diagnosis of IDA in CKD on HD. Similar results were found by (Cai *et al.*, 2017; Dignass *et al.*, 2018; Kariyawasan and Samarasekara, 2020 and Suari and Ariawati, 2015) [7] [31] [34] [35]. Other studies recorded that the cutoff point of CHr to diagnose absolute iron deficiency anemia in hemodialysis patients ranges from 27.2 pg to 33 pg (Davidkova *et al.*, 2016; Dalimunthe and Lubis, 2016; Buttarello *et al.*, 2010; Ogawa *et al.*, 2020) [15] [16] [26] [36]. The use of different CHr cut-off values in different studies for discriminating iron deficiency anemia in both non-dialysis and dialysis populations has led to variations in the specificities and sensitivities as a marker of IDA.

There was no significant difference between the study cases and their controls regarding NGAL in the current study. In addition, there was no significant correlation with the study parameters that include hematological parameters, which suggests NGAL has no role in the diagnosis of IDA on the patient of CKD on regular hemodialysis. Our result, in agreement with Kim *et al.* (2018) [37], suggests that NGAL confirms acute kidney injury diagnosis but does not diagnose IDA in CKD on regular hemodialysis.

Our result disagrees with (Ismail *et al.*, 2015) [38] that reported NGAL has a significant correlation with iron status and has a significant role in diagnosing IDA in CKD on hemodialysis.

5. Conclusion

Reticulocyte Hb content level is an early predictor of iron deficiency anemia in children on regular hemodialysis and is possibly better than TSAT% and ferritin

in predicting the response to iron administration. Further studies on many children are required to confirm and determine the diagnostic implications of CHr and its relation to iron deficiency anemic patients on regular hemodialysis. Indeed from the current study results, NGAL seems unreliable in diagnosing iron deficiency anemia in CKD children on regular hemodialysis.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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