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Empirical evaluation of MCV in differentiating sickle cell and β-thalassemia phenotypes in Western Kenya: a receiver operating characteristic (ROC) analysis

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Abstract

Mean corpuscular volume (MCV) is commonly employed in resource-limited settings as a preliminary screening tool for haemoglobinopathies, based on the assumption that these disorders typically present with microcytosis. However, there is a lack of locally validated MCV cut-offs for haemoglobinopathy detection in Kenya, which limits its reliability for both clinical and research applications. In this study, we retrospectively analysed 773 participants, including 388 HbAA controls and 385 individuals with confirmed haemoglobinopathies, whose haemoglobin phenotypes were determined using high-performance liquid chromatography (HPLC). Receiver operating characteristic (ROC) analysis demonstrated poor discriminatory performance of MCV for combined haemoglobinopathies, with an area under the curve (AUC) of 0.528. The optimal MCV threshold of 75.3 fL yielded 50.4% sensitivity and 42.8% specificity, indicating limited diagnostic utility. Sickle cell disease (SCD) phenotypes in this cohort were predominantly normocytic when interpreted using population-based Kenyan reference ranges, contrasting with iron deficiency and other microcytic anemias that typically reduce MCV. The ROC-derived optimal threshold for SCD detection (76.9 fL) fell within the population reference distribution (median 76.8 fL; IQR 11.6), indicating that maximum detection occurs within normocytic ranges. Sickle cell trait and thalassemia traits were sometimes microcytic but overlapped with other conditions. These findings emphasize the importance of using locally derived MCV reference ranges rather than conventional 80–100 fL thresholds. While MCV may assist in triaging suspected cases in resource-limited settings, it cannot reliably identify carrier states, and definitive haemoglobin characterization remains essential for accurate diagnosis and effective screening.

Keywords Empirical · ROC curve · Haemoglobinopthies · Mean curpuscular volume · Sickle cell · Western Kenya

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Introduction

Distinguishing iron deficiency anaemia (IDA) from haemoglobinopathies remains a persistent diagnostic challenge in sub-Saharan Africa, where both conditions are highly prevalent and frequently coexist [1, 2]. The clinical overlap between these disorders is particularly problematic because both may present with similar hematological patterns, especially microcytic hypochromic red cell indices. In many healthcare settings across the region, access to definitive diagnostic technologies such as high-performance liquid chromatography (HPLC), capillary electrophoresis, or molecular testing remains limited due to financial constraints, inadequate laboratory infrastructure, and shortages of trained personnel [3]. As a result, clinicians frequently depend on routinely available laboratory parameters from the complete blood count (CBC) to guide preliminary diagnostic decisions [2].

Among these parameters, mean corpuscular volume (MCV) is one of the most widely interpreted indices and forms a cornerstone of the morphological classification of anaemia [4]. Clinically, microcytosis is often interpreted as suggestive of iron deficiency or thalassaemia trait, whereas normocytic or macrocytic indices are commonly assumed to exclude these conditions. However, MCV values are influenced by numerous physiological and pathological factors, including age, sex, genetic background, erythropoietic activity, nutritional status, and coexisting disease states [5]. This complexity becomes particularly evident in haemoglobinopathies, where chronic haemolysis, increased reticulocyte production, and genetic modifiers can substantially alter red cell indices, resulting in MCV values that overlap with those observed in unaffected individuals. However, variations in MCV may still provide useful preliminary indicators for haemoglobin disorders. For instance, a study conducted in Cambodia demonstrated that combined red cell indices, particularly MCV and RBC count, can help flag potential haemoglobin disorders in population screening settings [6].

Despite these limitations, MCV continues to serve as an important triage marker in resource-limited settings because it is inexpensive, rapidly obtainable, and universally reported as part of routine hematology testing [2]. The clinical usefulness of MCV in screening or early diagnostic evaluation therefore depends largely on the validity of the reference thresholds applied in practice. Currently, many laboratories in Africa continue to apply conventional cut-offs derived largely from European and North American populations, typically defining microcytosis as an MCV below 80 fL [5]. Emerging evidence suggests that such thresholds may not accurately reflect the hematological characteristics of African populations, particularly in malaria-endemic regions where genetic diversity, environmental exposures, and nutritional factors can influence baseline red cell indices.

Receiver operating characteristic (ROC) curve analysis provides an objective statistical framework for evaluating the diagnostic performance of continuous laboratory variables such as MCV and for identifying optimal cut-off values that balance sensitivity and specificity [7]. Through ROC analysis, population-specific thresholds can be empirically derived, allowing more accurate discrimination between disease states and normal physiological variation [8]. Although ROC-based approaches have been applied to certain red blood cell indices in selected African studies, locally validated MCV thresholds for haemoglobinopathy screening remain scarce, particularly within Kenya.

Currently, no empirically validated mean corpuscular volume (MCV) cut-off exists for screening haemoglobinopathies in Kenya or the wider East African region, despite the widespread reliance on this parameter in routine clinical practice. The continued use of conventional thresholds such as MCV 80-100fL may therefore lead to misclassification or reduced diagnostic accuracy when applied to populations with different hematologic profiles [9]. This lack of region-specific diagnostic thresholds represents an important gap in laboratory medicine and highlights the need for population-based evaluation of CBC parameters within African settings.

This study was therefore designed to evaluate the diagnostic performance of MCV using receiver operating characteristic (ROC) analysis and to determine locally relevant MCV cut-off values for the identification of haemoglobinopathies and their specific phenotypes in a Western Kenyan population, thereby providing region-specific evidence on the reliability and limitations of red cell indices in haemoglobinopathy screening and addressing an important gap in the literature within Kenya and the broader Eastern African context.

Methodology

Ethical considerations

Ethical clearance for this study was obtained from the Masinde Muliro University of Science and Technology Ethical Review Committee (MMU/COR:403012 Vol. 3(03)) and from the National Commission for Science, Technology and Innovation (NACOSTI; Reference No. 407653). Additional authorization to access and utilize laboratory records was granted by the Aga Khan Hospital, Kisumu Ethics Committee (ADM/007/089). As this research involved only retrospective review of existing records, the need for individual informed consent was formally waived. Participant confidentiality was safeguarded by limiting access to physical files, which were securely stored in locked cabinets under the oversight of the principal investigator, and by anonymizing electronic datasets, which were maintained on password-protected computers.

Sample size determination

The study's minimum sample size was determined using Cochran's formula, assuming a hemoglobinopathy prevalence of 18.7% among children in Western Kenya, based on previous reports [10]. This calculation indicated that at least 204 participants per study group were needed, yielding a total minimum of 408 participants for the two-arm design.

Ultimately, 773 participants were included in the analysis, with 388 in the control group and 385 in the case group. Instead of limiting recruitment to the minimum calculated sample, all eligible cases available during the study period were included to enhance statistical precision and better reflect the population. This comprehensive inclusion strategy minimized sampling bias and increased the generalizability of the findings to children in Western Kenya.

Study design

This investigation employed a hospital-based, retrospective, cross-sectional comparative design. Laboratory records were examined using a census approach, encompassing 773 participants in total. Among these, 385 individuals (49.8%) were identified as cases, having confirmed haemoglobinopathies via high-performance liquid chromatography in the absence of iron deficiency, while 388 participants (50.2%) without haemoglobinopathies or anaemia served as controls. Corresponding complete blood count data for all participants were retrieved from the haematology databases of Aga Khan Hospital, Kisumu, including its affiliated satellite laboratories across western Kenya.

Data collection

Laboratory data were obtained from the hospital haematology database for patients evaluated over a five-year period, spanning 1 January 2015 to 9 November 2021. Only records containing complete haematological parameters along with confirmed diagnostic outcomes were considered eligible. Full blood count analyses were conducted using multiple Sysmex automated haematology systems, including the KX-21 N, XP-300, XN-L 330, XS-500i, and XS-1000i models (Sysmex Corporation, Kobe, Japan). Participants classified as cases were those with haemoglobinopathies definitively diagnosed via high-performance liquid chromatography using the Bio-Rad D10 analyser (Bio-Rad Laboratories, Hercules, California, USA). Records were excluded if full blood count data were missing, or if the patient had a confirmed leukaemia diagnosis, received a blood transfusion within the prior three months, or had documented antinuclear antibody positivity or clinical sepsis.

The control group included individuals without clinical or laboratory evidence of conditions affecting red blood cell morphology or indices, such as haemolytic disorders, macrocytic anaemia, iron deficiency anaemia, or

haemoglobinopathies. Controls represented multiple age categories and were confirmed by haemoglobin electrophoresis to possess normal adult haemoglobin (HbAA genotype). To minimise the inclusion of participants with anaemia, the normal HbAA control group was selected using the facility-specific haemoglobin reference ranges. Participants with documented iron deficiency based on available serum iron and ferritin results were excluded. However, because this was a retrospective study, iron studies were not routinely available for all participants.

Data analysis

Data were processed and analysed using the Statistical Package for the Social Sciences (SPSS), version 20 (SPSS Inc., Chicago, IL, USA). The distribution of continuous variables was assessed with the Kolmogorov–Smirnov and Shapiro-Wilk tests for normality. These tests indicated that values in the control group were not normally distributed ($p<0.05$); therefore, non-parametric statistical methods were applied throughout. Associations between demographic variables were evaluated using the chi-square test.

Comparisons of mean corpuscular volume (MCV) among the different haemoglobinopathy subtypes were conducted with the Kruskal-Wallis H test, while MCV differences between case and control groups were assessed using the Mann-Whitney U test. Reference intervals for normal MCV were defined based on the control group, specifically using the upper limit of the 95% confidence interval of the median.

Receiver operating characteristic (ROC) curve analysis was employed to evaluate the diagnostic accuracy of MCV for distinguishing participants with haemoglobinopathies from controls, as well as for differentiating among specific haemoglobin phenotypes. The area under the curve (AUC) with 95% confidence intervals was calculated. Optimal MCV thresholds were determined using the Youden index, and diagnostic performance metrics—including sensitivity, specificity, positive and negative predictive values, and likelihood ratios—were subsequently computed.

Results

Distribution of MCV by study group and demographic characteristics

The control group comprised 388 participants (50.2%), while the case group included 385 participants (49.8%). There was no statistically significant difference in distribution between the two groups ($p=0.914$), as shown in Table 1.

Table 1 Median (IQR) MCV (fL) by Study group and demographic characteristics

Variable	Overall Median (IQR)			*p*
		Control ($n=388$; 50.2%)	**Case** ($n=385$;49.8%)	0.914
Median (IQR)		Median (IQR)		
Overall MCV (fL)		76.8 (11.6)	75.3 (18.0)	**0.172**
Gender				
Male ($n=373$; 48.4%)	75.2 (15.0)	76.2 (11.6)	74.4 (21.0)	0.004
Female ($n=399$; 51.6%)	77.3 (12.8)	77.6 (10.2)	76.1 (15.8)	
Age Group				
<2 years ($n=246$; 31.8%)	69.1 (16.2)	69.5 (12.3)	67.9 (21.2)	<0.0001
3–5 years ($n=120$; 15.5%)	74.6 (11.3)	74.5 (6.8)	74.6 (15.7)	
6–11 years ($n=119$; 16.4%)	76.6 (9.3)	75.8 (4.5)	78.6 (13.7)	
≥12 years ($n=288$; 37.3%)	79.6 (10.0)	81.1 (7.7)	77.1 (13.2)	

The Control and case significance was derived using chisquare. MCV Values are presented as Median (interquartile range, IQR). *P*-values were derived using the Mann–Whitney U test for two-group comparisons and the Kruskal–Wallis test for multiple group comparisons. Control=HbAA; Case=participants with confirmed hemoglobinopathies. Statistical significance was set at $p<0.05$.

The overall median MCV was comparable between controls (76.8 fL) and cases (75.3 fL), with no statistically significant difference observed ($p=0.172$). When stratified by sex, males had a significantly lower median MCV compared to females (75.2 fL vs. 77.3 fL, $p=0.004$). This pattern was consistent in both the control and case groups.

A significant age-related trend was also identified ($p<0.0001$), with median MCV increasing progressively with age. Children under 2 years had the lowest median MCV (69.1 fL), whereas participants aged ≥ 12 years had the highest median values (79.6 fL). This age-dependent increase in MCV was consistent across both study groups.

These findings are presented in Table 1.

ROC-based determination of optimal MCV thresholds for hemoglobinopathies

Receiver operating characteristic (ROC) curve analysis was performed to assess the diagnostic performance of MCV in detecting hemoglobinopathies, as summarized in Table 2.

Using a cut-off value of 76.9 fL, the AUC for normal HbAA ($n=388$) was 0.529 (SE = 0.021; 95% CI: 0.487–0.570; $p=0.166$), with its ROC curve crossing the diagonal line (Fig. 1a).

For HbSS ($n=73$), the AUC was 0.591 (SE = 0.035; 95% CI: 0.523–0.659; $p=0.010$), and the ROC curve flowed above the diagonal line (Fig. 2a). Participants with HbSS/HPFH ($n=59$) had an AUC of 0.750 (SE = 0.034; 95% CI: 0.683–0.816; $p<0.001$), with the highest ROC curve among SCD phenotypes (Fig. 2b). For HbSS/β-thalassemia ($n=63$), the AUC was 0.682 (SE = 0.036; 95% CI: 0.611–0.753; $p<0.001$), with its ROC curve above the diagonal line (Fig. 2c).

Among HbAS participants ($n=165$), the AUC was 0.254 (SE = 0.021; 95% CI: 0.212–0.295; $p<0.001$), with its ROC curve below the diagonal line (Fig. 3a). For HbAS/HPFH ($n=3$), the AUC was 0.106 (SE = 0.036; 95% CI: 0.036–0.177; $p=0.018$), with the ROC curve also below the diagonal line, though construction was limited due to the small sample size. HbAS/β-thalassemia ($n=11$) had an AUC of 0.464 (SE = 0.102; 95% CI: 0.263–0.665; $p=0.681$), with the ROC curve along the diagonal line (Fig. 3b). For β-thalassemia alone, the AUC was 0.298 (SE = 0.081; 95% CI: 0.139–0.458; $p=0.022$), with the ROC curve along the diagonal line (Fig. 1b).

The overall AUC for all hemoglobinopathies combined was 0.528 (SE = 0.021; 95% CI: 0.487–0.568; $p=0.172$), with an optimal cut-off value of 75.3 fL. The ROC curve lay close to the diagonal reference line, indicating poor discriminatory ability of MCV in distinguishing hemoglobinopathies from normal HbAA (Fig. 1c).

Table 2 Receiver operating characteristic (ROC) curve analysis of MCV for the detection of hemoglobinopathies

	Cut-off values	Area	Std. Error	Asymptotic Sig.	Asymptotic 95% Confidence Interval	
					Lower Bound	Upper Bound
Normal HbAA ($n=388$)	76.9	0.529	0.021	0.166	0.487	0.570
HbSS ($N=73$)	76.9	0.591	0.035	0.010	0.523	0.659
HbSS/HPFH ($n=59$)	76.9	0.750	0.034	0.000	0.683	0.816
HbS/β-thalassaemia ($n=63$)	76.9	0.682	0.036	0.000	0.611	0.753
HbAS ($n=165$)	76.9	0.254	0.021	0.000	0.212	0.295
HbAS/HPFH ($n=3$)	76.9	0.106	0.036	0.018	0.036	0.177
HbAS/β-thalassaemia ($n=11$)	76.9	0.464	0.102	0.681	0.263	0.665
β-Thalassemia ($n=11$)	76.9	0.298	0.081	0.022	0.139	0.458
Overal Hemoglobinopathies	75.3	0.528	0.021	0.172	0.487	0.568

AUC area under the receiver operating characteristic (ROC) curve, *CI* confidence interval, *HbAA* normal adult haemoglobin, *HPFH* hereditary persistence of fetal haemoglobin, *HbAS* sickle cell trait, *HbSS* sickle cell disease, *β-thal* beta-thalassaemia. For each haemoglobinopathy subgroup, the AUC represents the ability of MCV to discriminate that subgroup from normal HbAA. An AUC of 0.50 indicates no discrimination, whereas an AUC of 1.00 indicates perfect discrimination. The asymptotic p-value tests the null hypothesis that the AUC equals 0.50. Standard errors and 95% CIs were estimated using the nonparametric assumption. The MCV cut-off was 76.9 fL for individual haemoglobinopathy subgroups and 75.3 fL for overall haemoglobinopathies.

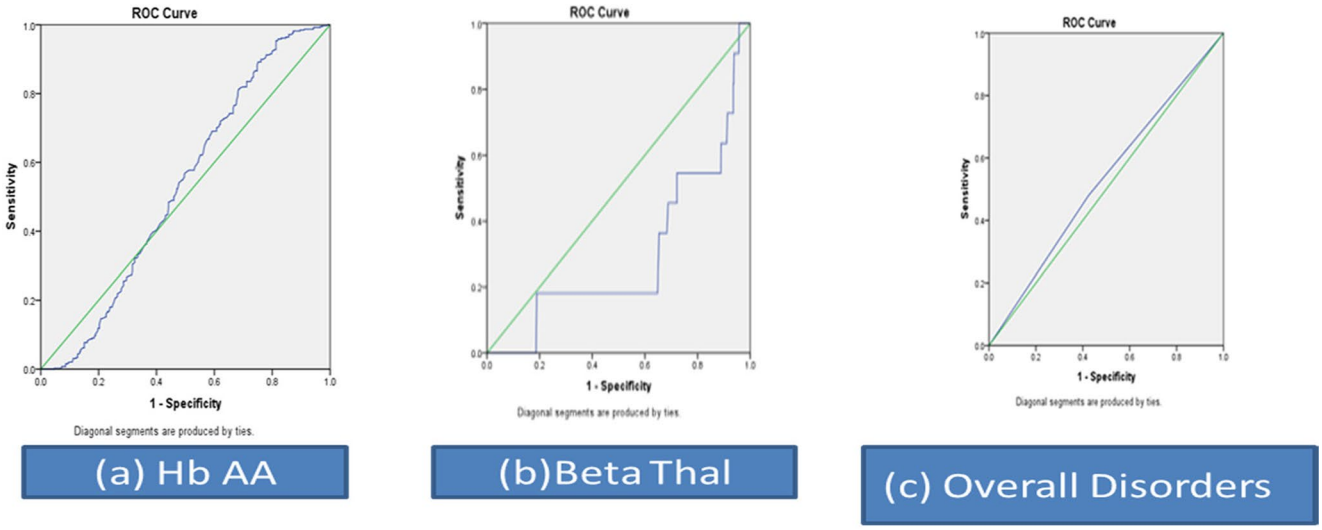

Fig. 1 MCV ROC curves presented in three panels: panel (**a**) shows the ROC curve for normal HbAA, panel (**b**) for β-thalassemia, and panel (**c**) for overall hemoglobinopathies

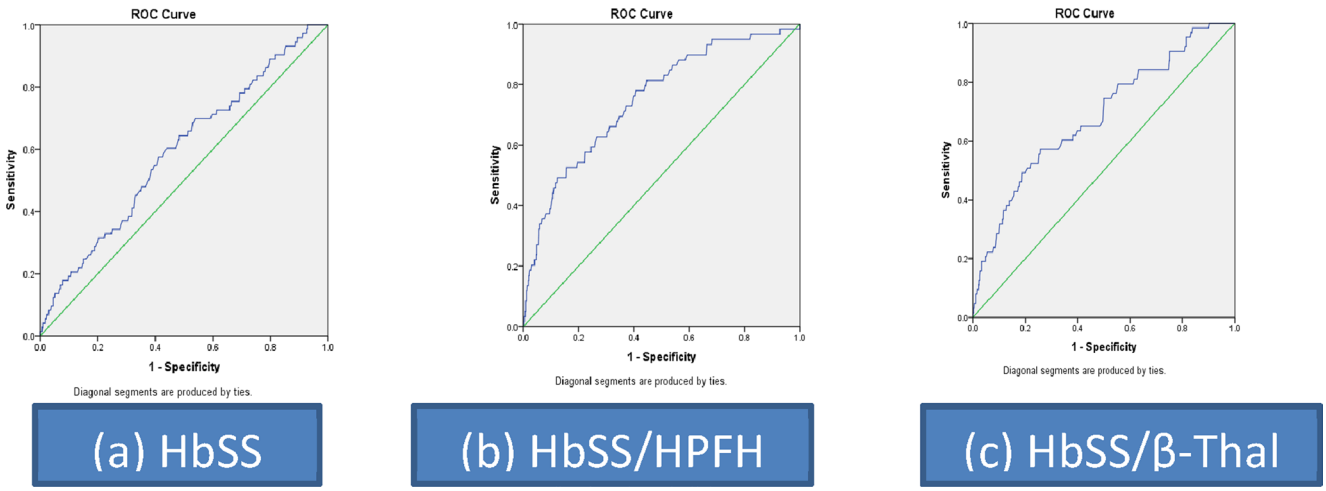

Fig. 2 MCV ROC curves for SCD phenotypes in three panels: panel (**a**) shows the ROC curve for homozygous HbSS, panel (**b**) for HbSS/HPFH, and panel (**c**) for HbSS/β-thalassemia

All results are detailed in Table 2.

ROC-derived cut-off and diagnostic accuracy of MCV across haemoglobinopathy phenotypes

Mean corpuscular volume (MCV) varied significantly across haemoglobinopathy phenotypes, with corresponding differences in diagnostic performance at the optimal ROC cut-off values. The p-values for comparisons between each case subgroup and the control group are presented in Table 3.

For normal HbAA ($n=388$), the median MCV was 76.8 fL (IQR 11.6). Using the ROC-derived cut-off value of 76.9 fL, sensitivity was 49% and specificity 54%, with a positive predictive value (PPV) of 15.8% and negative predictive value (NPV) of 84.9%. The positive likelihood ratio (LR+) was 1.07 and negative likelihood ratio (LR−) was 0.94, indicating limited discriminatory ability (Table 3).

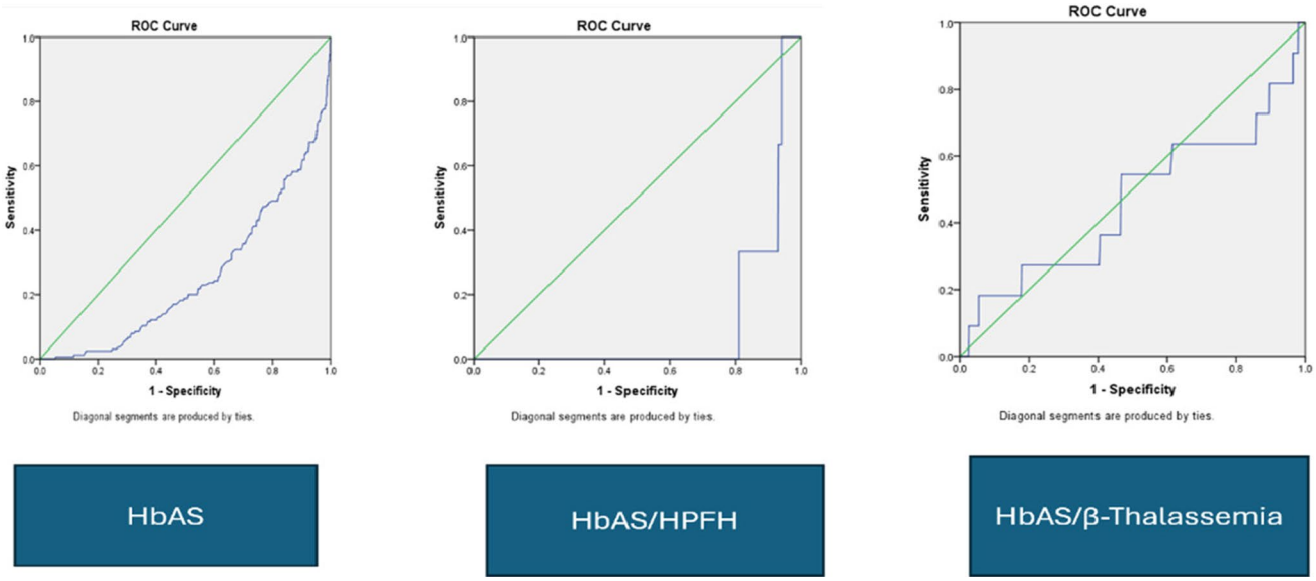

Fig. 3 MCV ROC curves for sickle cell trait variants. Panel (**a**) shows HbAS, panel (**b**) shows HbAS/HPFH, and panel (**c**) shows HbAS/β-thalassemia

Table 3 Median corpuscular volume and diagnostic accuracy across haemoglobinopathy phenotypes

Hb Phenotypes	Mediun (IQR)		*p*-value	Optimal ROC Cut-off (fL)	Sensitivity (%)	Specificity (%)	Positive Predictive Value (PPV)	Negative Predicitive Value	Positive Likelihood ratio	Negative Likelihood ratio
	CONTROL	CASE								
Normal HbAA ($n=388$)	76.8 (11.6)			76.9	49%	54%	15.8%	84.9%	1.07	0.94
HbSS ($N=73$)		78.8 (13.7)	1.000	76.9	60.3%	53.9%	18%	88%	1.31	0.74
HbSS/HPFH ($n=59$)		84.4 (12.5)	<0.001	76.9	81.4%	55.3%	21.7%	95.6%	1.82	0.34
HbS/β-thalassaemia ($n=63$)		83.2 (13.1)	0.234	76.9	65.1%	54.1%	19%	90.5%	1.42	0.65
HbAS ($n=165$)		69.1 (16.5)	0.001	76.9	21.8%	45.6%	14.5%	57.6%	0.40	1.72
HbAS/HPFH ($n=3$)		57.8	1.000	76.9	00%	52.3%	0%	98.5%	0	1.91
HbAS/β-thalassaemia ($n=11$)		77.1 (22.9)	1.000	76.9	54.5%	52.6%	3%	99.1%	1.15	0.86
β-Thalassemia ($n=11$)		70.0 (15.5)	1.000	76.9	18.2%	52.1%	1%	96%	0 0.38	1.57
Overall Hemoglobinopathies($n=385$)		75.3 (18.0)	0.172	75.3	50.4%	42.8%	46.7%	46.5%	0.88	1.16

Values are presented as median (interquartile range [IQR]). The p-values represent comparisons of MCV between each haemoglobinopathy phenotype and the normal HbAA control group. Optimal MCV cut-off values were determined using receiver operating characteristic (ROC) curve analysis and the Youden Index. Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), positive likelihood ratio (LR+) and negative likelihood ratio (LR−) were calculated at the corresponding optimal cut-off. *HbAA* normal adult haemoglobin, *HPFH* hereditary persistence of fetal haemoglobin, *HbSS* sickle cell disease, *HbAS* sickle cell trait, *β-thal* beta-thalassaemia. The overall haemoglobinopathy analysis includes all case phenotypes combined.

Among participants with HbSS($n=73$), the median MCV was 78.8 fL (IQR 13.7). The comparison with the control group was not statistically significant ($p=1.000$). At the same ROC-derived cut-off value of 76.9 fL, sensitivity was 60.3% and specificity 53.9%, with PPV 18% and NPV 88%, and LR + 1.31, LR − 0.74, demonstrating modest diagnostic performance (Table 3).

Participants with HbSS/HPFH ($n=59$) had the highest median MCV of 84.4 fL (IQR 12.5). This was significantly higher than the control group ($p<0.001$). Using the ROC-derived cut-off value of 76.9 fL, sensitivity was 81.4% and specificity 55.3%, with PPV 21.7% and NPV 95.6%, and LR + 1.82, LR − 0.34, representing the best diagnostic performance among all phenotypes (Table 3).

For HbSS/β-thalassemia ($n=63$), the median MCV was 83.2 fL (IQR 13.1). The comparison with controls was not statistically significant ($p=0.234$). At the ROC-derived cut-off value of 76.9 fL, sensitivity was 65.1%

Table 4 Univariate analysis of the effects of age and haemoglobin phenotype on MCV

Parameter	Age F-value	Age *p*-value	Phenotype F-value	Phenotype *p*-value
MCV (fL)	122.759	<0.001	35.308	<0.001

Univariate ANCOVA was performed with MCV as the dependent variable, age as a covariate, and haemoglobin phenotype as the fixed factor. Statistical significance was set at $p<0.05$

and specificity 54.1%, with PPV 19% and NPV 90.5%, and LR + 1.42, LR − 0.65, showing moderate diagnostic ability (Table 3).

In contrast, HbAS ($n=165$) had a significantly lower median MCV of 69.1 fL (IQR 16.5) compared to controls ($p=0.001$). Using the ROC-derived cut-off value of 76.9 fL, sensitivity was 21.8% and specificity 45.6%, with PPV 14.5% and NPV 57.6%, and LR + 0.40, LR − 1.72, indicating poor discrimination (Table 3).

HbAS/HPFH ($n=3$) and HbAS/β-thalassemia ($n=11$) demonstrated very limited diagnostic utility. HbAS/HbF had a median MCV of 57.8 fL, with the comparison to controls not reaching statistical significance ($p=1.000$). At the ROC-derived cut-off value of 76.9 fL, it showed 0% sensitivity, 52.3% specificity, PPV 0%, NPV 98.5%, LR + 0, LR − 1.91. HbAS/β-thalassemia had a median MCV of 77.1 fL (IQR 22.9), also with a non-significant p-value ($p=1.000$). Using the same ROC-derived cut-off value of 76.9 fL, it showed sensitivity 54.5%, specificity 52.6%, PPV 3%, NPV 99.1%, LR + 1.15, LR − 0.86 (Table 3).

For β-thalassemia alone ($n=11$), the median MCV was 70.0 fL (IQR 15.5). The comparison with controls was not statistically significant ($p=1.000$). At the ROC-derived cut-off value of 76.9 fL, sensitivity was 18.2% and specificity 52.1%, with PPV 1% and NPV 96%, LR + 0.38, LR − 1.57, again reflecting poor discriminatory performance (Table 3).

When all haemoglobinopathies were combined ($n=385$), using a different ROC-derived cut-off value of 75.3 fL, sensitivity was 50.4% and specificity 42.8%, with PPV 46.7% and NPV 46.5%, LR + 0.88 and LR − 1.16. The overall comparison between cases and controls was not statistically significant ($p=0.172$). These results indicate that, at this cut-off, MCV provides minimal ability to distinguish overall haemoglobinopathies from normal HbAA (Table 3).

Association of age and haemoglobin phenotype with MCV

Age was significantly associated with MCV ($F(1,764)=122.759$, $p<0.001$), indicating that MCV varied significantly with age. Haemoglobin phenotype was also significantly associated with MCV after adjustment for age ($F(7,764)=35.308$, $p<0.001$), demonstrating that differences in MCV between haemoglobin phenotype groups persisted independently of age as shown on Table 4.

Discussion

The present study demonstrated that both age and haemoglobin phenotype were significantly associated with MCV. Age had a significant effect on MCV ($F(1,764)=122.759$, $p<0.001$), while haemoglobin phenotype remained significantly associated with MCV after adjustment for age ($F(7,764)=35.308$, $p<0.001$). This indicates that differences in MCV across haemoglobin phenotype groups were not explained by age alone, suggesting a phenotype-related variation in red cell size. However, the significant association does not necessarily imply adequate diagnostic discrimination, highlighting the need to assess the phenotype-specific diagnostic performance of MCV.

Our initial analysis confirmed expected biological variations in MCV, which lends internal validity to our dataset. The finding that males had a significantly lower median MCV compared to females (75.2 fL verses 77.3 fL, $p = 0.004$) is consistent with established hematological norms. While the difference is small, it is a well-recognized phenomenon, often attributed to differences in iron stores and hormonal influences on erythropoiesis [11].

More pronounced was the strong age-related trend we observed ($p < 0.0001$), with MCV increasing progressively from infancy (median 69.1 fL in children < 2 years) to adolescence and adulthood (median 79.6 fL in those ≥ 12 years). This pattern is physiologically normal and well-documented. Infants and young children typically have lower MCV values as part of the physiological adaptation after birth and during the period of iron-dependent growth [12].The rise in MCV with age reflects the transition to adult erythropoiesis and the stabilization of iron stores. The consistency of these sex- and age-related patterns across our control and case groups reinforces the representativeness of our sample and confirms that these factors must be accounted for when interpreting MCV in clinical practice [6].

The primary objective was to assess MCV's ability to distinguish between normal individuals (HbAA) and those with any hemoglobinopathy. Using receiver operating characteristic (ROC) curve analysis, the overall area under the curve (AUC) for all hemoglobinopathies combined was 0.528 (95% CI: 0.487–0.568; $p = 0.172$), with an empirically derived optimal cut-off of 75.3 fL. At this threshold, sensitivity was 50.4%, specificity 42.8%, positive predictive value (PPV) 46.7%, negative predictive value (NPV) 46.5%, with likelihood ratios LR + 0.88 and LR − 1.16. These metrics indicate that MCV performs no better than chance in discriminating hemoglobinopathy cases from HbAA controls. Using MCV in isolation as a general screening tool would therefore result in a high rate of misclassification, missing nearly half of the affected individuals while incorrectly flagging a substantial proportion of healthy controls. This finding aligns with recent large-scale studies advocating for alternatives to a complete blood count (CBC)-first approach. For example, a 2025 multicenter study in India reported that relying on MCV and mean corpuscular hemoglobin (MCH) cut-offs alone would miss 14.1% of hemoglobinopathy carriers, whereas high-performance liquid chromatography (HPLC) as a first-line test provided more reliable detection [13].

The true value of our study lies in the stratification of results by specific hemoglobinopathy phenotype, which reveals a heterogeneous diagnostic profile for MCV across sickle cell–related conditions. Overall, MCV demonstrated modest discriminatory ability for homozygous sickle cell disease (HbSS), consistent with the biological nature of SCD as primarily a normocytic hemolytic disorder rather than a microcytic condition [14].

In our cohort, SCD exhibited only mild separation from HbAA controls, reflected by the modest diagnostic accuracy (AUC = 0.59). Although not statistically distinct from controls, the directional shift toward higher MCV values supports the interpretation that erythrocyte size in SCD tends to remain normal or slightly elevated rather than reduced. This pattern is biologically plausible given the chronic hemolytic state characteristic of SCD, which stimulates compensatory erythropoiesis and increases circulating reticulocytes—cells that are larger than mature erythrocytes and therefore elevate the overall MCV [15]. Similar observations have been reported in pediatric SCD cohorts, where mean MCV values were significantly higher in HbSS patients compared with HbAA controls, supporting the classification of SCD as a normocytic anemia associated with hemolysis and increased red cell turnover [16]. These findings reinforce the notion that MCV alone is unlikely to serve as a strong diagnostic discriminator for isolated SCD but may still capture subtle hematologic shifts reflecting disease physiology.

The diagnostic profile becomes more distinct when SCD occurs in combination with other modifying factors. In particular, the HbSS/HPFH phenotype demonstrated the strongest discriminatory performance among all phenotypes examined. Elevated fetal hemoglobin is known to modify erythropoiesis and red cell survival in SCD, often resulting in larger red cells and a macrocytic tendency. Previous work has demonstrated that increasing HbF levels are independently associated with higher MCV values in individuals with sickle cell anemia, reflecting altered erythropoietic kinetics in HbF-modulated states [17]. Our findings are consistent with this mechanism, showing a clear upward displacement of MCV in individuals with elevated HbF compared with HbAA controls. Clinically, this translated into the highest diagnostic accuracy observed among the sickle cell phenotypes and a particularly strong negative predictive value, suggesting that normal or higher MCV levels may help rule out this phenotype when screening populations relative to HbAA.

HbSS/β-thalassemia demonstrated a similar but less pronounced pattern compared with the HbF-modified phenotype. Although MCV tended to be higher than in HbAA controls, the discriminatory performance remained moderate, indicating limited separation between this compound phenotype and normal individuals. This pattern

likely reflects the interaction between two opposing hematologic processes: chronic hemolysis and reticulocytosis in SCD, which increase red cell size, and impaired globin synthesis in β-thalassemia, which promotes microcytosis. The coexistence of these mechanisms produces intermediate erythrocyte indices and reduces the diagnostic precision of MCV. These findings are consistent with reports from African cohorts showing that sickle cell–related disorders frequently present with normocytic rather than microcytic red cell indices, supporting the interpretation that MCV alone has only moderate diagnostic utility for identifying SCD + β-thalassemia relative to HbAA [18].

It is also important to distinguish the present application of MCV from other clinical contexts within SCD management. While this study evaluated MCV as a marker to differentiate hemoglobinopathy phenotypes from normal HbAA individuals, other investigations have used low MCV within SCD populations to predict complications such as iron deficiency. Evidence suggests that this approach performs poorly. For example, reduced MCV has been reported to have a sensitivity of only 40% for detecting iron deficiency among patients with SCD (Joshi and Joshi, 2024). This contrast highlights that erythrocyte indices in SCD are influenced by multiple competing physiological processes including hemolysis, reticulocytosis, nutritional status, and co-inherited hemoglobin variants thereby limiting the reliability of MCV as a standalone indicator for secondary conditions [19].

These findings empirically demonstrate that MCV has limited diagnostic value for distinguishing SCD phenotypes from normal HbAA. Instead, the results show that SCD-related anemia in this cohort is predominantly normocytic, challenging the common assumption that these patients are typically microcytic. This distinction is clinically important in resource-limited settings, as recognizing the normocytic pattern of SCD can help differentiate it from iron deficiency and other microcytic anemias when advanced diagnostic tests are unavailable [18].

In contrast to the SCD phenotypes, MCV demonstrated virtually no diagnostic utility for detecting sickle cell trait (HbAS). The ROC performance was markedly poor (AUC ≈ 0.25), indicating that MCV does not reliably discriminate HbAS from normal HbAA. This finding is biologically expected because individuals with HbAS are typically asymptomatic and lack the chronic hemolysis and reticulocytosis observed in SCD that would otherwise influence red cell size. Consequently, their erythrocyte indices largely overlap with those of the normal population, making MCV an ineffective screening parameter for HbAS [20, 21].

Interestingly, the present cohort showed a tendency toward lower MCV values among HbAS individuals, suggesting a predominantly microcytic pattern. However, this pattern overlaps substantially with iron deficiency anemia and other causes of microcytosis, limiting the interpretive value of CBC parameters alone. Similar observations have been reported in regional studies, where red cell indices demonstrated poor predictive performance for identifying hemoglobinopathies, reinforcing the need for confirmatory diagnostic methods such as hemoglobin electrophoresis or HPLC [22].

A comparable limitation was observed for β-thalassemia and related coinheritance states. Although β-thalassemia trait is classically associated with microcytosis, the diagnostic performance of MCV was poor in this dataset. This likely reflects the high prevalence of other causes of microcytosis in many populations, particularly iron deficiency, which reduces the specificity of MCV when used as a standalone screening marker [23, 24]. Additionally, the small number of individuals with β-thalassemia and compound phenotypes (e.g., HbAS/β-thalassemia or HbAS/HPFH) limits precise characterization of erythrocyte indices in these subgroups. Previous screening studies have similarly shown that although low MCV may demonstrate reasonable sensitivity for β-thalassemia carriers, its specificity remains limited and therefore requires confirmatory testing such as HbA_2 quantification or HPLC [25, 26].

Taken together, these findings highlight an important diagnostic distinction: while SCD phenotypes in this cohort tend to present with normocytic indices, HbAS and certain thalassemia-related phenotypes may appear microcytic, yet this pattern overlaps substantially with other common hematologic conditions. As a result, erythrocyte indices alone have limited reliability for detecting these carrier states, underscoring the continued importance of definitive hemoglobin characterization methods in screening programs [24].

Limitation

Some subgroups, particularly HbAS/HPFH and HbAS/β-thalassemia, had very small sample sizes, which reduced statistical power and limited the precision of diagnostic estimates. Larger studies are therefore recommended to provide more reliable estimates and improve the accuracy of findings for these phenotypes. Another limitation is that alpha-thalassaemia could not be comprehensively assessed in this study. The Bio-Rad D-10 HPLC method used for haemoglobinopathy identification does not reliably detect alpha-globin gene deletions, including the common $-\alpha^{3.7}$ deletion reported in Western Kenya. Because molecular testing for alpha-thalassaemia was not performed, some participants with co-existing or isolated alpha-thalassaemia may have been missed. This may have influenced the observed MCV distributions and the diagnostic performance estimates of MCV. Additionally, iron deficiency could not be comprehensively excluded in all controls because iron studies were not routinely performed as part of the retrospective laboratory assessment. Although controls were selected within the facility-specific haemoglobin reference ranges and participants with available evidence of iron deficiency were excluded, undetected iron deficiency may have remained among some controls and could have influenced MCV measurements and the diagnostic performance estimates.

Conclusion

Sickle cell disease (SCD) phenotypes in this cohort were predominantly normocytic when interpreted using population-based Kenyan reference ranges, contrasting with iron deficiency and other microcytic anemias that typically reduce MCV. ROC analysis showed poor discriminatory performance of MCV for combined haemoglobinopathies (AUC 0.528), with an optimal cut-off of 75.3 fL yielding 50.4% sensitivity and 42.8% specificity. The ROC-derived optimal threshold for SCD detection (76.9 fL) fell within the population-based reference distribution (median 76.8 fL; IQR 11.6), indicating that maximum detection occurs within normocytic ranges. Sickle cell trait and thalassemia traits were sometimes microcytic but overlapped with other conditions. These findings highlight the importance of using locally derived MCV reference ranges rather than conventional 80–100 fL thresholds. While MCV may help triage suspected cases in resource-limited settings, it cannot reliably identify carrier states, and definitive hemoglobin characterization remains essential for accurate diagnosis and effective screening.

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Author contributions BM conceptualization the study, collected and analysed data and wrote the original manuscriptRC conducted the literature review as well as carried out reviewsPO Supervised the study and carried critical reviews.

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Data availability The datasets generated and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Declarations

Competing interests The authors declare no competing interests.

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