

Prevalence of HPLC-Detected Hemoglobin Patterns and ABO/Rh(D) Blood Group Distribution Among Male Blood Donors at Al-Gumhuri Teaching Hospital, Sana'a, Yemen

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Abstract

Background: Inherited hemoglobin disorders remain common across the Middle East; however, contemporary donor-based data from Yemen are limited. Characterizing HPLC-detected hemoglobin patterns together with ABO/Rh(D) blood group distribution may provide valuable information for local blood bank planning.

Methods: A cross-sectional study was conducted among 339 apparently healthy male blood donors attending the blood bank of Al-Gumhuri Teaching Hospital, Sana'a, Yemen, between 11 May and 5 June 2024. ABO and Rh(D) blood groups were determined using the standard slide agglutination method. Hemoglobin concentration was measured photometrically, and hemoglobin variants were identified by cation-exchange HPLC. Descriptive statistics were used to summarize the findings, and the Fisher-Freeman-Halton exact test assessed the association between age group and hemoglobin phenotype.

Results: Most donors exhibited a normal HPLC hemoglobin pattern. An HbS-containing HPLC pattern consistent with sickle cell trait was detected in 16 donors (4.72%), whereas an elevated HbA2 screening pattern suggestive of β -thalassemia trait was identified in 4 donors (1.18%). Variant HPLC patterns were more frequently observed among donors aged 20–29 years, although this finding should be interpreted cautiously because adjusted analyses accounting for potential selection factors were not feasible. The ABO/Rh(D) distribution was dominated by O+, followed by A+. RhD-negative phenotypes accounted for 7.37% of donors.

Conclusion: HbS-containing HPLC patterns and elevated HbA2 screening patterns were detected in a minority of eligible male blood donors. These facility-specific findings should not be generalized to the wider population. Larger multicenter studies including female donors and molecular confirmation are needed to better define the epidemiology of inherited hemoglobin disorders in Yemen.

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Introduction

Inherited hemoglobin disorders are among the most consequential monogenic conditions worldwide. Recent Global Burden of Disease (GBD) estimates show a sustained rise in the number of people living with sickle cell disease (SCD) and births affected by SCD between 2000 and 2021, underscoring the large, often hidden mortality in early childhood and the need for stronger screening and care systems (Thomson et al., 2023). In parallel, updated GBD analyses for thalassemia report substantial prevalence and disability in many low- and middle-income countries, despite gradual declines in age-standardized rates, reflecting population growth and uneven access to preventive and comprehensive care (Tuo et al., 2024).

Population genetics further shapes the local disease patterns. The sickle cell trait (HbAS) confers partial protection against severe *Plasmodium falciparum* malaria, a classic example of balanced selection that helps maintain the Hemoglobin S (HbS) allele in malaria-endemic regions across Africa, the Middle East, and parts of South Asia (Ngou et al., 2023). These host-pathogen dynamics influence the geographic distribution and clinical expression of hemoglobinopathies, with implications for screening strategies in endemic settings (Ngou et al., 2023).

In Yemen, reliable, contemporary data are emerging but remain limited relative to the regional burden. Community surveys from Taiz highlight awareness gaps and mixed attitudes toward premarital screening for inherited conditions including SCD and thalassemia pointing to opportunities to strengthen public education and policy implementation (Almoliky et al., 2022). More recently, a donor-based study from Sana'a identified hemoglobinopathy traits among volunteer blood donors using standardized screening tools (Complete Blood Count (CBC) indices, NESTROF, and confirmatory High-performance liquid chromatography (HPLC)), supporting the feasibility of structured screening in routine blood bank workflows (Almorish et al., 2024). Unlike that study, which focused on screening performance, our study also describes the ABO/Rh(D) profile and donation-history stratification in this cohort.

Transfusion safety and inventory planning in Yemen also depend on up-to-date knowledge of ABO and Rhesus (RhD) distributions. Recent Yemeni data from the Amran Governorate (2020–2021) show O as the most common ABO phenotype ($\approx 51\%$) with RhD positivity around 87% (Al-Sormy, 2023), while a large series from the Ibb Governorate (2021–2023; $n \approx 4,800$ donors) reported O predominance ($\sim 56\%$) and a higher proportion of RhD-negative donors ($\sim 20\%$), illustrating intra-country variation that can affect the availability of compatible units and rare phenotypes (Al-Mohani & Al-Nawd, 2024). These patterns align with broader observations from the Arabian Peninsula, where O and RhD-positive phenotypes predominate, but with meaningful regional heterogeneity relevant to donor recruitment and contingency planning (Belali, 2022).

Against this backdrop, our study characterizes ABO/RhD distributions and common hemoglobinopathy traits in a contemporary Yemeni donor cohort to inform transfusion services and scalable screening efforts in Yemen.

Materials and Methods

Study Design

This was a cross-sectional study conducted to assess the prevalence of hemoglobin variants and the distribution of ABO/Rh blood groups among male blood donors. A quantitative approach was used to characterize hemoglobin phenotypes and blood group patterns in the study population.

Study Setting and Participants

The study was carried out at the blood bank of Al-Gumhouri Teaching Hospital in Sana'a City, Yemen, between 11 May and 5 June 2024. A non-probability convenience sampling approach was used. Eligible male blood donors who were available and willing to participate during the study period were recruited. In the local blood-donation context, donor attendance at the participating blood bank was restricted in practice to male donors because of prevailing sociocultural and operational norms regarding blood donation. Therefore, female donors were not available for recruitment during the study period. A total of 339 apparently healthy male donors were included. The study was designed as a time-bound, single-facility descriptive cross-sectional investigation; therefore, an a priori sample-size calculation was not undertaken. The precision achieved with the available sample is described in the Statistical Analysis section. All participants met the national eligibility criteria for safe blood donation, including age between 18–65 years, body weight ≥ 50 kg, hemoglobin level ≥ 12.5 g/dL, normal blood pressure, and absence of communicable diseases.

Data and Sample Collection

Sociodemographic and clinical data were collected using a structured questionnaire, which was pretested and refined before the main study. The questionnaire captured information such as age, type of donation, number of previous donations, and other relevant variables. One to two milliliters of venous blood were collected aseptically from each participant into ethylenediaminetetraacetic acid (EDTA) tubes, labeled with a unique code.

Laboratory Analysis

Blood Grouping

ABO and Rh blood groups were determined using the standard serological slide agglutination method. Commercially prepared anti-A, anti-B, and anti-D reagents were used according to the manufacturer's instructions. Quality control (QC) procedures included the use of positive and negative control sera for each antigen, with calibration verified through daily monitoring of reagent potency and performance, ensuring consistent results.

Hematological Analysis

Hemoglobin levels were measured using the Hemo Control System (Germany), which employs a photometric method at dual wavelengths (570 nm and 880 nm) to compensate for turbidity and ensure accuracy.

Hemoglobin Variant Detection

HPLC was performed using the H8 system (Lifotronic, China) to detect and quantify hemoglobin fractions. The assay was carried out according to the manufacturer's instructions. The HPLC system separates normal and variant hemoglobins based on retention time, and chromatographic peaks were interpreted with reference to predefined windows. HPLC findings were interpreted as screening classifications rather than definitive molecular diagnoses. An HbS-containing chromatographic pattern with an HbS proportion below 50% was classified as consistent with an HbAS pattern, while Hemoglobin A2 (HbA2) values between 4% and 9% were classified as elevated HbA2 screening patterns suggestive of β -thalassemia trait. Molecular testing was not performed; therefore, genotype confirmation was not available. For each run, quality-control samples representing known HbAA, HbAS, and HbA2 patterns were included to verify the separation process, and atypical chromatograms were flagged for repeat analysis.

Statistical Analysis

Data were analyzed using Statistical Package for Social Sciences (SPSS) version 26 (IBM Corp., Armonk, NY, USA). Categorical variables were summarized as frequencies and percentages, while continuous variables were expressed as means $\pm$ standard deviations. No missing data were identified; therefore, a complete-case analysis was performed.

Given the sparse counts in the age-stratified subgroups, the association between age group and hemoglobin phenotype was assessed using the Fisher–Freeman–Halton exact test instead of the Pearson chi-square test. For an exploratory secondary analysis, hemoglobin phenotype was dichotomized (any variant vs. HbAA) and age was grouped as 20–29 versus ≥ 30 years to estimate prevalence ratios, odds ratios, and their 95% confidence intervals. A two-sided p-value < 0.05 was considered statistically significant. The precision of the prevalence estimates is described by their 95% confidence intervals as presented in the Results section.

Results

A total of 339 male blood donors were included in this cross-sectional study conducted at Al-Gumhouri Teaching Hospital in Sana'a City, Yemen. All participants met the national eligibility criteria for safe blood donation. The study assessed the prevalence of hemoglobin variants and blood group phenotypes using HPLC and serological typing, respectively.

Distribution of HPLC-Detected Hemoglobin Patterns

The majority of donors showed a normal HPLC hemoglobin pattern (HbAA). Sixteen donors (4.72%; 95% confidence interval (CI), 2.7%–7.6%) had an HbS-containing HPLC pattern consistent with sickle cell trait. Four donors (1.18%; 95% CI, 0.3%–3.0%) had an elevated HbA2 screening pattern suggestive of β -thalassemia trait. These findings are summarized in Table 1.

Table 1: Distribution of HPLC-detected hemoglobin patterns among male blood donors at Al-Gumhouri Teaching Hospital, Sana'a, Yemen (n = 339)

HPLC-detected hemoglobin pattern	No. (%)	95% CI
Hb AA	319 (94.10%)	91.0–96.4
Hb AS (Sickle Cell Trait)	16 (4.72%)	2.7–7.6
Elevated HbA2 (suggestive of β -thalassemia trait)	4 (1.18%)	0.3–3.0

HPLC-Detected Hemoglobin Patterns by Age Group

Variant HPLC patterns were observed more frequently among donors aged 20–29 years than among donors aged 30 years or older. However, this descriptive pattern should not be interpreted as evidence of an age-related biological association because donation history and other donor-selection factors were not available for age-stratified adjustment. Among donors aged 20–29 years, 13 (10.5%) had an HbS-containing HPLC pattern consistent with sickle cell trait and 4 (3.2%) had an elevated HbA2 screening pattern suggestive of β -thalassemia trait, compared with 3 donors (2.1%) with an HbS-containing pattern and no donors with an elevated HbA2 pattern among those aged 30–39 years. No variant HPLC patterns were observed among donors aged 40 years or older. The distribution of HPLC patterns differed across age groups on Fisher–Freeman–Halton exact testing ($p = 0.0013$), but this unadjusted result should be interpreted cautiously. Detailed stratification is shown in Table 2.

Table 2. Distribution of HPLC-detected hemoglobin patterns by age group among male blood donors at Al-Gumhouri Teaching Hospital, Sana'a, Yemen (n = 339)

Age group (years)	Total donors	Hb AA, No. (%)	Hb AS, No. (%)	Elevated Hb A2, No. (%)	Any hemoglobin variant, No. (%)
20–29	124	107 (86.3)	13 (10.5)	4 (3.2)	17 (13.7)
30–39	143	140 (97.9)	3 (2.1)	0 (0.0)	3 (2.1)
40–49	62	62 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)
>49	10	10 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)
Total	339	319 (94.1)	16 (4.7)	4 (1.2)	20 (5.9)

Note: Values are presented as n (row %). Because several cells contained small counts or zero observations, the association between age group and HPLC pattern was assessed using the Fisher–Freeman–Halton exact test (exact $p = 0.0013$). This unadjusted comparison should be interpreted cautiously because donation history and other potential donor-selection factors were not available for age-stratified adjustment.

HPLC-Detected Hemoglobin Patterns by Donation History

Donation history was available as an overall summary. Among 112 first-time donors, 10 (8.9%) had any variant HPLC pattern, including 8 (7.1%) with an HbS-containing HPLC pattern and 2

(1.8%) with an elevated HbA2 screening pattern. Among 227 repeat donors, 10 (4.4%) had any variant HPLC pattern, including 8 (3.5%) with an HbS-containing HPLC pattern and 2 (0.9%) with an elevated HbA2 screening pattern. These findings are shown descriptively in Table 3. Because the individual-level linkage between age group, donation history, and HPLC pattern was unavailable, donation history could not be incorporated into an age-adjusted model.

Table 3. Distribution of HPLC-detected hemoglobin patterns according to donation history among male blood donors at Al-Gumhouri Teaching Hospital, Sana'a (n = 339)

Donation history	HbAA, No. (%)	HbS-containing HPLC pattern, No. (%)	Elevated HbA2 screening pattern, No. (%)	Any variant HPLC pattern, No. (%)
First-time donor	102 (91.1)	8 (7.1)	2 (1.8)	10 (8.9)
Repeat donor	217 (95.6)	8 (3.5)	2 (0.9)	10 (4.4)
Total	319 (94.1)	16 (4.7)	4 (1.2)	20 (5.9)

Note: Values are presented as n (row %). Donation history was available as an overall summary. The individual-level linkage between age group, donation history, and HPLC pattern was unavailable; therefore, no age-adjusted or donation-history-adjusted model was performed.

ABO and Rh Blood Group Distribution

Blood group analysis showed that the O+ phenotype was the most common among participants (50.74%), followed by A+ (30.97%) and B+ (7.67%). Less frequent groups included AB+, O-, A-, B-, and AB-, each comprising less than 4% of the donor population. When phenotypes were combined by RhD status, 314 donors were RhD-positive (92.63%) and 25 were RhD-negative (7.37%). Although RhD-negative phenotypes represented a minority of the cohort, this proportion remains operationally relevant for local inventory awareness and compatible-unit availability. These distributions are illustrated in Table 4.

Table 4: Distribution of ABO and Rh(D) blood groups among male blood donors at Al-Gumhouri Teaching Hospital, Sana'a, Yemen (n = 339)

Blood Group	No. (%)	95% CI, Wilson score
O+	172 (50.74%)	45.44–56.02
A+	105 (30.97%)	26.29–36.09
B+	26 (7.67%)	5.29–11.00
AB+	11 (3.25%)	1.82–5.72
O-	11 (3.25%)	1.82–5.72
A-	9 (2.65%)	1.40–4.97
B-	4 (1.18%)	0.46–2.99
AB-	1 (0.29%)	0.05–1.65

Discussion

In this cohort of 339 eligible male donors attending Al-Gumhouri Teaching Hospital, most participants showed normal HPLC hemoglobin patterns, while a minority showed HbS-containing HPLC patterns consistent with the sickle cell trait or elevated HbA2 screening patterns suggestive of β -thalassemia trait. Variant HPLC patterns were more frequently observed in donors aged 20–29 years. This finding should be interpreted cautiously because the cross-sectional design of the study cannot establish an age-related biological pattern. Donation history, previous screenings, donor self-selection, and deferral practices may have influenced the observed age distribution. Framed against global estimates, these findings sit within a broader landscape where sickle cell disease and thalassemia remain important inherited hemoglobin disorders, particularly in malaria-endemic and Middle Eastern regions ([Thomson et al., 2023](#); [Tuo et al., 2024](#)).

Regionally, our results are consistent with Yemen-specific evidence: a 2024 donor study from Sana'a reported detectable burdens of hemoglobinopathy traits (β -thalassemia, suspected α -thalassemia, and HbAS) using standardized screening and confirmatory HPLC, underscoring the feasibility and value of integrating such screening into routine blood-bank workflows ([Almorish et al., 2024](#)). Local ABO/Rh distributions also align with contemporary Yemeni reports from Amran and Ibb O predominance and relatively low Rh-negative frequency although intra-country variation is evident and operationally important for inventory planning ([Al-Mohani & Al-Nawd, 2024](#); [Al-Sormy, 2023](#)). Patterns across the Arabian Peninsula show broadly similar ABO/Rh profiles, with regional heterogeneity that can affect rare-type availability ([Belali, 2022](#)).

From a transfusion practice perspective, an HbS-containing donor pattern may be operationally relevant because whole-blood units from donors with sickle cell trait have been associated with higher leukoreduction filter failure rates in some settings. However, the present study did not evaluate blood-component processing, leukoreduction performance, or recipient outcomes; therefore, this observation is presented only as a potential operational consideration rather than a local practice recommendation ([Gehrie et al., 2022](#)).

Transfusion remains essential for recipients with SCD but carries an elevated risk of alloimmunization and delayed hemolytic transfusion reactions. Recent reviews and guidance support strategies, such as extended antigen matching and early recognition/management of hyperhemolysis, have been published ([Chou et al., 2020](#); [Pirenne & Pondarré, 2023](#)). Newer consensus work further argues for matching beyond ABO/RhD (e.g., RhCE/K and selective extended antigens) to mitigate alloimmunization, practices that are particularly relevant in regions with limited phenotyped inventories and diverse donor–recipient antigen profiles ([Wolf et al., 2025](#)).

Strengths and limitations

The use of standardized serological typing and HPLC-based screening strengthened the internal consistency of laboratory assessment. However, this was a single-facility study conducted at Al-Gumhouri Teaching Hospital using a non-probability convenience sample of eligible male donors.

Therefore, the findings should be interpreted as facility-specific estimates and should not be generalized directly to all blood donors in Sana'a City or to the wider Yemeni population. Donor characteristics may differ across blood-collection facilities because of differences in catchment populations, donor-recruitment practices, donor motivation, repeat-donation patterns, and eligibility practices. In addition, recruitment occurred over an approximately four-week period; therefore, the study may not capture temporal or seasonal variation in donor characteristics, blood-group distribution, or HPLC-detected hemoglobin patterns. Because donor attendance at the participating blood bank was restricted to male donors, the study could not estimate HPLC-detected hemoglobin patterns or ABO/Rh(D) distribution among female donors. Although overall donation-history information was available, the original individual-level dataset linking age, donation history, and HPLC findings was unavailable; consequently, donation history could not be incorporated into age-stratified adjusted analyses. Finally, molecular testing was not performed; therefore, elevated HbA2 findings should be interpreted as screening patterns suggestive of β -thalassemia trait rather than confirmed genotypes.

Implications

These findings provide descriptive information on HPLC-detected hemoglobin patterns and ABO/Rh(D) phenotypes among eligible male donors attending Al-Gumhouri Teaching Hospital. These results may support local inventory awareness and help identify priorities for future donor surveillance research. However, the present study did not evaluate clinical outcomes, blood-component quality, cost-effectiveness, screening performance, or policy implementation; therefore, it does not support changes to national screening or transfusion policy ([Al-Mohani & Al-Nawd, 2024](#); [Almorish et al., 2024](#); [Al-Sormy, 2023](#); [Chou et al., 2020](#); [Pirenne & Pondarré, 2023](#); [Wolf et al., 2025](#)).

Conclusions

In summary, this study describes HbS-containing HPLC patterns consistent with sickle cell trait and elevated HbA2 screening patterns suggestive of β -thalassemia trait among eligible male donors attending Al-Gumhouri Teaching Hospital in Sana'a. The ABO/Rh(D) profile in this facility-based donor sample was dominated by O-positive blood group, while RhD-negative phenotypes accounted for a minority of donors but remained relevant for local inventory awareness. These findings should not be interpreted as citywide or national prevalence estimates. Larger multicentre studies involving diverse blood-collection facilities, female donors, longer recruitment periods, and molecular confirmation are needed before broader epidemiological or policy conclusions can be drawn.

Abbreviations

ABO	ABO blood group system
CBC	Complete Blood Count
CI	Confidence Interval

EDTA	Ethylenediaminetetraacetic acid
GBD	Global Burden of Disease
HbA2	Hemoglobin A2
HbAA	Normal hemoglobin (homozygous HbA)
HbAS	Sickle cell trait (hemoglobin A/S)
HbS	Hemoglobin S
HPLC	High-Performance Liquid Chromatography
NESTROF	NESTROF screening test (for hemoglobin S detection)
QC	Quality Control
Rh(D)	Rhesus D antigen
SCD	Sickle Cell Disease
SPSS	Statistical Package for Social Sciences

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Declarations

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Author Contributions

Conceptualization: N.M.A.-H. and K.A.A.-M.; Methodology: N.M.A.-H. and K.A.A.-M.; Investigation: A.A.A. and S.M.A.A.; Data curation: A.A.A. and S.M.A.A.; Formal analysis: N.M.A.-H.; Resources: K.A.A.-M.; Writing—original draft preparation: N.M.A.-H.; Writing—review and editing: N.M.A.-H., K.A.A.-M., A.A.A., and S.M.A.A.; Supervision: K.A.A.-M.; Project administration: K.A.A.-M. All authors reviewed and approved the final manuscript and agree to be accountable for the integrity of the work.

Competing Interests

The authors declare no competing interests.

Ethics Statement

The study protocol was reviewed and approved before participant recruitment by the Yemeni Council for Medical and Health Specializations, Ministry of Health and Population, Sana'a, Yemen. No formal approval reference number was issued by the reviewing body. All procedures

complied with the Declaration of Helsinki and applicable national regulations. Written informed consent was obtained from all participants before enrolment. Identifiable information was kept confidential; records were coded and stored securely, and analyses were conducted on de-identified datasets.

Data Availability Statement

All data generated or analyzed during this study are included in this published article.

AI disclosure

ChatGPT (OpenAI) was used solely for language editing. No artificial intelligence tool was used to generate, analyze, or interpret study data. All scientific content, analyses, interpretations, and final revisions were independently reviewed and approved by the authors.

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