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ORIGINAL ARTICLE

Expression of major hemoglobin haplotypes in the first twenty-four months of life suggests a gradual decline of normal hemoglobin A among infants of African descent

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Summary

Introduction: Hemoglobin is the principal protein in red blood cells and is responsible for delivering oxygen from the lungs to other body parts. Understanding the hemoglobin type profile of infants and the patterns of expression in the first twenty-four months of life is a significant area of research that can provide crucial insights into infant health and development.

Material and Methods: The study population consisted of 147 infants (male and female) aged 9 to 24 months. Participants were recruited from the pediatric and sickle cell clinics and the medical laboratory department of Rivers State University Teaching Hospital (RSUTH) in Port Harcourt, Nigeria. The hemoglobin type was determined using high-performance liquid chromatography (HPLC) (D-10, Bio-Rad).

Results: The median (range) values of the hemoglobin types were: HbA 70% (22-98), HbF 10% (0-50), HbS 0% (0-78)and A2 CE 0% (0-50). Hemoglobin A expression was 65% at nine months, 79% at 12.5 months, 46% at 22 and 60% at 24 months. HbF expression was 21% at nine months, 10% at 12 months, 24% at 15.5 months, 0.25% at 21 months, and 12% at 24 months. HbS was 0.8% at nine months and 0% at 16 months. 50% at 22 months and lastly 22% at 24 months. The HbA2 was 0.5% at nine months and 12% at 11 months, 0% at 21 months and lastly 0.2% at 24 months. HbA, HbF, and HbA2 were negatively correlated with age, while HbS was positively correlated with age.

Conclusion: The pattern of expression of the four hemoglobin types in this study was age-dependent. Sex was not found to influence the expression of hemoglobin types in infants. There is a gradual reduction in the expression of normal hemoglobin A and a gradual increase in abnormal hemoglobin S among infants of African descent.

Keywords: hemoglobin types, percentage expression, sickle cell disease, beta thalassaemia, HbA, HbF, HbS, HbA2

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INTRODUCTION

Hemoglobin, the principal protein in red blood cells, carries oxygen in the blood. In infancy, the natural replacement of fetal hemoglobin (HbF) by adult hemoglobin (HbA) is a normal developmental process. HbF, comprising two alpha-globulin and two gamma-globulin chains ($\alpha_2\gamma_2$), is the major hemoglobin in fetal red blood cells during gestation, constituting 60 to 80 percent of total hemoglobin in full-term newborns. Around 6 to 12 months of age, HbF is gradually and naturally replaced by adult hemoglobin (HbA; $\alpha_2\beta_2$). This natural transition is a part of an infant's development, as HbA becomes the predominant type, gradually replacing HbF (1,2).

At Birth, HbF (fetal hemoglobin) accounts for approximately 80% of total hemoglobin while HbA (adult hemoglobin) accounts for 20%. The transition from gamma-globin synthesis (HbF) to beta-globin synthesis (HbA) begins before birth and continues in the first 24 months of life. By approximately six months, healthy infants will have mostly HbA, a small amount of HbA2, and negligible HbF. Therefore, HbA2 levels in infancy are typically as follows:0-30 days: 0.0-2.1%, 1-2 months: 0.0-2.6%, 3-5 months: 1.3-3.1%,> 6 months: 2.0-3.3%2 (3).

Hemoglobin S (HbS), an abnormal variant associated with sickle cell disease, is primarily expressed in infancy but its levels are typically low. If one parent carries the sickle cell trait (HbAS) and the other has normal hemoglobin (HbAA), the child will have HbAS (sickle cell trait). HbAS (sickle cell trait) is characterized by one normal beta-globin gene (HbA) and one sickle beta-globin gene (HbS). At six months, the levels of HbS decrease significantly, and over six months, the HbS level remains at a low percentage (3,4).

Studying the expression and pattern of development of hemoglobin types in people of African descent is crucial for several reasons, namely:

a) Sickle cell disease (SCD) is prevalent among people of African descent. Over 75% of the approximately 300,000 children born with sickle cell anemia each year are in sub-Saharan Africa (5). The mutation responsible for sickle cell disease originated in Africa and persists due to its protective effect against severe Plasmodium falciparum malaria. In some sub-Saharan African areas, up to 2% of all children are born with this condition (6). Understanding hemoglobin variants, such as HbS (sickle cell hemoglobin), helps diagnose and manage SCD effectively.
b) Thalassemia is an inherited blood disorder affecting hemoglobin production. Some thalassemia variants are more common in African populations (3). Studying these variants aids in early detection and appropriate management.
c) African populations exibit high genetic diversity. Investigating hemoglobin variants contributes to our understanding of human evolution and adaptation.
d. Accurate diagnosis and treatment depend on recognizing specific hemoglobinopathies. Research informs guidelines for prenatal screening, genetic counseling, and disease prevention;
e. In medical laboratories, understanding hemoglobin types ensures accurate results. Quality control measures prevent misdiagnoses and improve patient care.

In infants, the interaction between hemoglobins HbA, HbF, HbS, and HbA2 plays a crucial role in understanding various hemoglobinopathies and disorders. HbF, the predominant fetal hemoglobin, declines after birth, while HbA becomes the primary hemoglobin in adults, along with HbA2 and trace amounts of HbF (7). Hemoglobin variants like HbS can affect the determination of HbA2 levels, leading to biases in measurement (8). The precise measurement of HbA2 is crucial for diagnosing beta thalassemia trait, but other hemoglobin variants like HbS, HbC, HbE, or HbD can complicate the interpretation of results (9,10). Understanding the intricate balance and interactions between these hemoglobin types is essential for diagnosing and managing infant hemoglobin disorders.

Most of the values encountered in the literature concerning the pattern of expression of these hemoglobin types are often Caucasian values with minimal reference to people in the sub-Saharan Africa. This study was aimed at detecting various hemoglobin types and studying the patterns of expression of these hemoglobin types in the first twenty-four months of the life of infants of African descent.

MATERIAL AND METHODS

Study Area

This study was conducted at Rivers State University Teaching Hospital in Port Harcourt, Rivers State, Nigeria. The geographical coordinates of Rivers State are approximately latitude 4.7497 and longitude 6.8277. The hospital, formerly known as Braithwaite Memorial Specialist Hospital (BMSH), is a government-owned facility in Old GRA, Port Harcourt, Rivers State, Nigeria. The hospital serves as a state-of-the-art teaching facility for medical students and other health professionals from Rivers State University. With the capacity of 375 beds, RSUTH houses various departments, including Medicine, Pediatrics, Laboratories, Radiology, Family Medicine, Obstetrics & Gynecology Anesthesia, Surgery, Chemical Pathology, Hematology & Blood Transfusion, Medical Microbiology, Anatomical Pathology, Ophthalmology, and Accident and Emergency.

SAMPLE SIZE CALCULATION

The minimum sample size was calculated using the Cochran standard formula as described by Patra (11).

$$n = \frac{Z^2Pq}{d^2}$$

Where,

n= minimum sample size required

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

P= Proportion of sickle cell disease patients = 10% = 0.1

q= 1-P = 1-0.1 = 0.9

d = Level of precision = 0.05

Applying this formula,

$$n= \frac{1.96^2 \times 0.1 \times 0.9}{0.05^2} = 138.3$$

Therefore, the minimum sample size for this study = 139.

STUDY POPULATION

The study population consisted of 147 infants (male and female) aged 6 to 24 months. Participants were recruited from the pediatric and sickle cell clinics and the laboratory department of Rivers State University Teaching Hospital in Port Harcourt, Rivers State, Nigeria.

Ethical Approval; This study received ethical approval from the Rivers State University Teaching Hospital (RSUTH) ethics and research committee, Port Harcourt, Nigeria.

Informed Consent: The participants' parents voluntarily signed written informed consent forms in their handwriting as proof of their willingness to provide samples for the tests.

Collection of Sample: Two milliliters of whole blood were collected using an S-monovette vacutainer syringe. The blood was deposited into an anticoagulant containing ethylenediaminetetraacetic acid (EDTA) and used for the HPLC analysis.

Study Design. The study was cross-sectional.

Procedure

The hemoglobin type was determined using high-performance liquid chromatography (HPLC) (D-10 instrument;Bio-Rad).

PRINCIPLE

In this method phosphate buffers at different concentrations (mobile phase), pass under pressure through an ionic exchange column (stationary phase). The stationary phase consists of a temperature controlled analytical cartridge containing a resin of anionic or cationic particles (3-5 µm). The chromatographic station delivers a programmed buffer gradient of increasing ionic strength and pH to the cartridge by two dual-piston pumps, and the hemoglobin variants are separated according to their ionic interaction with the stationary phase.

The separated hemoglobin then pass through the flow cell of the filter photometer, where changes in the absorbance (415 nm) are measured; background variations are corrected by an additional filter at 690 nm. Each hemoglobin is characterized by a specific retention time, the elapsed time from the sample injection to the apex of a hemoglobin peak.

The calibration factors for HbA2, F, A1C are automatically calculated by processing a calibration sample at the beginning of each run. Specific software turns the raw data collected from each analysis into a report showing the chromatogram, with all the hemoglobin fractions eluted, the retention times, the areas of the peaks and the values (%) of the different hemoglobin components. The report presents the percentages of hemoglobin types F, A1C, A and A2 and provides qualitative and quantitative determination of abnormal hemoglobin types.

Procedures were followed as contained in the standard operating procedures.

STATISTICAL ANALYSIS

A structured approach was adopted for statistical analysis, beginning with an initial assessment of the distribution of hemoglobin variants (HbA, HbF, HbS, HbA2), including evaluations of normality and variability. Subsequently, the median and range for each measure were determined overall and stratified by sex (female vs. male) and age group (0–9, 10–19, 20–29 years). Box plots were used to illustrate the distribution of data points across the variables. For parameters that did not follow a normal distribution, the Mann–Whitney U test was used to compare two groups (sex), while the Kruskal–Wallis test was applied for comparisons involving more than two groups (age group). Spearman's correlation analysis was performed to assess the strength and direction of relationships between hemoglobin variants, as represented by the correlation coefficient (rho). All statistical tests were two-tailed, with a significance threshold set at $p < 0.05$. Data management, statistical analysis, and visualizations were conducted using SAS JMP Statistical Discovery Software (version 16.2; SAS Institute Inc., Cary, NC, USA).

RESULTS

Table 1 presents the key findings on the median (range) value of hemoglobin parameters of 147 participants, categorized by sex and age groups. The median (range) values were: HbA 70% (22-98), HbF 10% (0-50), HbS 0% (0-78) and A2 CE 0% (0-50). The p-value for hemoglobin A

Table 1. Comparison of Mean Parameters of hemoglobin variants by demographics

Characteristic	n	HbA (96.8-97.8)	HbF (0.8-2.0)	HbS (0.0-0.5)	A2 CE (2.2-3.2)
		median (range)	median (range)	median (range)	median (range)
Overall	147	70 (22-98)	10 (0-50)	0 (0-78)	0 (0-50)
Sex					
Female	77	71 (30-95)	10 (0-50)	0 (0-61)	0 (0-50)
Male	70	67 (22-98)	10 (0-50)	0 (0-78)	0 (0-30)
p-value		**0.041**	0.795	0.063	0.070
Age group (months)					
0-9	21	70 (30-92)[a]	21 (6-50)[a]	0 (0-45)[a]	0 (0-20)[a]
10-19	85	75 (39-98)[a]	10 (0-50)[a]	0 (0-61)[a]	0 (0-50)[a]
20-29	41	51 (22-91)[b]	1 (0-42)[b]	42 (0-78)[b]	0 (0-8)[ab]
p-value		**<0.001**	**<0.001**	**<0.001**	**0.001**

p-values in bold *are statistically significant at the level of probability indicated.*

type in relation to sex was 0.041. No statistically significant differences were observed with other hemoglobin types in relation to sex. Age was found to exert significant differences in all the hemoglobin types with a p-value of less than 0.001.

Table 2 shows the Spearman's pairwise correlation Analysis of the hemoglobin variants. A significant negative correlation exists between HbA and Age (rho=-0.37, p<0.001), HbF and Age (rho=-0.32, p=0.001), and a significant positive correlation between HbS and Age (rho=0.49, p<0.001). A significant negative correlation also existed between HbA2 and age (rho=-0.34, p<0.001). There is a negative correlation between HbS and HbA2 (rho=-0.29, p<0.001), as well as between HbS and HbF (rho=-0.58, p<0.001). HbS is the only hemoglobin type that showed positive correlation with age (rho=0.49, p<0.001)

Table 3 shows the pairwise correlation analysis of the hemoglobin variants by Age Groups. HbF vs. HbA shows a negative correlation in the 0-9 months group (rho=-0.45, p=0.040) and 10-19 months (rho=-0.46, p<0.001). No significant correlation was found in the 20-29-month groups. HbS vs. HbA exhibits a negative correlation in groups 0-9- and 20-29-months respectively (-0.61, p=0.004) and (-0.86, p<0.001). HbS vs. HbF shows no significant correlation in the 0-9 months group but exhibits a negative correlation in the 10-19- and 20-29-month groups, (rho=-0.43, p<0.001 and rho=-0.56, p<0.001 respectively). A2 CE vs. HbF has a positive correlation in the 20-29 months group rho=0.36, p=0.019 and negative correlations in the 0-9- and 10-19-months group (rho=-0.46, p=0.035 and rho=-0.37, p<0.001 respectively).

Box Plots presents the parameters' distribution by age and sex, highlighting variations and trends as shown in **Figures 1-3.**

Figure 1 shows the trend in the development of hemoglobin types according to the age of the infants. There was a gradual rise in the percentage rise in the hemoglobin A type which peaked at 79% by the 12.5 month. After that, there was a decline in the HbA, up to 46%, at 22 months, which was the lowest point. From this point, a steady rise in the percentage of HbA further increased and peaked at 60% at 24 months.

For HbF, at nine months, it was 21%. This value dropped to 10% at 12 months, then assumed a steady increase and peaked at 24% by 15.5 months. Another drop was observed, which settled at 0.25% by 21 months. The value resumed another rise and peaked at 12% by 24 months.

HbS at nine months was 0.8%, which dropped slightly and settled at 0% at 16 months. After that, there was a steady increase, which peaked at 50% by 22 months and later drastically rose to 22% at 24 months.

The HbA2 value at 9 months was 0.5%. This value increased and peaked at 12% by 11 months. After that, it gradually fell to 0% by 21 months and increased steadily to 0.2% by 24 months.

Table 2. Pairwise Correlation Analysis of the hemoglobin variants

Spearman's correlations		Age (months)	HbA (%)	HbF (%)	HbS (%)
HbA (%)	rho	-0.37			
	p-value	**<0.001**			
HbF (%)	rho	-0.32	-0.03		
	p-value	**<0.001**	0.728		
HbS (%)	rho	0.49	-0.63	-0.58	
	p-value	**<0.001**	**<0.001**	**<0.001**	
A2 CE (%)	rho	-0.34	0.19	-0.12	-0.29
	p-value	**<0.001**	**0.022**	0.164	**<0.001**

rho - Spearman's correlation coefficient
p-values in bold *are statistically significant at the level of probability indicated*

Table 3. Pairwise Correlation Analysis of the hemoglobin variants by Age Group

Variable	by Variable	0-9 (months)		10-19 (months)		20-29 (months)	
		rho	p-value	rho	p-value	rho	p-value
HbA (%)	HbF (%)	-0.45	**0.040**	-0.46	**<0.001**	0.17	0.295
HbA (%)	HbS (%)	-0.61	**0.004**	-0.17	0.123	-0.86	**<0.001**
HbA (%)	A2 CE (%)	0.41	0.068	-0.13	0.253	0.12	0.449
HbF (%)	HbS (%)	-0.21	0.351	-0.43	**<0.001**	-0.56	**<0.001**
HbF (%)	A2 CE (%)	-0.46	**0.035**	-0.37	**<0.001**	0.36	**0.019**
HbS (%)	A2 CE (%)	-0.31	0.167	0.01	0.911	-0.29	0.062

p-values in bold *are statistically significant at the level of probability indicated*

Figure 2 shows a Box Plot showing trends of hemoglobin types development by Age and Sex. The pattern of expression was similar in both male and female participants.

Box Plot of hemoglobin variants showing trends by Age Group and Sex is shown in **Figure 3**. The trend of expression and development is similar in both groups

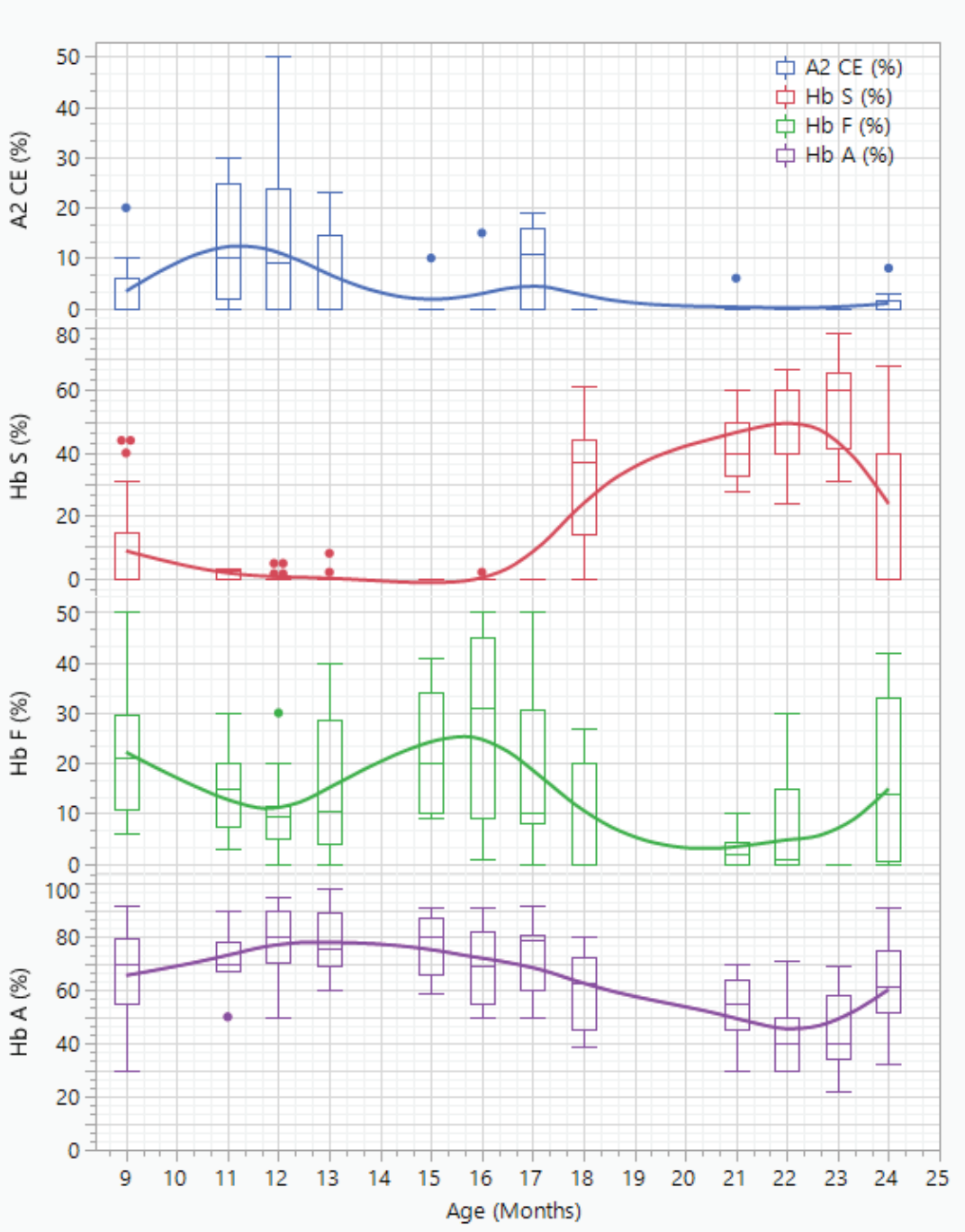

Figure 1. Box Plot of hemoglobin variants showing the trend of development by Age

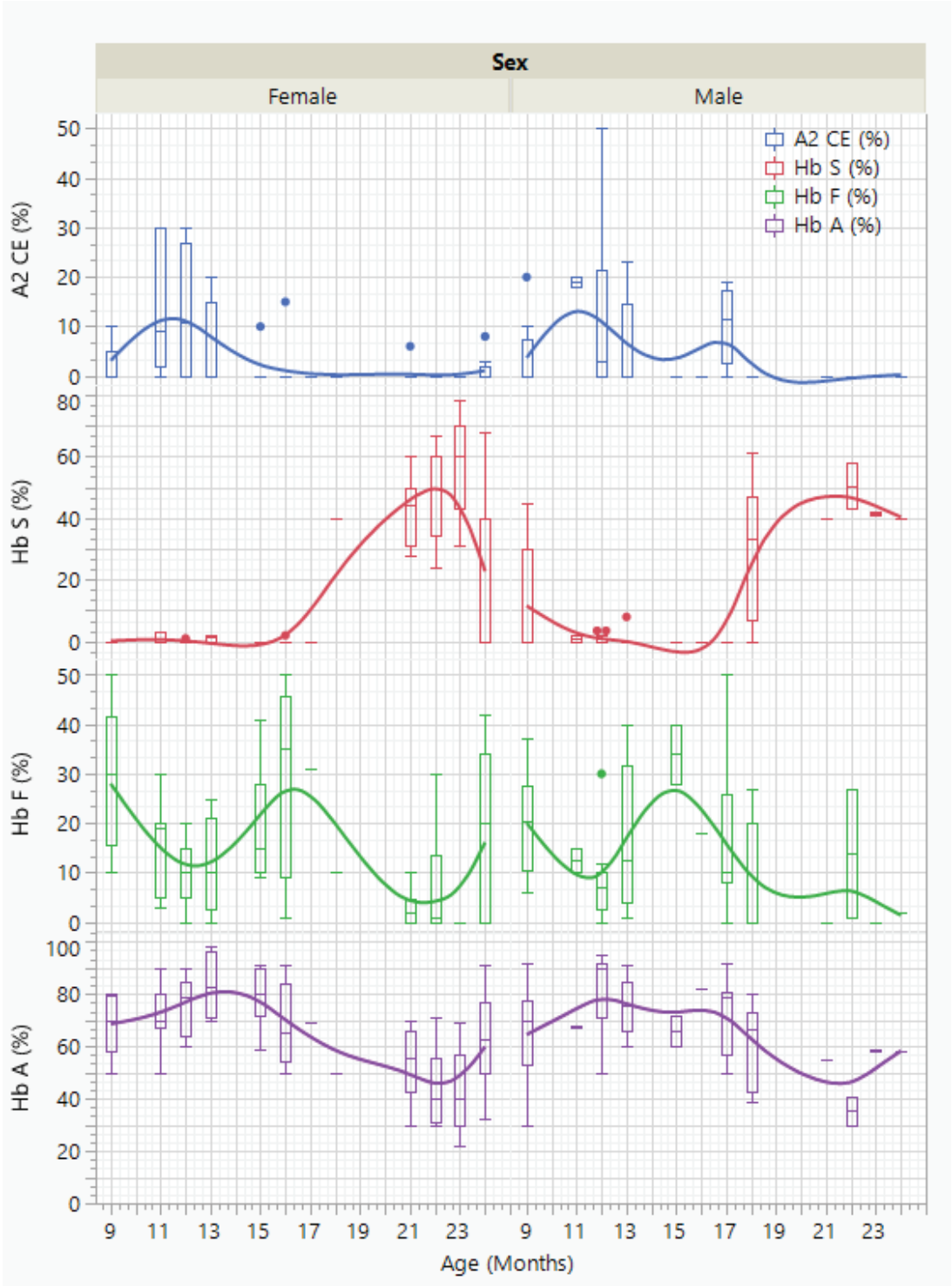

Figure 2. Box Plot showing trends of hemoglobin development by Age and Sex

DISCUSSION

This study aimed to detect the major hemoglobin types and investigate the pattern of expressions of these napisati hemoglobins in infants during their developmental ages of nine to twenty-four months. The key findings of the study are as follows: the average percentage expressions of HbA, HbF, HbS, and HbA2 were 70% (22-98), 10% (0-50), 0% (0-78) and 0% (0-50) respectively. The result shows normal HbA levels, which signify normal hemoglobin switching and erythropoiesis maturation. In cord blood, HbA levels are 21.14±7.04% and increase to 83.38±1.31% in the sixth month. In contrast, HbF levels are 78.39±7.59% and rapidly decrease in the first six months, according to Wong et al. (12). This is consistent with our findings that HbA levels are predominant in this age group, reflecting the transition from fetal hemoglobin (HbF) to adult hemoglobin (HbA).

Elghetany and Banki (1) had earlier reported that, from birth to adulthood, there are notable shifts in the relative amounts of different hemoglobin fractions, such as HbA, HbF, and HbA2. Specifically; HbF, the predom-

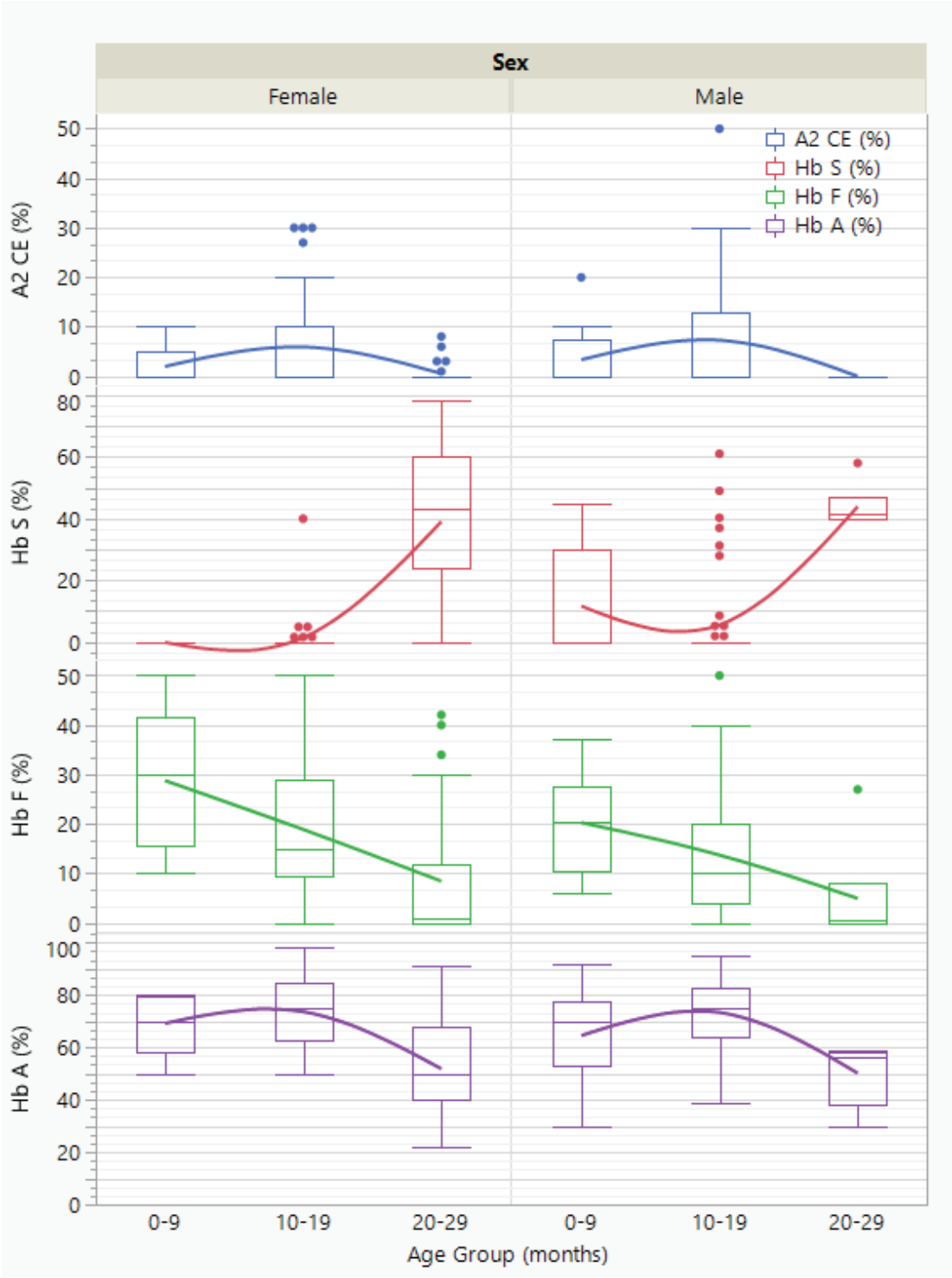

Figure 3. Box Plot of hemoglobin variants showing trends by Age Group and Sex

inant hemoglobin in fetal life, gradually decreases from 50-80% at birth to 1-2% by six months of age and beyond. HbA, the primary adult hemoglobin, increases from low levels at birth to 95-98% by adulthood. HbA2, a minor adult hemoglobin, increases from low levels at birth to 2-3% by adulthood.

The notable shifts in hemoglobin types with age are attributed to the regulation of globin gene expression during the developmental stage of erythropoiesis from fetal to adult. This process, as detailed in references (13,14), plays a crucial role in the transition from fetal to adult hemoglobin types.

HbS levels were observed to increase significantly with age, with the highest levels in the 20–29-month group, which also aligns with a study (4,16). The increased HbS levels with age may be linked to the genetic expression of sickle cell traits becoming more pronounced over time. This finding reiterates the importance of early detection for managing HbS, and underscores the potential for this research to contribute to improved management strategies for sickle cell disease.

It was observed in this study that females had elevated HbF and reduced HbS values than their male coun-

terparts. Several studies have reported sex-related differences in the percentage expression of hemoglobin types, particularly HbF. According to a survey by Bain (17), globin gene expression may be influenced by hormonal and genetic factors, which may explain why females typically have somewhat greater HbF levels than males. These differences persist until approximately 6 to 12 months of age, when HbF is almost entirely replaced by HbA (18). Risoluti *et al* (19) opined that the potential impact of sex-specific factors, such as androgen levels, on the regulation of hemoglobin synthesis and the variations in hemoglobin profiles between males and females must have been responsible for these gender differences in the expression of the hemoglobin types. These sex-related differences are often insignificant in magnitude, and their clinical significance may vary depending on the particular situation and underlying hemoglobin disorders under investigation (18,20). The lower HbS values in female infants are because female infants inherit one HbS gene from their affected parent (carrier mother) and one normal Hb gene from their unaffected parent (carrier father). Male infants inherit one HbS gene from their affected parent (carrier mother) and one Y chromosome from their father. The resultant effect is that female infants have lower overall HbS levels due to the presence of one normal Hb gene. Lower HbS levels in female infants reduce the risk of SCD symptoms (21).

The higher HbS levels among the male infants could be attributed to male infants inheriting one HbS gene from their affected parent (carrier mother) and one Y chromosome from their father. Female infants inherit one HbS gene from their affected parent (carrier mother) and one normal Hb gene from their unaffected parent (carrier father). As a result, male infants tend to have higher overall HbS levels due to the presence of one normal Hb gene. The implication is that the elevated HbS levels in male infants may increase the risk of SCD symptoms if both HbS genes are inherited (20, 21).

A negative correlation existed between HbA and HbS levels. This inverse relationship is supported by studies on sickle cell disease, where HbA and HbS levels are often inversely related due to the competitive synthesis pathways. Dan et al. (22) stated that in sickle cell-beta thalassemia (HbS/β-thal), the amount of HbA produced varies depending on the type of beta-thalassemia mutation. In HbS/β^0-thalassemia, HbA production is abolished, resulting in a phenotype similar to sickle cell anemia (HbSS). In HbS/β^+-thalassemia, variable amounts of HbA (ranging from <5% to 45%) dilute HbS and inhibit polymerization, leading to a milder clinical phenotype (23).

HbF and HbS showed a negative correlation, which explains Al-Shuelli *et al* (24) findings that higher HbF levels can mitigate the clinical severity of sickle cell disease by inhibiting the polymerization of HbS. This protective effect explains the negative correlation observed in our study, suggesting a potential clinical benefit of higher HbF levels in individuals with higher HbS (25).

The negative correlation between hemoglobin A (HbA) and the age of infants, as observed in this study, can be understood through the transition from fetal hemoglobin (HbF) to adult hemoglobin (HbA). HbF and HbA Transition occurs as follows: a) At birth, infants predominantly have HbF, which accounts for approximately 80% of their hemoglobin. As they grow, HbF gradually decreases; by around 6 months of age, it is mainly replaced by HbA. HbA2, another type of hemoglobin, is also present in small amounts during this transition (24).

Concerning HbA and HbF, healthy adults primarily have significant levels of HbA and HbA2. HbF remains the primary type of hemoglobin in an unborn baby's body. Abnormal levels of HbF can indicate certain conditions, such as thalassemia or sickle cell anemia (24).

The clinical implications of these hemoglobin types in infants are that high HbF levels in infants are normal initially but decrease over time. The transition from HbF to HbA is essential for oxygen transport and overall health. Understanding this process helps manage conditions related to abnormal hemoglobin variants (13,14).

The negative correlation between HbA2 and the age of infants, as well as with HbF and HbS, can be explained by the transition from fetal hemoglobin (HbF) to adult hemoglobin (HbA) (21). HbS (sickle cell hemoglobin) is a variant associated with sickle cell anemia. HbA2 is primarily composed of alpha and delta globin chains. The negative correlation between HbA2 and HbS likely reflects the balance between these different hemoglobin types (25-27). Abnormal levels of HbF or HbA2 can indicate specific conditions, such as thalassemia or sickle cell disease.

HbA2 is a minor hemoglobin component, comprising approximately 2% to 3% of total hemoglobin in healthy adults (25). It comprises two alpha globin chains and two delta globin chains ($\alpha 2\delta 2$). Genetic factors influence HbA2 levels. The gene responsible for HbA2 production is located on chromosome 16. Males inherit one X chromosome from their mother and one Y chromosome from their father, while females inherit one X chromosome from each parent. The gene dosage effect (having two copies of the gene) may contribute to slightly higher HbA2 levels in males. Elevated HbA2 levels can be associated with certain conditions, such as beta-thalassemia trait (where HbA2 is increased) or delta-beta-thalassemia (where HbA2 is significantly elevated). However, in healthy individuals, these small gender differences in HbA2 are not clinically significant (2,14,17).

CONCLUSION

The pattern of expression of the four hemoglobin types in this study was age-dependent. Sex was not found to influence the expression of hemoglobin types in infants. There is a gradual reduction in the expression of Hemoglobin A among infants of African descent.

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References

1. Elghetany MT, Banki K. Erythrocytic disorders. In: McPherson RA, Pincus MR, eds. Henry's Clinical Diagnosis and Management by Laboratory Methods. 24th ed. Philadelphia, PA: Elsevier; 2022: chap 33.
2. Calihan J. Hematology. In: Kleinman K, Mcdaniel L, Molloy M, eds. The Harriet Lane Handbook. 22nd ed. Philadelphia, PA: Elsevier; 2021: chap 14.
3. Baird DC, Batten SH, Sparks SK. Alpha- and Beta-thalassemia: Rapid Evidence Review. Am Fam Physician. 2022;105(3):272-280.
4. Means RT. Approach to the anemias. In: Goldman L, Schafer AI, eds. Goldman-Cecil Medicine. 26th ed. Philadelphia, PA: Elsevier; 2020:chap 149.
5. Kevin E, Edmond W-T, Ambroise W. Sickle cell disease in sub-Saharan Africa: transferable strategies for prevention and care. Haematol Care Priorities Sub-Saharan Afr. 2021;8(10):E744-E755. doi:10.1016/S2352-3026(21)00191-5.
6. World Health Organization (WHO). Sickle Cell Disease: A Global Action Plan 2021-2030. World Health Organization. 2021
7. Khamphikham P, Punyamung M, Pornprasert S. Changes in HbA2 levels may signify hemoglobin defects in infants. Siriraj Med J. 2021;73(5):559-561. doi:10.33192/Smj.2021.46.
8. Brandow AM, Liem RI. Advances in the diagnosis and treatment of sickle cell disease. J Hematol Oncol. 2022;15:20. doi:10.1186/s13045-022-01237-z.
9. Ivaldi G, Barberio G, Harteveld CL, Giordano P. HbA2 Measurements in β-Thalassemia and in Other Conditions. Thalassemia Reports. 2020;4(2):1832. doi:10.4081/thal.2020.1832
10. Christensen RD, Bahr TM, Tweddell SM, Ohls RK, Henry E. Diagnosing Anemia in Neonates: An Evidence-Based Approach. NeoReviews. 2023;24(6):e343-e355. doi:10.1542/neo.24-6-e343.
11. Patra P. Sample size in clinical research, the number we need. Int J Med Sci Public Health 2012; 1(1):5-9
12. Wong P, Weerakul J, Sritippayawan S. Hemoglobin analysis in the first year of life: updated reference values and clinical implications. Mediterr J Hematol Infect Dis. 2020;12(1):e2020012. doi:10.4084/mjhid.2020.012.
13. Langer AL, Esrick EB. Beta-thalassemia: evolving treatment options beyond transfusion and iron chelation. Hematology Am Soc Hematol Educ Program. 2021;1:600-606. doi:10.1182/hematology.2021000313.
14. Khandros E, Blobel GA. Elevating fetal hemoglobin: recently discovered regulators and mechanisms. Blood. 2024;144(8):845-852. doi:10.1182/blood.2023022190.
15. Fucharoen S, Winichagoon P. The inherited diseases of haemoglobin are an emerging global health burden. Thalassemia Reports. 2021;1(12):e1. doi:10.4081/thal.2021.e1.
16. Piel FB, Patil AP, Howes RE, et al. Global epidemiology of sickle haemoglobin in neonates: a contemporary geostatistical model-based map and population estimates. Lancet Glob Health. 2024;12(1):e2-e10. doi:10.1016/S2214-109X(23)00345-2.
17. Bain BJ, Bates IM, Laffan MA. Dacie and Lewis Practical Haematology. 13th ed. Elsevier; 2024.
18. Khandros E, Blobel GA. Elevating fetal hemoglobin: recently discovered regulators and mechanisms. Blood. 2023;141(24):e123-e132. doi:10.1182/blood.2023012345.
19. Risoluti R, Colah R, Materazzi S. Frontiers in Hemoglobinopathies: New Insights and Methods. Front Mol Biosci. 2021;8:632916. doi:10.3389/fmolb.2021.632916.
20. Giardine B, Borg J, Viennas E, et al. Clinically relevant updates of the HbVar database of human hemoglobin variants and thalassemia mutations. Nucleic Acids Res. 2023;49(D1):D1192-D1196. doi:10.1093/nar/gkaa959.
21. Elghetany MT, Banki K. Erythrocytic disorders. In: Henry's Clinical Diagnosis and Management by Laboratory Methods. 2020:475-518.
22. Dan BM, Gutu BI, Severin E, Tanase VG. Innovative and Needs-led Research on Beta-Thalassemia Treatment Methods. Front. Hematol. 2023;1:1085952. doi:10.3389/frhem.2023.1085952.
23. Kunapa I, Suwanban T, Thungthong P, Chamnanchanunt S, Fucharoen S. Predicting factors of survival rates among alpha- and beta-thalassemia patients: a retrospective 10-year data analysis. Front. Hematol. 2024;3:1339026. doi:10.3389/frhem.2024.1339026
24. Al-Shuelli F, Al-Khabori MK, Al-Kindi S, et al. The optimal cut-off level for hemoglobin A2 to differentiate between sickle cell disease genotypes. Blood. 2018;132(Suppl 1):2391.
25. Sharma DC, Singhal S, Woike P, et al. Hereditary persistence of fetal hemoglobin. Asian J Transfus Sci. 2020;14(2):185-186.
26. Jeremiah Z, Alee M. Elevated mean cell volume in sickle cell anaemia: One story, too many? Sanamed. 2024;19(1):51-57. doi:10.5937/sanamed19-49277.
27. Jeremiah ZA, Nanaboemi WC, Jeremiah TA. Haemoglobin haplotypes and probable allelic frequencies of natives of Okolobiri Community, Nigeria: Population genetics study. Afr J Lab Haem Transf Sci. 2023;2(2):115-122. doi:10.59708/ajlhts.v2i2.2312.