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ORIGINAL ARTICLE

Role of Reticulocyte Hemoglobin Content in Prediction of Iron Deficiency Anemia in Pediatric Congenital Heart Diseases at Zagazig University Hospitals

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ABSTRACT

Background: Iron deficiency anemia is common in congenital heart disease (CHD) children. The recent development of automated analyzers has made it possible to measure reticulocyte hemoglobin content (RET-He), which is thought to reflect iron content in reticulocytes. this study aims to investigate whether or not RET-He is a good indicator in comparison with other hematimetric and biochemical iron parameters in terms of diagnosing iron deficiency (ID) and iron deficiency anemia (IDA) in children with CHDs.

Methods: This is a case-control study conducted in the pediatric cardiology clinic of Zagazig University hospitals on 60 patients with CHD (study group) and 30 ages– and sex-matched healthy children (control group) during the period of October 2018 to May 2019. The participants were subdivided according to iron status (serum iron and transferrin saturation) and blood hemoglobin levels(HGB). All subjects underwent history taking, clinical examination, echocardiography, and lab investigations for complete blood count (CBC), iron profile, and reticulocyte profiles.

Results: RET-He shows a significant reduction in patients with CHD. It can be used to differentiate between Iron deficiency and Iron Deficiency Anemia. That was confirmed using ROC and the decision tree.

Conclusions: The prediction of IDA in CHD children can be made based on the RET-He provided by automated analyzers without the requirement of additional biochemical investigations.

Keywords: Congenital heart disease , reticulocyte hemoglobin content, Case control ,Iron deficiency anemia.

INTRODUCTION

Congenital heart disease (CHD) is defined as a structural cardiac or great vessels anomaly that is or could be of functional significance, which presents at birth even though the diagnosis may come years, or even decades, later. This term does not include arrhythmias in common usage, even if they are noted at birth(1). CHD represents the most common single organ anomalies in humans; moreover, it significantly contributes to infant mortality and morbidity. In recent decades, significant developments in the early diagnostics, surgical, and interventional therapeutic methods reduce mortality and morbidity in these patients(2). Iron deficiency (ID) is a common complication of CHD(3). It leads to increased morbidity and psychomotor development impairment in children. For that reason, the early detection and treatment of ID are essential. However, the diagnosis of ID is considerably complex, which necessitates multiple blood tests in combination to provide enough evaluation. Laboratory indicators of iron status such as serum iron and serum ferritin are detected at earlier stages of ID, followed by erythrocyte-related measurements on complete blood counts (CBC). Classically, ID is manifested as reduced mean cell hemoglobin (MCH), which indicates hypochromia, and a decreased mean cell volume (MCV), which means microcytosis (4).

The frequency of IDA is associated with definite essential aspects of iron metabolism and nutrition. IDA is specifically often existing in children with CHD caused by many different causes. This is a reason for repeated hematological investigations and iron supplements in children in need, especially in those with central cyanosis(5).

Reticulocytes are immature red blood cells (RBC)s that circulate for 18 to 36 hours after release from the bone marrow and before maturing into RBCs. Their characteristics (such as absolute counts,

volume, and hemoglobin concentration/content [RET-He or CHr]) reflect the status of the bone marrow erythropoiesis (RBCs production), which is particularly relevant in ID (6). Serum ferritin levels can also evaluate ID. However, serum ferritin is a positive acute phase response protein, making it less specific as its levels may be increased due to chronic inflammation or infection. Meanwhile, the RET-He levels are not affected by inflammation, malignancy, or anemia caused by chronic illness and might be desirable. The RET-He assess measures the iron available to reticulocytes recently left the bone marrow(7).

This study is a case-control study conducted in the outpatient department of the pediatric cardiology clinic in Zagazig University Hospitals. It involved sixty pediatric patients with CHD in the period from December 2018 up to May 2019. Further, age– and gender-matched controls (thirty) completely healthy children were included.

Children of age between 6 months and 18 years with a diagnosis of CHD confirmed by echocardiography formed the case group, and matched healthy children formed controls. Participants with coexisting hemolytic anemia, acute infection, chronic illness, chronic kidney diseases, liver disorders, connective tissue disorders, or iron supplements were excluded. All participants were subjected to detailed history taking, complete clinical examination, echocardiography, and lab investigations that included CBC, iron profile, transferrin saturation (TSAT), and reticulocyte profile.

The subjects were further classified depending on their TSAT, Serum Ferritin, and HGB into four classes: Normal Iron profile, ID, IDA, and non-iron deficiency anemia (Non-IDA) as described before (8) following the criteria in Table 1.

The results of other investigations, including echocardiography (M mode, 2D, pulsed), color, and continuous Doppler echocardiography, were obtained for the diagnosis and assessment of the severity of CHD. Blood samples were collected for CBC) and reticulocyte indices involving RET-He, (XN-2000, Sysmex). Serum iron, total iron-binding capacity (TIBC), serum ferritin, and other biochemical parameters were measured using Cobas e 602. (TSAT) was calculated as (serum iron /TIBC) × 100.

Ethical declaration: Written informed consent was obtained from all participants, and the research's ethical committee of the Faculty of Medicine, Zagazig University, approved the study. The work has been carried out following The Code of Ethics of the World Medical Association (Declaration of Helsinki) for human studies.

STATISTICAL ANALYSIS

Data were analyzed using Microsoft Excel and Statistical Package for the Social Sciences (SPSS version 20.0) software. Qualitative data are represented as numbers and ratios. Continuous quantitative data are represented as mean ± SD, Chi-square test (X2), t-test, Mann Whitney, ANOVA, Kruskal Wallis, Pearson's and Spearman's correlations, and ROC curve analysis was used accordingly. P values less than 0.05 were considered significant, while P values less than 0.001 were considered highly significant.

Decision tree: Using the R statistical package "rpart", the decision trees, three models were created, tree plotted, and variable importance is calculated.

RESULTS

study population: The research population consisted of 90 participants in total; 30 normal children compared with 60 patients with congenital heart diseases. Seventeen patients were diagnosed with Ventricular septal defect(VSD), seven patients with Atrial septal defect (ASD), four patients with Transposition of great arteries (TGA), four patients with Teratology of Fallot (TOF), and one patient with aortic regurgitation (AR) (Table 2). To show the structure of both patients and control groups regarding their iron deficiency and anemia status, we sub-classify both groups according to iron status(8), and HGB for age (as described in methods Table 1). The patients' group (group I) participants were further subdivided into four subgroups, normal iron profile (NIP): 25 patients (41.7%). Iron deficiency (ID) : 20 patients (33.3%). IDA: 11 patients (18.3%), (Non-IDA :4 patients (6.7%). Similarly group II (controls) were subdivided into 3 subgroups, Normal iron profile: 16 children (53.3%), ID: 10 children (33.3%), IDA: 4 children(13.4%) (Figure 1).

RET-He is lower in congenital heart disease patients and especially those with Iron deficiency anemia

To show the importance of RET-He in the diagnosis of anemia and iron storage assessment in children with CHD, we compared both groups regarding the reticulocyte-related parameters using a t-test. Figure2:A shows that regardless of their anemia and Iron storage status, patients of the group (I) show increased levels of high fluorescence ratio (HFR), which is suggestive of the presence of more abundant immature reticulocytes corresponding with the anemia status in CHD patients. Conversely, the reticulocyte hemoglobin (RET-He) showed a significant decrease in CHD patients (p 0.0001), which entails

that the availability of the functional iron storage essential for the production of the new RBCs is compromised (Figure2:B). Simple correlation analysis shows that RET-He is significantly correlated with many other parameters used in the diagnosis and assessment of iron deficiency anemia and iron storage, namely (HGB, HCT, MCV, and MCHC). It also shows a significant correlation with Serum ferritin, which suggests a more robust reflection of the status of iron storage and requirement (Figure 3).

RET-He alone can diagnose IDA

To show the robustness of using these parameters in diagnosing ID and IDA, we fitted a general linear model for each parameter alone. We used it to predict the anemia status. Figure 4 shows the performance of these models. Some models show inconsistent performance in CHD patients compared to controls. However, RET-He and HFR showed consistent and exemplary performance in both patients and control groups. Meanwhile, other parameters like serum Iron, platelets, and lymphocytes performed poorly. Figure 5 shows more details in about RET-He, it shows that RET-He alone could diagnose IDA with high accuracy in both controls and CHD patients.

Decision tree: We further examined the importance of RET-He in ID and IDA using the decision tree model along with other reticulocyte parameters mentioned in Table 3. This approach utilizes Artificial intelligence and machine learning methods to determine each variable's contribution (parameter) in the decision-making process when IDA and related states are diagnosed. Figure 6 and table 3 show that in differentiation between NIP and IDA, RET-He could be used with a threshold value of 28 with the contribution of 32% in the decision process. This means that this model gives a weight of 32% out of 100% to the information obtained from RET-He to make the diagnosis. This weight reaches 57.5% when it comes to the differentiation of ID and IDA.

This means that RET-He has high importance in diagnosing IDA, while in the case of ID, other parameters like reticulocyte count (RET%), low fluorescence Ratio LFR and medium fluorescence Ratio (MFR) are more informative

Table1: Diagnostic criteria used to classify participants in regard to their Anemia and Iron storage status.

Class	TSAT	Ferritin	Hb
Normal Iron profile	> 20 %	> 30 ng/ml	Normal for age
Iron deficiency	< 20 %	> 30 ng/ml	Normal for age
Iron deficiency anemia	< 20 %	< 20 ng/ml	< Normal for age
Non-Iron deficiency anemia	> 20 %	> 30 ng/ml	< Normal for age

Table 2: Count and percentage of different diagnoses in the patients group.

Diagnosis	No.	(%)
Ventricular Septal Defect	17	(28.4%)
Atrial Septal Defect (ASD)	7	(11.6%)
ASD + Patent Ductus Arteriosus (PDA)	5	(8.5%)
Transposition of Great Arteries (TGA)	4	(6.7 %)
ASD + VSD	4	(6.7 %)
Tetralogy of Fallot (TOF)	4	(6.7 %)
Coarctation of Aorta (COA)	3	(5 %)
Pulmonary Atresia (PA)	2	(3.4 %)
Double outlet right ventricle (DORV)	2	(3.4 %)
Atrioventricular Canal Defect (AVC)	2	(3.4 %)
Tricuspid Atresia	2	(3.4 %)
Aortic stenosis (AS) + Pulmonary stenosis (PS)	1	(1.6 %)
ASD + Total anomalous pulmonary venous return (TAPVR)	1	(1.6 %)
Dextrocardia + Double inlet left ventricle (DILV) + Double Outlet Left Ventricle (DOLV)	1	(1.6 %)
Aortic Regurgitation (AR)	1	(1.6 %)
VSD + Pulmonary arterial hypertension (PAH) + severe Right Ventricular Outflow Tract (RVOT) obstruction	1	(1.6 %)
Moderate Mitral Regurgitation (MR)	1	(1.6 %)
AS	1	(1.6 %)
Mitral Valve Prolapse (MVP)	1	(1.6 %)
Total	60	(100 %)

Table 3: Variable importance of reticulocyte-related parameter in differentiation of (NIP: Noraml Iron Profile, IDA: Iron Deficiency Anemia, ID: Iron Deficiency, RET%: Reticulocyte percentage, LFR: Low fluorescence ratio, MFR: Medium fluorescence ratio, IRF: Immature reticulocyte fraction, HFR: High fluorescence ratio, RET-He: Reticulocyte hemoglobin content). All the values are percentage of importance.

variable	NIP~IDA	NIP~ID	ID~IDA
RET%	4	28.5	15.7
LFR	18	19.3	6.7
MFR	18	19.3	6.7
IRF	16	17.2	6.7
HFR	12	10	6.7
RET-He	32	5.7	57.5

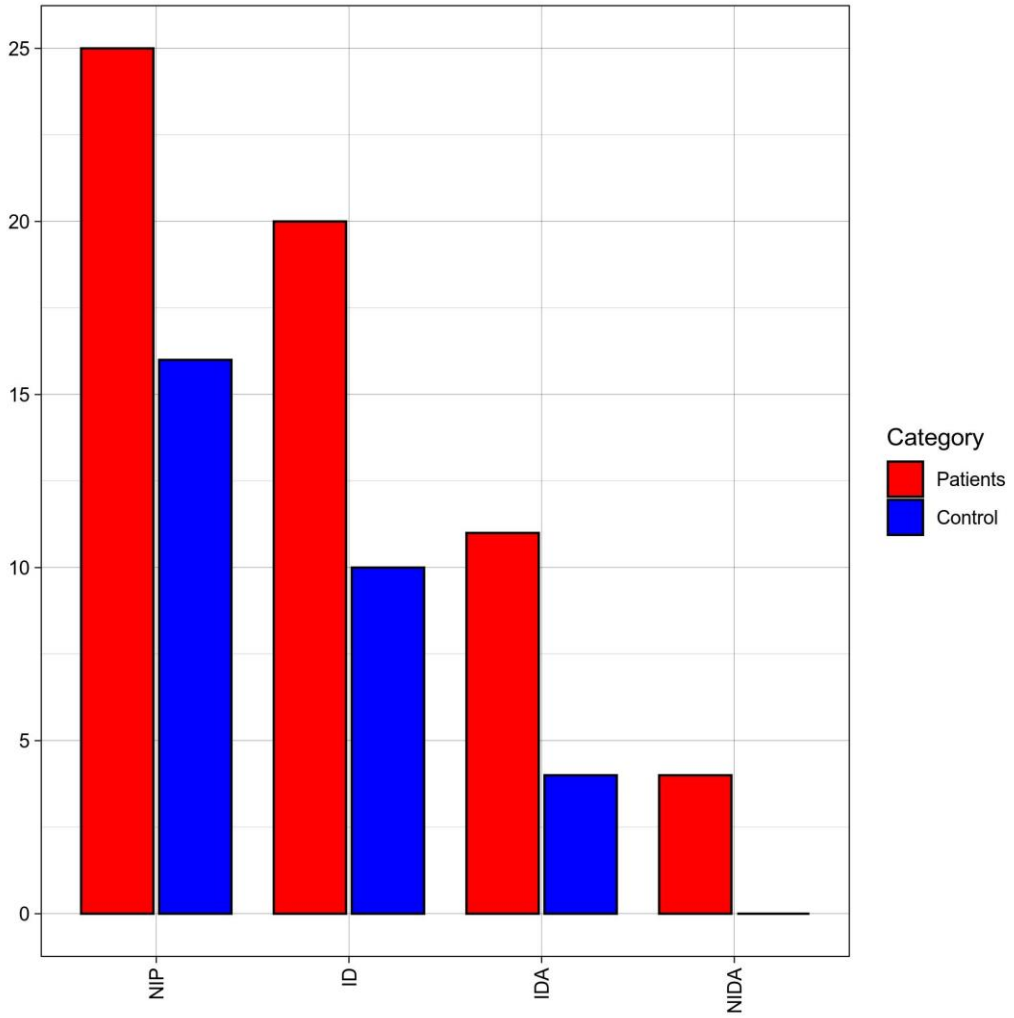

Figure1: The status of study groups in regard to their iron and anemia status; following the criteria in methods (NIP: Normal Iron profile, ID: Iron deficiency, IDA: Iron deficiency anemia, NIDA: Non-iron deficiency anemia).

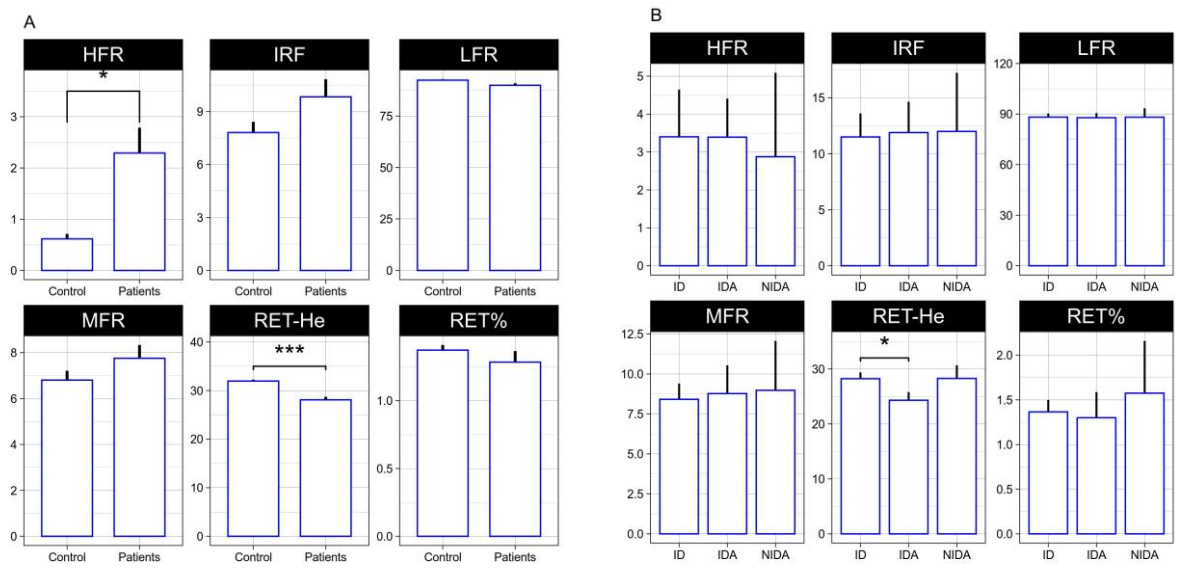

Figure 2: Reticulocyte related parameters in (A: patients compared to controls, and B: Patients with Different Anemia and Iron status); (HFR: High Fluorescence Ratio, IFR: Immature Reticulocyte Fraction, LFR: Low Fluorescence Ratio, MFR: Medium fluorescence Ratio, RET-He: Reticulocyte Hemoglobin Content, RET %: Reticulocyte Count, ID: Iron Deficiency, IDA: Iron Deficiency Anemia, NIDA: Non-Iron Deficiency Anemia) *: $p < 0.05$, ***: $p< 0.001$).

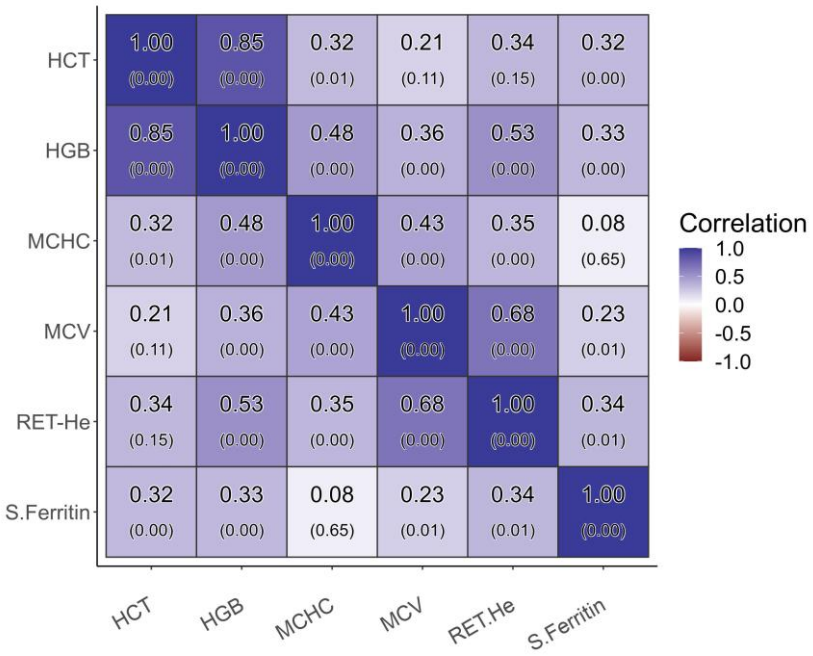

Figure 3: Correlation matrix of RET-He with selected other parameters: upper numbers are correlation coefficients, while small numbers in brackets are p values; (HCT: Hematocrit, HGB: Hemoglobin, MCHC: Mean corpuscular hemoglobin concentration, MCV: Mean corpuscular volume, RET-He: Reticulocyte Hemoglobin Content, S.Ferritin: Serum Ferritin).

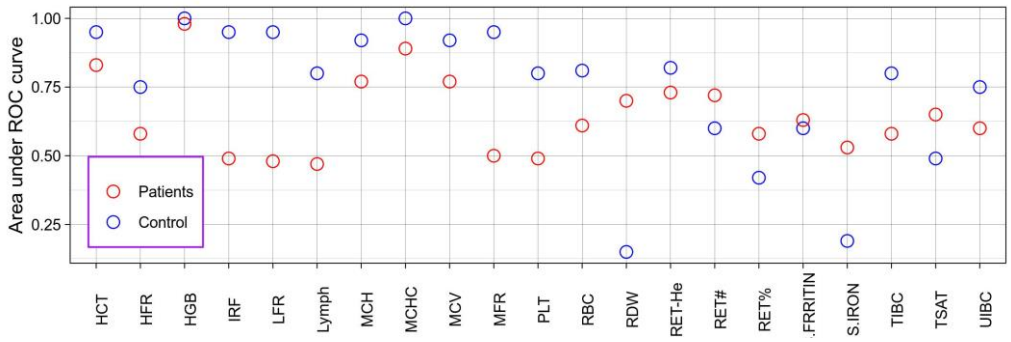

Figure 4: Area under ROC curve for all the parameters, comparing using these parameters to diagnose (IDA) Iron deficiency anemia (differentiate IDA from ID). RET-He consistent AUC in both groups; and it is the highest next to the performance of HGB, MCHC, MCV, and HCT; (RBC: Red blood cells, HCT: Hematocrit, HGB: Hemoglobin, MCHC: Mean corpuscular hemoglobin concentration, MCV: Mean corpuscular volume, PLT: Platelet count, RDW: Red Cell distribution width , RET-He: Reticulocyte hemoglobin content, TIBC: Total iron binding capacity, TSAT: Transferrin saturation, UIBC, Unsaturated iron binding capacity, HFR: High fluorescence ratio, IFR: Immature reticulocyte fraction, LFR: Low fluorescence ratio, MFR: Medium fluorescence Ratio, RET%: Reticulocyte percentage , RET#: Absolute reticulocyte count, Lymph: lymphocyte count).

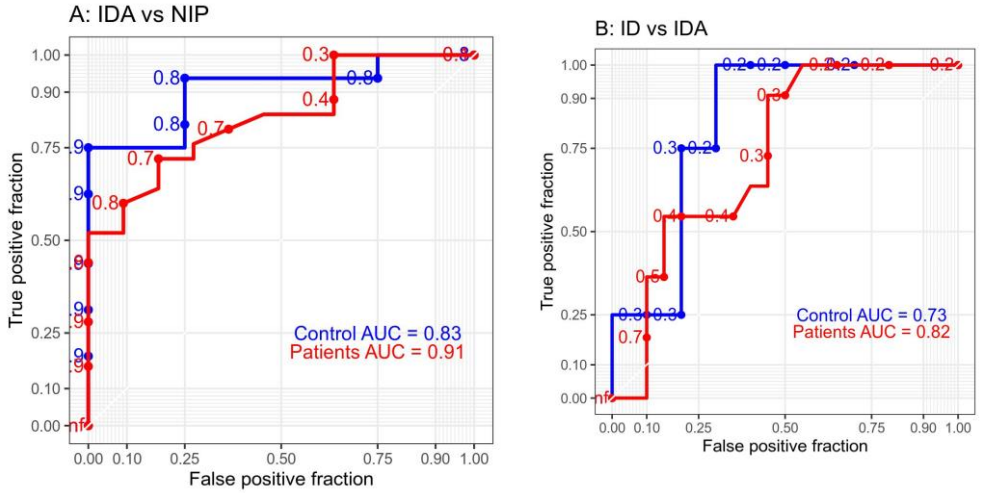

Figure 5: Area under ROC curve using RET-He alone to differentiate (A: Iron deficiency anemia from Normal Iron Profile patients and B: Iron deficiency anemia from Iron deficiency) (IDA: Iron deficiency anemia, ID: Iron deficiency, NIP: Normal Iron profile).

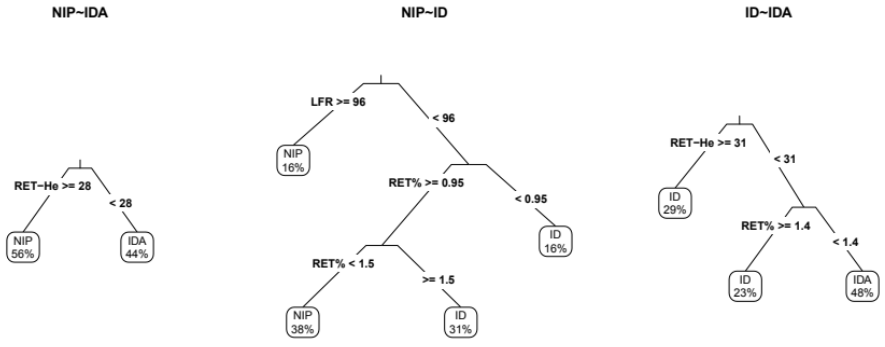

Figure 6: Decision tree illustration, the numbers on the branches shows the values used to differentiate cases, the percentage on leaves represents the percentage of the class in the whole data set. Total of percentages of each model is 100%. (NIP: Normal Iron Profile, IDA: Iron Deficiency Anemia, ID: Iron Deficiency, RET-He: Reticulocyte hemoglobin content, LFR: Low fluorescence ratio, RET%: Reticulocyte percentage).

DISCUSSION

We showed that RET-He alone could diagnose IDA and differentiate it from ID in CHD children through a case-control study and a novel statistical approach. In this approach, we supported our results with simple statistical testing between the study groups regarding RET-He and general linear modeling and the machine learning technique, decision tree.

The study group is comparable to previously published reports. For instance, the percentage of VSD in (9), and TOF and TGA in (10) are similar to this study. In the same context, RET-He was useful in detecting ID in control group, which is corroborate with previous findings by Karagülle et al. who stated that in female patients with IDA and without CHD, the RET-He displayed a significant positive correlation with HGB (r =0.775), MCV (r =0.868), MCH (r=0.883), MCHC (r=0.685), S.iron (r=0.648), and TSAT (r=0.764), and a significant negative correlation with TIBC (r= -0.613) (11).

Multiple observations support the importance of RET-He in the diagnosis of IDA in this study. Using general linear modeling (Figure 3), RET-He constitutes one of the best–performing parameters. Moreover, the decision tree shows the high variable importance of RET-He in differentiating IDA from ID and NIP patients. Decision trees show that IDA can be diagnosed with a cut-off value of 28 pg/l (Figure 4). Similar findings have been reported in adult patients with renal failure on dialysis, showing that RET-He has high sensitivity and specificity in predicting the need for iron supplement to those patients (12).

Previous studies concluded that RET-He is a good indicator of ID in children, pregnant ladies, neonates, aging, and even in the general people (10, 13); however, our results suggest that it has limited application in the case of CHD children. Figure 2:A shows that regardless of the iron profile of the patients assessed by TIBC, serum ferritin, and HGB level, the patients of CHD showed a significant reduction in RET-He levels, which seemingly suggested that it is difficult to use this parameter to detect ID.

Due to the decrease of oxygen and erythropoietic effect on patients with cyanotic CHD, they are prone to have IDA even with increased HGB concentration which could explain why RET-He could not differentiate ID from NIP. The reasons mentioned above make assessing anemia and especially iron storage status for CHD patients complex, and probably more invasive assessment is required. For a definitive ID diagnosis, the body iron stores need to be assessed using bone marrow aspiration followed by microscopical examination with iron staining.

Because bone marrow aspiration is invasive and time-consuming, looking for more accessible, reliable alternatives using blood samples is mandatory and may reduce the necessity to perform bone marrow aspiration(9, 14, 15).

The correlation between RET-He and other blood parameters in adults has been reported previously (16), showing that correlation is higher and more significant with IDA patients. However, the presence of CHD in children makes this correlation less prominent in our study. RET-He is also reported to be lower in non-IDA compared to ID adults (17).

Diagnosing ID in CHD children is challenging. Even though previous reports suggest that RET-He is a good indicator for Iron stores in children's bone marrow without CHD (8, 18–21) with variable diagnostic cut-offs and criteria.

Limitations of the study:

There is a possibility that even non-invasive measurements of iron stores in our study did not show ID. However, ID is there, and probably RET-He is a more sensitive indicator of iron stores than TIBC, serum Iron, and Serum Ferritin. Due to the limitation of this study, to conclude, more investigation is required. To understand the importance of RET-He in diagnosing ID in children with CHD, a more invasive approach with measurement of bone marrow storage is needed.

CONCLUSION

The prediction of IDA in CHD children can be made based on the RET-He provided by automated analyzers without additional biochemical investigations. Even with the limitation of this study of not including bone marrow storage measurement, we could show the tremendous importance of RET-He in IDA diagnosis in CHD patients. We clarified the specific challenges, and we recommend evaluating them with more invasive approaches, including bone marrow aspiration and iron storage measurements.

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