AJMB Reports

https://doi.org/10.58460/ajmbs.v2i01.183
https://doi.org/10.58460/ajmbs.v2i01.183

RESEARCH ARTICLE

# Establishing Hemoglobin Variant Confidence Intervals to Improve Sickle Cell Anemia and Related Hemoglobinopathies Classification in Western Kenya

Martin Ngutuku Maratani[*1,2,3,4], Benard Mutua[3,4]

[1] Department of Medical Laboratory Sciences, School of Medicine and Health Sciences, Kabarak University, Nakuru, Kenya
[2] Department of Biological Sciences, Youngstown State University, Ohio, USA
[3] Department of Medical Laboratory Sciences, Masinde Muliro University of Science and Technology (MMUST), Kakamega, Kenya
[4] SMARTPATH Diagnostic Laboratory and CPD Webinar Services LTD, Nakuru, Kenya

*Corresponding Author: mmaratani@kabarak.ac.ke



*Submitted: 25th September 2025 | Accepted: 12th October 2025 | Published Online: 29th October 2025*

ABSTRACT

Sickle cell disease (SCD) is one of the most prevalent inherited hemoglobinopathies in sub-Saharan Africa and remains a major cause of morbidity and mortality. In many resource-limited settings, diagnosis continues to rely on the sickling test, which cannot reliably distinguish SCD from sickle cell trait (SCT). Although hemoglobin phenotyping offers superior diagnostic accuracy, confidence intervals (CIs) for hemoglobin variants have not been established in Western Kenya, leading to challenges in accurate classification and patient management. This study aimed to establish 95% confidence intervals for hemoglobin variants to improve classification of SCD, SCT, and related hemoglobinopathies in Western Kenya. A retrospective descriptive study was conducted using hematology records of 385 individuals tested between January 2015 and November 2021. Hemoglobin variant distributions (HbA1, HbS, HbA2, and HbF) were analyzed using Chi-square tests for categorical associations and One-way ANOVA to assess significant differences across diagnostic groups. The findings showed that SCD patients had markedly reduced HbA1 (<25%) and elevated HbS (>52%), while SCT cases presented with HbA1 >33% and HbS <33%. Pure beta-thalassemia (β-Thal) was characterized by HbA1 >70%, and heterozygous combinations such as HbSS/β-Thal, HbAS/β-Thal, and β-Thal minor consistently showed elevated HbA2 fractions. However, HbA2 and HbF were of limited value in distinguishing SCD from SCT. Establishing phenotype-specific confidence intervals for HbA1 and HbS enhances diagnostic accuracy, minimizes misclassification, and strengthens hemoglobinopathy diagnosis in resource-limited settings such as Western Kenya.

**Keywords:** *sickle cell disease, sickle cell trait, phenotyping, hemoglobin variants, Western Kenya*

**How to Cite this Paper:** MARATANI, M., & MUTUA, B. (2025). Establishing Hemoglobin Variant Confidence Intervals to Improve Sickle Cell Disease and Trait Classification in Western Kenya. *African Journal of Medical Biosciences*, 2(01). https://doi.org/10.58460/ajmbs.v2i01.183

# INTRODUCTION

Sickle cell disease (SCD) is a hereditary hemoglobinopathy caused by a point mutation in the β-globin gene, resulting in the substitution of valine for glutamic acid at the sixth position of the polypeptide chain. This structural alteration produces abnormal hemoglobin S (HbS) molecules that polymerize under deoxygenated conditions, distorting erythrocytes into the characteristic sickle shape. The deformation impairs oxygen transport, promotes vaso-occlusion, and leads to recurrent clinical complications including anemia, pain crises, and organ damage (Williams et al., 2018). Sickle cell disease (SCD) was first described clinically in 1910 by James B. Herrick, who reported the peculiar elongated and sickle-shaped erythrocytes in a dental student with anemia (Herrick, 1910). The molecular basis of the disorder was later elucidated by Pauling, Itano, Singer, and Wells (1949), marking SCD as the first identified molecular disease. Recent reviews by Williams and Thein (2018) and Naik and Haywood (2015) have expanded on these foundational discoveries in the context of modern genetic and clinical understanding.

Globally, SCD represents the most common monogenic disorder, affecting millions of individuals, particularly those of African, Middle Eastern, and Indian descent, as well as populations of African origin residing in the Americas and Europe (Kato et al., 2018; Vargas-Hernández et al., 2023). The World Health Organization (WHO) and the United Nations have identified SCD as a significant public health burden, noting that undiagnosed or misdiagnosed cases contribute substantially to childhood mortality in endemic regions (WHO, 2021 and UN 2020). Mortality is highest in low-resource settings, where diagnostic infrastructure remains limited, and universal newborn screening programs widely adopted in high-income countries are rarely implemented (Uyoga et al., 2019). Consequently, many affected children die before their fifth birthday, while survivors face lifelong morbidity.

In sub-Saharan Africa, the prevalence of sickle cell trait (SCT) is estimated at 10–40%, with SCD accounting for a significant proportion of pediatric hospital admissions (Ndeezi et al., 2016). The high burden is partly driven by malaria endemicity, as SCT confers partial protection against *Plasmodium falciparum* infection, maintaining the gene within populations through balanced polymorphism (Alegana et al., 2021). However, despite this evolutionary advantage, the clinical burden of homozygous SCD remains severe, contributing to increased mortality and reduced life expectancy (Kosiyo et al., 2021). Regional studies have shown alarming trends of under-diagnosis and misdiagnosis, leading to inappropriate clinical management and increased risk of transmission among offspring (Adekunle et al., 2021).

In Kenya, the prevalence of SCD is particularly high in malaria holoendemic regions such as the Lake Victoria basin, which includes much of Western Kenya (Mutua, Sowayi, & Okoth, 2022). Despite this, large-scale population screening and reliable phenotyping programs remain limited, largely due to resource constraints and reliance on outdated or suboptimal diagnostic tools. Most clinical laboratories continue to depend on qualitative methods such as sickling tests, solubility tests, and peripheral blood smears, which, while affordable, cannot distinguish between SCD and SCT with sufficient accuracy (Naik & Haywood, 2015; Arishi, Al-Hadrami, & Zourob, 2021). More recently, rapid test kits such as Sickle SCAN have been introduced, but they remain qualitative and prone to misclassification (Ndeezi et al., 2016).

By contrast, gold-standard methods including high-performance liquid chromatography (HPLC), hemoglobin electrophoresis, and DNA-based polymerase chain reaction (PCR) allow for precise phenotyping of hemoglobin variants and are critical for guiding clinical management, genetic counseling, and prognostic decisions (Shah et al., 2021; Baig et al., 2021). Among these, HPLC has been recognized as a highly sensitive and reproducible tool, second only to molecular genotyping (Khera et al., 2015). Machines such as the Bio-Rad D10 have become increasingly utilized in specialized laboratories, offering quantitative determination of hemoglobin fractions, yet their adoption in most Kenyan health facilities remains limited (Mutua et al., 2022).

The consequences of relying on non-standardized or imported reference ranges are profound. Studies have documented significant misclassification of hematological disorders in Africa when reference intervals derived from non-African populations are applied (Boyce, Sokolowski, & Robinson, 2020; Omarine Nlinwe, Kumenyuy, & Funwi, 2021). In Nigeria, for instance, nearly one-third of hemoglobin phenotype diagnoses were found to be erroneous due to reliance on outdated electrophoresis techniques, inadequate control materials, and absence of population-specific confidence intervals (Adekunle et al., 2021). Such diagnostic inaccuracies not only jeopardize individual patient care but also compromise public health interventions, registry accuracy, and genetic counseling.

In Western Kenya, where the burden of

hemoglobinopathies is high, the absence of locally established confidence intervals for hemoglobin variant phenotypes represents a critical gap in laboratory medicine. Misclassification of SCT as SCD or vice versa can lead to unnecessary treatment, psychological distress, or failure to prevent transmission to offspring through appropriate counseling (Mrazek et al., 2020). Moreover, erroneous phenotyping undermines clinicians' ability to tailor therapeutic and prognostic pathways, placing patients at risk of both under-treatment and overtreatment.

To address this gap, the present study developed population-specific 95% confidence intervals for hemoglobin variants using HPLC among individuals with SCD and related hemoglobinopathies in Western Kenya. To our knowledge, this is the first study in Kenya and possibly across sub-Saharan Africa to generate locally validated confidence intervals for hemoglobin phenotypes. Establishing such intervals is expected to improve diagnostic accuracy, enhance patient safety, inform appropriate treatment strategies, and strengthen population-level disease surveillance.

# METHODS

## *Study Design*

The study adopted a retrospective descriptive hospital-based design. A retrospective approach was appropriate as it enabled the inclusion of a sufficiently large dataset spanning multiple years, increasing statistical power and the reliability of confidence intervals developed for hemoglobin phenotypes. Descriptive studies of this type have been widely applied in hemoglobinopathy research, particularly in resource-limited settings, as they allow efficient utilization of hospital laboratory data to characterize disease burden and diagnostic challenges (Mutua et al., 2022).

## *Study Location*

This study was conducted at the Aga Khan Hospital in Kisumu, along with its satellite centers across Western Kenya, including Busia, Bungoma, Kitale, Kakamega, Kisii, and Migori. The hospital serves as a key referral facility for the Lake Victoria basin, a malaria holoendemic region where sickle cell disease (SCD) and related hemoglobinopathies remain highly prevalent (Kosiyo et al., 2021; Uyoga et al., 2019). Kisumu County and the broader Western Kenya region were selected as the study location due to their disproportionately high burden of hemoglobin disorders, which are further compounded by socioeconomic and infrastructural constraints that limit access to specialized diagnostic services (Mutua, Sowayi, & Okoth, 2022). The hospital laboratories are among the few in the region equipped with advanced diagnostic technologies, including high-performance liquid chromatography (HPLC), enabling accurate phenotyping of hemoglobin variants. This setting therefore, provided an ideal environment for conducting a large-scale, hospital-based retrospective study to generate confidence intervals for hemoglobin variant phenotypes.

## *Sampling Framework*

The study population comprised confirmed cases of sickle cell anemia (SCA), sickle cell trait (SCT), and beta-thalassemia (β-Thal) hemoglobinopathies that had been diagnosed using HPLC at Aga Khan Hospital, Kisumu and its affiliated centers including Busia, Bungoma, Kitale, Kakamega, Kisii, and Migori. Eligible study subjects were retrospectively identified from the hematology laboratory database between January 1, 2015, and November 9, 2021. The inclusion criteria specified individuals of all ages and both sexes with complete HPLC diagnostic profiles for hemoglobin variants. Subjects who had received blood transfusions within the three months prior to testing were excluded to avoid confounding from donor hemoglobin fractions.

The minimum required sample size was determined using Cochran's formula, assuming a 17.1% prevalence of hemoglobinopathies among children in Western Kenya, as previously reported (Mutua et al., 2022). This calculation yielded a minimum of 237 participants. However, to enhance precision and ensure broader representation, all eligible cases recorded during the study period were included, resulting in a final sample of 385 participants. This census approach minimized sampling error and increased the external validity of findings for the Western Kenya population.

## *Data Collection Tools*

Data were extracted using a structured data abstraction form developed by the research team. Information collected included demographic characteristics (age, sex), type of hemoglobinopathy, and quantitative HPLC results for hemoglobin fractions (HbA1, HbA2, HbS, HbF, and P-window). The HPLC machine used was the Bio-Rad D10, a widely validated instrument for hemoglobinopathy screening that has demonstrated high reproducibility and sensitivity in both African and international settings (Khera et al., 2015; Baig et al., 2021).

## *Data Collection Procedures*

Data were retrospectively retrieved from the electronic hematology laboratory records. Each eligible participant's record was reviewed, and relevant variables were transcribed into Microsoft

Excel spreadsheets. Quality control procedures were implemented to ensure accuracy and completeness of data entry. These included double-checking of entries by two independent researchers and resolving discrepancies through consensus.

All HPLC analyses had been carried out during routine diagnostic workup by qualified laboratory technologists at Aga Khan Hospital, Kisumu. The Bio-Rad D10 system was operated according to manufacturer instructions, with routine calibration and use of internal controls to ensure reliability of results. Hemoglobin fractions were expressed as percentages of total hemoglobin, and the data captured reflected the original diagnostic output from the machine. The study did not involve re-testing of samples but relied entirely on archived diagnostic records.

### *Data Analysis*

Data were coded and exported from Microsoft Excel into IBM SPSS Statistics version 23.0 (IBM Corp., Armonk, NY, USA) for analysis. Demographic variables such as sex and age were summarized using frequencies and proportions, while hemoglobin fractions were described using means and standard deviations.

The chi-square test was applied to assess variations among categorical variables, while one-way analysis of variance (ANOVA) was used to evaluate differences in mean hemoglobin fractions across phenotypes. Confidence intervals (95% CI) were calculated for each hemoglobin fraction, stratified by phenotype, age, and sex. Statistical significance was determined at $p < 0.05$.

The choice of chi-square and ANOVA was guided by their suitability for categorical and continuous variables, respectively, and their widespread application in hemoglobinopathy research for establishing diagnostic cutoffs (Vargas-Hernández et al., 2023; Mutua et al., 2022).

These analyses enabled the derivation of robust confidence intervals, essential for distinguishing between SCD, SCT, and β-Thal phenotypes.

### *Ethical Considerations*

The study adhered to ethical principles of biomedical research involving human participants. Ethical approval was obtained from Masinde Muliro University of Science and Technology Ethics Review Committee (Ref: MMU/COR:403012 Vol 3(03)). Additional clearance was granted by the National Commission for Science, Technology and Innovation (NACOSTI) (Permit No: 407653). Authorization for data access was provided by Aga Khan Hospital, Kisumu Ethics and Research Review Committee (Ref: ADM/007/089).

Patient confidentiality was maintained throughout the study. All records were anonymized by assigning unique identification codes, and data were stored in password-protected computers located in secure laboratory offices. Since this was a retrospective analysis of existing hospital records, informed consent was waived by the ethics committees, but strict confidentiality safeguards were implemented in accordance with institutional guidelines (Mrazek et al., 2020).

## RESULTS

### *Socio-demographic Characteristics of Study Participants*

A total of 385 participants were included in the analysis, with nearly equal representation of males (49.1%) and females (50.9%). The majority of participants were children under two years (33.5%), followed by those older than 12 years (29.1%). Participants aged 3–5 years and 6–11 years accounted for 18.4% and 19.0%, respectively. No statistically significant difference was observed in hemoglobin phenotype distribution by sex ($p = 0.101$) or age group ($p = 0.068$). Table 1 summarizes the distribution of participants by gender and age.

*Table 1:*

*Socio-demographic Characteristics of Study Participants (N = 385)*

| Variable | Category | Frequency (n) | p-value |
|---|---|---|---|
| Gender | Male | 189 | 0.101 |
| | Female | 196 | |
| Age | < 2 years | 129 | 0.068 |
| | 3–5 years | 71 | |
| | 6–11 years | 73 | |
| | > 12 years | 112 | |

*Note: P-values in bold indicate significant differences ($p < 0.05$). The table shows participants' socio-demographic characteristics (N = 385) by gender and age, with subgroup frequencies, percentages, and corresponding p-values for hemoglobin fraction variations*

The age distribution of the 385 participants included in the study is also illustrated on figure 1. Four age categories are shown: <2 years, 3–5 years, 6–11 years, and >12 years. The largest proportion of participants falls within the <2 years category, followed by those aged >12 years. The remaining participants are distributed fairly evenly between the 3–5 years and 6–11 years groups. The bar chart illustrates the relative frequency of each age group as a percentage of the total study population.

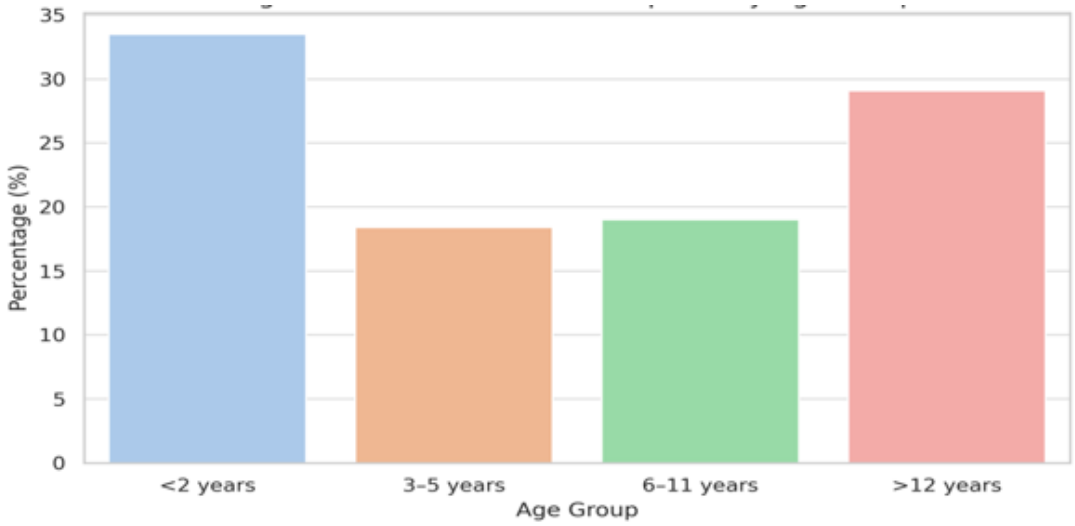


*Figure 1. Distribution of Participants by Age Group*

## *Distribution of Framingham Risk Scores*

The overall prevalence of hemoglobinopathies was dominated by sickle cell trait (SCT), which accounted for 42.9% of cases (n = 165). Homozygous sickle cell disease (HbSS) represented 19.0% (n = 73), while SCD with elevated fetal hemoglobin (HbSS+HbF) and SCD with beta-thalassemia (HbSS+β-Thal) accounted for 15.3% (n = 59) and 16.4% (n = 63), respectively. Less common phenotypes included SCT with HbF (0.8%, n = 3), SCT with β-thalassemia (2.9%, n = 11), and pure β-thalassemia (2.9%, n = 11). The differences in distribution of hemoglobin phenotypes were statistically significant ($p < 0.0001$). Table 2 presents these findings.

*Table 2:*

*Distribution of Hemoglobin Phenotypes (N = 385)*

| Hemoglobin Phenotype | Frequency (n) | Percentage (%) | p-value |
|---|---|---|---|
| HbSS (SCD) | 73 | 19.0 | <0.0001 |
| HbSS + HbF | 59 | 15.3 | |
| HbSS + β-Thal | 63 | 16.4 | |
| HbAS (SCT) | 165 | 42.9 | |
| HbAS + HbF | 3 | 0.8 | |
| HbAS + β-Thal | 11 | 2.9 | |
| β-Thal | 11 | 2.9 | |

*Note: P-values in bold indicate statistically significant differences ($p < 0.05$). This table presents the distribution of hemoglobin phenotypes among study participants (N = 385), showing their frequencies, percentages, and corresponding p-values for variation across phenotypic groups.*

The overall frequency of the identified hemoglobin phenotypes in the study cohort is displayed on figure 2. The phenotypes include HbSS (sickle cell disease), HbSS+HbF, HbSS+β-Thal, HbAS (sickle cell trait), HbAS+HbF, HbAS+β-Thal, and β-Thal. Each bar represents the proportion of cases attributed to a specific phenotype among the total participants. HbAS accounts for the largest proportion, while β-Thal and other compound phenotypes appear in lower frequencies. The figure provides a visual summary of the relative distribution of hemoglobinopathies detected.

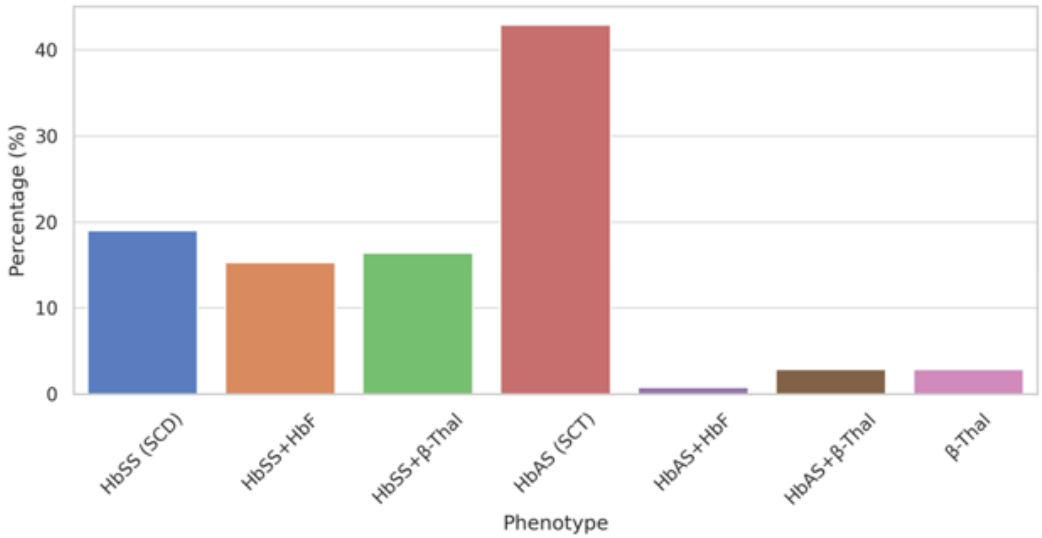


*Figure 2: Prevalence of Hemoglobin Phenotypes*

## *Hemoglobin Fractions across Phenotypes*

As seen in table 3 below, the mean levels of hemoglobin fractions varied significantly across the different phenotypes of sickle cell disease (SCD), sickle cell trait (SCT), and beta-thalassemia, with all differences reaching statistical significance ($p < 0.0001$). In homozygous SCD (HbSS), the total hemoglobin phenotype was 98.9%, with HbA1 showing the lowest mean proportion among all fractions at 4.9 ± 4.0 (95% CI = 0–14.7). HbA2 averaged 3.1 ± 1.2 (95% CI = 0.7–5.5), while HbS was highest across all phenotypes, with a mean of 81.2 ± 9.9 (95% CI = 61.4–100). HbF levels averaged 9.6 ± 7.7 (95% CI = 0–25.0), and the P-window fraction was minimal at 0.1 ± 0.5 (95% CI = 0–1.1).

For participants with SCD and elevated fetal hemoglobin (HbSS+HbF), the total hemoglobin phenotype was 98.7%. The mean HbA1 level was 6.2 ± 9.4 (95% CI = 0–25.0), while HbA2 was 2.5 ± 1.0 (95% CI = 0.5–4.5). HbS levels declined compared to HbSS alone, averaging 69.1 ± 12.3 (95% CI = 44.5–93.7). By contrast, HbF levels were the highest recorded in this phenotype, with a mean of 18.5 ± 8.1 (95% CI = 2.3–34.7). The P-window remained negligible at 0.1 ± 0.4 (95% CI = 0–0.9).

In SCD with beta-thalassemia (HbSS+β-Thal), total hemoglobin phenotype was 99.9%. HbA1 levels were low, at 4.9 ± 6.1 (95% CI = 0–17.1), while HbA2 averaged 3.8 ± 1.4 (95% CI = 1.0–6.6). HbS accounted for 72.8 ± 13.0 (95% CI = 46.8–98.8), the second-highest among phenotypes, while HbF was also elevated, with a mean of 17.4 ± 10.5 (95% CI = 0–38.4). The P-window in this group was consistent at 1.0 ± 0.01 (95% CI = 0.98–1.02).

In SCT, the total hemoglobin phenotype was 99.4%. HbA1 contributed the largest fraction, averaging 58.0 ± 8.9 (95% CI = 40.2–75.9), while HbA2 was 3.6 ± 1.1 (95% CI = 1.4–5.8).

HbS levels were much lower compared to SCD phenotypes, averaging 32.3 ± 6.6 (95% CI = 19.1–45.5). HbF remained minimal at 1.8 ± 4.2 (95% CI = 0–10.2), while the P-window was higher in this phenotype at 3.7 ± 0.6 (95% CI = 2.5–4.9).

Among participants with SCT and elevated HbF (SCT+HbF), the total hemoglobin phenotype was 94.1%. The mean HbA1 was 63.0 ± 15.0 (95% CI = 33.0–93.0), HbA2 was 2.6 ± 1.0 (95% CI = 0.6–4.6), and HbS was 15.8 ± 12.8 (95% CI = 0–41.4). HbF was relatively high in this phenotype, averaging 12.7 ± 9.6 (95% CI = 0–31.9). No P-window was detected in this subgroup.

For SCT with beta-thalassemia (SCT+β-Thal), the total hemoglobin phenotype was the lowest among all phenotypes at 90.0%. HbA1 averaged 36.9 ± 27.4 (95% CI = 0–91.7), while HbA2 was markedly elevated at 9.3 ± 11.6 (95% CI = 0–32.5). HbS levels averaged 31.7 ± 17.6 (95% CI = 0–66.9), and HbF was 8.7 ± 14.2 (95% CI = 0–37.1). The P-window was 3.4 ± 2.0 (95% CI = 0–7.4).

Finally, in pure beta-thalassemia, the total hemoglobin phenotype was 96.7%. HbA1 was highest across all phenotypes, with a mean of 79.9 ± 12.6 (95% CI = 54.7–100). HbA2 averaged 5.3 ± 3.6 (95% CI = 0–12.5), while HbS was minimal, averaging only 0.5 ± 1.8 (95% CI = 0–4.1). HbF levels averaged 5.8 ± 8.8 (95% CI = 0–23.4). The P-window was highest in this phenotype, with a mean of 5.2 ± 0.8 (95% CI = 3.6–6.8). Overall, the comparative analysis confirmed that the distribution of HbA1, HbA2, HbS, HbF, and P-window varied significantly across hemoglobin phenotypes, with $p < 0.0001$ for all fractions.

**Table 3**:
*Sickle Cell and Beta Thalassemia Hemoglobin Variants Confidence Intervals in Western Kenya*

| Phenotype | HbA1(Mean±SD, 95% CI) | HbA2 (Mean±SD, 95%CI) | HbS (Mean ± SD, 95% CI) | HbF(Mean±SD ,95%CI) | P-window (Mean±SD, 95% CI) | Total Hb (%) |
|---|---|---|---|---|---|---|
| HbSS | 4.9 ± 4.0 (0–14.7) | 3.1 ± 1.2 (0.7–5.5) | 81.2 ± 9.9 (61.4–100) | 9.6 ± 7.7 (0–25.0) | 0.1 ± 0.5 (0–1.1) | 98.9 |
| HbSS+HbF | 6.2 ± 9.4 (0–25.0) | 2.5 ± 1.0 (0.5–4.5) | 69.1 ± 12.3 (44.5–93.7) | 18.5 ± 8.1 (2.3–34.7) | 0.1 ± 0.4 (0–0.9) | 98.7 |
| HbSS+β-Thal | 4.9 ± 6.1 (0–17.1) | 3.8 ± 1.4 (1.0–6.6) | 72.8 ± 13.0 (46.8–98.8) | 17.4 ± 10.5 (0–38.4) | 1.0 ± 0.01 (0.98–1.02) | 99.9 |
| SCT | 58.0 ± 8.9 (40.2–75.9) | 3.6 ± 1.1 (1.4–5.8) | 32.3 ± 6.6 (19.1–45.5) | 1.8 ± 4.2 (0–10.2) | 3.7 ± 0.6 (2.5–4.9) | 99.4 |
| SCT+HbF | 63.0 ± 15.0(33.0–93.0) | 2.6 ± 1.0 (0.6–4.6) | 15.8 ± 12.8 (0–41.4) | 12.7 ± 9.6 (0–31.9) | Not detected | 94.1 |
| SCT+β-Thal | 36.9 ± 27.4 (0–91.7) | 9.3 ± 11.6 (0–32.5) | 31.7 ± 17.6 (0–66.9) | 8.7 ± 14.2 (0–37.1) | 3.4 ± 2.0 (0–7.4) | 90.0 |
| β-Thal | 79.9 ± 12.6 (54.7–100) | 5.3 ± 3.6 (0–12.5) | 0.5 ± 1.8 (0–4.1) | 5.8 ± 8.8 (0–23.4) | 5.2 ± 0.8 (3.6–6.8) | 96.7 |
| p-value | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | — |

***Note**: P-values in bold indicate statistically significant differences ($p < 0.05$). This table shows the mean ± SD and 95% confidence intervals of hemoglobin fractions (HbA1, HbA2, HbS, HbF, P-window) and total hemoglobin (%) for sickle cell and beta thalassemia phenotypes in Western Kenya.*

The mean percentages of hemoglobin fractions (HbA1, HbA2, HbS, and HbF) across the analyzed phenotypes are illustrated in Figure 3. Each bar cluster represents a distinct phenotype and displays the corresponding mean levels of each hemoglobin fraction. This visualization facilitates comparison of quantitative differences in hemoglobin composition among phenotypes, based on data obtained through high-performance liquid chromatography (HPLC) analysis.

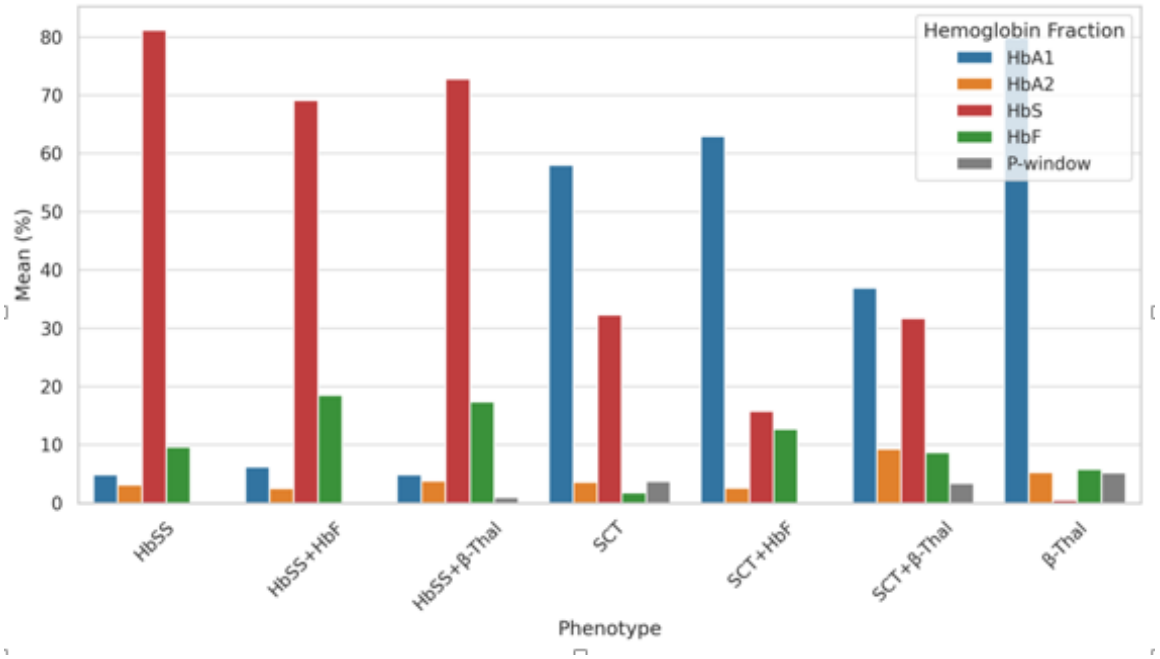


*Figure 3: Mean Hemoglobin Fractions Across Phenotypes*

### *Hemoglobin Variants by Gender and Age*

Table 4 below saws comparison of hemoglobin fractions by gender revealing significant differences in several variants. HbA1 was significantly higher in males (34.6 ± 27.9; 95% CI = 0–90.4) compared to females (28.5 ± 28.6; 95% CI = 0–85.7; p = 0.037). Similarly, HbA2 levels were significantly elevated in males (3.9 ± 3.3; 95% CI = 0–10.5) compared to females (3.2 ± 1.2; 95% CI = 0.8–5.6; p = 0.010). In contrast, HbS was significantly higher among females (55.7 ± 24.9; 95% CI = 5.9–100.0) compared to males (49.8 ± 25.2; 95% CI = 0–100.2; p = 0.022). Although fetal hemoglobin (HbF) levels appeared slightly higher in females (9.8 ± 9.9; 95% CI = 0–29.6) than in males (7.8 ± 10.2; 95% CI = 0–28.2), this difference was not statistically significant (p = 0.055). The P-window fraction, however, was significantly higher in males (2.4 ± 1.9; 95% CI = 0–6.2) than in females (1.4 ± 1.8; 95% CI = 0–5.0; p = 0.002).

When analyzed by age group, HbA1 varied significantly (p = 0.028). Children younger than 2 years recorded a mean of 34.5 ± 27.9 (95% CI = 0–90.3), while those aged 3–5 years and 6–11 years had lower but similar levels at 25.8 ± 28.1 (95% CI = 0–82.0) and 25.8 ± 28.3 (95% CI = 0–82.4), respectively. Participants older than 12 years had the highest mean HbA1 levels, averaging 35.2 ± 28.4 (95% CI = 0–92.0).

By contrast, HbA2 did not show significant variation across age groups (p = 0.294). Mean values were stable at 3.4 ± 1.5 (95% CI = 0.4–6.4) in those <2 years, 3.4 ± 1.2 (95% CI = 1.0–5.8) in 3–5 years, 3.4 ± 1.6 (95% CI = 0.2–6.6) in 6–11 years, and 3.9 ± 4.1 (95% CI = 0–12.1) in those >12 years.

HbS showed significant age-related differences (p = 0.019). The lowest mean level was observed in children <2 years (48.3 ± 22.9; 95% CI = 2.5–94.1), while progressively higher levels were recorded in children aged 3–5 years (57.2 ± 26.3; 95% CI = 4.6–109.8) and 6–11 years (58.3 ± 25.9; 95% CI = 6.5–110.1). Participants older than 12 years had slightly lower HbS levels at 51.7 ± 25.9 (95% CI = 0–103.5). HbF levels also varied significantly with age (p < 0.001). The highest levels were observed in children aged 6–11 years (10.6 ± 11.1; 95% CI = 0–32.8) and 3–5 years (10.5 ± 10.8; 95% CI = 0–32.4), followed by those <2 years (9.7 ± 9.9; 95% CI = 0–29.5). Participants older than 12 years had the lowest HbF levels (5.3 ± 8.2; 95% CI = 0–21.7).

The P-window did not show statistically significant variation across age groups (p = 0.483). Children younger than 2 years had a mean of 1.8 ± 1.9 (95% CI = 0–5.6), those aged 3–5 years had slightly higher levels at 2.3 ± 2.1 (95% CI = 0–6.5), while children aged 6–11 years recorded 1.5 ± 1.9 (95% CI = 0–5.3). Participants older than 12 years had comparable levels at 2.2 ± 1.8 (95% CI = 0–5.8).

**Table 4**:

*Haemoglobin Variants Confidence Intervals Based on Gender and Age in Western Kenya*

| Hemoglobin Fraction | Gender (Mean ± SD; 95% CI) | p-value | Age Group (Mean ± SD; 95% CI) | p-value |
|---|---|---|---|---|
| **HbA1** | Male: 34.6 ± 27.9 (0–90.4)<br>Female: 28.5 ± 28.6 (0–85.7) | 0.037 | <2 yrs: 34.5 ± 27.9 (0–90.3)<br>3–5 yrs: 25.8 ± 28.1 (0–82.0)<br>6–11 yrs: 25.8 ± 28.3 (0–82.4)<br>>12 yrs: 35.2 ± 28.4 (0–92.0) | 0.028 |
| **HbA2** | Male: 3.9 ± 3.3 (0–10.5)<br>Female: 3.2 ± 1.2 (0.8–5.6) | 0.010 | <2 yrs: 3.4 ± 1.5 (0.4–6.4)<br>3–5 yrs: 3.4 ± 1.2 (1.0–5.8)<br>6–11 yrs: 3.4 ± 1.6 (0.2–6.6)<br>>12 yrs: 3.9 ± 4.1 (0–12.1) | 0.294 |
| **HbS** | Male: 49.8 ± 25.2 (0–100.2)<br>Female: 55.7 ± 24.9 (5.9–100.0) | 0.022 | <2 yrs: 48.3 ± 22.9 (2.5–94.1)<br>3–5 yrs: 57.2 ± 26.3 (4.6–109.8)<br>6–11 yrs: 58.3 ± 25.9 (6.5–110.1)<br>>12 yrs: 51.7 ± 25.9 (0–103.5) | 0.019 |
| **HbF** | Male: 7.8 ± 10.2 (0–28.2)<br>Female: 9.8 ± 9.9 (0–29.6) | 0.055 | <2 yrs: 9.7 ± 9.9 (0–29.5)<br>3–5 yrs: 10.5 ± 10.8 (0–32.4)<br>6–11 yrs: 10.6 ± 11.1 (0–32.8)<br>>12 yrs: 5.3 ± 8.2 (0–21.7) | 0.000 |
| **P-window** | Male: 2.4 ± 1.9 (0–6.2)<br>Female:1.4 ± 1.8 (0–5.0) | 0.002 | <2 yrs: 1.8 ± 1.9 (0–5.6)<br>3–5 yrs: 2.3 ± 2.1 (0–6.5)<br>6–11 yrs: 1.5 ± 1.9 (0–5.3)<br>>12 yrs: 2.2 ± 1.8 (0–5.8) | 0.483 |

***Note**: P-values in bold indicate statistically significant differences (p < 0.05). This table shows the mean ± SD and 95% confidence intervals of hemoglobin fractions (HbA1, HbA2, HbS, HbF, P-window) and total hemoglobin (%) for sickle cell and beta thalassemia phenotypes in Western Kenya.*

# DISCUSSION

This study revealed that sickle cell trait (SCT) was the most prevalent hemoglobin phenotype in Western Kenya, followed by homozygous sickle cell disease (HbSS), SCD with elevated fetal hemoglobin (HbSS+HbF), and SCD with beta-thalassemia (HbSS+β-Thal). Less frequent phenotypes included SCT with HbF, SCT with β-thalassemia, and pure β-thalassemia. The frequency of SCT observed in this cohort (42.9%) aligns with earlier studies across East and West Africa that reported prevalence rates ranging between 20–40%, depending on geographic and ecological settings (Makani et al., 2011; Ndeezi et al., 2016). These findings reaffirm the evolutionary interplay between malaria and hemoglobinopathies in sub-Saharan Africa.

The prevalence of homozygous SCD (19%) observed here is consistent with hospital-based reports from Tanzania and Uganda, where SCD represents a major burden of childhood morbidity and mortality (Makani et al., 2011; Ndeezi et al., 2016). The relatively high frequencies of HbSS+HbF (15.3%) and HbSS+β-Thal (16.4%) indicate phenotypic diversity of hemoglobin disorders in African populations, which likely reflects underlying genetic heterogeneity. These observations mirror findings from other regions where compound phenotypes are increasingly recognized (Serjeant, 2013, Kim et al, 2017)
Importantly, the detection of β-thalassemia, both as isolated cases and in combination with SCT or SCD, suggests that thalassemia may be more prevalent in East Africa than historically assumed (Chakravorty & Williams, 2015). This calls for renewed focus on thalassemia surveillance, as most genetic studies in the region have concentrated on SCD.

Hemoglobin fraction analysis highlighted distinct patterns across phenotypes. HbSS participants showed the highest HbS levels (81.2%) with nearly absent HbA1, consistent with the pathophysiology of SCD (Steinberg, 2009). Elevated HbF levels in HbSS+HbF and HbSS+β-Thal phenotypes (18.5% and 17.4%, respectively) are clinically significant, as HbF is protective against sickling and hemolysis (Steinberg, 2009; Thein, 2017). These results echo prior studies showing that naturally elevated HbF contributes to milder disease phenotypes and better survival outcomes among African children with SCD (Makani et al., 2011).

Age stratification revealed significant differences in hemoglobin fraction distribution. Younger children (<12 years) exhibited higher HbF levels, particularly within the 3–11-year age range, whereas adolescents and adults (>12 years) had markedly lower HbF concentrations. This pattern is consistent with previous findings indicating a natural developmental decline in HbF levels with increasing age (Steinberg, 2009; Thein, 2017).

HbS levels HbS levels also varied with age, showing relatively higher proportions among children aged 3–11 years compared to infants and adolescents. This pattern may be attributed to maturational changes in globin gene expression, particularly the physiological switch from γ-globin (HbF) to β^S-globin (HbS) synthesis that occurs during early childhood in individuals with sickle cell genotypes The gradual activation of the β-globin gene cluster leads to increased HbS production as HbF declines, reflecting the genetic regulation of hemoglobin switching Sankaran & Orkin (2013). In contrast, HbA2 levels remained relatively stable across all age groups, consistent with previous reports indicating minimal age-related variation in this fraction (Chakravorty & Williams, 2015).

The analysis revealed significant differences in hemoglobin fractions between males and females. HbA1 and HbA2 levels were higher in males, while HbS was significantly higher in females. HbF appeared slightly elevated in females compared to males, although this difference was not statistically significant. These sex-based differences have not been consistently reported in prior studies, and while they may reflect biological variation, sampling effects cannot be ruled out (Lauridsen et al., 2023; Urio et al., 2023).

The findings of this study carry important clinical and public health implications. The high burden of SCD and related hemoglobinopathies highlights the need for systematic newborn screening programs in Kenya. Evidence from Tanzania and Uganda shows that early identification of affected children, coupled with interventions such as penicillin prophylaxis, vaccination, and parental education, can significantly reduce SCD-related mortality (Makani et al., 2011; Ndeezi et al., 2016). Moreover, the variation in hemoglobin fractions across phenotypes underscores the necessity of advanced diagnostic tools like high-performance liquid chromatography (HPLC), which provide detailed quantification beyond conventional electrophoresis.

The detection of β-thalassemia in this population also signals the need for improved diagnostic awareness. Elevated HbA2 levels, as seen in SCT+β-Thal individuals in this study, highlight the role of HbA2 quantification in diagnosing thalassemia syndromes (Chakravorty & Williams, 2015). Misdiagnosis or under-recognition of these

conditions may lead to inappropriate treatment strategies, emphasizing the need for training and laboratory capacity building.

Several limitations should be considered when interpreting these results. First, the cross-sectional design restricts the ability to establish temporal relationships or assess long-term clinical outcomes. Second, phenotypic classification relied on HPLC rather than molecular diagnostics, which may have led to misclassification in compound heterozygous states. Previous research has highlighted that molecular characterization provides a more accurate picture of genotype–phenotype interactions (Chakravorty & Williams, 2015). Third, the study population was drawn from Western Kenya and may not be representative of other regions with different genetic and ecological contexts.

## Conclusions

This study established phenotype-specific confidence intervals for HbA1 and HbS, enhancing diagnostic accuracy and minimizing the risk of misclassifying sickle cell disease (SCD) and sickle cell trait (SCT) in Western Kenya. The high prevalence of SCT, along with notable frequencies of compound phenotypes involving elevated fetal hemoglobin and β-thalassemia, reflects the phenotypic diversity of hemoglobinopathies in the region. By applying high-performance liquid chromatography (HPLC) for precise hemoglobin fraction quantification, this study supports more reliable clinical decision-making and strengthens the diagnostic framework for hemoglobinopathies. These locally validated confidence intervals provide a model that can be adapted to other resource-limited settings to improve screening, diagnosis, and management of sickle cell disorders across sub-Saharan Africa.

## Recommendations

Based on these findings, we recommend that health facilities in Western Kenya and similar resource-limited regions adopt the established phenotype-specific confidence intervals for HbA1 d HbS as part of standard diagnostic interpretation. Integrating these locally validated reference ranges into routine laboratory practice will enhance diagnostic precision, minimize misclassification between SCD and SCT, and improve clinical decision-making. Wider implementation of high-performance liquid chromatography (HPLC) for hemoglobin fraction analysis should be prioritized within national screening and diagnostic programs. Furthermore, training laboratory personnel on interpretation of HPLC results using local reference data will strengthen diagnostic reliability. At the policy level, incorporating such context-specific diagnostic standards into national guidelines will advance equitable and accurate detection, management, and surveillance of hemoglobinopathies across sub-Saharan Africa.

We also recommend that future research builds upon the present study by incorporating post-hoc analyses, such as Tukey's HSD, to identify specific intergroup differences. While this study focused on overall variations in hemoglobin fractions across phenotypic groups, such additional analyses would provide deeper insights into how particular phenotypes differ in their hemoglobin fraction profiles

Finally, researchers should conduct longitudinal molecular and clinical studies to better understand genotype phenotype interactions and inform the development of region-specific management guidelines

## Conflict of Interest

The authors declare no conflict of interest.

## Author Contributions

[Martin Maratani] conceptualized and designed the study drafted the manuscript. [Benard Mutua] coordinated data collection and ensured quality control, conducted data analysis and interpretation. Both authors critically reviewed the manuscript, contributed to revisions, and approved the final version for submission.

## REFERENCES

Naik, R. P., & Haywood, C. Jr. (2015). Sickle cell trait diagnosis: Clinical and social implications. Hematology 2014, The American Society of Hematology Education Program Book, 2015(1), 160–167. https://doi.org/10.1182/asheducation-2014.1.160

Williams, T. N., & Thein, S. L. (2018). Sickle cell anemia and its phenotypes. Annual Review of Genomics and Human Genetics, 19, 113–147. https://doi.org/10.1146/annurev-genom-083117-021320

Kim, M., Odame, I., Little, J. A., & Gurkan, U. A. (2017). Emerging point-of-care technologies for sickle cell disease screening and monitoring. Expert Review of Medical Devices,13(12),1073–1093. https://doi.org/10.1080/17434440.2016.1254038

Arishi, W. A., Al-Hadrami, H. A., & Zourob, M. (2021). Techniques for the detection of sickle

cell disease: A review. Micromachines, 12(5), 519. https://doi.org/10.3390/mi12050519

Uyoga, S., Macharia, A. W., Mochamah, G., Ndila, C. M., Nyutu, G., Makale, J., … Williams, T. N. (2019). The epidemiology of sickle cell disease in children recruited in infancy in Kilifi, Kenya: A prospective cohort study. The Lancet Global Health, 7(10), e1458–e1466. https://doi.org/10.1016/S2214-109X(19)30328-6

Kato, G. J., Piel, F. B., Reid, C. D., Gaston, M. H., Ohene-Frempong, K., Krishnamurti, L., & Vichinsky, E. P. (2018). Sickle cell disease. Nature Reviews Disease Primers, 4(1), 1–22. https://doi.org/10.1038/nrdp.2018.10

Chakravorty, S., & Williams, T. N. (2015). Sickle cell disease: A neglected chronic disease of increasing global health importance.Archives of Disease in Childhood, 100(1), 48–53. https://doi.org/10.1136/archdischild-2013-303773

Kosiyo, P., Otieno, W., Gitaka, J., Munde, E. O., & Ouma, C. (2021). Haematological abnormalities in children with sickle cell disease and non-severe malaria infection in western Kenya.BMC Infectious Diseases, 21, 325. https://doi.org/10.1186/s12879-021-06025-7

Alegana, V. A., Macharia, P. M., Muchiri, S., Mumo, E., Oyugi, E., Kamau, A., Snow, R. W. (2021). Plasmodium falciparum parasite prevalence in East Africa: Updating data for malaria stratification. PLOS Global Public Health,1(12),e0000014. https://doi.org/10.1371/journal.pgph.0000014

Shah, N., Khonglah, Y., Raphael, V., Swer, B., Nath, C., & Singh, A. S. (2021). Antenatal screening for hemoglobinopathies with HPLC. Indian Journal of Pathology and Oncology, 8(3),481–486. https://doi.org/10.18231/j.ijpo.2021.116

Ndeezi, G., Kiyaga, C., Hernandez, A. G., Munube, D., Howard, T. A., Ssewanyana, I., & Aceng, J. R. (2016). Burden of sickle cell trait and disease in the Uganda Sickle Surveillance Study (US3): A cross-sectional study. The Lancet Global Health, 4(3), e195–e200. https://doi.org/10.1016/S2214-109X(16)00038-9

Adekunle, M. O., Ojewunmi, O., Animasahun, A. B., Lawani, F. O., & Ubuane, P. O. (2021). Prevalence, determinants and impact of haemoglobin phenotype misdiagnosis among parents of children living with sickle cell disease in Nigeria. Journal of Pediatric Research,8(3),180–187. https://doi.org/10.4274/jpr.galenos.2020.54366

Mrazek, C., Lippi, G., Keppel, M. H., Felder, T. K., Oberkofler, H., Haschke-Becher, E., & Cadamuro, J. (2020). Errors within the total laboratory testing process, from test selection to medical decision-making: A review of causes, consequences, surveillance and solutions. Biochemia Medica, 30(2), 215–233. https://doi.org/10.11613/BM.2020.020502

Boyce, W. T., Sokolowski, M. B., & Robinson, G. E. (2020). Genes and environments, development and time. Proceedings of the National Academy of Sciences, 117(38), 23235–23241. https://doi.org/10.1073/pnas.2016710117

Jovanov, P., Đorđić, V., Obradović, B., Barak, O., Pezo, L., Marić, A., & Sakač, M. (2019). Prevalence, knowledge and attitudes towards using sports supplements among young athletes. Journal of the international society of sports nutrition, 16(1), 27. https://doi.org/10.1186/s12970-019-0294-7

Mutua, B., Chelangat, R., Mustafa, B., Were, T., Makani, J., Sowayi, G., & Okoth, P. (2022). High-performance liquid chromatography local reference ranges of hemoglobin fractions (HbA, HbA2, and HbF) in detection of hemoglobinopathies in Western Kenya. The Egyptian Journal of Internal Medicine, 34(1), 95. https://doi.org/10.1186/s43162-022-00115-x

Thein, S. L. (2018). The molecular basis of β-thalassemia and sickle cell disease.Cold Spring Harbor Perspectives in Medicine, 8(12),a034983. https://doi.org/10.1101/cshperspect.a034983

Vargas-Hernández, D. A., Uscategui-Ruiz, A. C., De Avila, J., & Romero-Sánchez, C. (2023). Differences in the distribution of hemoglobin variants according to the geographic regions in a Colombian population. Hematology, Transfusion and Cell Therapy, 45(Suppl 2), S140–S147. https://doi.org/10.1016/j.htct.2022.11.012

Makani, J., Cox, S. E., Soka, D., Komba, A. N., Oruo, J., Mwamtemi, H., Snow, R. W. (2011). Nutritional status, hospitalization and mortality among patients with sickle cell anaemia in Tanzania. Haematologica, 96(7), 952–959. https://doi.org/10.3324/haematol.2010.028167

Steinberg, M. H. (2009). Genetic etiologies for phenotypic diversity in sickle cell anemia.The Scientific World Journal, 9,46–67. https://doi.org/10.1100/tsw.2009.10

Omarine Nlinwe, N., Kumenyuy, L., & Funwi, C. P. (2021). Establishment of hematological reference values among healthy adults in Bamenda, North West Region of Cameroon. Anemia, 2021, 1–7. https://doi.org/10.1155/2021/6691477

P. (2021). Establishment of hematological reference values among healthy adults in Bamenda, North West Region of Cameroon. Anemia,2021,1–7. https://doi.org/10.1155/2021/6691477

Baig, M. A., Swamy, K. B., Baksh, A. D., Bahashwan, A., Moshrif, Y., Al Sawat, A., Alharbi, N. (2021). Evaluation of the role of HPLC (merits & pitfalls) in the diagnosis of various hemoglobinopathies and thalassemic syndromes. Indian Journal of Pathology and Microbiology,64(3),518–523. https://doi.org/10.4103/IJPM.IJPM_126_20

Makani, J., Cox, S. E., Soka, D., Komba, A. N., Oruo, J., Mwamtemi, H., … Snow, R. W. (2011). Mortality in sickle cell anemia in Africa: A prospective cohort study in Tanzania. PLoS ONE, 6(2), e14699. https://doi.org/10.1371/journal.pone.0014699

Sankaran, V. G., & Orkin, S. H. (2013). The switch from fetal to adult hemoglobin.Cold Spring Harbor Perspectives in Medicine, 3(1), a011643. https://doi.org/10.1101/cshperspect.a011643

World Health Organization. (2021).Sickle-cell disease:A strategy for the African Region . World Health Organization. https://www.who.int/publications/i/item/sickle-cell-disease-strategy

United Nations. (2020).UN resolution on addressing sickle-cell anaemia as a public health priority. United Nations. https://www.un.org/en/observances/sickle-cell-day

Suchdev, P. S., Ruth, L. J., Earley, M., Macharia, A., & Williams, T. N. (2014). The burden and consequences of inherited blood disorders among young children in western Kenya. Maternal & Child Nutrition, 10(1), 135–144. https://doi.org/10.1111/j.1740-8709.2012.00454.x

Urio, F., Nkya, S., Mgaya, J., Rooks, H., Ponsian, P., El Hoss, S., Mselle, T., Makani, J., & Menzel, S. (2023). Gender effect on production and enrichment of F cell numbers in sickle cell disease patients in Tanzania. American Journal of Hematology, 98(6), E139–E141. https://doi.org/10.1002/ajh.26914

Lauridsen, K. M., et al. (2023). Pediatric reference intervals of the hemoglobin fractions using high-performance liquid chromatography and capillary electrophoresis. Clinical Biochemistry, 107, 1–8. https://doi.org/10.1016/j.clinbiochem.2023.09.001