PREVALENCE OF IRON DEFICIENCY ANAEMIA AMONG CHILDREN WITH CONGENITAL HEART DISEASE AND THE USEFULNESS OF ERYTHROCYTE INDICES AS A SCREENING TOOL

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ABSTRACT

Iron deficiency is thought to be the most common micronutrient deficiency worldwide, and is a preventable and treatable condition. Iron is an essential micronutrient playing a fundamental role in the formation of hemoglobin, myoglobin and numerous oxygen-carrier enzymes. In 2000, the results of the first study aimed at treating anemia in patients with heart failure. Anemia in children with CHD, especially in cyanotic children who show high hematocrit value due to chronic hypoxia, is frequently missed or under diagnosed in daily practice. From RBC indices, we can suspect iron deficiency anemia. This hospital based study was conducted with the aim and objectives of identifying iron deficiency anemia in children with congenital heart diseases, to treat, and to prevent its consequences & role of erythrocytes indices. It has been found that half (46.0%) of the children were between 6months-1year of age, and very less number (5.6%) of children were >10 years of age. Males were 54.0% and females were 46.0% with male: female ratio being 1.17:1. This study population includes 66.7% CAHD, and 33.3% CCHD with CAHD: CCHD ratio being 2:1. Among CAHD, 53.6% were VSD and among CCHD, 64.3% were TOF. The study shows statistical significance in erythrocyte indices, both in congenital acyanotic as well as cyanotic heart diseases. It has been concluded in the study that prevalence of iron deficiency anemia (IDA) in congenital heart diseases was 34.1%, which was more than one-third of the study population; and iron depleted stage was also found in another one-third of the study population. RBC indices were very useful as a screening tool to detect, prevent and treat iron deficiency or IDA in children with CHD.

KEYWORDS: Iron-Deficiency Anemia, Heart Diseases & Erythrocyte Indices

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1. INTRODUCTION

Iron deficiency is thought to be the most common micronutrient deficiency worldwide, and is a preventable and treatable condition [1]. Iron is an essential micronutrient playing a fundamental role in the formation of hemoglobin, myoglobin and numerous oxygen-carrier enzymes [1]. According to the World Health Organization (WHO), iron deficiency anemia (IDA) exerts negative impact on a great number of women and children in developing countries [2], and even in women of reproductive age in industrialized nations [1]. According to WHO for the year 2011, it is estimated that roughly 43% of children have anemia globally, corresponding to 273 million children [3]. Iron deficiency anemia was the most common cause and children of school age were the most vulnerable group [2].

Most children with IDA are asymptomatic. Pallor is the most recognized clinical sign of IDA, but is not usually visible until the hemoglobin (Hb) falls to 7-8gm/dl. Parents often fail to note the pallor because of the

typical slow decline of Hb over time. Older individuals may feel cold intolerance, fatigue, exercise induced dyspnea or decreased mental acuity. In mild to moderate IDA, compensatory mechanisms, including increased levels of 2,3-DPG and a shift of the oxygen dissociation curve, may be so effective that few symptoms of anemia aside from mild irritability are noted. When the Hb level falls to <5gm/dl, irritability, anorexia, lethargy develop, and systolic flow murmurs are often heard. If the Hb continues to fall, tachycardia and high output cardiac failure can occur. Both iron deficiency and IDA are associated with impaired neurocognitive function in infancy and exert negative impact on the psycho-motor development in children and adolescents [4, 5] and increased behavioral and social problems in adolescence [6].

Research has now focused on the roles of iron and iron deficiency in patients with cardiovascular disease. In 2000, the results of the first study aimed at treating anemia in patients with heart failure (HF) were published, showing that subcutaneous application of erythropoietin, combined with intravenous iron sucrose, improved haemoglobin levels, left ventricular ejection fraction (LVEF), and NYHA classification of HF [7]. The results of subsequent studies have shown that replenishing iron alone can be beneficial in patients with HF and iron deficiency, irrespective of anemia [8]. Of note, IDA has been clearly shown to be an important risk factor that increases morbidity and mortality in patients with congenital heart disease (CHD) [9].

Congenital heart diseases are of two types, congenital acyanotic heart disease (CAHD) and congenital cyanotic heart disease (CCHD). Severe iron deficiency anemia (IDA) increases cardiovascular stress in the form of tachycardia, hypotension. In children with CAHD who are at risk of heart failure, IDA increases the risk of heart failure due to hyperdynamic circulation; and similarly in congestive heart failure (CHF), IDA increases as CHF advances [10]. Its multifactorial etiology is not fully understood, but is postulated as: a general loss of appetite and poor nutrition; decreased gastrointestinal (GI) iron absorption due to edema; and, importantly, as a consequence of the chronic inflammatory state of these patients [11, 12]. The quality of life is significantly affected by iron deficiency. Iron deficiency can also negatively impact outcomes and increased mortality (by 40-60%) in CHF [13, 14, 15]. An early detection and an appropriate treatment of anemia in patients with CAHD have been strongly recommended. In children with CCHD, the right-to-left shunt leads to chronic arterial desaturation which acts as a trigger for increase in erythropoietin production and secondary erythropoiesis in an effort to maintain tissue oxygenation and consequent high blood viscosity. Hyper viscosity and polycythemia contribute to erythrocyte deformation, formation of cell aggregates and development of emboli. In addition, in patients with CCHD suffering from anemia, especially due to iron deficiency hypochromic anemia, red blood cells are less permeable. This phenomenon contributes in part to the increased rate of embolism and other cerebrovascular accidents [16]. There is also a tendency to develop metabolic acidosis and cyanotic spells. In children with CCHD, the prevention of thromboembolic events and cyanotic spells by giving iron supplementation has to be emphasized [17]. Polycythemia causes hemoglobin (Hb) and hematocrit (Hct) values to increase in children with CCHD. Iron deficiency anemia may develop although Hb and Hct values remain within normal range for that age group and this condition may have negative effects on morbidity and mortality [18, 19]. However, the MCV, MCH and serum ferritin are usually comparatively lower in IDA than their peers as shown by Onur CB et al, a phenomenon known as relative anemia [20].

In developing countries including India, the high prevalence of malnutrition is still a real public health concern [21]. We would speculate that IDA is of increased rate in this vulnerable group. Anemia in children with CHD, especially in cyanotic children who show high hematocrit value due to chronic hypoxia, is frequently missed or underdiagnosed in daily practice [22].

From RBC indices we can suspect iron deficiency anemia. But it needs confirmation. Ferritin is an iron-containing protein that functions in the body as an iron-storage compound. Serum ferritin provides a sensitive, specific, and reliable measurement for determining iron deficiency at an early stage, and it may be the best indicator of total body iron status. Further diagnostic tests are serum iron, TIBC and TIBC%.

We will conduct these investigations to assess the prevalence of IDA in children with CHD and to evaluate the usefulness of erythrocyte indices in screening IDA in this group with the hope that these indices would help the physicians to detect IDA and to start the iron supplement appropriately in resource limited health facilities, where the specialized tests of IDA is not routinely available.

2. AIMS AND OBJECTIVES

2.1 Aim: 1. To identify iron deficiency anemia in children with congenital heart diseases, to treat, and to prevent its consequences. 2. To establish the role of screening tool using erythrocyte indices for early recognition of iron deficiency anemia.

2.2 Objective: 1. To investigate the prevalence of IDA in children with CHD. 2. To assess the diagnostic values of hemogram, especially the erythrocyte indices as a simple screening tool for early recognition of IDA.

3. MATERIAL AND METHODS

This hospital based cross-sectional study was conducted in the Department of Pediatrics, S.C.B. Medical College & Hospital and SVPPGIP, Cuttack done from January 2017 to December 2019, after obtaining clearance from institutional ethical committee. 6 months to 14 years of age were taken for the study, as per inclusion and exclusion criteria; 126 patients with congenital heart disease proven by echocardiography as per inclusion and exclusion criteria.

3.1. Inclusion Criteria: Confirmed cases of congenital heart disease diagnosed by 2-D echocardiography, not following any exclusion criteria between 6 months to 14 years of age, who (1) visited OPD for the first time due to symptoms, or (2) incidental finding during routine check-up, or (3) came for follow up.

3.2 Exclusion Criteria: 1. Patients with signs and symptoms of hemolysis due to thalassemia and other hemolytic anaemia or other unknown mechanisms. 2. Candidates who will present with overt or suspected infection, fever, inflammatory diseases. 3. Infant less than 6 months. 4. Children already on Iron supplements.

3.3 Investigation Done: 1. Complete blood count i.e. Hb%, DC, TLC, RBC, TPC was done by automated hematology analyzer (SYSMEX). 2. Peripheral smear was examined by microscopy. 3. Red cell indices were also done with same automated hematology analyzer (SYSMEX). 4. Serum ferritin estimation was done using Electrochemiluminescence Immunoassay "ECLIA" using Elecsys and cobas e immunoassay analyzers. 5. Serum iron and TIBC were done by Ferrozine method using Robonik semi-auto-analyzer. 6. 2-D-Echocardiography.

3.4 Statistical Analysis: Categorical variables are expressed as number of patients and percentage of patients and compared across the groups, using Pearson's Chi Square test for Independence of Attributes/ Fisher's Exact Test as appropriate. Continuous variables are expressed as Mean, Median and Standard Deviation and compared across the groups using Mann-Whitney U test/Kruskal Wallis as appropriate. Non-parametric receiver operating characteristic (ROC) curve analysis was done, and ROC curves were used to evaluate the accuracy of observed parameters to detect true IDA and depleted state, indicated by the area under the curve (AUC). The "optimum cut-off point" was determined, as the cut-off

point with the highest [(sensitivity + specificity)/2] ratio, at which there was a maximal correct classification of the disease is done. The statistical software SPSS version 20 has been used for the analysis. An alpha level of 5% has been taken, i.e. if any p value is less than 0.05 it has been considered as significant.

4. OBSERVATION

In the present study, 126 cases of congenital heart disease (as per inclusion criteria) were taken randomly from OPD; hematological, biochemical parameters and results were statistically analyzed and compared.

Table1: Age Distribution of Study Population

Age Group	Frequency	Percentage
6months-1year	58	46
>1year-5years	46	36.5
>5years-10years	15	11.9
>10years -14years	7	5.6
Total	126	100

OBSERVATIONS

In the present study, 126 cases of congenital heart disease (as per defined criteria) were taken randomly from OPD; hematological, biochemical parameters and results were statistically analyzed and compared.

Table 1: Showing Age Distribution of Study Population

Age Frequency Percentage (%)

6 months - 1 Year 58 46.0

>1-5 Years 46 36.5

>5-10 Years 15 11.9

>10-14 Years 7 5.6

Total 126 100.0

Out of total 126 children, 58(46.0%) children were between 6months-1years

Of age, 46(36.5%) children were between >1-5 years of age, whereas 15(11.9%)

children were between >5-10 years of age & 7(5.6%) children were >10 years o

Out of total 126, 46% of the children are between 6months to 1year age group, and only 5.6% of children were between 10 and 14 years of age group.

Table 2: Gender Distribution

Gender	Frequency	Percentage
Female	58	46
Male	68	54
Total	126	100

Out of 126 children in the study group, 46% were male, 54 % were female with ratio of 1.17:1.

Table 3: Distribution of Type of Congenital Heart Diseases

Type of Heart Disease		Frequency	Percentage
Congenital Acyanotic Heart Diseases n=84 (66.7%)	VSD	45	56.3
	ASD	19	22.6
	PDA	4	4.8
	Combined	12	12.2
	Others	4	4.8
Congenital Cyanotic Heart Diseases n=42(33.3%)	TOF	27	64.3
	TGA	8	19
	Others	7	16.7
Total		126	100

Out of 126 children, 66.7% children were having Congenital Acyanotic Heart Diseases, most common being VSD, 33.3% were having Congenital Cyanotic Heart Diseases, most common being TOF with ratio being 2:1.

Table 4: Peripheral Smear Distribution

Peripheral Smear Distribution		Frequency	Percentage
Microcytic hypochromic		88	69.8
Normocytic normochromic		38	30.2
RDW	<14.5	30	23.8
	≥14.5	96	76.2
RBC indices	No sign of iron deficiency	16	15.2
	sign of iron deficiency	89	84.8
Total		126	100

Out of 126 children, 69.8% children showed microcytic hypochromic and 30.2% showed normocytic normochromic RBCs, 23.8% of children were having RDW <14.5% & 76.2% children showed RDW ≥ 14.5%, the RBC indices were suggestive of iron deficiency in 84.8% cases.

Table 5: Prevalence of Different Stage of Iron Deficiency in Different Age Group

Result	Age group				Total	p-value=0.007	Statistically significant
	6m -1yr	>1yr-5yr	>5yr-10yr	>10yr-14yrs			
True IDA	21(36.21%)	13(28.26%)	3(20%)	6(85.71%)	43(34.13%)		
Depletion of body iron storage	17(29.31%)	15(32.61%)	6(40%)	0(0%)	38(30.16%)		
At risk of iron deficiency	13(22.41%)	5(10.87%)	6(40%)	0(0%)	24(19.05%)		
Normal body iron storage	7(12.07%)	13(28.26%)	0(0%)	1(14.29%)	21(16.67%)		
Total	58(100%)	46(100%)	15(100%)	7(100%)	126(100%)		

Among 58 children between 6 month to 1 year, 36.21% children had True IDA, 29.31% children had depletion of body iron storage, 22.41% children were at risk of iron deficiency, and 12.07% children had normal body iron storage.

Among 46 children between >1 year to 5 years, 28.26% children had True IDA, 32.61% children had depletion of body iron storage, 10.87% children were at risk of iron deficiency, 28.26% children had normal body iron storage.

Among 15 children between >5 years to 10 year, 20% children had True IDA, 40% children had depletion of body

iron storage, 40% children were at risk of iron deficiency, and no children had normal body iron storage.

Among 7 children between >10years to 14 years, 85.71% children had True IDA, 14.29% children had normal body iron storage and no children had depletion of body iron storage & children were at risk of iron deficiency. The result was statistically significant (p value=<0.05)

Table 6: Prevalence of Different Stage of Iron Deficiency in Different Gender Group

Result	Gender		Total	p-value =0.535	Statistically not significant
	Female	Male			
True IDA	17(27.59%)	27(39.71%)	43(34.13%)		
Depletion of body iron storage	20(34.48%)	18(26.47%)	38(30.16%)		
At risk of iron deficiency	12(20.69%)	12(17.65%)	24(19.05%)		
Normal body iron storage	10(17.24%)	11(16.18%)	21(16.67%)		
Total	58(100%)	68(100%)	126(100%)		

Among 58 female children, 27.59% children had True IDA, 34.48% children had depletion of body iron storage, 20.69% children were at risk of iron deficiency, 17.24% children had normal body iron storage.

Among 68 male children, 39.71% children had True IDA, 26.47% children had depletion of body iron storage, 17.65% children were at risk of iron deficiency, 16.18% children had normal body iron storage. The result was statistically not significant (p value=>0.05)

Table 7: Prevalence of Different Stage of Iron Deficiency in Two Different Type of Heart Disease

Result	Disease		Total	p value =0.004	Statistically significant
	CAHD	CCHD			
True IDA	23(27.38%)	20(47.26%)	43(34.13%)		
Depletion of body iron storage	22(26.19%)	16(38.1%)	38(30.16%)		
At risk of iron deficiency	22(26.19%)	2(4.76%)	24(19.05%)		
Normal body iron storage	17(20.24%)	4(5.92%)	21(16.67%)		
Total	84(100%)	42(100%)	126(100%)		

Among 84 CAHD children, 27.38% children had True IDA, 26.19% each had depletion of body iron storage, children were at risk of iron deficiency, and 20.24% children had normal body iron storage.

Among 42 CCHD children, 47.26% children had True IDA, 38.1% children had depletion of body iron storage, 4.76% children were at risk of iron deficiency, and 5.92% children had normal body iron storage.

The result was statistically significant (p value=<0.05)

Table 8: Peripheral Smear Distribution in Different Stage of Iron Deficiency

Elements		True IDA	Depletion of body iron storage	At risk of iron deficiency	Normal body iron storage	Total	p-value
Peripheral smear	MC HCH	43(48.86%)	35(39.77%)	10(11.36%)	0(0)	88(100%)	<0.001 Statistically Significant
	NC HCH	0(3.79%)	3(7.89%)	14(36.84%)	21(55.26%)	38(100%)	
	Total	43(34.13%)	38(30.16%)	24(19.05%)	21(16.67%)	126(100%)	
RDW	<14.5	0(0)	3(10)	11(36.67%)	16(53.33%)	30(100%)	<0.001 Statistically Significant
	≥14.5	43(44.79%)	35(36.46%)	13(13.54%)	5(5.21%)	96(100%)	
	Total	43(34.13%)	38(30.16%)	24(19.05%)	21(16.67%)	126(100%)	
RBC indices	No sign of ID	0(0)	3(7.89%)	13(54.17%)	-	16(15.24%)	<0.001 Statistically

Sign of ID	43(100%)	35(92.11%)	11(45.83%)	-	89(84.76%)	Significant
Total	43(100%)	38(100%)	24(100%)		105(100%)	

Among 88 children having microcytic hypochromic RBCs, 48.86% had True IDA, 39.77% children had depletion of body iron storage, 11.35% children were at risk of iron deficiency, and no children had normal body iron storage.

Among 38 children having normocytic normochromic RBCs, no children had True IDA, 7.89% had depletion of body iron storage, 36.84% children were at risk of iron deficiency, 55.26% children had normal body iron storage. The result was statistically significant (p value=<0.05).

Among 30 children having RDW<14.5%, no one belong to True IDA, 10% children had depletion of body iron storage, 36.67% children were at risk of iron deficiency and 53.33% children had normal body iron storage.

Among 96 children having RDW≥14.5%, 44.79% of children had True IDA, 36.46% had depletion of body iron storage, 13.54% children were at risk of iron deficiency, and 5.21% children had normal body iron storage. The result was statistically significant (p value=<0.05).

Among 43 children with true IDA, all children have RBC indices suggestive of iron deficiency anemia.

Among 38 children having depletion of body iron store, 92.11% children were at risk of iron deficiency and 7.89% children had RBC indices not suggestive of iron deficiency.

Among 24 children with risk of iron deficiency, 54.17% children had RBC indices not suggestive of iron deficiency and 54.83% children had suggestive RBC indices. The result was statistically significant (p value=<0.05).

Table 9: Comparison of Variables in Different Stage of Iron Defficiency

Parameter	Result												Pvalue/ significance
	True IDA			Depletion of Body Iron Storage			At risk of Iron Deficiency			Normal Body Iron Storage			
	mn	me	sd	mn	me	sd	mn	me	sd	Mn	me	sd	
Hb(gm/dl)	11.3 2	9.8 0	3.5 8	13.0 8	10.90	4.67	11.0 4	10.5 0	1.5 4	12.7 0	12.1 0	2.84	0.025/ significant
HCT(%)	36.0 6	31. 20	11. 12	43.2 1	31.95	18.5 3	33.8 6	32.6 5	6.3 3	38.2 0	36.6 0	9.24	0.133/ not significant
MCV(fl)	66.3 9	67. 00	4.2 47	70.1 9	69.95	3.54	72.3 5	74.5 5	7.5 6	79.4 3	77.9 0	6.39	<0.001/ significant
MCH(pg)	21.0 0	21. 40	2.1 3	21.9 0	22.50	2.35	23.9 3	23.9 0	2.2 3	26.5 0	26.2 0	2.12	<0.001/ significant
MCHC(g/d l)	31.2 9	31. 70	2.1 2	31.1 2	31.25	2.86	32.7 6	33.0 0	1.7 3	33.3 0	33.6 0	1.25	0.001/ significant
S. Ferritin (ng/ml)	7.39	8.4 4	2.4 1	38.5 3	43.00	9.58	74.0 1	74.1 2	13. 67	166. 15	154. 50	68.1 8	0.001/ significant
S. Iron (µg/dl)	30.0 0	24. 76	21. 15	80.4 8	65.59	42.2 2	121. 28	119. 51	61. 81	142. 39	137. 05	51.9 4	0.001/ significant
TIBC (µg/dl)	439. 45	457 .30	94. 45	385. 45	389.4 6	95.5 7	349. 92	351. 61	77. 92	386. 76	373. 50	132. 0	0.001/ significant
TIBC %	6.80	5.8 9	4.4 4	23.1 3	17.10	18.4 5	34.8 0	37.5 0	15. 78	38.6 2	36.8 6	13.3 2	0.001/ significant
RBC (m/mm ³)	5.47	4.7 1	1.8 1	6.15	4.64	2.62	4.73	4.45	1.1 0	4.79	4.61	0.92	0.572/ not significant

RDW(%)	19.2 9	19. 9	3.8	16.1 5	16	1.56	14.4 4	14.5	2.1 9	13.9 8	15.5 5	0.79	0.001/ significant
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mn=mean, me : median, sd =standard deviation

The result was statistically significant (p value=<0.05) in all cases except HCT and RBC parameter.

Table 10: Comparison of Variables in Ida and Non-Ida Children with Cahd

Observed Parameters	CAHD						p-value/ significance
	True IDA			Non-IDA			
	Mean	Median	SD	Mean	Median	SD	
Hb(gm/dl)	8.67	8.80	0.91	10.59	10.50	1.28	<0.001/significant
HCT(%)	27.54	26.60	3.54	31.95	31.50	3.73	<0.001/significant
MCV(fl)	67.42	67.50	2.90	73.42	72.80	6.80	<0.001/significant
MCH(pg)	21.49	21.40	1.68	24.37	23.90	2.27	<0.001/significant
MCHC(gm/dl)	31.30	31.40	2.05	33.10	33.30	1.53	<0.001/significant
S. Ferritin (ng/ml)	8.17	8.44	2.39	87.38	70.55	61.23	<0.001/significant
S. Iron(µg/dl)	26.15	23.38	22.16	109.55	110.75	59.24	<0.001/significant
TIBC (µg/dl)	438.84	425.00	105.56	375.37	373.50	107.12	0.012/significant
TIBC(%)	5.78	4.76	4.42	30.26	28.40	15.51	<0.001/significant
RBC (m/mm ³)	4.09	3.90	0.69	4.36	4.38	0.37	0.002/significant
RDW(%)	16.56	15.80	2.11	14.53	14.50	1.60	<0.001/significant

mn=mean, me : median, sd =standard deviation

The result was statistically significant (p value=<0.05) in all cases .

Table 11: Comparison of Variables in Ida and Non-Ida Children with Cchd

Observed parameters	CCHD						p-value/ significance
	True IDA			Non-IDA			
	Mean	Median	SD	Mean	Median	SD	
Hb(gm/dl)	14.37	13.75	3.02	17.39	17.80	3.38	<0.001/significant
HCT(%)	45.86	48.10	8.36	59.45	60.45	13.05	0.002/significant
MCV(fl)	65.20	65.40	5.22	72.42	70.50	6.84	<0.001/significant
MCH(pg)	20.45	19.80	2.47	21.67	20.80	3.59	0.820/not significant
MCHC(g/dl)	29.50	29.30	2.62	31.28	31.80	2.24	0.036/significant
S. Ferritin (ng/ml)	7.65	7.77	2.46	63.61	46.34	64.95	<0.001/significant
S. Iron (µg/dl)	34.42	25.00	19.54	103.49	88.98	50.93	<0.001/significant
TIBC (µg/dl)	440.16	459.67	82.42	375.88	390.24	87.20	0.016/significant
TIBC (%)	7.98	5.89	4.27	30.89	21.40	23.20	<0.001/significant
RBC (m/mm ³)	7.06	7.50	1.32	8.28	8.28	1.97	0.018/significant
RDW%	22.44	21.35	1.74	16.71	16.7	1.70	<0.001/significant

The result was statistically significant (p value=<0.05) in all cases, except MCH parameter

5. DISCUSSIONS

This was a cross-sectional study of children between 6months to 14 years of age with echocardiography proven congenital heart disease, who came to OPD for follow up or diagnosed as congenital heart disease for the first time as per inclusion and exclusion criteria, conducted in the Department of Pediatrics, S.C.B. Medical College & Hospital and SVPPGIP, Cuttack, in which different hematological and biochemical parameters were analyzed and their relationship were found out. There were 126 cases in the present study.

Out of total 126 children, 58(46.0%) children were of 6months-1year of age, 46(36.5%) children were between >1-5 years of age, whereas 15(11.9%) children were between >5-10 years of age & 7(5.6%) children were >10 years of age.

In the study by Mukherjee Set al[23] (2017),60.78% children were <1year of age, 35.29% children were between 1-5 years, which is almost similar to our study, and 3.92% children were >5 years of age.

The study population was dominated by infants, who comprised of half of the total. This is probably due to symptomatic manifestations of congenital heart disease seeking medical attention.

Out of 126 children, females were 58(46.0%) in number and males were 68(54.0%) in number with male:female being 1.17:1.

In the study by Mukherjee S et al (2017),[23]two-thirds of the study population comprised of male children, whereas in the study by Meshram RM et al (2018),[24] male:female 1.3:1, in the study by Binh TQ, et al[25], the ratio was 1.16:1 which were almost similar to our study.

In this study, out of 126 children, 84(66.7%) children were having CAHD, 42(33.3%) were having CCHD with CAHD: CCHD being 2:1. Similar findings were observed in the study by Meshram RM et al (2018),[24] having 66.74%CAHD and 33.26% CCHD, Shah GS et al[26] having 69% and 31%, Pate et al[27]having 60.6% and 38.6%, and Sani et al[28]having 68.9% and 31.1% CAHD and CCHD respectively.

Few studies have shown a greater number of CAHD compared to CCHD, those studies include Wanni et al[29] 88.6% and 11.4%, Saxena et al[30] 79.88% and 20.12% CAHD and CCHD, respectively.

Bakhtyar Zahid et al[31]in their study had CAHD & CCHD 52.8% and 47.2% respectively; whereas in study by Binh TQ et al[25] 47.82% was CAHD and 52.17% was CCHD.

This is probably due to increased prevalence of CAHD, of which most common is VSD.

Out of 84 children with CAHD,45(53.6%) were VSD, 19(22.6%) were ASD, 4(4.8%) were PDA, 12(14.2%) were combined disease, and 4(4.8%) were other types. Out of 42 children with CCHD, 27(64.3%) were TOF, 8(19.0) were TGA, and 7(16.7%) were other types. VSD (35.7%) was the most common CHD, followed by TOF (21.4%), ASD (15.1%).In the study of Meshram RM et al. (2018)[24] VSD (30.01%) was the most common CHD, followed by ASD (20.70%), TOF (16.05%), and PDA (10.23%). VSD was the most common CAHD while TOF was the most common CCHD. Most of the studies in the literature reported similar observation from India and other countries;[26-29,32-34]however, few studies reported a higher incidence of PDA compared to ASD.110,112In the study by Mukherjee Set al.105 74.5% children had TOF among all CCHDs.

As we know, VSD is the most common CAHD, while TOF is the most common CCHD in children, so its number in this population is more.

Out of 126 children with congenital heart disease (CHD), 43(34.1%) children had true IDA, 38(30.2%) children had depletion of body iron storage, 24(19.0%) children were at risk of iron deficiency, and 21(16.7%) children had normal body iron storage. So, the prevalence of IDA in CHD was 34.1%.

47.06% were iron deficient in CCHD in the study by Mukherjee S et al (2017)[23]. In another study, done by

Olçay et al[18] the prevalence of IDA in CCHD was found to be 52.2%. However, one study in children with CCHD done by West et al[35] showed much lower prevalence of IDA. In study done by Binh TQ et al[25] 11.1% of children with CCHD demonstrated true IDA, but 3% of the CAHD group showed true IDA.

Since serious complications in CCHD patients are more frequent, all the studies in India were done in CCHD patients and not in CAHD.

The higher prevalence of true IDA and depleted stage in our population is probably due to a larger subset of infants and toddlers in whom nutritional iron deficiency was coexistent. No patient in our study, who had serum ferritin above 300 ng/mL, which considered to be the upper normal range.

Out of 126 children, 88(69.8%) children showed microcytic, hypochromic (MC, HCH) RBCs, and 38(30.2%) children showed normocytic, normochromic (NC, NCH) RBCs in peripheral smear examination.

Out of 126 children, only 30(23.8%) children were having RDW is $<14.5\%$ and 96 (76.2%) children were having $RDW \geq 14.5\%$.

Among 105 children with different stages of iron deficiency, RBC indices were suggestive of iron deficiency in 89(84.8%) children, and not suggestive of iron deficiency in 16(15.2%) children. This is because of body iron depletion, which is reflected by changing the values of RBC indices after a certain point.

Among 58 children between 6 months-1 year, 21(36.21%) children had true IDA, 17(29.31%) children had depletion of body iron storage, 13(22.41%) were at risk of iron deficiency, and 7(12.07%) children had normal body iron storage.

Among 46 children between >1-5 years, 13(28.26%) children had true IDA, 15(32.61%) children had depletion of body iron storage, 5(10.87%) were at risk of iron deficiency, and 13(28.26%) children had normal body iron storage.

Among 15 children between >5-10 years, 3(20%) children had true IDA, 6(40%) children had depletion of body iron storage, 6(40%) were at risk of iron deficiency, and no children had normal body iron storage.

Among 7 children of >10 years, 6(85.71%) children had true IDA, 1(14.29%) child had normal body iron storage and no child had iron depletion stage, and no child was at risk of iron deficiency.

This result was statistically significant ($p < 0.05$).

IDA and depleted stage were more among children <1 year of age probably due to coexistence of micronutrient deficiency. Most of the children of >1-5 years of age showed depleted stages compared to true IDA. This showed a trend towards IDA in this age group. In children of >10 years, more than 85% showed IDA.

Among 58 female children, 16(27.59%) children had true IDA, 20(34.48%) children had depletion of body iron storage, 12(20.69%) children were at risk of iron deficiency, and 10(17.24%) children had normal body iron storage.

Among 68 male children, 27(39.71%) children had true IDA, 18(26.47%) children had depletion of body iron storage, 12(17.65%) children were at risk of iron deficiency, and 11(16.18%) children had normal body iron storage.

This result was statistically not significant ($p > 0.05$).

In the study on CCHD children by Lang'o MO et al[36], prevalence of IDA was more in males (61.1%) which was similar to our study which had prevalence of IDA among males at 62.8%. This result even in their study was statistically not significant ($p=0.301$). In the study by Animasahun BA et al. (2019)[37] no significant difference was observed in iron status of subjects and gender.

In our study, males and females were having almost equal percentage of various stages of iron deficiency except true IDA which showed more in case of males (62.8% of total IDA). Thus, it implies no gender difference in manifestation of iron deficiency anemia.

Among 84 CAHD children, 23(27.38%) children had true IDA, 22(26.19%) children had depletion of body iron storage, similar percentage of children were at risk of iron deficiency, and 17(20.24%) children had normal body iron storage.

Among 42 CCHD children, 20(47.62%) children had true IDA, 16(38.1%) children had depletion of body iron storage, 2(4.76%) children were at risk of iron deficiency, and 4(9.52%) children had normal body iron storage.

Significant difference was found between these groups ($p<0.05$).

In a study by Binh TQ et al[25] prevalence of IDA was more in CCHD than CAHD; prevalence of iron depleted stage and at-risk group were also high in CCHD children.

Various degrees of iron deficiency were almost equally distributed among CAHD patients, but most of the CCHD patients were having true IDA followed by depleted stage.

CCHD children were having a greater number of true IDA than CAHD, and they are at increased risk of iron deficiency due to polycythemia.

Among 88 children having microcytic hypochromic RBCs, 43(48.86%) children had true IDA, 35(39.77%) children had depletion of body iron storage, 10(11.36%) children were at risk of iron deficiency and no one had normal body iron stores.

Among 38 children having normocytic normochromic RBCs, no child had true IDA, 3(7.89%) children had depletion of body iron storage, 14(36.84%) children were at risk of iron deficiency, and 21(55.26%) children had normal body iron.

This result was statistically significant ($p<0.05$).

In the study by Mukherjee S et al[23], all children with iron deficiency had microcytic, hypochromic picture on peripheral smear.

In our study, there was an increasing trend of number of microcytic, hypochromic RBCs on peripheral smear from at risk group to true IDA probably due to the decreasing trend of iron store in the body. All IDA children had microcytic, hypochromic RBCs, and all non-iron deficient children had normocytic, normochromic RBCs.

Among 30 children having $RDW < 14.5\%$, no one belonged to true IDA, 3(10%) children belonged to depleted stage, 11(36.67%) children belonged to at risk group, 16(53.33%) children belonged to normal body iron group.

Among 96 children having $RDW > 14.5\%$, 43(44.79%) children belonged to true IDA group, 35(36.46%) children

belonged to depleted group, 13(13.54%) children belonged to at risk group, 5(5.21%) children belonged to normal body iron storage group.

This result was statistically significant ($p < 0.05$).

As changes in RDW was reflected by the changes in body iron status, all true IDA and most of the depleted stage were having increased RDW suggestive of iron deficiency. RDW is of prime importance in the diagnosis of iron deficiency. Previous studies have deduced that iron deficiency can be diagnosed with 98% accuracy by evaluating RDW and MCV together.[38,39]

Among 43 children with true IDA, all (100%) children had RBC indices suggestive of iron deficiency.

Among 38 children having depleted iron store, 35(92.11%) children had RBC indices suggestive of iron deficiency and 3(7.89%) children had RBC indices not suggestive of iron deficiency.

Among 24 children with risk of developing iron deficiency, 13(54.17%) children had RBC indices not suggestive of iron deficiency and 11(45.83%) children had suggestive RBC indices.

This result was statistically significant ($p < 0.05$).

As the depletion of iron store progresses in the body, serum ferritin starts decreasing first followed by serum iron, followed by changes in RBC indices occurs. So, here in case of all true IDA and most of the depleted group showed RBC indices suggestive of iron deficiency.

In true IDA mean Hb, Hct were 11.32 g/dl (SD 3.58) and 36.06% (SD 11.12) respectively and in non-iron deficient children mean Hb, Hct were 12.70 g/dl (SD 2.84) and 38.20% (SD 9.24) respectively.

In true IDA mean MCV, MCH, MCHC, RDW were 66.39 fl (SD 4.24), 21 pg (SD 2.13), 31.29 g/dl (SD 2.12), 19.29% (SD 3.8) respectively and in non-iron deficient children 79.43 fl (SD 6.39), 26.50 pg (SD 2.12), 33.30 g/dl (SD 1.52), 13.98% (SD 0.79) respectively with significant difference between these two groups ($p < 0.05$).

In true IDA serum ferritin, iron, TIBC, TIBC% were 7.93 ng/ml (SD 2.41), 30.0 µg/dl (SD 21.15), 439.45 µg/dl (SD 94.45), 6.80% (SD 4.44) respectively and in non-iron deficient children 166.15 ng/ml (SD 68.18), 142.39 µg/dl (SD 51.94), 386.76 µg/dl (SD 132.0), 38.62% (SD 13.32) respectively with significant difference between these two groups ($p < 0.05$).

In the study of Mukherjee S et al[23] mean serum ferritin was 7.63 ng/ml (SD 3.4) in iron deficient group as compared to 33.53 ng/ml (SD 10.51) in non-iron deficient group; serum iron and transferrin saturation were lower and TIBC higher in the iron deficient group; increased RDW and low MCV in iron deficient group.

CAHD children with IDA were having mean Hb, Hct and RBC count of 8.67 g/dl (SD 0.91), 27.54% (SD 3.54) and 4.09 M/mm³ (SD 0.69); whereas non-IDA group were having 10.50 g/dl (SD 1.28), 31.95 % (SD 3.73) and 4.36 M/mm³ (SD 0.37), respectively.

Among CAHD children, mean MCV, MCH, MCHC, and RDW in IDA group were 67.42 fl (SD 2.90), 21.49 pg (SD 1.68), 31.30 g/dl (SD 2.05), and 16.56% (SD 2.11); whereas in non-IDA group mean of 73.42 fl (SD 6.80), 24.37 pg (SD 2.27), 33.10 g/dl (SD 1.53) and 14.53% (SD 1.60), respectively.

CAHD children with IDA were having mean serum ferritin, iron, TIBC and TIBC% of 8.17 ng/ml (SD 2.39), 26.15 µg/dl (SD 22.16), 438.84 µg/dl (SD 105.65) and 5.78% (SD 4.42); whereas non-iron deficient group were having 87.38 ng/ml (SD 61.23), 109.55 µg/dl (SD 59.24), 375.37 µg/dl (SD 107.12) and 30.26% (SD 15.51), respectively.

All these results were statistically significant ($p < 0.05$).

CCHD children with IDA were having mean Hb, Hct and RBC count of 14.37 g/dl (SD 3.02), 45.86% (SD 8.36) and 7.06 M/mm³ (SD 1.32); whereas non-IDA group were having 17.39 g/dl (SD 3.38), 59.45% (SD 13.05) and 8.28 M/mm³ (SD 1.97), respectively.

Among CCHD children, mean MCV, MCH, MCHC, and RDW in IDA group were 65.20 fl (SD 5.22), 20.45 pg (SD 2.47), 29.50 g/dl (SD 2.62) and 22.44% (SD 1.32); whereas in non-IDA group mean of 72.42 fl (SD 6.84), 21.67 pg (SD 3.59), 31.28 g/dl (SD 2.24) and 16.71% (SD 1.97), respectively.

CCHD children with IDA were having mean serum ferritin, iron, TIBC and TIBC% of 7.65 ng/ml (SD 2.46), 103.49 µg/dl (SD 19.54), 375.88 µg/dl (SD 82.42) and 7.98% (SD 4.27); whereas non-iron deficient group were having 63.61 ng/ml (SD 64.95), 109.55 µg/dl (SD 50.93), 375.37 µg/dl (SD 87.20) and 30.89% (SD 23.20), respectively.

These results were statistically significant ($p < 0.05$) except MCH value.

These two tables showed presence of much higher Hb, Hct, RBC count and RDW but slightly lower MCV, MCH, MCHC in CCHD children with IDA compared to CAHD children with IDA.

In a study by Binh TQ, et al [25] children with CCHD showed clearly higher values of RBC counts, hemoglobin, hematocrit compared to children with CAHD; whereas MCV, MCH, and MCHC showed almost similar values between these two groups.

6. SUMMARY

In this study, almost half (46.0%) of the children were between 6 months-1 year of age, and very less number (5.6%) of children were >10 years of age. Males were 54.0% and females were 46.0% with male: female ratio being 1.17:1. This study population includes 66.7% CAHD, and 33.3% CCHD with CAHD:CCHD ratio being 2:1. Among CAHD, 53.6% were VSD and among CCHD, 64.3% were TOF. Prevalence of iron deficiency anemia (IDA) was 34.1% in congenital heart diseases; and depleted stage was 30.2%, at risk group was 19.0% and normal body iron storage was 16.7%. 69.8% children showed microcytic, hypochromic (mc, hch) RBCs in peripheral smear and 76.2% children had RDW $\geq 14.5\%$ suggestive of iron deficiency state. Among 105 children with different stages of iron deficiency, RBC indices were useful as a screening tool in 84.8% children. 36.21% children of ≤ 1 year of age, 28.26% children of >1-5 years of age, 20% children of >5-10 years of age, and 40% children of >10 years of age had true IDA which was statistically significant ($p < 0.05$). Males were having a greater number of IDA (62.8%) than females (37.2%). Prevalence of IDA is more in CCHD (47.62%) than CAHD (27.38%). Only 9.52% CCHD had normal body iron stores when compared with 20.24% of CAHD having normal iron stores. This result was statistically significant ($p < 0.05$). All children with true IDA had microcytic, hypochromic (mc, hch) picture and all children with normal body iron had normocytic, normochromic (nc, nch) picture in peripheral smear. This result was statistically significant ($p < 0.05$). All children with true IDA had RDW $> 14.5\%$ and more than half of the children with normal body iron had RDW $< 14.5\%$. This result was statistically significant ($p < 0.05$). All children with true IDA had RBC indices suggestive of iron deficiency and more than half (54.17%) of at-risk group had RBC indices not suggestive of

iron deficiency. This result was statistically significant ($p < 0.05$). Mean MCV & MCH in true IDA were 66.39 ± 4.24 fl, 21 ± 2.13 prospectively, which was less than mean of non-iron deficient group. This result was statistically significant. The mean RDW among true IDA $19.29 \pm 3.8\%$ was more than the mean RDW of non-iron deficient group. Mean serum ferritin, serum iron, TIBC% in true IDA were 7.93 ± 2.41 ng/ml, 30.0 ± 21.15 μ g/dl and $6.80 \pm 4.44\%$ respectively, which was less than mean of non-iron deficient group. This result was statistically significant. Mean serum TIBC was 439.45 ± 94.45 μ g/dl in true IDA which was more than the mean of TIBC in non-iron deficient group. This result was statistically significant. Mean values of Hb, Hct, RBC count and RDW were higher in CCHD children with IDA (14.37 g/dl, 45.86% and 7.06 M/mm³, 22.44%) compared to CAHD children with IDA. Presence of slightly lower values of MCV, MCH, MCHC in CCHD children with IDA compared to CAHD children with IDA. CAHD children with IDA were having mean serum ferritin, iron, TIBC and TIBC% of 8.17 ng/ml, 26.15 μ g/dl, 438.84 μ g/dl and 5.78% ; whereas CCHD children with IDA had mean of 7.65 ng/ml, 103.49 μ g/dl, 375.88 μ g/dl and 7.98% .

7. CONCLUSIONS

Iron deficiency is an inadequately addressed and significant public health problem among Indian infants and children, which has negative effect on congenital heart diseases resulting in serious consequences. Hb, RBC indices, and iron status of the patients were analyzed and following inferences were drawn:

- Prevalence of iron deficiency anemia (IDA) in congenital heart diseases was 34.1% , which was more than one-third of the study population; and iron depleted stage was also found in another one-third of the study population.
- Though one-third of all congenital heart diseases had IDA, half (47.62%) of all CCHD were having IDA; whereas, 27.38% of CAHD were suffering from IDA.
- RBC indices were very useful as a screening tool to detect, prevent and treat iron deficiency or IDA in children with CHD. All children with true IDA, and almost all children with depleted stage had RBC indices suggestive of iron deficiency, whereas more than half of at-risk group can also be detected, making it a very good tool in resource limited settings.
- Among all RBC indices, RDW, MCV, MCH, MCHC were highly sensitive tests to detect iron deficiency in both CAHD and CCHD groups; which can be confirmed by doing serum ferritin which is test of choice in detecting IDA.

ABBREVIATIONS

CAHD- Congenital Acyanotic Heart Disease

CCHD- Congenital Cyanotic Heart Disease

IDA- Iron Deficiency Anemia

Hb- Hemoglobin

Hct- Hematocrit

MCV- Mean Corpuscular Volume

MCH- Mean Corpuscular Hemoglobin

MCHC- Mean Corpuscular Hemoglobin Concentration

CPS- Complete Peripheral Smear

MC, HCH- Microcytic Hypochromic

NC, NCH- Normocytic, Normochromic

RDW- Red Blood Cell Distribution Width

TIBC- Total Iron Binding Capacity

RBC- Red Blood Cell Count

WBC- White Blood Cell Count

TPC- Total Platelet Count

S/O- Suggestive Of

Sr- Serum

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