Original Article

Comparison of erythrocyte indices and haematological indices as markers of iron status of lagos children with cyanotic congenital heart disease

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Background: There are very few reports on the erythrocyte indices of children with cyanotic congenital heart disease in Nigeria. The study aims to document and compare the erythrocyte indices of children with cyanotic congenital heart disease attending the cardiology clinic of the department of Paediatrics, Lagos State University Teaching Hospital, Ikeja, Lagos.

Methods: The study was part of a large cross-sectional study involving children with cyanotic congenital heart disease and apparently healthy controls of the same age, sex and socioeconomic class. Full blood count (FBC), haemoglobin concentration, mean corpuscular volume (MCV), red blood cell (RBC) count, mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), serum Ferritin and blood film were done. Tests of statistical significance between subjects and controls included Student *t*-test for mean of continuous data while Fisher exact test was used for categorical data. Level of significance was set at P<0.05.

Results: One hundred and fifty children were studied over a period of six months. The mean age and standard deviation was 47.5±2.9 months. Mean PCV and the haemoglobin concentration and RBC count were significantly higher in CCHD across all age categories (P=0.000, P<0.05 respectively). The mean MCV was significantly higher in subjects older than 24 months compared with controls (P=0.039). There was no significant difference in the mean MCH across all age sub categories.

Conclusions: Erythrocyte indices were lower in iron deficient children compared to iron sufficient children with cyanotic congenital heart disease but the difference was not significant.

Keywords: Comparison; erythrocyte indices; children; lagos; cyanotic congenital heart disease

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Introduction

Among other conditions, uncorrected cyanotic congenital heart lesions may potentially have adverse effects on iron balance (1). These cardiac lesions keep the body in a state of constant hypoxia (2). Unabated hypoxias and attendant secondary erythrocytosis lead to polycythemia and depletion of iron stores (1). An additional cause of iron depletion is the bleeding tendency consequent upon thrombocytopenia and haemostatic abnormalities (2,3).

Reduction of body iron has three main stages, which constitute a continuum from iron depletion, through iron deficiency to iron deficiency anaemia (4). With iron depletion, iron requirement exceeds intake causing progressive depletion of iron store (4), is reflected as a reduction in

serum ferritin concentration (4). Iron deficiency refers to the stage when storage iron is depleted and there is insufficient iron absorption to counteract normal body losses (4). This is characterized by a reduction in serum ferritin, mean corpuscular volume (MCV) and mean corpuscular haemoglobin (MCH) (4). Iron deficiency anaemia is the most severe stage in which there is a decrease of iron in the red blood cells (4). Features include a reduction in serum ferritin, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and haemoglobin level (4).

The identification of iron deficiency in children with cyanotic congenital heart disease (CCHD) is important, as the condition contributes to worsening clinical outcome and may have negative long-term consequences on neurocognitive development (5) and growth (6). Clinical pallor is a reliable sign of moderate to severe anaemia (4). However, this feature is usually masked in patients with cyanotic congenital heart disease due to polycythemia (2), thus requiring a more objective analysis for early detection and prompt treatment. Hence, the diagnosis of iron deficiency is based primarily on laboratory measurements (7). A recent study reported a high prevalence of iron deficiency in children with cyanotic congenital heart disease (8). Other authors reported that the expected microcytic, hypochromic erythrocyte picture of iron deficiency anaemia is often absent in cyanotic patients with iron deficiency (9).

In the current study iron status of children with cyanotic congenital heart disease was described using a combination of haematological (MCV, MCH, MCHC) and biochemical indices (serum iron, TIBC and transferrin saturation), and the prevalence of ID in study subjects was determined by the serum ferritin status.

There is a paucity of studies on erythrocyte indices and iron deficiency among children with cyanotic congenital heart disease in Nigeria. It is expected that the data generated will further describe the magnitude of iron deficiency in children with cyanotic congenital heart disease, and increase the awareness on the need for routine monitoring of iron status and prompt treatment, as this will improve morbidity in the subjects.

This study aims to determine and compare the erythrocyte indices of children with cyanotic congenital heart disease and apparently healthy age, sex and socio-economic status matched controls.

Specific objectives

The specific objectives of the study are to:

(I) Document the erythrocyte indices of children with cyanotic congenital heart disease and apparently healthy controls.

(II) Compare erythrocyte indices of iron status, (haemoglobin concentration, mean corpuscular volume (MCV), red blood cell count (RBC), Mean corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC) of patients with cyanotic congenital heart disease with appropriate age and sex matched controls.

(III) Determine the relationship between erythrocyte indices (haemoglobin, MCV, MCHC) and iron status (ferritin level) in subjects and controls.

Methods

Study site

The study was carried out among children with cyanotic congenital heart disease attending the Paediatric cardiology clinic of Lagos State University Teaching Hospital, Ikeja, Lagos (LASUTH), Nigeria. The Lagos State University Teaching Hospital is an urban tertiary health center in Lagos State, South Western Nigeria. It is a major referral center serving the whole of Lagos State, which is a major point of entry into Nigeria from different parts of the world, and the economic nerve center of country. The Department of Paediatric Cardiology Unit runs a weekly clinic and attends to children up to twelve years of age according to LASUTH hospital policy.

An average of 25 children with acquired or congenital heart diseases are seen in the clinic. Cyanotic congenital heart disease accounts for about 20% of the total patients. Children with congenital and acquired heart disease are followed up once in 3 months in most cases. However, the clinical status of each patient determined how often they are seen. Patients were recruited consecutively until the desired sample 150 was attained from May 2015 through October 2015. Approval for the study was obtained from the Ethics Committee of LASUTH, consent and assents were obtained as necessary.

Approval for the study was obtained from the Ethics Committee of Lagos State University Teaching Hospital, Ikeja, Lagos State. Written and informed consent was obtained from the parents/caregivers of the subjects and controls.

The study was prospective cross sectional and analytical, involving consecutive children with cyanotic congenital

Table 1 The grading scale used for erythrocyte morphology

Grading scale	% of the cells vary from a normal red blood cell
Normal	5%
1+	5–10%
2+	10–25%
3+	50–75%
4+	75% and above

heart disease confirmed by echocardiography. Subjects and controls were matched for gender, sex and socioeconomic class. The inclusion criteria for subjects were: Confirmed case of cyanotic congenital heart disease by echocardiography that has not had corrective surgery aged 6 months to 12 years, subjects who have no symptoms or signs attributable to an acute illness in the preceding 4 weeks.

Patients whose parents denied consent, those who had a partial exchange transfusion, blood transfusion or repeated phlebotomy in 3 months, those on iron supplementation prior to recruitment and those diagnosed sickle cell anaemia and other chronic illnesses were excluded from the study. The estimated sample size was determined using the standard statistical formula for comparative design studies (10).

Those who were recruited had venepuncture don according to the WHO guidelines (11). A maximum of 5 mL of blood was collected: 3 mL into a plain bottle and 2 mL into Na EDTA bottle. The specimen containers were labelled using codes to avoid mix up. The 2 mL samples in the SODIUM EDTA bottle were protected from light at all times using sheets of black polythene and was transported to the laboratory within 4 to 6 hours where complete blood count (CBC), and blood film for erythrocyte morphology were done. A SYSMEX XS-500 analyser was used to provide an automated complete blood count. Whole blood (EDTA added) samples were used. Quality assurance was ensured by running two sets of complete blood count for the first five study subjects. One sample was run at the Research Laboratory of the Lagos State College of Medicine (LASUCOM) and the second sample was run at the laboratory of the Department of Haematology, of LASUTH for corroboration. The CBC includes the haemoglobin concentration, haematocrit, total white blood cell count and differential, platelet count, mean cell volume, mean cell haemoglobin, mean cell and the red cell distribution width. The wedge slide technique was the method employed for the preparation of peripheral blood smears and staining of the peripheral blood smear was by Wright–Giemsa staining method. The red blood cells stained a pink colour. The morphology of the blood film was described by the trained laboratory technologist using the operational definitions stated below and compared the findings to an atlas of haematology (11).

Operational Definitions used to describe the erythrocyte morphology were:

- Anisocytes—are red blood cells with variable size (12).
- Elliptocytes—are red blood cells that are elliptical in shape due to cell membrane defect (12).
- Hypochromia—are red blood cells with defective haemoglobinization so that they are abnormally pale (12).
- Macrocytes—are red blood cells that are large in size (diameter >8 μm) (12).
- Microcytes—are red blood cells that are small in size (diameter <5 μm) (12).
- Normocytes—are normal red blood cells with normal size (diameter 5–8 μm) (12).
- Poikilocytes—are red blood cells with variably shaped cells (12).
- Spherocytes—are red blood cells that are spherical in shape due to cell membrane Defect (12).
- Target cells—are red cells with central staining with precipitated haemoglobin that resembles a bull's eye (12).

Using the above operational definitions, the percentage of cells that vary in size from a normal red blood cell was observed in at least 10 oil immersion fields. The erythrocyte morphology was graded using the scale listed in *Table 1* (12):

Serum iron was measured by using Iron Ferrozine test kit.

Data analysis was done using Microsoft Excel statistical package and the Statistical Package for Social Sciences® version 20.0. Measures of statistical location like mean, median standard deviation and range were derived for continuous variables. Categorical variables were represented using frequency and percentage. Continuous variables were compared using Student t-test, while test of association for categorical variables were tested with Fisher exact test. Kolmogorov–Smirnov test was used to test for normality assumption of continuous data. Student t-test was used to compare mean values of normally distributed data while Mann Whitney U test was used for skewed or not normally distributed data. The degree of agreement between different methods used to evaluate the same parameter was estimated using Kappa statistics. Probability value (P value) less than 5% (0.05) was accepted as statistically significant.

The study subjects were classified as having:

(I) Iron deficiency if:
- ❖ MCV < expected range for age of the study subjects (13).
- ❖ MCHC < expected range for age of the study subjects (13).
- ❖ Serum ferritin less than 12 ng/L for study subjects less than 5 years or less than 15 ng/L for subjects aged 5 years or more (7).

(II) Iron Deficiency Anaemia if:
- ❖ Haemoglobin < expected range for age of the study subjects (13).
- ❖ Plus, features of Iron deficiency.

Results

Characteristics of the study populations

A total of 150 children who met the study criteria was recruited over a period of 6 months (May to October 2015): 75 with cyanotic congenital heart disease and 75 apparently healthy controls respectively.

In the distribution of heart lesions among the subjects, tetralogy of Fallot was the most common heart lesion as it occurred in 39 out of 75 (52%) patients recruited. The least common echocardiography diagnosis among the subjects was Ebstein anomaly and complex heart disease which accounted for 1.3% of the subjects.

Haematological profile of study subjects

The haematological profile of the study subjects were categorized according to age groups. The comparisons of the mean values of red blood cell indices between children with cyanotic congenital heart disease and apparently healthy controls aged 6 to 23 months are shown in *Table 2*. The mean values for packed cell volume was significantly higher in children with cyanotic congenital heart disease (P=0.00). Similarly, the haemoglobin concentration, red blood cell count, RDWSD and RDWCV were also significantly higher in cases than controls (P<0.05). On the other hand, the platelet concentration was higher in the controls compared to the cases (P=0.00). Comparing the MCV, MCH and Mean corpuscular haemoglobin concentration there was no significant difference between cases and controls.

The comparisons of the mean values of red blood cell indices between children with cyanotic congenital heart disease and apparently healthy controls aged 24 to 59 months are shown in *Table 3*. The mean values for packed cell volume was significantly higher in children with cyanotic congenital heart disease (P=0.00). Similarly, the haemoglobin concentration, red blood cell count, RDWSD and MCV were also significantly higher in cases than controls (P=0.000, 0.003, 0.000, 0.039 respectively). On the other hand, the MCH and Mean corpuscular haemoglobin concentration and platelet concentration was not significantly different between cases and controls. (P=0.092, 0.069, 0.072 respectively).

The comparisons of the mean values of red blood cell indices between children with cyanotic congenital heart disease and apparently healthy controls aged 60 to 144 months are shown in *Table 4*. The mean values for packed cell volume was significantly higher in children with cyanotic congenital heart disease (P=0.00). Similarly, the haemoglobin concentration, red blood cell count, RDWSD and MCV were also significantly higher in cases than controls (P=0.00, 0.00, 0.002 and 0.006 respectively) On the other hand, the platelet concentration was higher in the controls compared to the cases (P=0.014). On the other hand, the MCH and Mean corpuscular haemoglobin concentration and RDWCV were not significantly different between cases and controls (P=0.159, 0.156, 0.120 respectively).

Erythrocyte morphology of study subjects

The comparisons of erythrocyte morphology distribution between CCHD and controls subjects are shown in *Table 5*. The proportion of patients with normocytosis and normochromia was more in controls than CCHD (P=0.02 respectively). There was no significant difference in the proportion of patients that had hypochromia, microcytosis, anisocytosis, poikilocytosis and target cells (P<0.05).

Comparison of the haematological indices of the study subjects

The comparison of haematological parameters between children with cyanotic congenital heart disease and controls is shown in *Table 6*. The haematological parameters were categorized as low, normal or high. There was a significant difference between CCHD and controls in the number of subjects who had normal and high MCV (P=0.000 respectively). On the contrary there was no significant difference between CCHD and controls in those that had low MCV (P=0.499). With respect to MCH, low values

Table 2 Comparison of mean haematological profile values of subjects in 6–23 months' age group

Parameters measured	Study subjects		t value	P value
	CCHD (n=75)	Control (n=75)		
PCV (%)				
Mean ± SD	45.7±12.4	33.9±2.9	4.51	0.000*
Median	42.1	34.6		
Range	28.6–74.1	27.9–38.7		
Haemoglobin concentration (g/dL)				
Mean ± SD	13.8±3.9	10.28±0.8	4.28	0.000*
Median	12.25	10.6		
Range	8.6–22.7	8.6–11.6		
Red blood cell count ($\times10^{12}$/L)				
Mean ± SD	5.4±1.6	4.4±0.3	3.073	0.000*
Median	5.2	4.5		
Range	2.9–8.9	3.6–5.1		
Platelet ($\times10^{3}$/μL)				
Mean ± SD	183.8±10.9	288.8±6.4	4.24	0.000*
Median	157	297		
Range	43–531	161–377		
MCV (fL)				
Mean ± SD	93.9±11.2	78.9±6.5	1.902	0.063
Median	84.2	80.4		
Range	65.7–100.0	63.9–86.6		
MCH (pg/cell)				
Mean ± SD	25.3±4.5	23.8±2.4	1.432	0.159
Median	26.1	23.5		
Range	17.1–31.9	17.7–28.6		
MCHC (g/dL)				
Mean ± SD	30.1±2.0	30.2±1.1	0.349	0.729
Median	30.4	30.5		
Range	24.8–33.7	27.7–31.6		
RDWSD (fL)				
Mean ± SD	51.7±8.4	43.2±3.3	4.615	0.000*
Median	51.4	44.1		
Range	38.8–67.4	32.9–48.5		
RDWCV (%)				
Mean ± SD	17.9±3.4	15.4±1.7	3.241	0.002*
Median	18.3	15.6		
Range	12.0–24.8	12.4–19.0		

*significant.

Table 3 Comparison of mean haematological profile values of subjects in 24–59 months' age group

Parameters measured	Study subjects		t value	P value
	CCHD (n=75)	Control (n=75)		
PCV (%)				
Mean ± SD	49.9±15.5	35.8±2.2	4.9	0.000*
Median	50.8	36.3		
Range	9.4–79.6	32.1–39.1		
Haemoglobin concentration (g/dL)				
Mean ± SD	15.3±5.8	10.6±0.6	4.28	0.000*
Median	15.4	10.9		
Range	2.6–24.9	9.6–11.5		
Red blood cell count ($\times10^{12}$/L)				
Mean ± SD	5.7±1.9	4.7±0.4	3.09	0.003*
Median	5.4	4.7		
Range	1.2–9.7	3.8–5.1		
Platelet ($\times10^{3}$/μL)				
Mean ± SD	195.7±92.2	235.1± 83.2	1.835	0.072
Median	193	225		
Range	37–351	66–379		
MCV (fL)				
Mean ± SD	82.1±7.9	78.6±4.9	2.118	0.039*
Median	83.1	78.9		
Range	65.7–102.0	69.6–85.8		
MCH (pg/cell)				
Mean ± SD	25.3±4.5	23.8±2.4	1.715	0.092
Median	26.1	23.5		
Range	17.1–31.9	17.7–28.6		
MCHC (g/dL)				
Mean ± SD	30.1±2.0	29.8±0.9	1.952	0.069
Median	30.4	29.9		
Range	24.8–33.7	27.6–31.6		
RDWSD (fL)				
Mean ± SD	50.9±8.0	44.4±4.1	3.999	0.000*
Median	48.4	45.7		
Range	36.7–67.4	36.3–51.9		
RDWCV (%)				
Mean ± SD	18.5±7.8	15.6±3.3	1.874	0.066
Median	16.1	14.6		
Range	12.9–54.3	13.2–24.8		

*significant.

Table 4 Comparison of mean haematological profile of subjects in 60-144 months' age group

Parameters measured	Study subjects		t value	P value
	CCHD (n=75)	Control (n=75)		
PCV (%)				
Mean ± SD	60.3±12.3	36.7±3.1	8.71	0.000*
Median	61.7	36		
Range	37.1–78.8	26.7–41.3		
Haemoglobin concentration (g/dL)				
Mean ± SD	18.8± 3.7	11.3±1.04	9.1	0.000*
Median	18.4	11.5		
Range	11.5–23.7	8.2–12.4		
Red blood cell count ($\times 10^{12}$/L)				
Mean ± SD	6.9±1.1	4.4±0.43	8.91	0.000*
Median	7.1	4.5		
Range	4.5–8.6	3.69–5.17		
Platelet ($\times 10^3$/μ L)				
Mean ± SD	170.3± 79.5	240.8±101.2	2.569	0.014*
Median	146.5	234		
Range	55–340	32–453		
MCV (fL)				
Mean ± SD	86.9±7.7	81.1±5.2	2.919	0.006*
Median	87.9	80.7		
Range	73.1–102	72.2–89.4		
MCH (pg/cell)				
Mean ± SD	27.1±2.7	15.2±2.4	1.432	0.159
Median	27.8	14.6		
Range	21.1–32.2	12.4–24.8		
MCHC (g/dL)				
Mean ± SD	30.4±10.6	29.9±2.4	1.444	0.156
Median	31.3	30.5		
Range	28.9–80.6	23.1–32.1		
RDWSD (fL)				
Mean ± SD	49.4±7.3	43.3±4.4	3.39	0.002*
Median	48.4	44.1		
Range	38.8–66.6	33.0–51.0		
RDWCV (%)				
Mean ± SD	15.1±1.8	14.7±1.3	1.589	0.120
Median	14.9	14.3		
Range	12.8–19.1	12.6–17.1		

*significant.

Table 5 Erythrocyte morphology distribution in the blood film among subjects

Erythrocyte morphology	Study subjects number (%)		OR	95% CI	P value
	CCHD	Control			
Hypochromia	51 (68.0)	46 (61.3)	1.62	0.81–3.21	0.167
Microcytic	18 (24.0)	21 (28.0)	0.812	0.39–1.68	0.578
Normocytic	9 (12.0)	21 (28.0)	0.35	0.14–0.83	0.020*
Normochromia	9 (12.0)	21 (28.0)	0.35	0.14–0.83	0.020*
Anisocytosis	32 (42.7)	30 (40.0)	0.99	0.51–1.91	0.981
Poikilocytosis	15 (20.0)	11 (14.7)	1.45	0.61–3.40	0.380
Target cells	4 (5.3)	0 (0.0)	9.50	0.50–179.7	0.133

*significant.

were significantly more frequently encountered among controls while normal and high values were significantly commoner among cases.

Comparison of the erythrocyte morphology and haematologic indices

Kappa statistics was used to assess the degree of agreement between microcytosis as determined by blood film and low MCV as determined by auto analyzed complete blood count. The same statistics was also used to assess the degree of agreement between hypochromia as determined by blood film and low MCH as determined by auto analyzed complete blood count.

Out of the 75 children with CCHD, 18 were identified as having microcytosis by blood film while 5 were identified as having low MCV. None of the subjects had both microcytosis and low MCV. When this was subjected to Kappa statistics of agreement the result was: k=0.12 (95% CI: 0.031–0.202). This was interpreted using the Kappa statistics grading scale as a poor agreement.

A similar analysis was done in the 75 controls, of which 4 subjects had microcytosis and 21 had low MCV but none had both microcytosis and low MCV. It yielded k=0.01 (95% CI: 0.013–0.184) denoting a poor agreement.

Further analysis was also done to assess the degree of agreement between hypochromia as determined by blood film and low MCH as determined by auto analyzed complete blood count. Out of the 75 children with CCHD 36 were identified as having hypochromia by blood film while 9 were identified as having low MCH, 15 of the subjects had both microcytosis and low MCH. When this was subjected to Kappa statistics of agreement the result was: k=0.062, (95% CI: 0.116–0.241). This was interpreted using the Kappa statistics grading scale, denoting a poor agreement. A similar analysis was done in the 75 controls, of which 29 subjects had microcytosis and 28 had low MCH, 17 subjects had both hypochromia and low MCH. It yielded k=0.592, (95% CI: 0.446–0.738) denoting a good agreement between hypochromia and low MCH in controls.

Discussion

In the present comparative study, the commonest cyanotic congenital heart disease is TOF. Tetralogy of Fallot has been consistently documented as the commonest cyanotic congenital heart disease (8,14-16). Ebstein's anomaly is a rare cyanotic congenital heart disease and being the least diagnosis in the present study is not surprising.

The packed cell volume and haemoglobin concentration are higher in cases with cyanotic congenital heart diseases irrespective of age strata than the controls in the present study. These blood parameters reflect red blood cell counts. Higher values of these parameters have been demonstrated in previous studies (17,18). Physiologic response to chronic hypoxemia in cyanotic congenital heart diseases includes increased erythropoietin production in the kidneys, increased bone marrow erythropoiesis and resultant increase in packed cell volume and hemoglobin concentration (19).

Lower values of platelet count were noticed in subjects in the present study in all age groups compared to the controls. Thrombocytopenia as a hematologic index in subjects with cyanotic congenital heart diseases have been previously documented (18,20). The pathogenesis of thrombocytopenia in cyanotic disease conditions are decrease platelet and megakaryocyte production,

Table 6 Comparison of the hematological indices of the study subjects

Hematologic indices	Study subjects, n (%)		Total, n=150, n (%)	P value
	CCHD	Controls		
MCV (fL)				
Low	5 (6.7)	4 (5.3)	9 (6)	0.499
Normal	49 (65.3)	69 (92.0)	118 (78.7)	0
High	21 (28.0)	2 (2.7)	23 (15.3)	0
MCH (pg/cell)				
Low	24 (32.0)	45 (60.0)	69 (46)	0
Normal	43 (57.3)	30 (40.0)	73 (48.7)	0.02
High	8 (10.7)	0 (0)	8 (5.3)	0.003
MCHC (g/dL)				
Low	26 (34.7)	37 (49.3)	63 (42.0)	0.049
Normal	47 (62.7)	38 (50.7)	85 (56.7)	0.093
High	2 (2.7)	0 (0)	2 (1.3)	0.248
Platelet ($\times 10^3/\mu L$)				
Thrombocytopenia	34 (45.3)	7 (9.3)	41 (27.3)	4.768
Normal	41 (54.7)	68 (90.7)	109 (72.7)	4.768
PCV (%)				
Anemia	8 (10.7)	32 (42.7)	40 (26.7)	0
Normal	19 (25.3)	43 (57.3)	62 (41.3)	0
Polycythemia	48 (64.0)	0 (0)	48 (32)	3.834
RDWCV (%)				
Normal	34 (45.3)	18 (24)	52 (34.7)	0.004
Raised	41 (54.7)	57 (76)	98 (65.3)	0.004
RDWSD (fL)				
Low	3 (4.0)	1 (1.3)	4 (2.7)	0.309
Normal	19 (25.3)	74 (98.7)	93 (62)	1.773
Raised	53 (70.7)	0 (0.0)	53 (35.3)	3.717

*, statistically significant P<0.05. Statistical test used Fisher exact. NB: values in parenthesis are % of number in group.

increase platelet activation and destruction (20). These mechanisms occur largely from reduced platelet production in the lung beds from less shunting of blood through the pulmonary veins in cyanotic heart diseases and delivery of megakaryocytes to the systemic arterial circulation through the shunt (20).

The mean values of mean corpuscular volume were normal in both cases and controls. A significant higher value was however noticed in older cases than controls. Mean corpuscular volume, is the volume of red cells. Higher mean MCV has also been reported in subjects with cyanotic congenital heart diseases (9,21). Low mean corpuscular volume has also been reported in subjects with cyanotic congenital heart diseases that had iron deficiency anaemia (22). MCV has been documented not to be a sensitive indicator of iron deficiency in cyanotic patient (9). The trend of higher mean MCV in CCHD in the present study is most likely as a consequence of unabated hypoxia

Table 7 Comparison between haematological indices and iron status among CCHD subjects

Hematological indices	Iron status		t value	P value
	Iron deficient	Iron sufficient		
PCV (%)	48.8±12.6	50.9±14.8	0.379	0.706
Hb concentration (g/dL)	15.0±4.0	15.6±4.8	0.308	0.759
MCV (fL)	79.6±5.4	84.9±9.4	1.588	0.117
MCH (pg/cell)	25.1±2.6	26.0±3.7	0.701	0.486
MCHC(g/dL)	30.6±1.5	31.3±6.6	0.287	0.775
RDWSD (fL)	49.7±5.9	51.2±8.2	0.499	0.619
RDWCV (%)	17.2±3.4	16.6 ±3.2	0.394	0.473

stimulating haemopoiesis thus providing more rapid supply of young red cells which are likely to have higher MCV (22).

No significant difference was noticed in the mean corpuscular haemoglobin values of cases and controls across all age strata. Mean corpuscular haemoglobin, the amount of haemoglobin per red cell is a haematologic parameter for diagnosis of iron deficiency anaemia. MCV and MCH have been shown to be normal red cell indices in early phases of iron deficiency anaemia and are not specific markers in making the diagnosis (23).

RDW was significantly higher in children with cyanotic congenital heart disease compared to the controls. This is in keeping with previous documented findings (8,24). Increased RDW is an indicator of the variation in erythrocyte volume which is expected to be increased when there is an exaggerated hematopoietic response as found in children with cyanotic congenital heart disease (25). More so, increased RDW is a sensitive marker for diagnosing iron deficiency anaemia.

A significant higher proportion of controls had normochromic and normocytosis red cell distribution. Target cells were solely seen in subjects with cyanotic heart diseases. No significant difference in proportion of subjects with hypochromic and microcytic cells. Examination of the morphology of the peripheral blood smears is not specific in diagnosing IDA and this can explain the result seen in the present study. Poor agreement was noticed between microcytosis and low MCV in both cases and controls in the current study. Poor agreement was also noticed between hypochromia and MCH in the cases.

Mean haematologic parameters (PCV, haemoglobin concentration, MCV, MCH, RDWSD, and RDWCV) was lower in iron deficient subjects with cyanotic heart diseases compared to those with sufficient iron status. This finding was however not statistically significant (*Table 7*). Similar finding was with significant difference was previously reported by Onur *et al.* (24) and Mujib *et al.* (26).

The mean serum iron in children with cyanotic congenital heart disease was significantly lower than controls across the age subcategories. This finding is consistent with previously documented studies (21,24) The explanation for the low serum iron in CCHD is the presence of chronic hypoxemia that triggers increase in erythropoietin from the kidneys with subsequent bone marrow stimulation for production of red cells, polycythemia, increase in iron demand and its depletion (27,28).

In conclusion, the mean packed cell volume, haemoglobin concentration and red blood cell count were significantly higher in CCHD than controls in the current study. Erythrocyte indices were lower in iron deficient children compared to iron sufficient children with cyanotic congenital heart disease but the difference was however not statistically significant.

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Footnote

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at http://dx.doi.org/10.21037/aob.2020.01.01). The authors have no

conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). Approval for the study was obtained from the Ethics Committee of Lagos State University Teaching Hospital, Ikeja, Lagos State. Written and informed consent was obtained from the parents/caregivers of the subjects and controls.

References

1. Rosove MH, Perloff JK, Hocking WG, et al. Chronic hypoxaemia and decompensated erythrocytosis in cyanotic congenital heart disease. Lancet 1986;2:313-5.
2. Verel D. Blood volume changes in cyanotic congenital heart disease and polycythemia rubra vera. Circulation 1961;23:749-53.
3. Tempe DK, Virmani S, Rigler B, et al. Coagulation abnormalities in patients with cyanotic congenital heart disease. J Cardiothorac Vasc Anesth 2002;16:752-65.
4. Oechslin E. Hematological management of the cyanotic adult with congenital heart disease. Int J Cardiol 2004;97:109-15.
5. Lozoff B, Jimenez E, Wolf AW. Long-Term Developmental Outcome of Infants with Iron Deficiency. N Engl J Med 1991;325:687-94.
6. Kochanowski BA, Sherman AR. Cellular growth in iron-deficient rats: effect of pre- and postweaning iron repletion. J Nutr 1985;115:279-87.
7. World Health Organization. Iron Deficiency Anaemia: Assessment, Prevention, and Control- A Guide for Program Managers. Geneva, Switzerland: World Health Organization; 2001. WHO/NHD/01.3.
8. Ogunkunle O. Erythrocyte indices of iron status in children with cyanotic congenital heart disease at the University College Hospital, Ibadan. Niger J Paediatr 2012;40:75-8.
9. Kaemmerer H, Fratz S, Braun SL, et al. Erythrocyte indexes, iron metabolism, and hyperhomocysteinemia in adults with cyanotic congenital cardiac disease. Am J Cardiol 2004;94:825-8.
10. Kirkwood BR, Sterne JAC. Calculation of required sample size. Essential Medical Statistics, 2nd. Oxford: Blackwell, 2003:422-8.
11. WHO guidelines on drawing blood : best practices in phlebotomy. World Heal Organ. 2010:1-105.
12. Erythrocyte morphology and inclusions. In: Turgeon ML. editor. Textbook of Clinical Hematology: Theory and Procedures. 4th ed. Baltimore: Lippincott Williams and Wilkins; 2005:99-111.
13. Pesce MA. Reference Ranges for Laboratory Tests and Procedures. In: Kliegman RM, Behrman RE, Jenson HB, Stanton BF. editors. Nelson Textbook of Pediatrics, 18th ed. Philadelphia: Saunders, 2007:2939-54.
14. Bakhtyar Zahid S, Jan AZ, Ahmed S, et al. Spectrum of congenital heart disease in children admitted for cardiac surgery at Rehman Medical Institute, Peshawa, Pakistan. Pak J Med Sci 2013;29:173-6.
15. Jatav RK, Kumbhare MB, Srinivas M, et al. Prevalence and pattern of congenital heart diseases in Karimnager, Andhra Pradesh, India: diagnosed clinically and by trans-thoracic-two-dimensional echocardiography. Int J Res Med Sci 2014;2:186-92.
16. Animasahun BA, Adekunle MO, Kusimo OY. Time to diagnosis, socioeconomic and clinical features of patients with congenital heart diseases in a teaching hospital in Nigeria. J Public Health Emerg 2018;2:1-7.
17. Westbury SK, Lee K, Reilly-Stitt C, et al. High haematocrit in cyanotic congenital heart disease affects how fibrinogen activity is determined by rotational thromboelastometry. Thromb Res 2013;132:e145-51.
18. Chamanian S, Maleki M, Rohani A, et al. Haematologic abnormalities in cyanotic congenital heart diseases patients. J Cardiothorac Med 2015;3:270-2.
19. Warrell DA, Cox TM, Firth JD, et al. Cyanotic heart disease: A multisystemic disorder. Oxford Textbook of Medicine, 4th edition. London: Oxford University Press; 2003.
20. Lill MC, Perloff JK, Child JS. Pathogenesis of thrombocytopaenia in cyanotic congenital heart disease. Am J Cardiol 2006;98:254-8.
21. Tay ELW, Peset A, Papaphylactou M, et al. Replacement

therapy for iron deficiency improves exercise capacity and quality of life in patients with cyanotic congenital heart disease and/or the Eisenmenger syndrome. Int J Cardiol 2011;151:307-12.
22. Onur CB, Sipahi T, Tavil B, et al. Diagnosing iron deficiency in cyanotic heart disease. Indian J Pediatr 2003;70:29-31.
23. Perloff JK, Rosove MH, Child JS, et al. Adults with cyanotic congenital heart disease: hematologic management. Ann Intern Med 1988;109:406-13.
24. Sultana GS, Haque SA, Sultana T, et al. Value of red cell distribution width (RDW) and RBC indices in the detection of iron deficiency anaemia. Mymensingh Med J 2013;22:370-6
25. Aulakh R, Sohi I, Singh T, et al. Red cell distribution width (RDW) in the diagnosis of iron deficiency with microcytic hypochromic anemia. Indian J Pediatr 2009;76:265-8.
26. Mujib ASM, Mahmud ASM, Halder M, et al. Study of haematologic parameters in children suffering from Iron deficiency anaemia in Chattagram Maa-o-Shishu general hospital, Chittagong, Bangladesh. Anaemiaa.2014,Article ID 503981, 10 pages
27. Lang'o MO, Githanga JN, Yuko-Jowi CA. Prevalence of iron deficiency in children with cyanotic heart disease seen at Kenyatta National hospital and Mater hospital Nairobi. East African Medical Journal 2009;86:S47-51
28. Guyatt GH, Oxman AD, Ali M, et al. Laboratory diagnosis of iron-deficiency anemia: an overview. J Gen Intern Med 1992;7:145-53.

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