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Original article

CLINICAL UTILITY OF RETICULOCYTE INDICES IN THE DIAGNOSIS AND MANAGEMENT OF PAEDIATRIC SICKLE CELL DISEASE PATIENTS IN PORT HARCOURT, NIGERIA

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Abstract: **Introduction:**Sickle cell anemia (SCA) is a hereditary blood disorder resulting from a point mutation in the β-globin gene, leading to the production of abnormal hemoglobin S that distorts red blood cell morphology and impairs their function. Reticulocyte indices, which measure immature red blood cells in circulation, are key indicators of bone marrow response and erythropoietic activity in SCA patients.

Materials and Methods: This cross-sectional study examined reticulocyte indices in individuals with sickle cell anemia. It involved 45 children aged 2 to 19 at the Rivers State University Teaching Hospital. Blood samples were collected from each patient and placed in EDTA bottles. Reticulocyte counts were performed using the New Methylene Blue staining method and counted under a light microscope. Hemoglobin (Hb) and packed cell volume (PCV) were measured with a Veri-Q RED Hemoglobin meter. Reticulocyte indices, including absolute reticulocyte count (ARC), reticulocyte index (RI), and reticulocyte production index (RPI), were calculated using MDCalc.

Results: The mean reticulocyte count was 1.33 ± 0.22%, while the mean reticulocyte production index was 0.40 ± 0.09. Reticulocyte production index (RPI) showed a positive and significant correlation with Hb and PCV in both crisis and steady states ($r = 0.820$, $p = 0.02$). Additionally, RPI and RC were significantly correlated in patients on and off hydroxyurea ($p < 0.01$). Only 2 (4.4%) SCA patients demonstrated appropriate bone marrow response, while the remaining 43 (95.6%) SCA patients were hypoproliferative.

Conclusion: This study found derangements in the reticulocyte parameters indicating hypoproliferative anaemia. There is a need to monitor the patients closely by periodic reticulocyte counts for overall improved patient outcomes and quality of life in SCA patients.

Keywords: Sickle Cell Anaemia, hydroxyurea, reticulocyte count, reticulocyte production index, hypoproliferative anaemia, absolute reticulocyte count.

INTRODUCTION

Sickle cell disease (SCD) encompasses a spectrum of inherited hemoglobinopathies characterized by mutations in the HBB gene, which encodes the β-globin subunit of hemoglobin (1). Sickle cell disease (SCD) comprises a heterogeneous group of hemoglobinopathies, including sickle cell anemia (SCA), hemoglobin SC disease (HbSC), and hemoglobin S/β-thalassemia, which may present as either β^+ or β^0 thalassemia depending on the nature of the β-globin gene mutation. Several other minor variants exist within the group of SCDs, albeit not as common as the varieties mentioned above. It is essential to mention the sickle cell trait (HbAS), which carries a heterozygous mutation and seldom presents clinical signs or symptoms. Sickle cell anaemia is the most common form of SCD, with a lifelong affliction of haemolytic anaemia requiring blood transfusions, pain crises, and organ damage (2).

Sickle cell anaemia (SCA) involves mutations in haemoglobin (Hb), a protein in red blood cells (RBCs). Normal adult haemoglobin includes HbA1 (95%), HbA2 (less than 4%), and HbF (mostly in fetuses). The sickle cell mutation occurs when valine replaces glutamine at the beta-globin chain's sixth position (3). The sicklecell mutation follows an autosomal codominant inheritance pattern. Homozygosity for the

HbS allele (HbSS genotype) leads to the most severe form of sickle cell anemia, whereas heterozygosity (HbAS genotype) typically results in a carrier state with minimal or no clinical manifestations. Additional genotypic variants within the sickle cell disease spectrum include HbS/β^0-thalassemia and HbSC disease, which arise from compound heterozygosity involving the HbS allele and either β-thalassemia or hemoglobin C mutations (4, 5).

Sickle cell anaemia is characterized by haemolysis and vaso-occlusive crises (VOC). The mutation in the beta-globin gene causes haemoglobin S (HbS) to form rigid polymers when deoxygenated, leading to the cyclical sickling of red blood cells (RBCs). Over time, this sickling becomes irreversible, increasing the risk of haemolysis and VOC. Factors like the low oxygen affinity of HbS, high 2,3-diphosphoglycerate, and increased sphingokinase-1 activity contribute to HbS polymerization (6). Oxidative stress from auto-oxidation of HbS damages RBC membranes (7). Haemolyzed cells release free haemoglobin and arginase 1, reducing nitric oxide and increasing oxidative stress and vascular remodeling. Vaso-occlusion is further promoted by interactions between sickle RBCs, free haeme, reactive oxygen species, the endothelium, neutrophils, and platelets (8).

Globally, 20 to 25 million people are affected by SCD, with 12 to 15 million residing in Africa, while developed countries account for only 10% of cases (9). Sub-Saharan Africa accommodates 75% of all patients with SCD and 70% of all SCD births globally, with many affected children dying before the age of 5 (10).

Nigeria bears the highest burden of sickle cell disease (SCD) in Sub-Saharan Africa, with an estimated 2–3% of its population affected. Globally, approximately 300,000 infants are diagnosed with SCD each year, with Sub-Saharan Africa accounting for nearly 75% of these cases. Nigeria alone contributes between 100,000 and 150,000 newborns annually, representing roughly one-third of the global incidence (11). The prevalence of SCD within Nigerian states ranges from 1% to 3%, with HbSS being the predominant haemoglobin variant. A 2.4% prevalence of the HbSS genotype was reported in southwestern Nigeria (11, 12). In North Central Nigeria, a 5% prevalence rate of SCD among children was identified by Diwe et al. (13).

In patients with sickle cell anaemia (SCA), a peripheral blood smear shows elongated red blood cells (RBCs) with tapering ends (drepanocytes). Additional findings can include Howell-Jolly bodies (DNA remnants, indicating autosplenectomy), target cells (seen in thalassaemia and sickle-thalassaemia syndromes), polychromatic cells (reticulocytes, indicating a marrow response to haemolysis), and sometimes nucleated red blood cells (14). These findings are not confirmatory for SCA. Confirmation requires haemoglobin electrophoresis, high-performance liquid chromatography, or isoelectric focusing. DNA-based techniques are used for uncertain diagnoses and prenatal testing through amniocentesis, while foetal DNA capture from maternal blood remains investigational (15).

Treatment and management of sickle cell anaemia focus on alleviating symptoms, preventing complications, and improving the quality of life. This typically involves regular blood transfusions to manage anaemia, pain management strategies including medications and hydration, and the use of hydroxyurea to reduce the frequency of pain crises and the need for transfusions. Preventive measures include vaccinations and antibiotics to reduce infection risks. In severe cases, haematopoietic stem cell transplantation may be a potential cure (5).

Reticulocytes, the earliest form of erythrocytes released by the bone marrow into peripheral circulation, serve as a dependable indicator of recent erythropoietic activity. Under physiological conditions, nucleated erythroid precursors complete clonal maturation within the bone marrow over a period of 1–3 days (16). Following nuclear extrusion, reticulocytes—immature erythrocytes—are released into peripheral circulation, where they persist for approximately 1–2 days before maturation into fully developed erythrocytes. These cells can be detected using supravital staining techniques for manual reticulocyte enumeration or via automated methods that quantify residual ribonucleic acid (17).

The reticulocyte index (RI) is a calculated value employed in haematology to assess bone marrow function and erythropoiesis, the body's process of producing red blood cells (RBCs). Reticulocytes are immature RBCs recently released from the bone marrow into the peripheral blood. The reticulocyte count is a critical parameter that reflects the rate of RBC production. The RI is particularly significant in the evaluation of anaemia. It aids in differentiating between anaemias caused by decreased production of RBCs and those caused by increased destruction or loss of RBCs. By assessing the RI, healthcare professionals can determine whether the bone marrow responds appropriately to the body's demand for RBCs (18).

The reticulocyte index (RI) is calculated by multiplying the reticulocyte percentage by the patient's haematocrit, dividing this product by a normal haematocrit value of 45%, and then adjusting this result by a correction factor to account for early reticulocyte release in severe anaemia. This formula accounts for the fact that in severe anaemia, reticulocytes are released into the bloodstream earlier than usual and thus spend more time maturing in the peripheral circulation. The correc-

tion factor adjusts for this premature release, typically set at 1 for a haematocrit of 45% but varies depending on the actual haematocrit level (19).

Interpretation of the RI provides insights into bone marrow activity. An RI less than 2% suggests decreased RBC production, characteristic of conditions such as aplastic anaemia, iron deficiency anaemia, and chronic kidney disease. Conversely, an RI of 2% or higher indicates increased RBC production, seen in conditions involving accelerated RBC destruction, such as haemolytic anaemias, or following acute blood loss (19).

Sickle cell anemia (SCA) poses significant socio-economic challenges globally, including in Nigeria, deeply impacting individuals, families, and the healthcare system (20). The disease causes chronic anaemia, severe pain crises, and complications like acute chest syndrome, stroke, and organ damage, which frequently necessitate hospitalization and long-term medical care (5).

These health issues lead to substantial financial strain for families due to ongoing medical costs and lost income from missed work and school. The economic burden is particularly severe in rural areas, where access to healthcare is limited, resulting in long travel distances for treatment. Social stigma further exacerbates the challenges, leading to discrimination and mental health issues for those affected.

Nigeria's healthcare system is overwhelmed by the demand for SCA care, with resource limitations and a shortage of trained healthcare professionals resulting in suboptimal treatment. Monitoring the reticulocyte indices in SCA patients is crucial, as it helps assess bone marrow function and the body's response to anaemia, providing essential information for effective disease management.

The reticulocyte index (RI) measures the production of new red blood cells (reticulocytes) in the bone marrow. It provides valuable information about the bone marrow's response to anaemia and can help assess the effectiveness of treatments. However, the dynamics of reticulocyte production in SCA patients are complex and poorly understood. Sickle cell anemia patients often experience chronic haemolytic anaemia due to the rapid destruction of sickle cells (21). The bone marrow compensates by increasing reticulocyte production (22). Studying the RI in these patients can provide insights into the bone marrow's ability to respond to anaemia, which is critical for effective disease management.

Various treatments, such as hydroxyurea and blood transfusions, aim to reduce haemolysis and improve haemoglobin levels in SCA patients (23). Monitoring the response to intervention (RI) can help evaluate the efficacy of these treatments and inform therapeutic decisions. High or low RI levels in SCA patients can indicate different complications. For instance, a very high RI may suggest severe haemolysis, while a low RI could indicate bone marrow failure or aplastic crisis. Understanding these patterns can aid in the early detection and intervention of complications.

By establishing the significance of the RI in SCA patients, healthcare providers can develop better strategies for monitoring and managing the disease. This can lead to more personalized and effective treatment plans, ultimately improving patient outcomes and quality of life. Despite the importance of the RI, limited research specifically focuses on its role in SCA. This study aims to fill this gap by providing information on reticulocyte production in SCA patients, thereby contributing to the broader understanding of the disease.

MATERIALS AND METHODS

Study Area

This investigation was carried out at Rivers State University Teaching Hospital (RSUTH), formerly known as Braithwaite Memorial Specialist Hospital, located at 5–8 Harley Street, Old Government Reservation Area (GRA), Port Harcourt, Rivers State, Nigeria (coordinates: 4.7843°N, 7.0104°E). RSUTH is a government-owned tertiary healthcare facility and ranks among the largest in the Niger Delta region, with a bed capacity of 375 and accreditation across the majority of clinical departments. Port Harcourt, the capital and most populous city of Rivers State, lies along the Bonny River and has an estimated population of 1,148,665. As a major Nigerian city, it has experienced rapid urbanization driven by the country's social and economic history. Port Harcourt is a significant hub for various economic, social, and political activities, offering new opportunities across these sectors.

Study Population

The study population comprised 45 pediatric patients, aged 2 to 19, who attended the sickle cell clinic at Rivers State University Teaching Hospital (RSUTH). These individuals were referred to the haematology laboratory for diagnostic confirmation of sickle cell disease. Participants included both male and female children.

Study Design

This descriptive cross-sectional study evaluated the reticulocyte indices in sickle cell anaemia patients in a single-point measurement of reticulocyte indices in paediatric sickle cell disease patients attending the sickle cell disease clinic in a tertiary health institution in Port Harcourt, Nigeria.

Sample Size Calculation

To determine the minimum sample size of the subjects recruited in the study, the global prevalence of sickle cell disease, as reported by Naing et al. (11), was 3%. This value was used to calculate the minimum sample size as follows.

Using the formula:

$n = Z^2P(1-P)/d^2$

Where n is the minimum sample size

Z = Standard normal deviation corresponding to 95% confidence level set at 1.96

p = 3% = 0.03

1-P = 0.97

d = desired precision, 5% (0.05)

$n \approx 45$

According to the calculation, 45 samples were used in this study.

Ethical Considerations

Ethical clearance for the study was granted by the Research Ethics Committee of Rivers State University Teaching Hospital, Port Harcourt, Nigeria.

Inclusion and Exclusion Criteria

Inclusion Criteria

- Children between the ages of 2 and 19 who are sickle cell clinic attendees of RSUTH.
- Individuals residing in Port Harcourt and its surrounding regions seeking medical attention at RSUTH sickle cell clinic.
- Those whose parents/guardians have given written informed consent for their children/ward to participate in the study.

Exclusion Criteria

- Individuals who are not within the age range of the study participants.
- Individuals who are not sickle cell clinic attendees of RSUTH.
- Those whose parents/guardians did not give written informed consent for their children/ward to participate in the study.

Sample Collection and Storage

Aseptic venipuncture of the cubital vein was performed using sterile, disposable Vacutainer® needles and tubes to collect 5 mL of blood from each patient. Samples were transferred into EDTA-containing tubes and analyzed for reticulocyte counts and haematological parameters within three hours of collection.

Procedures

Determination of Reticulocyte Count: New Methylene Blue Staining Method

Principle: An isotonic solution of a supravital stain (i.e., one that stains living material), such as new methylene blue, is incubated with a few drops of blood. The red blood cells must be stained while they are alive to detect ribosomal RNA in reticulocytes. A thin blood film preparation is made and stained, and the reticulocytes are counted microscopically. Reticulocytes are recognized by the violet-blue stained granules of ribosomal RNA (reticulin) they contain. The reticulocyte count is expressed as a percentage or preferably in absolute numbers when an electronic analyzer RBC count is available.

Procedure

Reticulocyte staining was performed using the supravital technique. Three drops of new methylene blue solution were combined with three drops of a well-mixed EDTA-anticoagulated blood sample in a test tube and thoroughly mixed. The mixture was incubated either at ambient temperature for 20 minutes or at 35–37 °C for 10–15 minutes. Following incubation, the red blood cells were gently resuspended, and a drop of the stained sample was applied to each of two microscope slides using a capillary or plastic bulb pipette. The blood was evenly spread to create thin films and air-dried by gentle waving. One of the slides was subsequently counterstained with Leishman stain and stored in a dust- and insect-free environment. Microscopic evaluation was carried out the following day. Initial assessment was conducted using the 10× objective lens with a partially closed condenser iris diaphragm to identify suitable areas of cell distribution. A drop of immersion oil was placed on the selected field, and detailed examination was performed with the oil immersion objective (100×), adjusting the diaphragm accordingly. Reticulocytes were enumerated by systematically scanning consecutive microscopic fields, counting 500 red blood cells—extended to 1,000 cells in cases of low reticulocyte prevalence. The reticulocyte percentage was calculated based on the total number of cells counted.

Determination of Haemoglobin and Packed Cell Volume: Veri-Q RED Haemoglobin Meter

Principle

Veri-Q test strips for Haemoglobin and Packed Cell Volume (PCV) are designed to quantify the concentration of red blood cells in whole blood through advanced biochemical and optical detection mechanisms. When a

blood sample is applied, capillary action draws it into the reaction zone, where reagents interact with haemoglobin or cellular components to generate a measurable signal. This signal may be electrochemical, based on changes in conductivity, or photometric, using light absorbance to determine red cell concentration. The paired device processes the signal using calibrated algorithms to calculate and display the PCV as a percentage and haemoglobin as g/dl.

Procedure

Whole blood samples, anticoagulated with EDTA, were gently mixed to ensure homogeneity before analysis. A precise volume of 20 µL of each sample was applied to the reaction zone of the test strip, ensuring complete coverage without overflow. The test strip was then inserted into the Veri-Q device, which quantified PCV by detecting electrochemical or photometric signals generated from the interaction of blood components with the strip's reagents. The device processed these signals using proprietary algorithms to calculate and display PCV as a percentage. Results were recorded for each sample, and quality control was conducted using standardized control samples to ensure accuracy.

Sickle SCAN RDT Test

Principle

The Sickle SCAN® test kit is a rapid, qualitative lateral flow immunoassay designed for the detection of haemoglobin variants A, S, and C associated with sickle cell disorders. A 5 µL blood sample is obtained via venipuncture using the provided capillary sampler and introduced into a buffer-loaded pretreatment module, facilitating erythrocyte lysis and haemoglobin release. Subsequently, four drops of the treated sample are dispensed into the sample inlet of the Sickle SCAN cartridge. The sample migrates through the cartridge via capillary action over a five-minute period, during which antibody-conjugated colorimetric nanoparticles interact with the haemoglobin variants. Capture zones yield up to four distinguishable lines, including a control (Ctrl) line that confirms successful fluid migration. The presence of blue indicator lines within the designated regions signifies detection of haemoglobin A, S, and C variants.

Procedures

Blood samples were obtained following standard laboratory procedures. A 5 µL sample was collected using a sterile capillary sampler and introduced into the buffer-loaded pretreatment module, which contains a premeasured volume of extraction buffer. Care was taken during module handling to avoid contamination. The module was inverted and gently mixed three times to ensure thorough haemoglobin extraction via erythrocyte lysis. The tip of the colored cap was broken off, and four drops of the treated sample were dispensed into the inlet port of the Sickle SCAN® cartridge, which was placed on a level surface at ambient temperature. The lateral flow assay was allowed to proceed for five minutes, after which results were interpreted via the cartridge's detection window. Any assays exceeding 10 minutes of run time were deemed invalid. This test served to confirm the sickle cell status of patients with a prior diagnosis of sickle cell anaemia.

Reticulocyte count was performed using light microscopy at 100× objective. A minimum of 500 red blood cells were counted, and the number of reticulocytes encountered was recorded and calculated as follows:(Number of reticulocytes seen / 500 cells) × 100%.

After obtaining the percentage of the reticulocyte count by microscopic examination of the reticulocyte film stained with New Methylene Blue and counterstained with Leishman stain, the reticulocyte indices—absolute reticulocyte count (ARC), corrected reticulocyte percentage/reticulocyte index (RI), and reticulocyte production index (RPI)—were calculated using MDCalc on the website www.mdcalc.com/calc/1667/absolute-reticulocyte-count-reticulocyte-index.

A reticulocyte production index of less than 2.0 defines a hypoproliferative bone marrow response.

Data Analysis

Data obtained from this study were analyzed using SAS statistical software, version 9.4 (SAS Institute Inc., Cary, NC, USA). Statistical significance was defined as a p-value ≤ 0.05 at a 95% confidence interval.

RESULTS

This study was conducted on sickle cell anaemia (SCA) patients attending the sickle cell clinic of Riv-

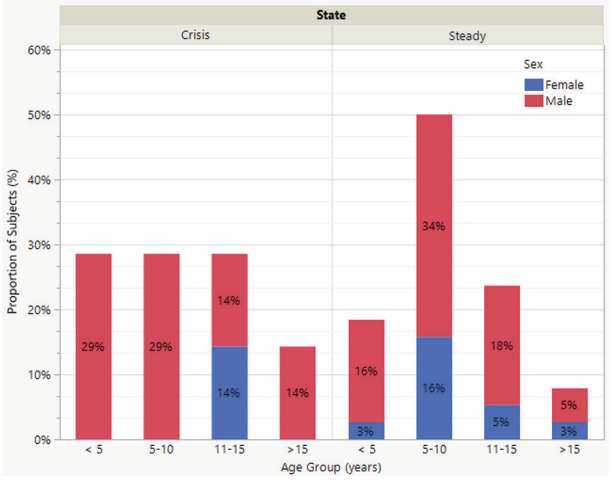


***Figure 1**. Distribution of Sickle Cell Anaemia patients by Age Group, Sex and the Disease States of the patients*

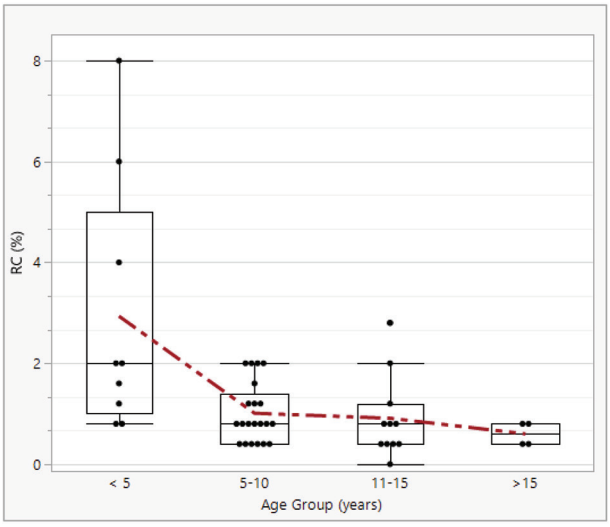


Figure 2. *Box Plot of Reticulocyte Count (RC) by Age Group of Sickle Cell Subjects*

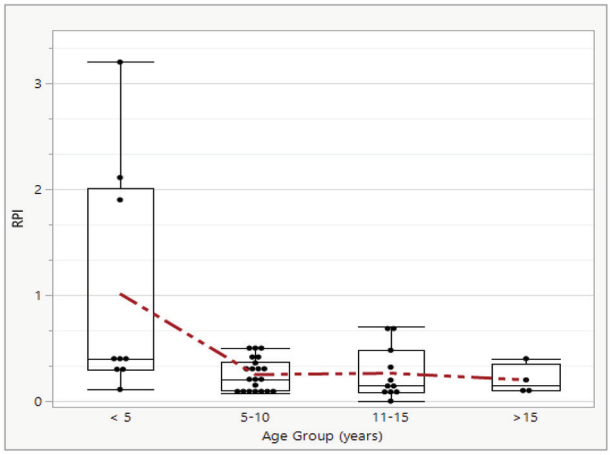


Figure 3. *Box Plot of Reticulocyte Production Index (RPI) by Age Group of Sickle Cell Subjects*

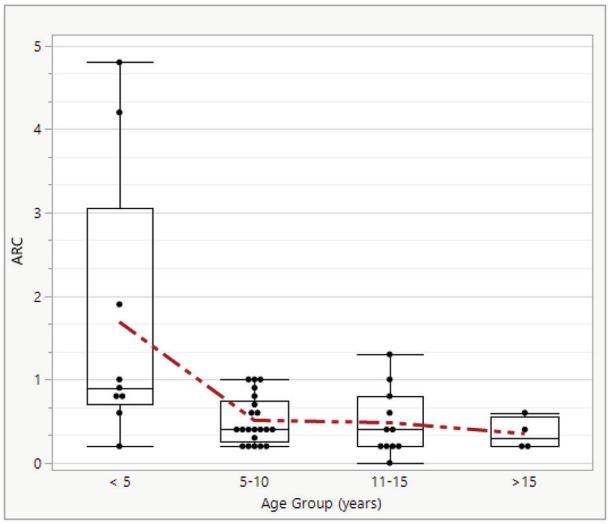


Figure 4. *Box Plot of Absolute Reticulocyte Count (ARC) by Age Group of Sickle Cell Subjects*

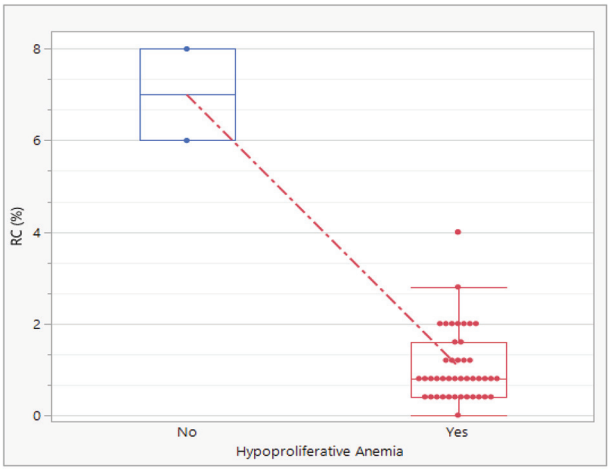


Figure 5. *Box Plot of Reticulocyte Count by Hypoproliferative Anemia Status Among Sickle Cell Subjects*

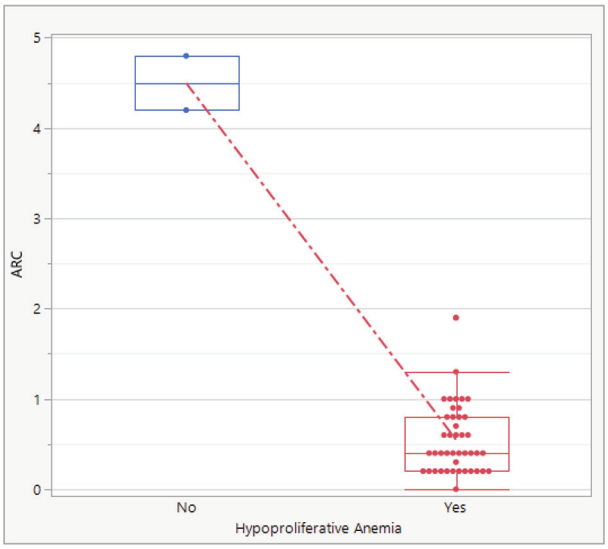
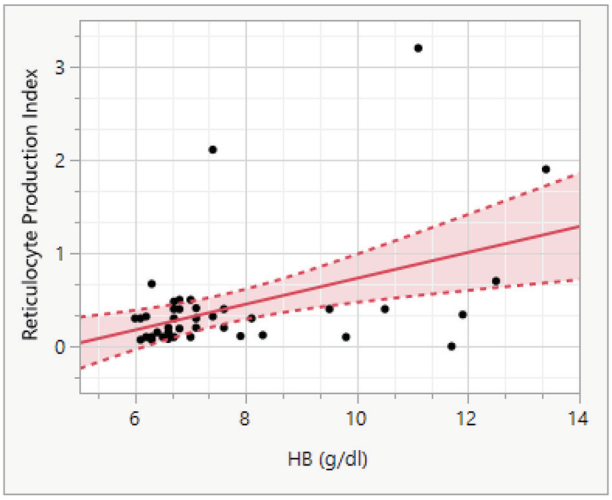


Figure 6. *Box Plot of Absolute Reticulocyte Count (ARC) by hypoproliferative Anemia Status Among Sickle Cell Subjects*

ers State University Teaching Hospital (RSUTH), Port Harcourt, to determine their reticulocyte indices.

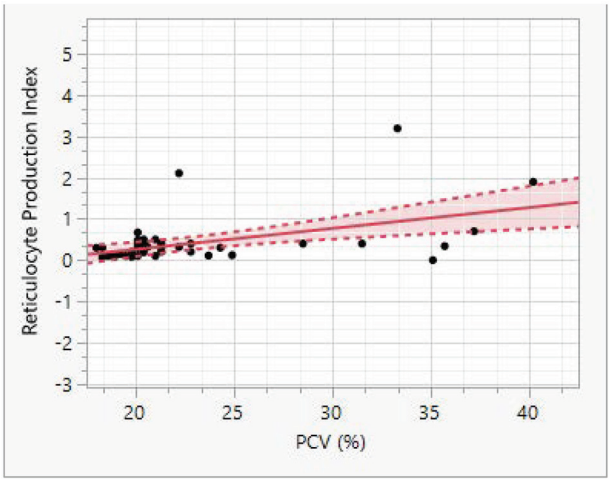
Figure 1 shows the distribution of SCA patients by age group, sex, and disease state. Most SCA patients in crisis were aged 11–15, while those in the steady state were aged 5–10. This is only referring to the age of the SCA patients who were in crisis and those in steady states as shown in the figures. It doesn't refer to the overall age of the SCA patients in the study.

Figure 2 presents a graphical representation of the reticulocyte count by age group for patients with sickle cell disease. The reticulocyte count was higher in children under 5 years old than in other age groups. The reticulocyte count was lowest among children 15 years and older. A similar pattern was observed with the Reticulocyte Production Index (RPI) and absolute Reticulocyte Count (ARC), as shown in Figures 3 and 4.



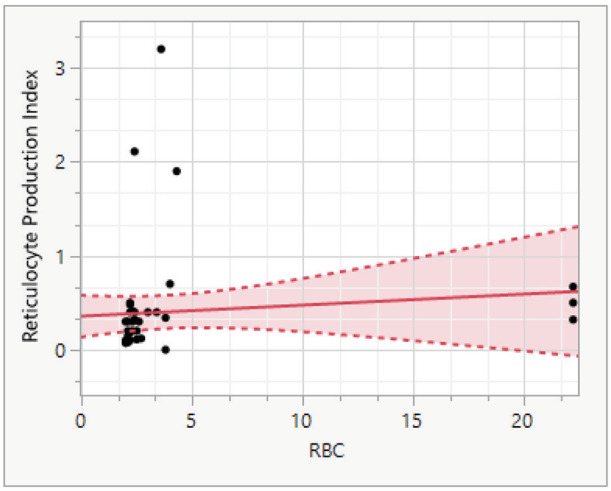
Term	Estimate	Std Error	t Ratio	Prob > |t|
Intercept	-0.657066	0.334679	-1.96	0.0561
HB (g/dl)	0.1387649	0.042628	3.26	0.0022**
Equation	RPI = -0.657066 + 0.1387649*HB (g/dl)			
Correlation	0.4446, 95%CI: 0.174-0.653, p=0.0022			
R^2	0.1977			

Figure 7. *Relationship Between Hemoglobin and Reticulocyte Production Index*



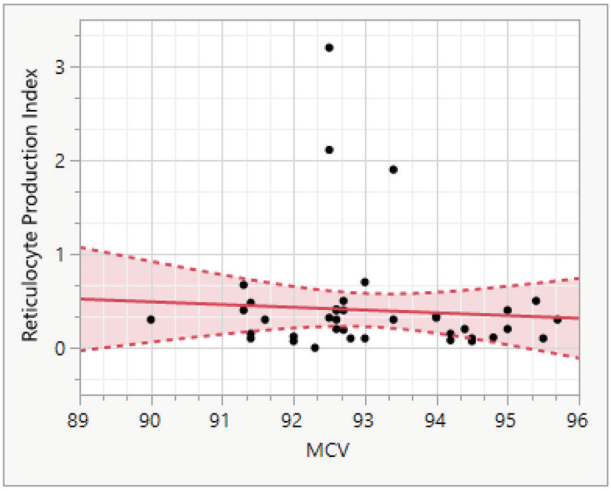
Term	Estimate	Std Error	t Ratio	Prob > |t|
Intercept	-0.751659	0.334236	-2.25	0.0297*
PCV (%)	0.0507031	0.014293	3.55	0.0010***
Equation	RPI = -0.751659 + 0.0507031*PCV (%)			
Correlation	0.4758, 95%CI: 0.211-0.675, p=0.0010			
R^2	0.2264			

Figure 8. *Relationship Between Packed Cell Volume (PCV) and Reticulocyte Production Index*



Term	Estimate	Std Error	t Ratio	Prob > |t|
Intercept	0.3571768	0.110437	3.23	0.0023**
RBC	0.0117404	0.017717	0.66	0.5111
Equation	RPI = 0.3571768 + 0.0117404*RBC			
Correlation	0.1005, 95%CI: -0.198-0.383, p=0.5111			
R^2	0.0101			

Figure 9. *Relationship Between Red Blood Cell (RBC) and Reticulocyte Production Index*

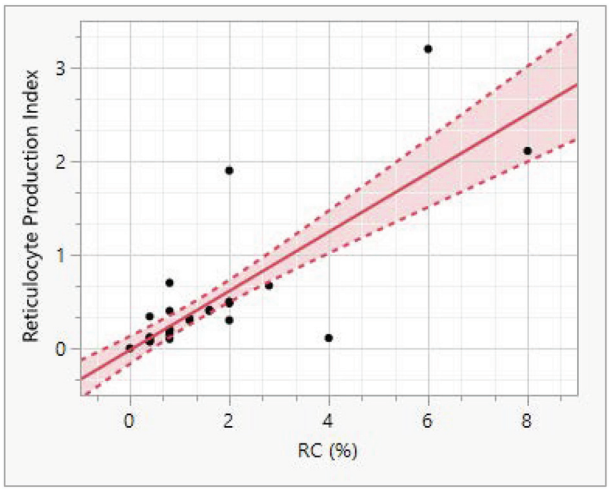


Term	Estimate	Std Error	t Ratio	Prob > |t|
Intercept	3.1320241	5.990534	0.52	0.6038
MCV	-0.029351	0.064389	-0.46	0.6508
Equation	RPI = 3.1320241 - 0.0293515*MCV			
Correlation	-0.069, 95%CI: -0.356-0.229, *p=0.6508*			
R^2	0.005			

Figure 10*. Relationship Between Mean Cell Volume (MCV) and Reticulocyte Production Index*

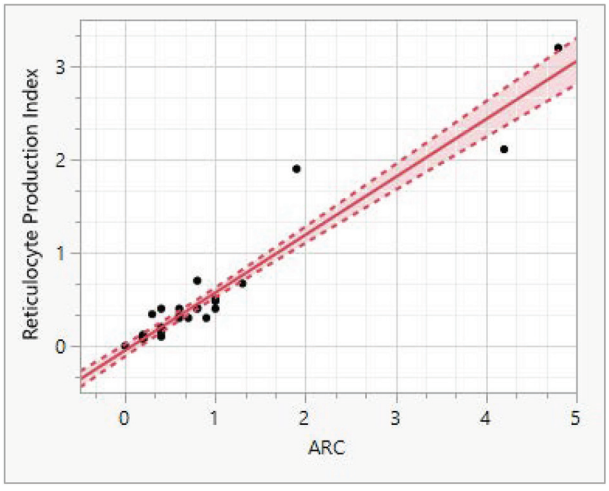
The Reticulocyte Production Index (RPI) measures the bone marrow response in patients with sickle cell disease. Patients with an RPI of less than 2.0 were interpreted as hypoproliferative. Only two of the SCA patients had an RPI of 2.0 or above, which is represented graphically in Figure 5. The same pattern follows with ARC in Figure 6.

Figure 7 graphically represents the relationship between haemoglobin (Hb) and RPI. There is a strong pos-



Term	Estimate	Std Error	t Ratio	Prob > |t|
Intercept	-0.019077	0.073107	-0.26	0.7954
RC (%)	0.3154742	0.037068	8.51	<.0001****
Equation	RPI = -0.019077 + 0.3154742*RC (%)			
Correlation	0.792, 95%CI: 0.650-0.881, p<.0001****			
R^2	0.627			

Figure 11. *Relationship Between Reticulocyte Count (RC) and Reticulocyte Production Index*



Term	Estimate	Std Error	t Ratio	Prob > |t|
Intercept	-0.047929	0.032234	-1.49	0.1443
ARC	0.6204544	0.028004	22.16	<.0001****
Equation	RPI = -0.047929 + 0.6204544*ARC			
Correlation	0.959, 95%CI: 0.926-0.977, p<.0001****			
R^2	0.919			

Figure 12. *Relationship Between Absolute Reticulocyte Count (ARC) and Reticulocyte Production Index*

itive relationship between these variables (p = 0.002). A similar pattern existed between RPI and packed cell volume (PCV) (Figure 8) and RPI and red blood cell count (RBC) (Figure 9). No significant relationship

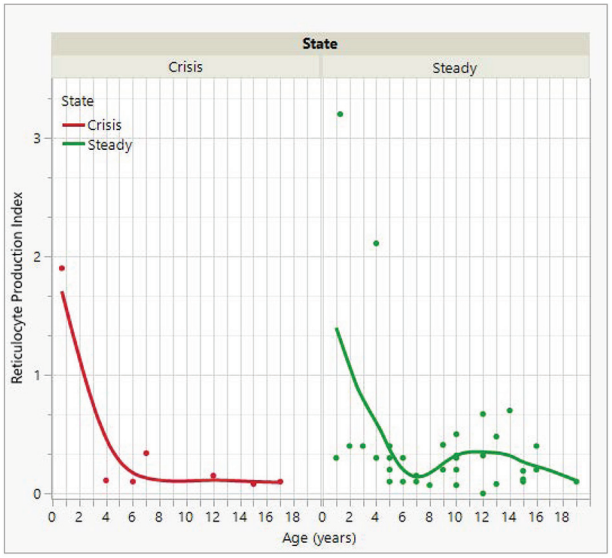


Figure 13. *Relationship Between Age and Reticulocyte Production Index for Sickle Cell Subjects in Crisis and Steady State*

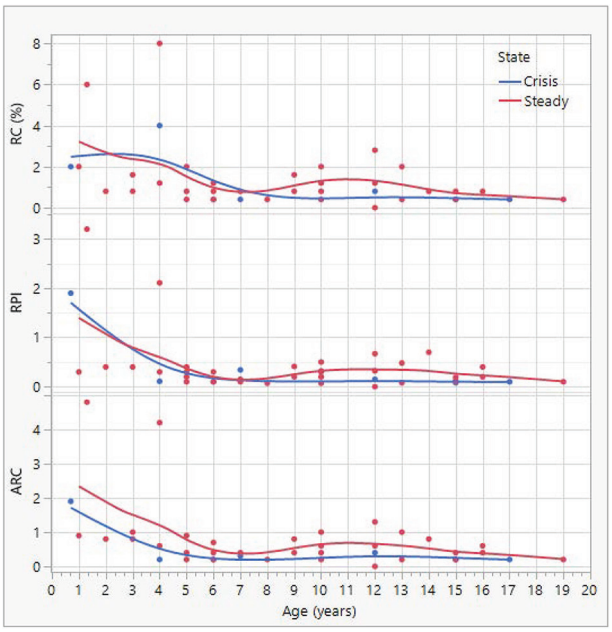


Figure 14. *Relationship Between ARC, RPI, and RC and Age by State of Sickle Cell Subjects*

existed between RPI and mean corpuscular volume (MCV), as shown in Figure 10 (p = 0.65).

Figure 11 graphically shows the relationship between reticulocyte count and reticulocyte production index. The relationship was highly significant and positive (p < 0.001). This suggests that an increase in reticulocytes leads to a corresponding increase in the production index, and vice versa. Similarly, the reticulocyte production index is significantly and positively related to the absolute reticulocyte count (p < 0.001). This relationship is graphically demonstrated in Figure 12.

Figure 13 shows the line graph of the RPI against the age of the SCA patients. The graph demonstrates

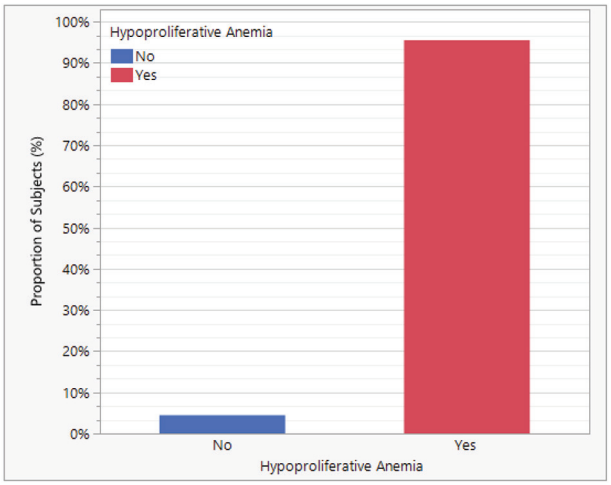


***Figure 15**. Distribution of Hypoproliferative Anemia Among Sickle Cell Subjects*

the trend of RPI across participants' ages. The RPI was higher in children aged 0–6 years in both crisis and steady states. The trend graphs of ARC, RPI, and reticulocyte count against the ages of SCA patients in different sickle cell states are represented together in Figure 14. The trend is consistent with that observed in Figure 13.

Figure 15 shows the proportion of SCA patients who demonstrate appropriate or hypoproliferative bone marrow response, as indicated by a cut-off RPI value of 2.0. SCA patients with an RPI less than 2.0 were classified as hypoproliferative, while those with RPI above 2.0 were classified as appropriate. Only 2 SCA patients demonstrated appropriate bone marrow response, while most were hypoproliferative.

DISCUSSION

Reticulocytes, the immature red blood cells produced by the bone marrow, play a crucial role in evaluating erythropoietic activity and bone marrow response in SCA patients. This study determined reticulocyte indices—including reticulocyte count, reticulocyte production index (RPI), and mean reticulocyte volume—to provide valuable insights into the dynamics of red blood cell production and destruction. These indices are vital for understanding the degree of haemolysis, compensatory erythropoiesis, and treatment response in SCA (24).

The mean reticulocyte count in children with sickle cell anaemia is typically around 12% of total red blood cells (RBCs), significantly higher than the normal range of approximately 0.5% to 2.5% in healthy children (25). In this study, the mean reticulocyte count of SCA patients was 1.33 ± 0.22%, while the mean RPI was 0.40 ± 0.09. The reticulocyte count observed here was lower than that reported by Sani et al. (26), who found 1.48 ± 1.46% in SCA patients. Similarly, the RPI in our study was lower than the 1.7/µL reported by Akingbola et al. (27). Our study's mean reticulocyte count and RPI fell within the normal reference range.

Sickle cell anaemia is known to cause reticulocytosis due to increased haemolysis of sickled red blood cells (28). However, our results showed a reduction in reticulocyte indices, likely due to hydroxyurea treatment received by some subjects, which is known to reduce erythropoiesis, as supported by the correlation analyses.

Although reticulocyte count, RPI, and absolute reticulocyte count were higher in males compared to females, these differences were not statistically significant. This finding aligns with Candar et al. (29), who reported no significant sex differences in platelet counts. Our study found a significant decrease in reticulocyte indices with increasing age, indicating age-related effects on erythropoiesis. Additionally, no significant differences were observed in reticulocyte indices between steady and crisis states, suggesting that disease state does not significantly affect these indices.

The haemoglobin levels, packed cell volume (PCV), red blood cell (RBC) counts, and mean corpuscular volume (MCV) in SCA subjects were 7.63 ± 0.28 g/dL, 22.74 ± 0.82%, 3.73 × 10^{12}/L, and 75.3 fL, respectively. These haemoglobin levels were higher than those reported by Jeremiah and Magnus (30), who found 5.68 ± 1.7 g/dL in SCA patients. Similarly, Akodu et al. (31) reported PCV, Hb, MCV, and RBC values of 20.9%, 6.9 g/dL, 75.3 fL, and 2.9 × 10^{12}/L, respectively, in SCA subjects.

Haemoglobin and PCV were significantly elevated in patients who did not receive hydroxyurea. According to study (32), hydroxyurea causes bone marrow suppression, which likely accounts for the reduced Hb and PCV observed in treated subjects due to delayed erythropoiesis.

Hydroxyurea (HU) is a widely used treatment for sickle cell anaemia, known to improve haemoglobin levels and reduce complications, thus decreasing the need for blood transfusions (33). HU increases foetal haemoglobin (HbF) levels, which reduces sickling of red blood cells and the frequency of vaso-occlusive crises (34, 35). Additionally, HU reduces leukocyte and platelet counts, potentially lowering inflammation and thrombotic risk (34).

In this study, an RPI of less than 2.0—indicative of a hypoproliferative bone marrow response—was observed in 95.5% of SCA patients on hydroxyurea, with only two patients showing an appropriate bone marrow response (RPI ≥ 2.0). This confirms that HU therapy can lead to hypoproliferative anaemia by in-

hibiting DNA precursor synthesis necessary for cell division (33, 36, 37).

While hydroxyurea effectively reduces sickle cell complications, it may exacerbate anaemia through bone marrow suppression. Therefore, careful monitoring and dose adjustments are essential to balance therapeutic benefits and side effects. Close collaboration between patients and healthcare providers is crucial for personalized management.

CONCLUSION

This study found derangements in reticulocyte parameters indicative of hypoproliferative anaemia in SCA patients. Regular monitoring of reticulocyte counts is necessary to improve patient outcomes and quality of life.

Abbreviations

EDTA - Ethylene diamine tetraacetic acid
RPI - Reticulocyte Production Index
SCA - Sickle Cell Anaemia
RC - Reticulocyte Count
RDT - Rapid Diagnostic Test
ARC - Absolute Reticulocyte Count
PCV - Packed Cell Volume
Hb - Haemoglobin

Conflict of Interest Statement

The authors declare that there is no conflict of interest related to this study. There are no financial relationships, employment, consultancy, stock ownership, honoraria, patents, or paid expert testimony that could influence the outcome of the research presented. Furthermore, there are no close relationships, competitive academic agendas, or philosophical biases that might have affected the conduct of the study.

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Ethical Approval and Informed Consent

All subjects included in the study gave informed consent to participate. Participant information was kept anonymous and in accordance with the International Committee of Medical Journal Editors (ICMJE) guidelines for the protection of research participants. The study was conducted in accordance with the Declaration of Helsinki. Ethical clearance for the study was granted by the Research Ethics Committee of Rivers State University Teaching Hospital, Port Harcourt, Nigeria.

Author Contributions & Responsibilities

The authors take full responsibility for the accuracy and integrity of the content, as well as the validity of institutional affiliations. The publisher remains neutral regarding jurisdictional claims in institutional affiliations. All authors have read and agreed to the published version of the manuscript. Author roles**:** ZAJ: Professor of Haematology and Blood Transfusion Science; designed and supervised the study; drafted the initial manuscript; CCO: Student researcher; collected samples and performed laboratory analysis; BNJ: Medical officer; assisted with sample collection; CO: Consultant paediatric haematologist; managed patients, obtained informed consent, and referred for blood sample collection.

Note: Artificial intelligence was not utilized as a tool in this study.

Sažetak

KLINIČKA PRIMENA RETIKULOCITNIH INDEKSA U DIJAGNOSTICI I LEČENJU PEDIJATRIJSKIH PACIJENATA SA SRPASTOM ANEMIJOM U PORT HARCORT-u U NIGERIJI

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Uvod: Srpasta anemija (SA) je nasledni hematološki poremećaj izazvan tačkastom mutacijom u genu za β-globin, koja dovodi do stvaranja abnormalnog hemoglobina S, što menja oblik eritrocita i narušava njihovu funkciju. Indeksi retikulocita, koji kvantifikuju nezrele eritrocite u perifernoj krvi, predstavljaju ključne markere aktivnosti koštane srži i eritropoeze kod pacijenata sa SA.

Materijali i metode: U ovoj studiji preseka analizirani su indeksi retikulocita kod 45 dece uzrasta od 2 do 19 godina sa SA, lečene u Univerzitetskoj bolnici Rivers State. Uzorci krvi su prikupljeni u EDTA epruvetama. Brojanje retikulocita izvršeno je metodom bojenja novim metilen-plavim i mikroskopskim pregledom. Hemoglobin (Hb) i hematokrit (PCV) određeni su korišćenjem Veri-Q RED merača. Indeksi retikulocita — apsolutni broj retikulocita (ARC), indeks retikulocita (RI) i indeks produkcije retikulocita (RPI) — izračunati su primenom MDCalc alata.

Rezultati: Prosečan retikulocitni broj iznosio je 1,33 ± 0,22%, dok je prosečni indeks produkcije retikulocita bio 0,40 ± 0,09. Indeks produkcije retikulocita (RPI) značajno je pozitivno korelisan sa vrednostima hemoglobina i hematokrita u kriznim i stabilnim fazama bolesti ($r = 0,820$, $p = 0,02$). Takođe, kod pacijenata na terapiji i bez terapije hidroksiureom zabeležena je značajna korelacija između RPI i retikulocitnog broja ($p < 0,01$). Samo 2 pacijenta (4,4%) su pokazala adekvatan odgovor koštane srži, dok je kod preostalih 43 (95,6%) utvrđena hipoproliferacija.

Zaključak: Uočene su promene u retikulocitnim parametrima koje ukazuju na hipoproliferativnu anemiju. Neophodno je redovno praćenje retikulocitnog statusa radi poboljšanja ishoda lečenja i kvaliteta života kod pacijenata sa srpastom anemijom.

Ključne reči: Srpasta anemija, hidroksiurea, broj retikulocita, indeks produkcije retikulocita, hipoproliferativna anemija, apsolutni broj retikulocita.

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