



DSPACE

<https://dspace.org/>

High-performance liquid chromatography local reference ranges of hemoglobin fractions (HbA, HbA2, and HbF) in detection of hemoglobinopathies in western Kenya

Mutua, Benard; Chelangat, Rose; Mustafa, Barasa; Were, Tom; Makani, Julie ...

2022

The Egyptian Journal of Internal Medicine

<https://doi.org/10.1186/s43162-022-00187-9>

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.

RESEARCH

Open Access



High-performance liquid chromatography local reference ranges of hemoglobin fractions (HbA, HbA2, and HbF) in detection of hemoglobinopathies in western Kenya

Benard Mutua^{1,2*} , Rose Chelangat³, Barasa Mustafa⁴, Tom Were^{4,5}, Julie Makani⁶, George Sowayi¹ and Patrick Okoth⁷

Abstract

Background: Western Kenya, being a malaria-endemic region, has a high prevalence of hemoglobinopathies mostly sickle cell and thalassemia. The hemoglobin fractions or variants, HbA, HbA2, and HbF, serve as biomarkers for the detection of hemoglobinopathies and are commonly used in laboratory screening and diagnosis of these diseases. Diagnosis of diseases entails accurate and precise representation of a patient's condition. This is the main aim of International Organization for Standardization (ISO) certified laboratories of offering a reliable diagnostic guide for the various diseases. For this to be realized, valid normal reference ranges are required. Such are reference values that are valid for local population of the setting where they are to be used is critical in quantitative diagnostic tests. Local normal reference ranges are necessary because research has revealed variations in the phenotypic expression of the genes for biological characteristics in humans inhabiting different geographical regions, owing to epigenetic differences imposed by physical environments, and associated sociocultural influences, even in cases of similarity in gene patterns. No local normal reference ranges for hemoglobin fractions are reported for Kenya and Africa as a whole. Laboratories therefore continue to use those found in textbooks and brochures from manufacturers of diagnostic reagents, which are derived from populations of geographical locations faraway and socioculturally different from Kenya. This could be misleading in diagnosis of hemoglobinopathies in western Kenya and indeed all of Kenya. Therefore, the present study aimed at exploring the possibility of developing local normal reference ranges for the concentrations of hemoglobin fractions, HbA, HbA2, and HbF, based on hemoglobinopathy-free, non-anemic subjects attending the Aga Khan Hospital Kisumu in western Kenya and its satellites. The hospital serves the populations inhabiting in and predominantly indigenous to western Kenya.

Objectives: To derive the 95% confidence intervals for hemoglobin fractions (HbA, HbA2, and HbF), evaluate the potential of these intervals as normal reference values for the local population by use of concentrations for non-anemic hemoglobinopathy-free subjects and compare the performance of the current HPLC normal ranges with those intervals we derived, based on receiver operating characteristic (ROC) curve.

Materials and methods: This was an analytical retrospective study using routine assay results from laboratory database for 386 non-anemic, HPLC-confirmed hemoglobinopathy-free subjects. Blood samples were obtained at the

*Correspondence: bensmutua@yahoo.com

² Department of Pathology, The Aga Khan Hospital, P.O Box 530-40100, Kisumu, Kenya

Full list of author information is available at the end of the article

Kisumu Aga Khan Hospital and its satellite sites in western Kenya, covering January 2015 to November 9, 2021. The data for Hb fractions were nonparametric, and so confidence intervals, together with the age of subjects, were thus expressed as the median and interquartile range (IQR). Data for the gender and other characteristics of study subjects were summarized in frequencies and proportions, Kruskal-Wallis *H*-test was used to test the significance of differences in Hb concentrations between stations and age groups, while Mann-Whitney *U*-test is between males and females. The receiver operating characteristic (ROC) curve was used to evaluate the potential of the derived confidence intervals as normal reference values in comparison with the commonly used normal values for hemoglobin fractions.

Results: The potential normal reference intervals were computed as 95% confidence intervals (CI) for median percentage levels for the concentrations of the Hb fractions HbA, HbA2, and HbF for the hemoglobinopathy-free patients. The overall confidence intervals were derived first for the combined sample of all the hemoglobinopathy-free patients combined together irrespective station where blood specimens were obtained, age or gender, and then followed by those for separate groups, stratified based on station, age, and gender. The overall median values for the hemoglobin fractions were hemoglobin: A (HbA) 87.7, *IQR* = 5.7, 95% *CI* = 76.3–99.1; hemoglobin A2 (HbA2), 3.0, *IQR* = 0.6; 95% *CI* = 1.8–4.2; and hemoglobin F (HbF), 0.8, *IQR* = 0.8; 95% *CI* = 0.00–2.4, with the P window, 4.98, *IQR* = 0.4; 95% *CI* = 4.18–5.78. The commonly used normal reference ranges for the hemoglobin fractions were as follows: HbA 95–98%, had an accuracy of 57.5%, HbA2 of 1.5–3.5%, had an accuracy of 95.9% in grading the presumed healthy population as hemoglobinopathy-free, while HbF 0–2.0 was equal to that established by the present study.

Conclusion: It is important to report that the use of normal range for HbA of 95–98% published by Kratz et al. [1] in western Kenya has a potential threat of misdiagnosis of normal population and thus needs urgent review as it lacked efficacy ($p = 0.795$) in grading hemoglobinopathy-free subjects as normal with a poor accuracy of 57.5%, a sensitivity of 100%, specificity of 0.3%, positive predictive validity of 15.1%, negative predictive validity of 1%, and 1.03 positive likelihood ratio. However, the traditional normal range for HbA2 of 1.5–3.5% on use in western Kenya may be retained as it was effective ($p < 0.0001$) in grading majority of study subjects as normal with an accuracy of 95.9%, sensitivity of 98.4%, specificity of 93.3%, positive predictive validity of 99.7%, negative predictive validity of 70.0%, 14.7 positive likelihood ratio, and 0.017 negative likelihood ratio. Similarly, the existing normal range for HbF of 0–2.0 on use was almost the same as the one we derived of 0–2.4 and therefore may be retained for use in western Kenya. It is anticipated that the finding of this study will help improve the management of hemoglobinopathies in Kenya and Africa at large, by contributing to improvement in the validity of the clinical-pathologic interpretation assay results for the percentage values for the Hb fractions.

Introduction

The hemoglobin fractions or variants, HbA, HbA2, and HbF, serve as biomarkers for the detection of hemoglobinopathy and are commonly used in the laboratory screening and diagnosis of these diseases. A case in point is those developed by Kratz et al. [1] in the USA as HbA 95–98%, HbA2 1.5–3.5%, and HbF 0–2.0. A normal reference range is an upper and lower limit at an appropriate confidence level of the quantity of a variable (in this case laboratory test results of a health status or disease biomarker) considered to indicate the absence of the target disease. It is therefore established from an appropriate confidence interval of the values for normal or other healthy individuals. They provide the cutoff values that enable interpretation of the test results of individuals, thereby serving as a criterion for diagnosing or ruling out the presence of disease as well as monitoring efficacy of therapy [2].

Research reports spanning several years show growing numbers of hemoglobinopathies in malaria holoendemic regions; thus, establishment of local reference

ranges for hemoglobin fractions is of great importance as it facilitates accurate diagnosis, due to widely and unanimously reported variations of phenotypic features in humans resulting from sociocultural epigenetic changes that influence genes affecting biological characteristics in human population in a given geographical location. These reports indicate that normal ranges for hematological, immunological, and biochemical parameters in Africans differ significantly from those of non-Africans [2]. This indicates that use in Africa of normal reference ranges from populations of non-African regions may lead to incorrect diagnosis of concerned diseases. Population-based reference ranges have been derived in several African Countries situated in different geographical regions including West Africa (Ghana, Cameroon, Nigeria, and Togo), Southern Africa (Mozambique), northeastern Africa (Ethiopian), and Eastern Africa (Uganda). All these studies have reported differences in the cutoff values for hematological, biochemical, and immunological parameters based on geographical locations [3–8]. Similarly, for Kenya, the literature reveals that comparison

of locally derived values for same parameters varied significantly from those reported from outside the country [9–13]. These previous African studies therefore underscore the need for African countries to develop their own local normal reference ranges for the various laboratory diagnostic biomarkers. However, none of them reported work on the derivation of local normal reference values for the hemoglobin fractions. This implies that countries in the continent continue to use normal reference ranges for this hematological parameter based on populations outside, such as those in textbooks or recommended by WHO (World Health Organization). A case in point is those developed by Kratz et al. [1] in the USA: HbA 95–98%, HbA2 1.5–3.5%, and HbF 0–2.0.

In addition, the reports show that reference ranges for the concentrations of these Hb fractions (HbA, HbA2, and HbF) widely on use were derived by Roa et al. (1995) done among African Americans and Kratz et al. (2004) also done in USA, which is as old as 27 and 18 years back [1, 14]. Over these years, significant changes in critically relevant sociocultural and demographic characteristics of the human populations and environmental conditions that can cause epigenetic changes affecting phenotypic expression of genes are likely to have occurred. These conditions have potential to alter the epidemiologic profiles of genetically determined biological characteristics, including hemoglobin fractions and their concentrations in affected individuals and, hence, normal reference ranges. Western Kenya shelters over 10 million multiethnic inhabitants and being one of the country's malaria-holoendemic geographical regions, consequently having a high burden of hemoglobinopathies [15]. There was need therefore, for this very first exploration the possibility of developing normal reference ranges for hemoglobin fractions, based on the local population. The objectives of this study were to derive 95% confidence limits/intervals for the hemoglobin fractions (HbA, A2, and HbF), evaluate their efficacy as potential normal reference values for use in the management of hemoglobinopathies in western Kenya patients attending the Aga Khan Hospital and its satellites in western Kenya, and compare their efficacy as such with the widely used ones adopted from Roa et al. [14] and Kratz et al. [1] in western Kenya patients attending Aga Khan Hospital and its satellites.

To derive normal reference values for the blood levels of various hemoglobin fractions, a population of humans with normal hemoglobin phenotype (HbAA), considered free from clinical conditions that might alter the values of these, is important [16]. Studies have described and prescribed two types of source populations for laboratory reference values for disease biomarkers. One is the healthy populations, as far as the disease for which the reference values are to be derived is concerned. This is

considered to be the ideal source for the requisite data, and use of this has been referred to as the direct method [17, 18]. However, gathering the requisite numbers of the necessary mix of healthy subjects is always a daunting task in terms of logistical feasibility and financial affordability. The second, and more readily obtainable population used as source or reference population for this purpose, is the hospital patients routinely investigated for the relevant analytes by the concerned laboratory and found free from the disease of interest. That is because laboratory data for patients confirmed free from a given pathological condition and any other known disease that can derange the values for the diagnostic biomarker for the condition tend to be similar to those of the healthy population [16, 19]. This is referred to as the indirect method of deriving normal reference values [17, 18, 20–22]. In this case, stored laboratory data for the analyte of interest from patients found free from the disease under investigation or, alternatively, residual appropriately archived assay specimens from the patients can be analyzed to provide the required data. Accordingly, assay results for hemoglobinopathy-status plus corresponding ones for the blood levels of hemoglobin fractions from the laboratory database were used for this purpose. Therefore, HPLC-generated percentage values of Hb fractions for HPLC-confirmed hemoglobinopathy-free patients investigated at the Aga Khan Hospital's Kisumu laboratory from 1st Jan 2015 to 9th November 2021 were used to derive the 95% confidence intervals evaluated as potential normal reference values for hemoglobin fractions. To enable evaluation of the sensitivity of the derived confidence intervals as potential normal reference intervals, as well as performance of the commonly used normal reference intervals, data from same laboratory database on percentage levels of same Hb fractions in HPLC-confirmed hemoglobinopathy cases were also obtained.

Methods

This was analytical retrospective study using archived routine laboratory assay results for patients attending the Aga Khan Hospital, Kisumu, and its western Kenya satellites, investigated for hemoglobinopathy at the hospital's Kisumu laboratory from 1st January 2015 to 9th November 2021. The eligible subjects were all the 386 non-anemic HPLC-confirmed hemoglobinopathy-free. The Hb concentrations (g/dl) of the Hb hemoglobinopathy-free patients were stratified based on age as follows: $\geq 9.5\text{g/dl}$ for ≤ 5 year olds, $\geq 10.5\text{g/dl}$ for ≤ 11 years old, and $\geq 11\text{g/dl}$ for ≥ 12 years old with values of their Hb fractions (HbA, HbA2, and HbF) HPLC-confirmed hemoglobinopathy-free.

Ethical considerations

This was part of wider study approved by Masinde Muliro University Ethical Review Committee (Registration number: MMU/COR: 403012 vol. 3 (03) and again licensed by National Commission of Science and Technology (NACOSTI) (applicant identification number: 407653). The Aga Khan Hospital, Kisumu Ethical Review Committee, gave out the permit through registration number ADM/007/089. The confidentiality of study subjects was maintained by password protection of data in computers that had stringent personal password, stored in restricted-entry rooms and coding of identities on documents with biodata.

Sample size determination and sampling

The study sample was obtained by census. Data for all the subjects who fulfilled the inclusion criteria were included in the study. These were hemoglobinopathy-free non-anemic study subjects (HbAA phenotypes) confirmed using HPLC (Bio-Rad D-10) machine together with their respective Hb (g/dl) concentrations. The exclusion criterion was individuals confirmed to have hemoglobinopathies, those that were transfused in the past 3 months, those that lacked hemoglobin (g/dl) or hemoglobin was reduced, those that were confirmed to have leukemias, and those that tested positive for blood cultures and antinuclear antibodies (ANA).

Data collection

The data were obtained from the hematology laboratory database at the Aga Khan Hospital in Kisumu for all the 386 eligible subjects.

Data analysis

The coded data in MS Excel was exported to SPSS (Statistical Package of Social Sciences) version 23 for analysis. Data for population characteristics other than age were summarized as frequencies and proportions. Kolmogorov-Smirnov and Shapiro-Wilks normality tests revealed that the data were skewed, thus necessitating use of the medians and interquartile range as descriptive statistics to derive the 95% confidence interval (CI) and related upper limit or cutoff value as recommended by a previous report [23]. Kruskal-Wallis *H*-test was used to evaluate variation of these medians among the stations and between the age groups, while Mann-Whitney *U*-test was used to assess the differences between the males and females. The receiver operating characteristic (ROC) curve was used to evaluate the efficacy of the derived confidence intervals as well as currently used normal reference intervals in evaluating

assay results for the concentrations of hemoglobin fractions.

Results

Overall confidence intervals of the levels of hemoglobin fractions for hemoglobinopathy-free patients in western Kenya

The overall hemoglobin fractions value meant the concentration of Hb fractions for all stations and population groups put together. For hemoglobin A (HbA), it had a median of 87.7, *IQR* = 5.7; 95% *CI* = 76.3–99.1, while hemoglobin A2 (HbA2) had a median of 3.0, *IQR* = 0.6; 95% *CI* = 1.8–4.2. Fetal hemoglobin (HbF) had an overall median of 0.8, *IQR* = 0.6; 95% *CI* = 0.0–2.4. The P window had a median of 4.98, *IQR* = 0.4; 95% *CI* = 4.18–5.78 (as detailed in Table 1).

Hemoglobin A (HbA) confidence intervals for hemoglobinopathy-free patients in western Kenya

Hemoglobin A (HbA) concentration varied significantly among the stations ($p = 0.016$) with Busia having a median of 86.3, *IQR* = 4.9; 95% *CI* = 76.5–96.1, while Bungoma station had a median of 88.4, *IQR* = 5.0; 95% *CI* = 78.4–98.4. Kitale recorded the lowest median of 86.2, *IQR* = 2.8; 95% *CI* = 80.6–91.8 with Kakamega having the highest HbA median of 90.4, *IQR* = 6.2; 95% *CI* = 78.0–102.8. Kisumu had HbA median concentration of 88.5, *IQR* = 6.0; 95% *CI* = 76.5–100.5 while Kisii region a median of 87.2, *IQR* = 3.5; 95% *CI* = 80.2–94.2. Homa Bay and Migori recorded the lowest HbA median of 85.5, *IQR* = 6.5; 95% *CI* = 72.7–98.5 and 85.0, *IQR* = 8.0; 95% *CI* = 69–101, respectively.

Hemoglobin A varied significantly ($p = 0.005$) between the sexes, with males having a lower median of 86.9, *IQR* = 5.2; 95% *CI* = 76.5–97.2, while females had median of 88.5, *IQR* = 5.4; 95% *CI* = 77.7–99.3. However, it did not vary significantly with age ($p = 0.106$) where children ≤ 2 years had a median of 87.7, *IQR* = 6.2; 95% *CI* = 75.3–100.1, and the age group 3–5 years had HbA median of 86.7, *IQR* = 5.8; 95% *CI* = 75.1–98.3. The age groups 6–11 years had HbA median of 87.8, *IQR* = 6.1; 95% *CI* = 75.6–100, and age group ≥ 12 years had median of 88.5, *IQR* = 5.4; 95% *CI* = 77.7–99.3.

The performance of 95–98% HbA normal range in western Kenya

The HbA normal range of 95–98% developed by Kratz et al. [1] was tested against the HbA of the 386 presumed hemoglobinopathy-free subjects generating an accuracy of 57.5% ($p = 0.795$), a high sensitivity of 100%, poor specificity of 0.3%, a positive likelihood ratio (LR+) of 1.03, and a negative likelihood ratio (LR–) of 0 (results summarized on Table 2).

Table 1 Levels of hemoglobin fractions of hemoglobinopathy patients attending Aga Khan Hospital Kisumu, western Kenya, and its satellites

Characteristics		Levels of hemoglobin fractions							
		HbA (%)	<i>p</i>	HbA2 (%)	<i>p</i>	HbF (%)	<i>p</i>	P window (%)	<i>p</i>
		Median (IQR) 95% CI		Median (IQR) 95% CI		Median (IQR) 95% CI		Median (IQR) 95% CI	
Overall (N = 386)		87.7 (5.7) 76.3–99.1		3.0 (0.6) 1.8–4.2		0.8 (0.8) 0.0–2.4		4.98 (0.4) 4.18–5.78	
Station	Busia (n = 52) 13.5%	86.3 (4.9) 76.5–96.1	0.016	3.2 (1.03) 1.14–5.26	< 0.0001	0.8 (0.8) 0.0–2.4	< 0.0001	5.1 (0.67) 3.76–6.44	0.020
	Bungoma (n = 49) 12.7%	88.4 (5.0) 78.4–98.4		3.3 (0.5) 2.3–4.3		0.8 (0.1) 0.6–1.00		4.9 (0.54) 3.82–5.98	
	Kitale (n = 18) 4.7%	86.2 (2.8) 80.6–91.8		3.2 (0.9) 1.4–5.0		0.8 (0.65) 0.0–2.1		4.99 (0.92) 3.15–6.83	
	Kakamega (n = 15) 3.9%	90.4 (6.2) 78.0–102.8		2.8 (1.3) 0.2–5.4		0.5 (1.10) 0.0–2.7		4.98 (0.44) 4.1–5.86	
	Kisumu (n = 182) 47.2%	88.5 (6.0) 76.5–100.5		3.0 (0.6) 1.8–4.2		0.8 (0.8) 0.0–2.4		4.98 (0.3) 4.38–5.58	
	Kisii (n = 32) 8.3%	87.2 (3.5) 80.2–94.2		3.0 (1.1) 0.8–5.2		0.8 (0.58) 0.0–1.96		5.0 (0.7) 3.6–6.4	
	Homabay (n = 32) 8.3%	85.5 (6.5) 72.7–98.5		3.0 (0.65) 1.7–4.3		0.8 (1.05) 0.0–2.9		5.0 (0.53) 3.94–6.06	
	Migori (n = 6) 1.6%	85.0 (8.0) 69–101		2.9 (0.5) 1.9–3.9		2.1 (0.7) 0.7–3.5		4.99 (1.8) 1.39–8.59	
Gender	Male (n = 185) 47.9%	86.9 (5.2) 76.5–97.3	0.005	3.0 (0.6) 1.8–4.2	0.901	0.8 (0.8) 0.0–2.4	0.075	4.98 (0.63) 3.72–6.24	0.514
	Female (n = 201) 52.1%	88.5 (5.4) 77.7–99.3		3.0 (0.6) 1.8–4.2		0.8 (0.8) 0.0–2.4		4.98 (0.34) 4.3–5.66	
Age	≤ 2 years (n = 115) 29.8%	87.7 (6.2) 75.3–100.1	0.106	3.0 (0.8) 1.4–4.6	0.219	1.0 (0.8) 0.0–2.6	< 0.0001	5.0 (0.5) 4.0–6.0	0.259
	3–5 years (n = 49) 12.7%	86.7 (5.8) 75.1–98.3		3.1 (0.8) 1.5–4.7		0.8 (0.8) 0.0–2.4		4.98 (0.8) 3.38–6.58	
	6–11 years (n = 46) 11.9%	87.8 (6.1) 75.6–100		3.15 (0.6) 1.95–4.35		0.8 (0.03) 0.74–0.86		4.98 (0.46) 4.06–5.9	
	≥ 12 years (n = 176) 45.6%	88.5 (5.4) 77.7–99.3		3.0 (0.7) 1.6–4.4		0.55 (0.8) 0.0–2.15		4.98 (0.4) 4.18–5.78	

Table 2 HbAA hemoglobin fractions

HbAA hemoglobin fractions	Western Kenya-derived normal CI (95% CI)	Normal range (95% CI)	AUC/Accuracy/ Youden index	Asymptotic sig. (p)	Sensitivity	Specificity	LR+	LR–
HbA	76.3–99.1	95–98	0.575	0.795	100	0.3	1.03	0
HbA2	1.8–4.2	1.5–3.5	0.959	0.000	98.4	93.3	14.7	0.017
HbF	0.0–2.4	0.0–2.0	N/A	N/A	N/A	N/A	N/A	N/A

Predictive validity of HbA normal range of 95–98% in western Kenya

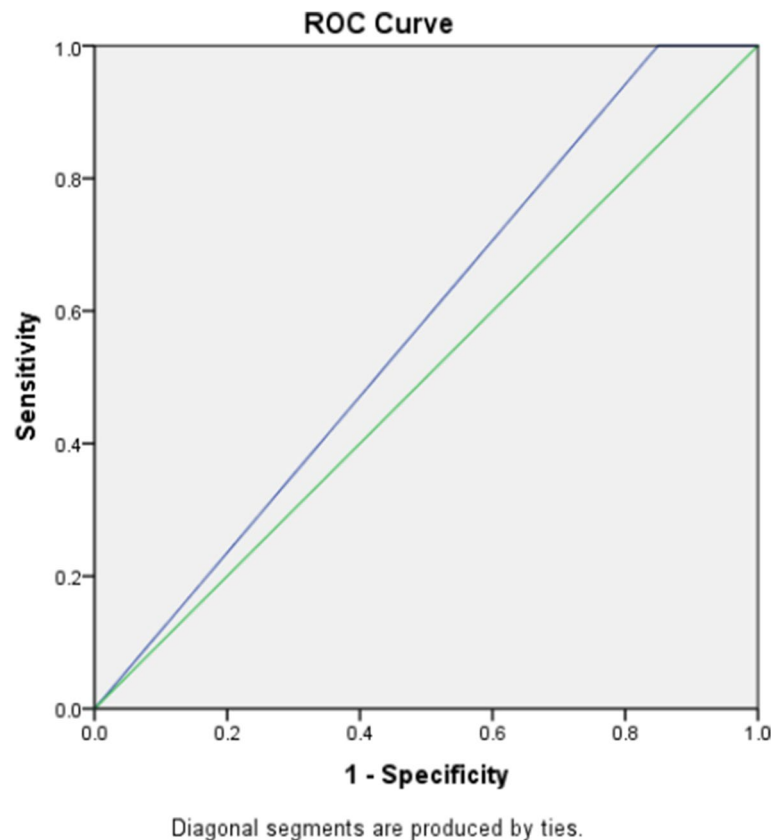
The HbA normal range of 95–98% was used as the gold standard against the 386 haemoglobinopathy-free study subjects, to determine its ability to grade the healthy population as normal. It was able to give a positive predictive validity of 15.1% and a negative predictive

validity of 1% whose ROC curve flowed slightly above the diagonal line (Table 3 and Fig. 1).

The ROC curve that analyzed the efficacy of HbA normal range of 95–98% in western Kenya flowed slightly above the diagonal line as shown on Fig. 1, generating accuracy (Youden Index), sensitivity, and specificity of

Table 3 Predictive validity of the currently HbA as a normal range in western Kenya

		Current HbA normal ranges		Total	Predictive values
		95–98 (positive)	< 95 (negative)		
Western Kenya HbA ranges	76.3–99.1 (positive)	58	327	385	15.1 (+)
	< 76.3 (negative)	0	1	1	1% (–)
Total		58	328	386	

**Fig. 1** ROC curve analysis for current HbA normal range of 95–98%

its ability to categorize healthy population as outline on Table 2.

Hemoglobin A2 (HbA2) confidence intervals for hemoglobinopathy-free patients in western Kenya

Similarly, hemoglobin A2 (HbA2) varied significantly ($p < 0.0001$) with the stations from where the samples were collected with Busia recording HbA2 median of 3.2, $IQR = 1.03$; 95% $CI = 1.14$ –5.26, while Bungoma had a median of 3.3, $IQR = 0.5$; 95% $CI = 2.3$ –4.3. These were the highest among the rest of the stations in western Kenya, together with Kitale that had HbA2 medians

of 3.2, $IQR = 0.9$; 95% $CI = 1.4$ –5.0. Kakamega recorded an HbA2 median of 2.8, $IQR = 1.3$; 95% $CI = 0.2$ –5.4, while Kisumu station had HbA2 median of 3.0, $IQR = 0.6$; 95% $CI = 1.8$ –4.2. Kisii station had a HbA2 median of 3.0, $IQR = 1.1$; 95% $CI = 0.8$ –5.2, while Homa Bay had a median of 3.0, $IQR = 0.65$; 95% $CI = 1.7$ –4.3. Migori station recorded a HbA2 median of 2.9, $IQR = 0.5$; 95% $CI = 1.9$ –3.9.

There was no statistical significance ($p = 0.901$) in gender with males having a median of 3.0, $IQR = 0.6$; 95% $CI = 1.8$ –4.2, while females had the same median and interquartile range, thus sharing the same cutoff value.

Hemoglobin A2 (HbA2) did not vary significantly ($p = 0.219$) with age where children ≤ 2 years had a HbA2 median of 3.0, $IQR = 0.8$; 95% $CI = 1.4$ – 4.6 , and age group 3–5 years had a median of 3.1, $IQR = 0.8$; 95% $CI = 1.5$ – 4.7 . The age group 6–11 years had HbA2 median of 3.15, $IQR = 0.6$; 95% $CI = 1.95$ – 4.35 . The study subjects ≥ 12 years had HbA2 median of 3.0, $IQR = 0.7$; 95% $CI = 1.6$ – 4.4 .

The performance of 1.5–3.5% HbA2 normal range in western Kenya

The HbA2 normal range of 1.5–3.5% developed by Kratz et al. [1] was tested against the HbA2 of the 386

healthy subjects generating a significant ($p < 0.0001$) accuracy of 95.9%, a high sensitivity of 98.4%, specificity of 93.3%, a positive likelihood ratio (LR+) of 14.7, and a negative likelihood ratio (LR–) of 0.017 (results summarized on Table 2).

Predictive validity of HbA2 normal range of 1.5–3.5% in western Kenya

The HbA2 normal range of 1.5–3.5% currently on use was used as the gold standard against the 386 hemoglobinopathy-free study subjects, to determine its ability to grade the healthy population as normal. It was able to give a positive predictive validity of 99.7% and a negative

Table 4 Predictive validity of currently HbA2 normal range in western Kenya

		Current HbA2 normal ranges		Total	Predictive values
		1.5–3.5 (positive)	< 1.5 (negative)		
Western Kenya HbA2 ranges	1.8–4.2 (positive)	365	1	366	99.7% (+)
	< 1.8 (negative)	6	14	20	70.0% (–)
Total		371	15	386	

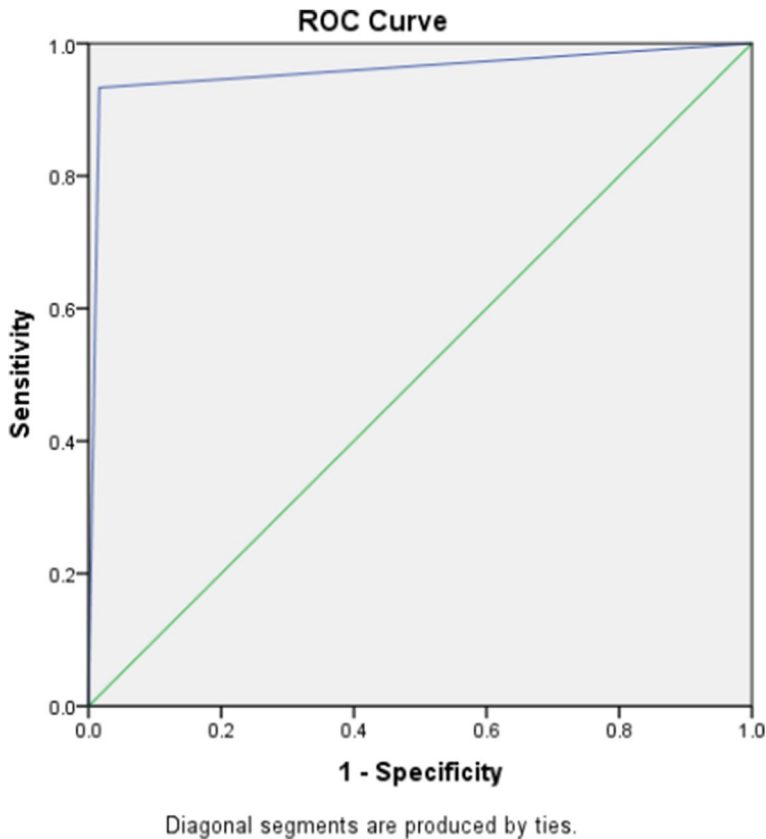


Fig. 2 ROC curve analysis for HbA2 hemoglobin

predictive validity of 70.0% whose ROC curve to the left upper corner, far away from the diagonal line (Table 4 and Fig. 2).

The ROC curve that analyzed the HbA2 confidence interval of 1.5–3.5% in western Kenya flowed close to the left upper corner, far away from the diagonal line which is an indicator of a potential biomarker for use in clinical practice, approved by a significant ($p < 0.0001$) accuracy, high sensitivity, and high specificity (Fig. 2, Table 2).

Hemoglobin F confidence intervals for hemoglobinopathy-free patients in western Kenya

Similarly, hemoglobin F varied significantly ($p < 0.0001$) from the station where samples were collected, with Busia and Kisumu region recording similar cutoff value of 0.8, $IQR = 0.8$; 95% $CI = 0.0$ –2.4. Bungoma had a median of 0.8, $IQR = 0.1$; 95% $CI = 0.6$ –1.00, while Kitale recorded a median of 0.8, $IQR = 0.65$; 95% $CI = 0.0$ –2.1. Kakamega had a HbF median of 0.5, $IQR = 1.10$; 95% $CI = 0.0$ –2.7, while Kisii station produced a cutoff value of 0.8, $IQR = 0.58$; 95% $CI = 0.00$ –1.96. Homa Bay recorded a median of 0.8, $IQR = 1.05$; 95% $CI = 0.0$ –2.9 with Migori having the highest HbF median of 2.1, $IQR = 0.7$; 95% $CI = 0.7$ –3.5.

There was no significance ($p = 0.075$) in fetal hemoglobin (HbF) between males and females who recorded the same HbF median of 0.8, $IQR = 0.8$; 95% $CI = 0.0$ –2.4.

Among the hemoglobin fraction, only fetal hemoglobin (HbF) varied significantly ($p < 0.0001$) with age where children ≤ 2 years had the highest HbF median of 1.0, $IQR = 0.8$; 95% $CI = 0.0$ –2.6, while children of age groups 3–5 years had a median of 0.8, $IQR = 0.8$; 95% $CI = 0.0$ –2.4. The age groups 6–11 years had HbF median of 0.8, $IQR = 0.03$; 95% $CI = 0.74$ –0.86, while study subjects ≥ 12 years recorded the lowest HbF median of 0.55, $IQR = 0.8$; 95% $CI = 0.0$ –2.15.

P-Window hemoglobin fraction in western Kenya

The P window is a segment of hemoglobin fractions that represents degraded products in HPLC results. The P window had an overall median of 4.98, $IQR = 0.4$; 95% $CI = 4.18$ –5.78 and varied significantly ($p = 0.020$) from different locations from where the samples were collected. P window did not vary significance ($p = 0.514$) with gender and neither did it vary ($p = 0.259$) with age.

Discussion

Normal reference range of a population remains to be a fundamental element in the accurate diagnosis of a variety of diseases in the human population with reports showing that use of reference range of a different population may result into a biased diagnosis

leading to improper clinical decisions that could harm a healthy population through inappropriate management and prognosis [2]. To avoid such grave errors in clinical practice, researchers have emphasized use of locally derived reference ranges due to reported wide variation in immune-hematological and biochemical parameters due to genetic, and sociocultural variations, as well as epigenetic changes resulting in diverse phenotypic features in different populations [24, 25]. Despite reports showing such enormous evidence-based findings, western Kenya is yet to derive hemoglobin fractions reference range and therefore continues to use those derived in Western countries. The literature reviewed also shows that similar data is lacking in the entire Kenyan population and indeed Africa as a whole. To date, few studies have described the pattern of production of common hemoglobin variants in Africa population [26]. Although the determinants of variations in the production of different forms of hemoglobin fractions remain poorly understood, factors such as age, sex, ethnicity, environment, and genetics play a significant role [26]. These conditions have potential to alter epidemiologic profiles of genetically affected health problems including hemoglobin variants reference range. There was need therefore to develop hemoglobin fractions reference range at 95% confidence interval from the normal non-anemic hemoglobinopathy-free subjects, which to the best of our knowledge was the first attempt ever to be carried out in this region.

It was important to note that hemoglobin A (HbA) that forms about 95% of the red cell hemoglobin fractions as reported by previous findings contrasted our findings of an overall median of 87.7%, $IQR = 5.7$; 95% $CI = 76.3$ –99.1 which was the first ever to be established in Africa but varied greatly from 95 to 98% reported by Kratz et al. [1]. This means that the overall median of 87.7% in HbA obtained from normal hemoglobinopathy-free subjects (Hb AA) would be out of range and possibly suggest a possibility of hemoglobinopathy if graded as per the reference range of 95–98% hemoglobin fraction reported by Kratz et al. [1]. HbA varied significantly ($p = 0.016$) from the location where the sample was collected, which may be suggesting each region to develop their own normal range as it has been recommended by the previous studies [27]. Hemoglobin A varied ($p = 0.005$) in gender where females had a higher HbA median of 88.5, $IQR = 5.4$; 95% $CI = 77.7$ –99.3, while males had a median of 86.9, $IQR = 5.2$; 95% $CI = 76.5$ –97.3, meaning that males and females need their own HbA reference range; however, we were unable to get similar findings in the literature; thus, to give credence to the present findings, validation exercise is imperative using a population-based study. We also discovered that the hemoglobin A

did not vary ($p = 0.106$) with age which means that all the age groups can share Hb A reference range provided they are of the same gender confirming that the fraction for HbA volume may be genetically determined compared to the quantitative hemoglobin (g/dl) in CBC analyzer outputs which vary greatly with age [28].

When the current used HbA normal range of 95–98% was computed against the HbA of the 386 presumed hemoglobinopathy-free subjects, it generated a nonsignificant ($p = 0.795$) accuracy of 57.5%, when it is widely known a normal reference range would need to have 95% confidence interval for it to be effective in grading a normal population [23]. With a sensitivity of 100% and a specificity of 0.3% is an indicator of a worthless diagnostic biomarker that should not be used in clinical practice [29]. Furthermore, it had 15.1% positive predictive validity, meaning that use of HbA range of 95–98% was able to grade only 15.1% of the population as normal while grading most of the population of 84.9% as abnormal. The other findings were negative predictive validity of 1%; 1.03 positive likelihood ratio and all of them are suggestive of a poor efficacy of 95–98% HbA normal range utility in western Kenya population. Similarly, its ROC curve flowed close to the diagonal line which indicates a worthless test that cannot be relied on in clinical diagnosis. With these findings, it is now profoundly clear use of the HbA range of 95–98% in western Kenya will grade majority of the population out of range that could be suggestive of hemoglobinopathy and thus may lead to erroneous treatment and prognosis. Therefore, the present study recommends that the HbA normal range of 95–98% needs to be replaced immediately by developing new normal range using a population-based study in western Kenya population and Africa at large. These findings also confirm the widely reported variations of immune-hematological and biochemical parameters in populations of different geophysical locations [2, 11] thus, for correct determination of hemoglobinopathies in western Kenya, hemoglobin fractions confidence intervals derived by the present study may be used to serve the population until when they are replaced by population-based study normal reference range.

The present study developed an Hb A2 cutoff value of 3.0% and 95% $CI = 1.8$ – 4.2 , which seemed to be higher than the HbA2 reference range of 1.5–3.5% developed by Kratz et al. [1], and 1.2% (0.5–3.4%) Hb A2 reference range reported among Saudi adults aged 18–50 years [30]. The HbA2 is regarded as an indicator of α - and β -thalassemia, but it is unreliable in differential diagnosis since it varies in different conditions; example, it may be reduced in iron deficiency anemia [30]. These reports indicate that α -thalassemia patients have HbA2 level that is normal or reduced (2.5%), while in β -thalassemia,

HbA2 is either normal or elevated (3.5%) where such cases are rarely reported in sub-Saharan Africa [26, 30]. A similar report done in Kenyan coast documented a higher HbA2 of $> 4.0\%$ in Hb AA infants, where an Hb A2 of $> 3.5\%$ is suggestive of heterozygous beta thalassemia, a disease now reported in low frequencies in western and coastal region of Kenya [15, 26]. However, having a higher HbA2 in normal HbAA may not necessarily mean the individual has beta thalassemia as this may be supporting the reported variation of hematological, biochemical, and immunological parameters due to geographical, ethnic, race, and genetic differentiated documented by various studies in African continent [25, 29]. Therefore, the HbA2 cutoff value of 1.8–4.2% developed by the present study emphasizes establishment of local reference ranges as those among the health population with HbA2 of $> 3.5\%$ may be erroneously graded as beta thalassemia.

However, when the HbA2 currently used normal range of 1.5–3.5 was used to grade the 386 hemoglobinopathy-free subjects, it generated an accuracy of 95.9% which was within the accepted confidence interval of a credible reference range [23]. The effective utility of the currently on use HbA2 normal range was supported by a statistical significance ($p < 0.0001$) of high sensitivity and specificity of 98.4% and 93.3%, respectively. This means that most of the 386 normal study subjects were within the current used normal reference range of 1.5–3.5. In addition, a strong positive likelihood ratio of 14.7 demonstrated the ability of 1.5–3.5 HbA2 normal range to grade western Kenya 14.7 more times likely to be free from hemoglobinopathy and a negative likelihood ratio of 0.017 [1]. Furthermore, it had 99.7% positive predictive validity and a negative predictive validity of 70.0%, which confirms use of HbA2 normal range of 1.5–3.5 would categorize majority of western Kenya population as hemoglobin-free. The HbA2 normal range of 1.5–3.5 ROC curve flowed on the upper left corner of the curve, which is a signal of an accurate biomarker effective in clinical diagnosis [29]. This finding serves as evidence that the HbA2 normal range of 1.5–3.5 which is currently on use may be retained as an accurate reference range in western Kenya population.

Various forms of hemoglobin are expressed at different stages of human development where HbF ($\alpha_2\gamma_2$) predominates in neonates and declines during the first year of life [26]. These findings were similar to the present study where there was significant different ($p < 0.0001$) of HbF among the four age groups with children ≤ 2 years having the highest median of 1.0 (0.8), while those individuals above 2 years recorded HbF of ≤ 1 among Hb AA population. Similarly, it was also reported that HbF level is a useful parameter for the diagnosis of thalassemia,

which is majorly expressed in fetal life and young children but is switched off by the age of 2 years and then remain more or less constant at a value less than 1%; thus, the authors noted a high HbF in the 1 year old and gradually reduced to around 1% in the 2 years old [30]. Weiner and Andrew [26] also documented an overall HbF of 95% $CI = 4.5\text{--}4.6$ among all the samples done which was higher compared to our overall finding of 0.8 (0.0–2.4). This variation may be due to the fact that the study collected blood sample from neonates and infants, while our study included all the study population. The authors also reported lower HbF in infants with HbAA (4.4%, 95% $CI = 4.3\text{--}4.4$) compared to infants with HbSS whose HbF was 21.9%; 95% $CI = 20.1\text{--}23.5$), and that HbF was significantly higher in females than males in all the three phenotypes (Hb AA, Hb AS, Hb SS) [26]. This was in contrast with our finding which found out that there was no significant difference in HbF between females and males who recorded similar medians of 0.8, $IQR = 0.8$; 95% $CI = 0.0\text{--}2.4$. The current normal range on use of HbF of 0–2.0 was similar to the reference range developed by the present study; therefore, we were unable to use ROC curve analysis which suggests that it is effective in grading healthy individual in western Kenya as normal; therefore, it may be retained.

Bio-Rad HPLC machine is in the process of analyzing different hemoglobin fractions, it also generates certain percentage of degraded products which are as a result of the age of the specimen or storage conditions of the sample before analysis; thus, these conditions must be adhered to, according to manufacturer's guidelines to be able to get an acceptable proportion of degraded products in P3 window [31, 32]. The overall P window obtained by the present study was 4.98, $IQR = 0.4$; 95% $CI = 4.18\text{--}5.78$. This is the first ever normal range for P window in African continent, which suggests that P window above 5.78 may result into an erroneous grading of hemoglobin fraction. The P window varied significantly from the stations where the sample were collected, and we were unable to figure out an explanation for this. There was no significant different in P window based on gender and age.

Based on our findings, it is hoped that establishment of local HbA normal reference range using population-based study in Kenyan population and Africa at large will be treated as a matter of great urgency to replace the currently used reference range of 95–98% as this could be a source of grave errors that could jeopardize the safety of a population. Replacement of the currently used reference range may involve validation of our findings using healthy volunteer blood donors whose hemoglobin fractions will be determined using HPLC or preferably molecular technology. Blood donors are required to have more than 12

years which may fail to capture children under the age of 12 years; however, as per our study findings, hemoglobin A and hemoglobin A2 did not vary with age, meaning reference range of such hemoglobin can be shared across all age groups; however, fetal hemoglobin (HbF) varied with age, but it is usually replaced with adult hemoglobin by 6 months making our derived reference range viable to serve majority of the western Kenyan population [33].

Conclusions

It is important to report that the use of normal range for HbA of 95–98% published by Kratz et al. [1] in western Kenya has a potential threat of misdiagnosis of normal population and thus needs urgent review as it lacked efficacy ($p = 0.795$) in grading hemoglobinopathy-free subjects as normal with a poor accuracy of 57.5%, a sensitivity of 100%, specificity of 0.3%, positive predictive validity of 15.1%, negative predictive validity of 1%, and 1.03 positive likelihood ratio. However, the traditional normal range for HbA2 of 1.5–3.5% on use in western Kenya may be retained as it was effective ($p < 0.0001$) in grading majority of study subjects as normal with an accuracy of 95.9%, sensitivity of 98.4%, specificity of 93.3%, positive predictive validity of 99.7%, negative predictive validity of 70.0%, 14.7 positive likelihood ratio, and 0.017 negative likelihood ratio. Similarly, the existing normal range for HbF of 0–2.0 on use was almost the same as the one we derived of 0–2.4 and therefore may be retained for use in western Kenya. It is anticipated that the finding of this study will help improve the management of hemoglobinopathies in Kenya and Africa at large, by contributing to improvement in the validity of the clinical-pathologic interpretation assay results for the percentage values for the Hb fractions.

Abbreviations

ISO: International Organization for Standardization; HbA: Hemoglobin A; HbA2: Hemoglobin A2; HbF: Hemoglobin F; HPLC: High-performance liquid chromatography; IQR: Interquartile range; ROC: Receiver operating characteristic curve; WHO: World Health Organization; Hb: Hemoglobin; HbAA: Hemoglobin AA; ANA: Antinuclear antibodies; SPSS: Statistical Package for Social Sciences; CI: Confidence interval; AUC: Area under curve; N/A: Not applicable; CBC: Complete blood count.

Acknowledgements

We would like to thank Mr. Raphael Kitonga for allowing us to use his home-based library and all its accessories in addition to his articulate moral support. We also pass our gratitude to the Aga Khan Hospital, Kisumu, Ethical Review Committee for granting the permit for this study.

Authors' contributions

BM, conceptualized the study, collected data, analyzed data, and wrote the original manuscript. RC, described the P window in HPLC in addition to critical reviews. BM, technical support and critical reviews. TW, technical support and critical reviews. GS, conceptualized the study, curated data, offered technical support, gave critical reviews, and supervised the project. JM, technical support and critical reviews. PO, conceptualized the study, curated data, provided

expert technical advice, offered critical reviews, and supervised the project. The authors read and approved the manuscript.

Funding

No funding.

Availability of data and materials

If data will be needed, it will be made available upon request from the corresponding author.

Declarations

Ethics approval and consent to participate

The study was approved by Masinde Muliro University Ethical Review Committee and by the National Commission for Science and Technology (NACOSTI). Permit to collect data was also granted by the Aga Khan Hospital, Kisumu, Ethics and Research Review Board.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Author details

¹Department of Medical Laboratory Sciences, School of Public Health, Biomedical Sciences and Technology, Masinde Muliro University of Science and Technology, P.O. Box 190 50100, Kakamega, Kenya. ²Department of Pathology, The Aga Khan Hospital, P.O. Box 530-40100, Kisumu, Kenya. ³Department of Biological Sciences, School of Science and Technology, University of Kabianga, P.O. Box 2030-20200, Kericho, Kenya. ⁴Department of Microbiology and Parasitology, School of Medicine, Masinde Muliro University of Science and Technology, P.O. Box 190-50100, Kakamega, Kenya. ⁵School of Medicine, Uzima University, P.O. Box 2502-40100, Kisumu, Kenya. ⁶Department of Haematology and Blood Transfusion, Muhimbili University of Health and Allied Sciences, Tanzania, P.O. Box 65001, Dar es Salaam, Tanzania. ⁷Department of Biological Sciences, School of Natural and Applied Sciences, Masinde Muliro University of Science and Technology, P.O. Box 190, 50100, Kakamega, Kenya.

Received: 28 October 2022 Accepted: 21 December 2022

Published online: 29 December 2022

References

- Kratz PM, Ph D, Lewandowski KB (2004) Laboratory reference values, pp 1548–1563
- Omarine Nlinwe N, Larissa Kumenyuy Y, Precious Funwi C (2021) Establishment of hematological reference values among healthy adults in Bamenda, north west region of Cameroon. *Anemia* 2021. <https://doi.org/10.1155/2021/6690926>
- Addai-mensah O, Gyamfi D, Duneeh RV, Danquah KO, Annani-akollor ME, Boateng L, Owiredu E, Amponsah FA, Afriyie EY, Asare R, Ofosu DN (2019) Determination of haematological reference ranges in healthy adults in three regions in Ghana, vol 2019
- Viegas E, Macovela E, Tembe N, Joaquim O, Alfai E, Jani I, Nilsson C, Gonc E, Osman N (2014) Reference values for clinical laboratory parameters in young adults in Maputo, Mozambique. 9(5). <https://doi.org/10.1371/journal.pone.0097391>
- Miri-dashe T, Osawe S, Tokdung M, Daniel N, Choji RP, Mamman I, Deme K, Damulak D, Abimiku A (2014) Comprehensive reference ranges for hematology and clinical chemistry laboratory parameters derived from normal Nigerian adults. 9(5). <https://doi.org/10.1371/journal.pone.0093919>
- Yalew A, Terefe B, Alem M, Enawgaw B (2016) Hematological reference intervals determination in adults at Gondar University Hospital, northwest Ethiopia. *BMC Res Notes*:1–9. <https://doi.org/10.1186/s13104-016-2288-8>
- Karita E, Ketter N, Price MA, Kayitenkore K, Kaleebu P, Nanvubya A, Anzala O, Jaoko W, Mutua G, Ruzagira E, Mulenga J, Sanders EJ, Mwangome M, Allen S, Bwanika A, Bahemuka U, Awuondo K, Omosa G, Farah B et al (2009) CLSI-derived hematology and biochemistry reference intervals for healthy adults in eastern and southern Africa. *PLoS One* 4(2). <https://doi.org/10.1371/journal.pone.0004401>
- Lugada ES, Mermin J, Kaharuza F, Ulvestad E, Were W, Langeland N, Asjo B, Malamba S, Downing R (2004) Population-based hematologic and immunologic reference values for a healthy Ugandan population. 11(1):29–34
- Kibaya RS, Bautista CT, Sawe FK, Shaffer DN, Saterien WB, Scott PT, Michael NL, Robb ML, Birx DL, Souza MSD (2008) Reference ranges for the clinical laboratory derived from a rural population in Kericho, Kenya. 3(10):1–7. <https://doi.org/10.1371/journal.pone.0003327> <https://doi.org/10.1128/CDLI.11.1.29>
- Ochola J, Id O, Id DHM, Otieno L, Owuoth J, Ogutu B, Oyieko J, Korir JC, Sifuna P, Singoei V, Owira V, Maureen S, Gondii O, Andagalu B, Otieno W (2021) Clinical laboratory hematology reference values among infants aged 1 month to 17 months in Kombewa sub-county, Kisumu : a cross sectional study of rural population in western Kenya, pp 1–17. <https://doi.org/10.1371/journal.pone.0244786>
- Zeh C, Amornkul PN, Inzaule S, Ondoa P, Oyaro B, Dufton M, Vandenhoude H, Gichangi A, Williamson J, Thomas T, Kevin M (2011) Population-based biochemistry, immunologic and hematological reference values for adolescents and young adults in a rural population in western Kenya. 6(6). <https://doi.org/10.1371/journal.pone.0021040>
- Gitaka J, Ogwang C, Ngari M, Akoo P, Olotu A, Kerubo C, Fegan G, Njuguna P, Nyakaya G, Otieno T, Mwambingu G, Awuondo K, Lowe B, Chilengi R, Berkley A (2017) Clinical laboratory reference values amongst children aged 4 weeks to 17 months in Kilifi, Kenya : a cross sectional observational study, pp 1–13
- Odhiambo C, Omolo P, Oyaro B, Williamson J, Kinuthia J, Matemo D, Drake A, John-stewart G, Zeh C (2017) Establishment of reference intervals during normal pregnancy through six months postpartum in western Kenya, pp 1–11
- Roa D, Turner EA, Aguinaga MDP (1995) Reference ranges for hemoglobin variants by HPLC in African Americans. *Ann Clin Lab Sci* 25(3):228–235
- Mutua B, Sowayi G, Okoth P (2022) Distribution of hemoglobinopathy phenotypes in western Kenya: a retrospective study done at Aga Khan Hospital, Kisumu. *Egypt J Internal Med* 34(1):1–9
- Bakan E, Polat H, Ozarda Y, Ozturk N, Baygultalp NK, Umudum FZ, Bakan N (2016) A reference interval study for common biochemical analytes in eastern Turkey: a comparison of a reference population with laboratory data mining. *Biochem Med* 26(2):210–223
- Arzideh F, Özcürümez M, Albers E, Haeckel R, Streichert T (2021) Indirect estimation of reference intervals using first or last results and results from patients without repeated measurements. *J Lab Med* 45(2):103–109
- Placzowska S, Terpińska M, Piwowar A (2022) Establishing laboratory-specific reference intervals for TSH and fT4 by use of the indirect Hoffman method. *Plos one* 17(1):e0261715
- Bhattacharya CG (1967) A simple method of resolution of a distribution into Gaussian components. *Biometrics*:115–135
- Farrell CJL, Nguyen L (2019) Indirect reference intervals: harnessing the power of stored laboratory data. *Clin Biochem Rev* 40(2):99
- Yan R, Li K, Lv Y, Peng Y, Halm-Lutterodt V, Song W et al (2022) Comparison of reference distributions acquired by direct and indirect sampling techniques: exemplified with the Pediatric Reference Interval in China (PRINCE) study. *BMC Med Res Methodol* 22(1):1–10
- Ozarda Y, Ichihara K, Jones G, Streichert T, Ahmadian R (2021) Comparison of reference intervals derived by direct and indirect methods based on compatible datasets obtained in Turkey. *Clin Chim Acta* 520:186–195
- Lee DK, In J, Lee S (2015) Standard deviation and standard error of the mean. *Korean J Anesthesiol* 68(3):220
- Mutua B, Sowayi G, Okoth P (2022) Prognostic potential of RDW in discriminating hemoglobinopathies among patients reporting to Aga Khan Hospital, Kisumu. *Egypt J Med Hum Genet* 23(1):1–9
- Boyce WT, Sokolowski MB, Robinson GE (2020) Genes and environments, development and time. *Proc Natl Acad Sci* 117(38):23235–23241
- Weiner RD, Andrew D (2011) Etters to the. *J ECT* 27(2):175–177
- Gachie, Nyambura R.N. Haematological reference intervals for adolescents and adults in Nakuru County, Kenya (doctoral dissertation, department of pathology a thesis submitted for the award of the degree of Doctor of Philosophy (cell haematology) in the school of medicine, Kenyatta university). 2018

28. Totan M, Gligor FG, Bojita M, Grigore C, Grigore C (2013) Determining hemoglobin reference values in children and teenagers from Sibiu area. *Rev Român Med Lab* 21(1/4)
29. Mutua BM, Sowayyi G, Okoth P (2022) Red cell distribution width as a surrogate marker of haemoglobinopathies in western Kenya. *Afr J Lab Med* 11(1):1–8
30. El-Hazmi MA, Warsy AS (2001) Normal reference values for hematological parameters, red cell indices, HB A2 and HB F from early childhood through adolescence in Saudis. *Ann Saudi Med* 21(3-4):165–169
31. Riou J, Godart C, Hurtrel D, Mathis M, Bimet C, Bardakdjian-Michau J et al (1997) Cation-exchange HPLC evaluated for presumptive identification of hemoglobin variants. *Clin Chem* 43(1):34–39
32. Szuberski J, Oliveira JL, Hoyer JD (2012) A comprehensive analysis of hemoglobin variants by high-performance liquid chromatography (HPLC). *Int J Lab Hematol* 34(6):594–604
33. Makani J, Ofori-Acquah SF, Nnodu O, Wonkam A, Ohene-Frempong K (2013) Sickle cell disease: new opportunities and challenges in Africa. *Scientific World J* 2013

Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Submit your manuscript to a SpringerOpen[®] journal and benefit from:

- Convenient online submission
- Rigorous peer review
- Open access: articles freely available online
- High visibility within the field
- Retaining the copyright to your article

Submit your next manuscript at ► [springeropen.com](https://www.springeropen.com)
