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Elevated Fetal Hemoglobin with Normal Hematological Indices in Kurdish Children: A Cross-Sectional Study

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ABSTRACT

Fetal hemoglobin (HbF) is the predominant hemoglobin during fetal development, usually replaced by adult hemoglobin after birth. In certain individuals, low levels of HbF production persist into adulthood, leading to a benign genetic condition referred to as Hereditary Persistence of Fetal Hemoglobin (HPFH). The aim is to investigate cases of elevated HbF in individuals with otherwise normal complete blood count (CBC) parameters by comparing hematological indices and hemoglobin fractions. A cross-sectional study was conducted on 50 children aged 1–10 years, with demographic data including age and gender recorded. Hematological parameters were measured using the NANA-5 Geno TEK analyzer, and hemoglobin fractions were determined with the Bio-Rad D-10 High-Performance Liquid Chromatography (HPLC) system. Statistical analysis was performed utilizing SPSS version 25. The methods employed included the Kruskal–Wallis test, Mann-Whitney U test, and Chi-square test. In this context, p-values less than 0.05 were regarded as statistically significant. A male predominance (60–73%) was noted across groups. The study group showed lower RBC counts (4.3 ×10⁶/µL) and Hb (11.5 g/dL) than controls but higher MCV (78.5 fL) and MCH (26 pg) than control positive group. HbF was intermediate in the study group (4.1%), significantly higher than control negative group (0.9%) but lower than control positive group (9.85%). HbA_2 was normal (2.45%) versus elevated in control positive group (4.9%). The findings indicate that elevated HbF with normal red cell indices represents a benign phenotype, most consistent with HPFH or regulatory variation rather than β-thalassemia minor. Normal HbA_2 and red cell indices distinguished the study group from β-thalassemia minor, which showed microcytosis, hypochromia, and elevated HbA_2. Integrating CBC parameters and hemoglobin fractionation is essential for accurate differentiation, particularly in regions with a high prevalence of thalassemia such as the Kurdistan Region of Iraq.

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1. INTRODUCTION

Hemoglobin is a protein containing iron that facilitates oxygen transport and imparts the red color to blood [1]. Structurally, it comprises 2 (α β)like globin chains, each joined to a clump of heme group, enabling reversible oxygen binding [2]. Hemoglobin expression evolves throughout development, with eight globin genes producing six major hemoglobin types. Embryonic hemoglobin—Hb Gower I ($\zeta_2\varepsilon_2$), Hb Gower II ($\alpha_2\varepsilon_2$), and Hb Portland ($\zeta_2\gamma_2$)—dominate early development, while HbF ($\alpha_2\gamma_2$) is predominant during fetal life [3, 4].

Hemoglobin switching is tightly regulated and occurs in phases. Initially, hematopoiesis occurs in the yolk sac, then shifts toward the fetal liver by weeks six to eight, leading to the replacement of embryonic ε-globin (HBE) by fetal γ-

globin (HBG1, HBG2) **[5, 6]**. Eventually, hematopoiesis transitions to the bone marrow, where γ-globin is suppressed and replaced by β-globin expression from the HBB gene on chromosome 11 **[7, 8]**.

At birth, HbF accounts for 60–80% of total hemoglobin but by six months, HbF levels drop to approximately 5%, and decline to <1% by age two, remaining confined to F cells in adults. Adult hemoglobin composition is primarily HbA (96–98%), with HbA_2 (2.5–3.5%) and HbF (<1%) **[9, 10]**. HbF's higher oxygen affinity, due to weak 2,3-BPG binding, facilitates placental oxygen transfer **[11]**. Postnatally, HbA-expressing erythroid cells expand, reducing HbF levels [12]. Elevated HbF levels in adults can lead to tissue hypoxia, triggering erythropoietin production and an increase in RBC mass as a compensatory response. Environmental and physiological factors can also elevate HbF, including hypoxia, stress erythropoiesis, anemia, lung diseases, cyanotic heart disease, bone marrow disorders, thyroid dysfunction, and high-altitude. These factors often activate the VHL-HIF1α signaling pathway, enhancing HbF production [13-15].

Hereditary Persistence of Fetal Hemoglobin (HPFH) is a harmless genetic situation in which fetal hemoglobin (HbF) production lasts to maturity. Normally, HbF constitutes 0.8% to 5% of total hemoglobin but can exceed 30% in some cases **[16]**. Large deletions in the β-globin gene cluster, change to the promoters in HBG1 and HBG2, or various in administrative genes like BCL11A and MYB, can all causes HPFH **[17]**. It is often detected incidentally during hemoglobinopathy screenings **[18]**. HPFH can ameliorate β-thalassemia and sickle cell illness by compensating for defective β-globin or reducing HbS polymerization **[19, 20]**. Molecular analysis is essential to differentiate HPFH from other etiologies of elevated HbF **[18]**.

Hemoglobinopathies, affect 300,000-400,000 infants annually, particularly in Southeast Asia, the Mediterranean, and sub-Saharan Africa **[21]**. These disorders arise from mutations in the HBB gene **[22]**. In Iraq, the β-thalassemia carrier rate is high, with a 4.1% prevalence reported in Sulaymaniyah **[23]**. Quantifying HbF is vital for diagnosing hemoglobinopathies and evaluating feto-maternal hemorrhage. While advanced diagnostic tools like HPLC, capillary electrophoresis, and molecular techniques are effective, many regions still rely on CBC and conventional electrophoresis, which offer limited sensitivity **[24-26]**.

Gap statement

Hemoglobinopathies are highly prevalent in the Middle East, including Iraq, and represent a major public health concern. In Sulaymaniyah, β-thalassemia is common and well-studied; however, a distinct phenotype has been observed in some individuals who present with elevated fetal hemoglobin (HbF) despite normal complete blood count (CBC) parameters. This finding does not fit the classical features of β-thalassemia minor or other hemoglobinopathies and remains unexplained.

Elevated HbF is typically linked to pathological conditions such as β-thalassemia, sickle cell disease, and hereditary persistence of fetal hemoglobin (HPFH), or to physiological states like pregnancy and infancy. Its occurrence in otherwise healthy individuals with normal hematological indices is uncommon and has not been systematically studied in Sulaymaniyah. Without molecular characterization, these cases may be misclassified as hemoglobinopathy carriers, leading to unnecessary anxiety and inappropriate counseling. Addressing this knowledge gap is essential to improve diagnostic accuracy, refine laboratory practices, and expand understanding of HbF regulation beyond disease contexts. This study aims to detect characterization of elevated HbF levels in individuals with normal CBC parameters by analyzing hematological parameters and hemoglobin fractionation.

2. METHOD

This present study is a cross-sectional comparative analysis conducted to examine the laboratory characteristics of individuals exhibiting elevated fetal hemoglobin (HbF) levels despite having normal complete blood count (CBC) parameters, at the Technical College of Health and Medicine, Polytechnic University of Sulaymaniyah, Iraq. Blood samples were collected from a private clinical laboratory in Sulaimani City from October 2024 to July 2025, over a ten-month period. Ethical approval was obtained at 15/11/2024, and written informed consent was obtained from all members or their legal guardians before admission.

The study included a total of 50 participants from the population aged 1 to 10 years (pediatric age group) recruited from the Sulaimani Governorate, divided into three groups. The study group (Group I) comprised 30 individuals with elevated HbF levels (>2%) in the presence of normal CBC parameters, including hemoglobin concentration, MCV, MCH, MCHC, RBC, and RDW, all within established reference ranges. This group included 22 males and 8 females all aged between 1-10 years and represented individuals with isolated elevation of HbF.

The control positive group (Group II) consisted of 10 individuals diagnosed with β-thalassemia minor, characterized by abnormal CBC indices and diagnostic HPLC patterns consistent with β-thalassemia trait. This group included 6 males and 4 females. Participants with other hemoglobinopathies, or other types of thalassemia, were excluded. The

control negative group (Group III) included 10 individuals (6 males and 4 females) with normal CBC parameters and normal HPLC findings, including normal HbF levels. Selection of both control groups was based on the earliest eligible individuals identified during the screening period. To ensure age comparability with the study group, inclusion was restricted to individuals aged ≤10 years. After hematological classification, all 50 individuals across the three groups demographic variables, including age and sex, were recorded for all participants to facilitate subgroup analysis and accurate interpretation of HbF variability.

From each participant, 5 mL of peripheral blood was drawn by venipuncture from the median cubital vein into K_3-EDTA tubes (manufacture in China), which were gently mixed to prevent clotting and were maintained at room temperature prior to analysis. All specimen were processed within three hours of collection. Hematological parameters, were measured using the NANA-5 Geno TEK (Nana American Origin 5-Differential system) automated blood cell counter. The hematology analyzer was calibrated daily using manufacturer-provided control materials.

This analyzer utilizes a combination of electrical impedance, flow cytometry, and light scattering technologies to differentiate and quantify blood cells. The results were displayed on the analyzer screen, printed. Then transferred to a Laboratory Information System (LIS) for further review by laboratory personnel. Then compared with Children's Reference Ranges (Reference range: Blood Sciences, Department of Hematology. (n.d.) Reference ranges for FBC. Q Pulse Reference No: HA/WE/003. Version 5. Authorized by: Paul Virgo), (Figure 5).

To investigate hemoglobin variants, hemoglobin fractionation was performed using high-performance liquid chromatography (HPLC) with the Bio-Rad D-10 automated ion-exchange system (USA) at accredited private laboratories. Samples were analyzed promptly after collection or, when immediate analysis was not possible, stored at 3–4°C for up to 7 days. The D-10 system separates hemoglobin fractions by cation-exchange chromatography according to their ionic charge. Fractions were eluted using buffers of increasing ionic strength and detected by absorbance at 415 nm. The resulting chromatograms allowed the qualitative identification and quantitative determination of hemoglobin fractions based on their retention times and peak areas. The principal fractions analyzed were HbA_0, HbA_2, and HbF. Typical adult reference values are HbA_0: 95–98%, HbA_2: 2.0–3.5%, and HbF: <2%.

The variables analyzed in this study included demographic factors (age and gender), hematological parameters (Hb, MCV, MCH, MCHC, RDW, and RBC count), and hemoglobin fractions (HbA0, HbA2, and HbF). All statistical analyses were conducted using IBM SPSS Statistics version 25 (IBM Corp., Armonk, NY, USA). The level of statistical significance was set at $p < 0.05$ for all tests.

Descriptive statistics were used to summarize both categorical and continuous variables. Categorical data, such as participant sex, were presented as frequencies and percentages. Continuous variables, including age, RBC count, Hb concentration, and Hb fractions (HbA0, HbA_2, and HbF), were expressed as median values. For more detailed age-related analysis, participants were stratified into four subgroups: 1 year, 2 years, 3 years, and ≥4 years.

Data normality was assessed using the Shapiro–Wilk test, which demonstrated that most continuous variables were not normally distributed; therefore, non-parametric tests were applied in most comparisons. Variables that met parametric assumptions, such as RBC count, were analyzed using the independent-samples t-test for two-group comparisons and one-way ANOVA for comparisons involving more than two groups.

Comparative analyses were conducted among three groups: individuals with elevated HbF and normal CBC (Study group), β-thalassemia minor (Control positive), and healthy individuals (Control negative). For non-normally distributed variables, the Kruskal–Wallis H test was used as a non-parametric alternative to one-way ANOVA. This test was appropriate for identifying statistically significant differences in hematological and biochemical parameters among the three groups. Gender-based differences in percentages HbF levels within each group were evaluated using the Mann–Whitney U test. This non-parametric method was chosen due to the non-normal distribution of HbF levels. All results were reported with corresponding descriptive measures and p-values.

3. RESULTS AND DISCUSSION

Fetal hemoglobin (HbF) is the predominant hemoglobin during fetal life and normally declines substantially after birth as γ-globin expression is progressively silenced and β-globin synthesis becomes predominant [27]. The present study evaluated individuals from Sulaimani City, Kurdistan Region of Iraq, who demonstrated elevated HbF despite relatively preserved hematological parameters. Participants were categorized into three groups: the study group, comprising individuals with elevated HbF and normal CBC parameters; the β-thalassemia minor control-positive group; and the control-negative group, consisting of individuals with normal HbF and hematological findings.

3.1: Demographic data

3.1.1 Gender and Age Distribution

Table 1 and **Figures 1–2** demonstrate that the groups differed in both gender and age composition; therefore, these demographic differences should be considered when interpreting subsequent HbF comparisons. The study included 30 children with elevated HbF and comparatively preserved hematological indices, 10 children with β-thalassemia minor (control-positive group), and 10 children with physiologically low HbF (control-negative group). The study group was predominantly male (73.3%), whereas males accounted for 60.0% of each control group **(Figure 1)**. The predominance of males in the study group is more likely to reflect the composition of this relatively small cohort than a biological difference, particularly because participant selection was not sex-specific. Similar male predominance has been reported in some pediatric hematological screening cohorts [28, 29].

Table 1. Distribution of age groups among three groups. This table that showed the number and percentage of children individuals in all three groups according to sub-age groups.

Variable	Study group (n=30) (Elevated HbF with normal CBC)		Control positive (n=10) (Beta thalassemia minor) group		Control negative group (n=10)	
Age groups (year)s	**Number of individuals**	**Percentage (%)**	**Number of individuals**	**Percentage (%)**	**Number of individuals**	**Percentage (%)**
1	10	33.3%	2	20.0%	1	10.0%
2	13	43.3%	2	20.0%	2	20.0%
3	4	13.3%	2	20.0%	3	30.0%
≥ 4	3	10.0%	4	40.0%	4	40.0%
Total	30	100%	10	100%	10	100%

Age distribution differed among the groups (Table 1; Figure 2). In the study group, 76.6% of participants were aged 1–2 years, with 33.3% aged 1 year and 43.3% aged 2 years. Only 23.3% were aged ≥3 years. In contrast, 40.0% of both control groups were aged ≥4 years. Thus, elevated HbF in the study group was predominantly identified during the first two years of life. Because HbF normally declines rapidly after birth as γ-globin expression is progressively suppressed, persistent elevation beyond infancy warrants consideration of inherited or regulatory mechanisms affecting HbF expression [27].

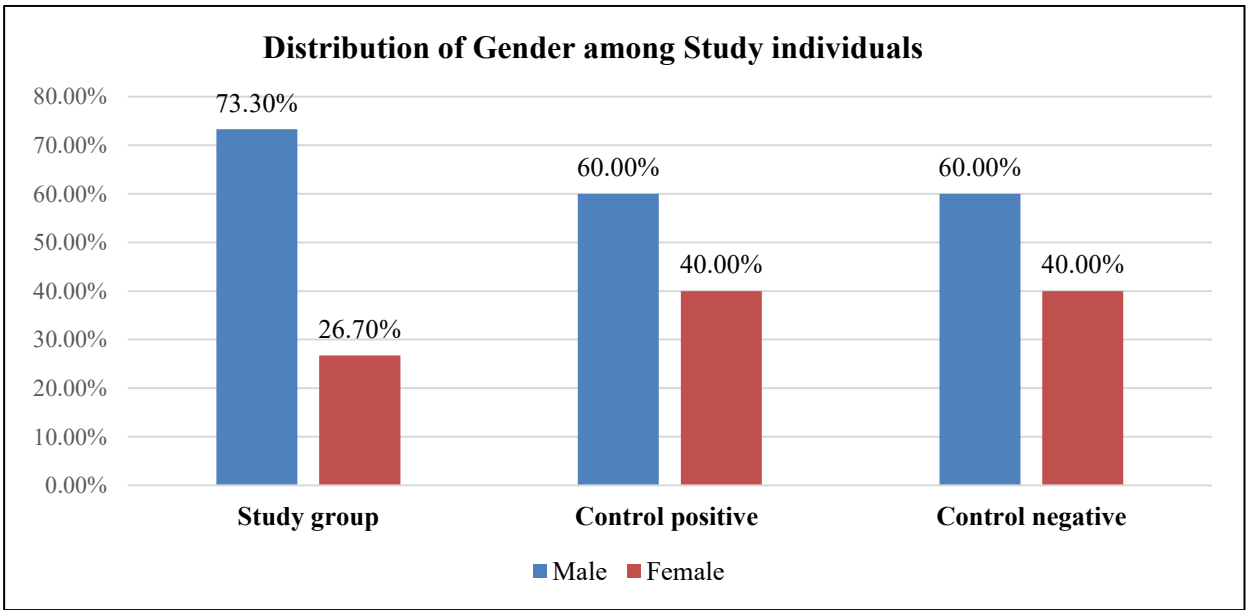

Figure 1. Distributions of gender among study individuals. This chart illustrating the proportion of male and female participants across the study group, control patient group, and control normal group, expressed as percentages.

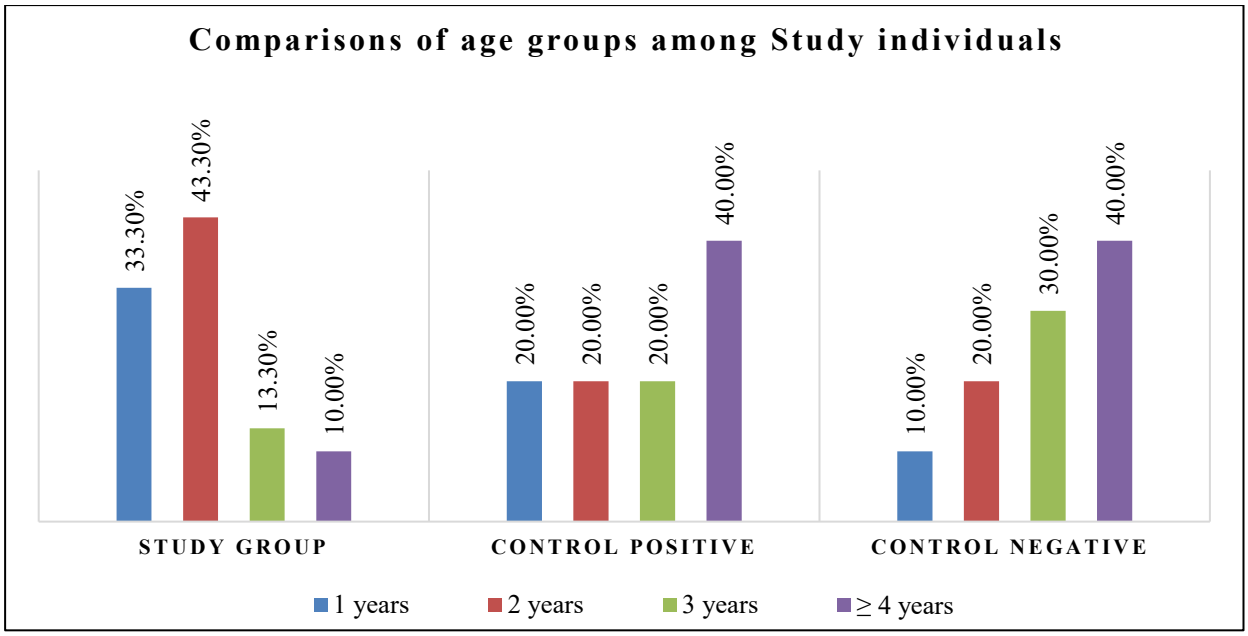

Figure 2. Comparisons of age groups among study individuals. This chart showing the percentage distribution of individuals across predefined age categories (1 year, 2 years, 3 years, and ≥4 years) within the study group, control patient group, and control normal group.

3.2: Comparison of Hematological Parameters

Marked differences in hematological indices were observed among the three groups **(Table 2–4).** Compared with the β-thalassemia minor group, the study group had a significantly lower RBC count (4.38 vs. 5.55 ×10⁶/µL), but significantly higher MCV (78.5 vs. 58.5 fL), MCH (26.0 vs. 19.0 pg), and Hb concentration (11.5 vs. 10.6 g/dL), with all differences reaching P = 0.0001. RDW was also substantially lower in the study group than in the β-thalassemia minor group (12.55% vs. 16.5%, P = 0.0001), whereas MCHC did not differ significantly (P = 0.548) **(Table 2)**. This pattern is consistent with the characteristic microcytic and hypochromic phenotype of β-thalassemia minor, in which reduced MCV and MCH and relatively increased RBC counts are commonly observed [30-32].

Table 2. Comparison of hematological parameters between Study group and Control positive group. These range compared with children references range.

	Study group (n=30) (Elevated HbF with normal CBC)	**Control positive (n=10) (Beta thalassemia minor) group**	
Variables	**Median**	**Median**	**P-value**
RBC (10^6 /µl)	4.38 (mean)	5.55 (mean)	0.0001*
MCV (fl)	78.5	58.5	0.0001*
MCH (pg)	26	19	0.0001*
MCHC (g/dl)	34	34	0.548
Hb (g/dl)	11.5	10.6	0.0001*
RDW (%)	12.55	16.5	0.0001*

P-value < 0.05 statistical significance.

Compared with the control-negative group, the study group had lower RBC count (4.38 vs. 4.95 ×10⁶/µL, P = 0.002), Hb concentration (11.5 vs. 13.4 g/dL, P = 0.001), and MCV (78.5 vs. 85.5 fL, P = 0.010), while MCH and RDW were not significantly different (P = 0.062 and 0.988, respectively). MCHC was significantly higher in the study group (34 vs. 32 g/dL, P = 0.001) **(Table 3).** These findings indicate that the study group was hematologically distinct from healthy controls but did not show the marked microcytosis, hypochromia, and increased RDW characteristic of β-thalassemia minor.

Table 3. Comparison of hematological parameters between Study group and Control negative. These range compared with children references range.

	Study group (n=30) (Elevated HbF with normal CBC)	Control negative group (n=10)	
Variables	**Median**	**Median**	**P-value**
RBC (10^6 /µl)	4.38 (mean)	4.95 (mean)	0.002*
MCV (fl)	78.5	85.5	0.010*
MCH (pg)	26	27	0.062
MCHC (g/dl)	34	32	0.001*
Hb (g/dl)	11.5	13.4	0.001*
RDW (%)	12.55	12.85	0.988

P-value < 0.05 statistical significance.

When all three groups were compared, significant differences were observed for RBC count, MCV, MCH, Hb, MCHC, and RDW **(Table 4)**. The β-thalassemia minor group showed the most pronounced microcytic–hypochromic profile, whereas the control-negative group had the highest MCV, MCH, and Hb values. The study group generally showed intermediate values. The relatively preserved MCV, MCH, and RDW in the study group, together with the absence of the typical β-thalassemia minor pattern, supports the interpretation that its elevated HbF is not simply a consequence of the hematological phenotype associated with β-thalassemia trait. Similar distinctions between HbF-persistent phenotypes and β-thalassemia carriers have been reported previously [30, 33, 34]. The higher RDW in β-thalassemia minor indicates greater red-cell size heterogeneity, although RDW is not specific for β-thalassemia and can also be affected by iron deficiency and other microcytic disorders [31, 35].

Table 4. Comparison of hematological parameters across three groups.

	Study group (n=30) (Elevated HbF with normal CBC)	Control positive (n=10) (Beta thalassemia minor) group	Control negative group (n=10)	
Variables	**Median**	**Median**	**Median**	**P-value**
RBC (10^6 /µl)	4.38 (mean)	5.55 (mean)	4.95 (mean)	0.0001*
MCV (fl)	78.5	58.5	85.5	0.0001*
MCH (pg)	26	19	27	0.0001*
MCHC (g/dl)	34	34	32	0.009
Hb (g/dl)	11.5	10.6	13.4	0.0001*
RDW (%)	12.55	16.5	12.85	0.0001*

These range compared with children references range. P-value < 0.05 statistical significance.

Importantly, the study group's Hb concentration was lower than that of the control-negative group. Therefore, rather than describing the participants categorically as having a completely "normal CBC," the findings are more appropriately interpreted as relatively preserved red-cell indices with no characteristic β-thalassemia minor pattern. This distinction is important for accurate journal reporting.

3.3 Comparison of hemoglobin Fractions

HPLC demonstrated clear differences in HbF, HbA_0, and HbA_2 among the three groups **(Tables 5–7)**. The study group had a median HbF of 4.1%, significantly higher than the control-negative group (0.9%, P = 0.0001) but significantly lower than the β-thalassemia minor group (9.85%, P = 0.0001) (Tables 5–7). Thus, the study group showed a consistent intermediate HbF phenotype between the two controls.

The HbA_0 concentration was significantly higher in the study group than in the β-thalassemia minor group (82.0% vs. 75.5%, P = 0.0001) but lower than in the control-negative group (86.0%, P = 0.0001) **(Tables 5)**. The relatively preserved HbA_0 concentration in the study group, together with its comparatively preserved CBC profile, does not suggest substantial impairment of β-globin production. In contrast, the lower HbA_0 in β-thalassemia minor is consistent with altered globin-chain synthesis associated with reduced β-globin production [36, 37]. HbA_2 further differentiated the groups, with a significantly higher median level in the β-thalassemia minor group (4.90%) than in the study group (2.45%, P = 0.0001), while no significant difference was observed between the study and control-negative groups (2.45% vs. 2.50%, P = 0.914) **(Tables 5–7)**. This elevated HbA_2, together with the microcytic and hypochromic profile described above, is consistent with the established laboratory phenotype of β-thalassemia trait, in which HbA_2 is generally elevated above approximately 3.5% [33, 38, 39]. In contrast, the normal HbA_2 level in the study group, comparable to that of the control-negative group, further supports the absence of a typical β-thalassemia trait phenotype and suggests that the observed HbF elevation may have a different underlying basis, such as HPFH or other HbF-regulatory mechanisms. Definitive identification of the underlying cause requires molecular analysis.

Table 5. Comparison of hemoglobin fractions between Study group and Control positive.

	Study group (n=30) (Elevated HbF with normal CBC)	Control positive (n=10) (Beta thalassemia minor) group	
Variable	**Median (%)**	**Median (%)**	**P-value**
HbA0	82	75.5	0.0001*
HbA2	2.45	4.9	0.0001*
HbF	4.1	9.85	0.0001*

*** P-value < 0.05 statistical significance.**

Table 6. Comparison of hemoglobin fractions between Study group and Control negative.

	Study group (n=30) (Elevated HbF with normal CBC)	Control negative group (n=10)	
Variable	**Median (%)**	**Median (%)**	**P-value**
HbA0	82	86	0.0001*
HbA2	2.45	2.5	0.914
HbF	4.1	0.9	0.0001*

*** P-value < 0.05 statistical significance.**

The combination of HbF elevation, normal-range HbA_2, and relatively preserved red-cell indices in the study group differentiates it from β-thalassemia minor. The HbF level of 4.1% is within the range reported in individuals with HPFH or other HbF-persistent phenotypes who lack major hematological abnormalities. Amato et al. (2014), for example, reported HbF values of approximately 5.9–6.5% among individuals with HPFH and normal hematological findings. Similarly, variation in HbF among individuals carrying β-globin-related or HbF-regulatory variants has been attributed to differences in γ-globin regulation and genetic modifiers [40-42].

Overall, the gradient of HbF—9.85% in β-thalassemia minor, 4.1% in the study group, and 0.9% in controls—indicates that elevated HbF can occur through different biological mechanisms **(Table 7)**. In β-thalassemia, increased HbF may partially compensate for reduced β-globin production, whereas persistent HbF in individuals without the characteristic β-thalassemia phenotype may reflect HPFH or other regulatory variation affecting γ-globin expression [37, 43]. Definitive discrimination between these possibilities, however, requires molecular characterization rather than hematological findings alone.

Table 7. Comparison of hemoglobin fractions across three groups.

	Study group (n=30) (Elevated HbF with normal CBC)	Control positive (n=10) (Beta thalassemia minor) group	Control negative group (n=10)	
Variable	**Median (%)**	**Median (%)**	**Median (%)**	**P-value**
HbA0	82	75.5	86	0.0001*
HbA2	2.45	4.9	2.5	0.0001*
HbF	4.1	9.85	0.9	0.0001*

*** P-value < 0.05 statistical significance.**

3.4 Gender- and Age-Based Analysis of Fetal hemoglobin Levels

Sex-stratified analysis showed relatively small differences in HbF within the study group, with median values of 4.10% in males and 4.05% in females. In the β-thalassemia minor group, females had higher HbF than males (12.35% vs. 8.90%), whereas HbF remained low in both sexes in the control-negative group (1.05% vs. 0.70%) **(Figure 3, Table 8)**. Given the small subgroup sizes, these differences should not be interpreted as evidence of a definitive sex-dependent effect. HbF variation is primarily influenced by developmental and genetic regulation of γ-globin expression, including BCL11A, KLF1, and HBS1L-MYB, rather than sex itself [43-45].

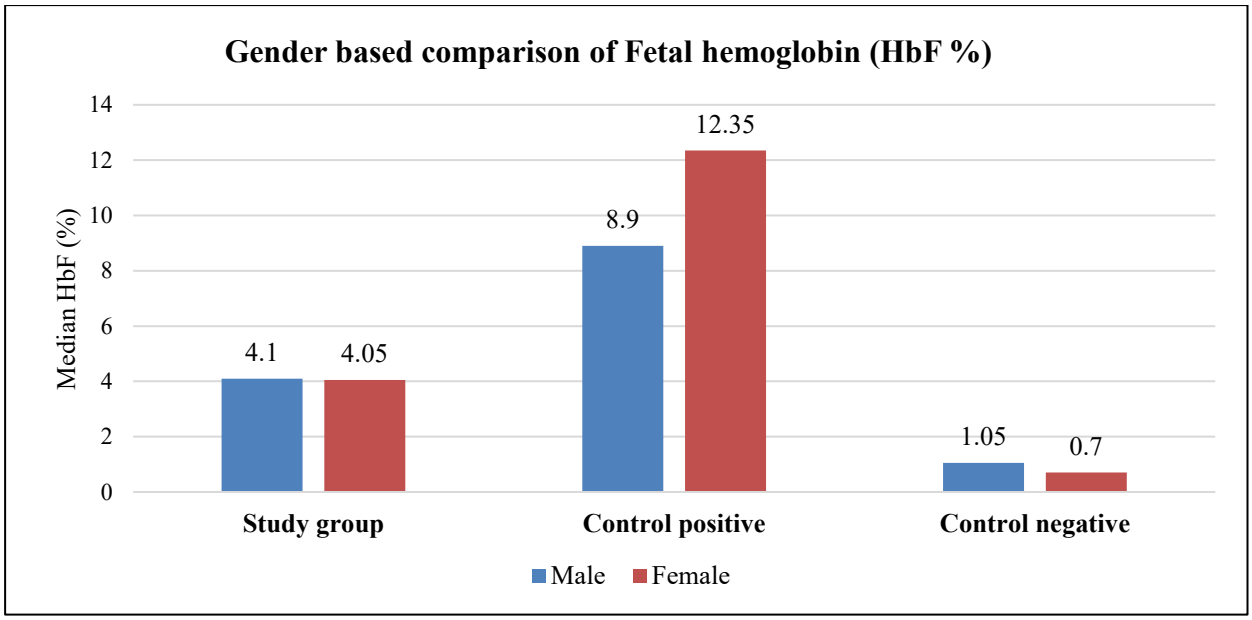

Figure 3. Gender based comparison of fetal hemoglobin levels across three groups**.** The figure illustrates differences in HbF distribution between males and females across all groups, highlighting gender-related variations in HbF expression among the study population.

HbA_2 showed the clearest sex-related difference in the β-thalassemia minor group, with values of 5.00% in males and 3.85% in females. In comparison, HbA_2 remained within the expected range in both sexes in the study and control-negative groups **(Table 8)**. The elevated HbA_2 in the β-thalassemia minor group is consistent with its established diagnostic value in β-thalassemia trait. Previous studies have reported sex-related variation in HbA_2 among β-thalassemia carriers, although such differences have not been consistently observed across populations [46-48].

HbA_0 showed the opposite pattern, being highest in the control-negative group (86.25% in males and 85.80% in females), intermediate in the study group (82.55% and 81.00%), and lowest in the β-thalassemia minor group (76.45% and 72.50%) **(Figure 3, Table 8)**. The reduction in HbA_0 in β-thalassemia minor is compatible with altered globin-chain synthesis associated with reduced β-globin production, whereas the relatively preserved HbA_0 in the study group further supports the absence of a typical β-thalassemia phenotype [36].

Table 8. Gender based Comparison of hemoglobin fractions across three groups. Median percentages of HbF, HbA_0, and HbA_2 are presented separately for males and females in the study group, control positive, and control negative.

	Study group (n=30) (Elevated HbF with normal CBC)		**Control positive (n=10) (Beta thalassemia minor) group**		**Control negative group (n=10)**		
	Male	**Female**	**Male**	**Female**	**Male**	**Female**	
Variable	**Median (%)**	**Median (%)**	**Median (%)**	**Median (%)**	**Median (%)**	**Median (%)**	**P-value**
HbF	4.10	4.05	8.90	12.35	1.05	0.70	0.0001*
HbA0	82.55	81.00	76.45	72.50	86.25	85.80	0.0001*
HbA2	2.45	2.60	5.00	3.85	2.50	2.45	0.0001*

*** P-value < 0.05 statistical significance.**

Because the present cohort consisted predominantly of young children, the observed sex differences should be interpreted cautiously. Hormonal influences on HbF are less likely to account for substantial variation before puberty; therefore, genetic and developmental factors may be more relevant in this age group. Importantly, the small sample size and unequal sex distribution limit the ability to draw definitive conclusions regarding sex-specific regulation of HbF.

HbF demonstrated distinct age-related patterns among the three groups **(Table 9, Figure 4)**. In the study group, median HbF remained relatively stable during the first three years of life, increasing slightly from 4.05% at one year to 4.40% and 4.60% at two and three years, respectively, before declining to 3.10% among children aged ≥4 years. This persistence of HbF beyond the period of physiological γ-globin suppression is consistent with a delayed or incomplete developmental switch from γ- to β-globin expression.

In contrast, the β-thalassemia minor group demonstrated a pronounced age-related decline in HbF, from 15.75% at one year to 10.90%, 8.90%, and 7.20% at ages 2, 3, and ≥4 years, respectively **(Table 9, Figure 4)**. This progressive decline is compatible with continued developmental suppression of γ-globin expression, although HbF remained substantially higher than in the control-negative group. Increased HbF in β-thalassemia may also partially compensate for reduced β-globin synthesis by increasing formation of HbF [37].

The control-negative group maintained consistently low HbF concentrations across age categories, ranging from 0.55% to 1.10%, consistent with the expected postnatal decline in HbF **(Table 9, Figure 4)**. The persistence of HbF at approximately 3–5% in the study group despite increasing age is therefore unlikely to represent normal physiological HbF alone. Similar persistent HbF phenotypes have been associated with HPFH and genetic variation involving HbF regulatory pathways, including BCL11A, HBS1L-MYB, and the HBG1/HBG2 loci [43, 49, 50].

Table 9. Age group-based Comparison of Fetal hemoglobin (HbF %) across three groups.

	Study group (n=30) (Elevated HbF with normal CBC)	Control positive (n=10) (Beta thalassemia minor) group	Control negative group (n=10)	
Age groups	**Median**	**Median**	**Median**	**P-value**
1	4.05	15.75	1.00	0.0001*
2	4.40	10.90	0.55	0.0001*
3	4.60	8.90	1.10	0.0001*
≥ 4	3.10	7.20	1.05	0.0001*

*** P-value < 0.05 statistical significance.**

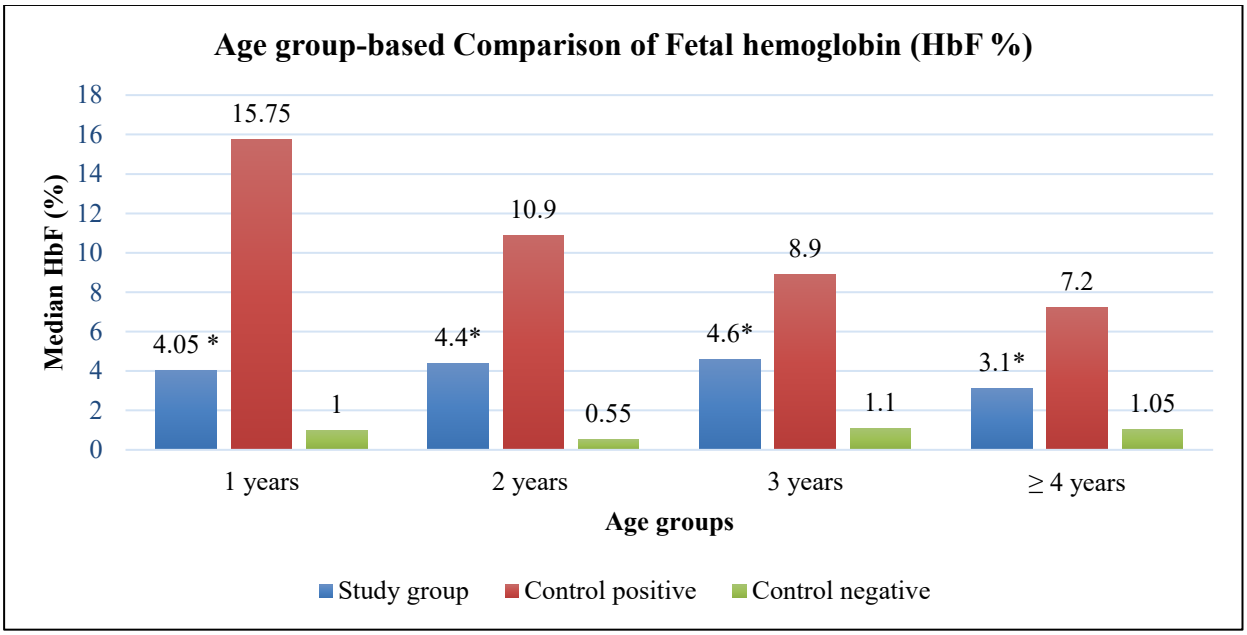

Figure 4. Age groups-based comparison of fetal hemoglobin levels across three groups**.** The figure presents median HbF values stratified by age in all groups, demonstrating age-related trends and differences in HbF expression among the groups.

Taken together, the hematological and HPLC findings distinguish the study group from both β-thalassemia minor and physiologically normal controls. The study group had intermediate HbF (4.1%), preserved HbA_0 (82%), normal-range HbA_2 (2.45%), and substantially less microcytosis and hypochromia than the β-thalassemia minor group **(Tables 2–7).** This combination suggests that the elevated HbF is unlikely to be explained by β-thalassemia minor alone.

The phenotype is compatible with persistent or increased γ-globin expression, potentially resulting from HPFH or other genetic modifiers of HbF production. Variants affecting transcriptional regulators such as BCL11A, KLF1, and HBS1L-MYB, as well as variants within the γ-globin regulatory regions, can produce increased HbF without the marked red-cell abnormalities characteristic of β-thalassemia [43, 45, 51]. Nevertheless, the present hematological and chromatographic findings cannot establish the underlying molecular cause.

Therefore, the combination of CBC parameters, HPLC-derived hemoglobin fractions, age-related HbF assessment, and targeted molecular analysis provides a more appropriate diagnostic framework for children with unexplained HbF

elevation. This is particularly relevant in populations with a high prevalence of hemoglobinopathies, where isolated elevation of HbF may otherwise be incorrectly attributed to β-thalassemia or another hemoglobin disorder [52-54].

The present findings consequently support further molecular investigation of the study group, particularly analysis of HBG1/HBG2 regulatory regions and established HbF-modifying loci, to determine whether the observed mild HbF elevation represents HPFH, a γ-globin regulatory polymorphism, or another inherited mechanism.

Children's Reference Ranges for Routine Hematology Tests

Age Group	Hb (g/L)	RBC ($\times 10^{12}$/L)	HCT
Birth	140–240	3.7–6.5	0.47–0.75
2 weeks	134–198	3.9–5.9	0.41–0.65
4 weeks	134–198	3.2–5.9	0.41–0.65
2–6 months	94–130	3.1–4.3	0.28–0.42
6 months–1 year	111–141	4.1–5.3	0.33–0.41
1–6 years	115–140	3.9–5.3	0.34–0.40
6–12 years	115–155	4.0–5.2	0.35–0.40
12–18 years (Female)	120–160	4.1–5.1	0.36–0.46
12–18 years (Male)	130–170	4.5–5.3	0.37–0.49

Age (years)	MCV (fL)	MCH (pg)	MCHC (g/L)
1	72–84	25.0–29.0	320–360
2	71–83	23.1–28.2	320–359
3	74–87	24.8–28.9	313–352
4	71–85	23.4–29.1	319–356
5	73–87	24.0–30.0	320–348
6	74–91	24.5–29.6	323–360
7	73–87	23.6–31.0	319–369
8	78–97	26.0–29.4	327–358
9	73–89	25.3–29.8	323–361
10	76–88	24.9–30.2	317–355
11	77–95	25.0–33.0	310–370

Figure 5. Children's Reference Ranges (Blood Sciences, Department of Hematology. (n.d.) **Reference ranges for FBC**. Q Pulse Reference No: HA/WE/003. Version 5. Authorized by: Paul Virgo).

4. CONCLUSION

The hematological findings indicate that individuals with elevated HbF and normal red cell indices represent a benign phenotype, most consistent with hereditary persistence of fetal hemoglobin (HPFH) or regulatory polymorphisms, rather than β-thalassemia minor. The study group showed normal hemoglobin levels, red cell indices, and HbA_2, in contrast to the β-thalassemia minor group, which demonstrated microcytosis, hypochromia, and elevated HbA_2. Persistent and relatively stable HbF levels, particularly during early childhood, further supported a benign pattern. These findings highlight the importance of integrating CBC parameters and hemoglobin fractionation to accurately distinguish benign HbF persistence from β-thalassemia carriers. This distinction is particularly important in the Kurdistan Region of Iraq, where thalassemia is prevalent and misclassification during screening may have important clinical and social consequences.

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REFERENCES

[1] A. N. Al-Shura, *Advanced Hematology in Integrated Cardiovascular Chinese Medicine: Volume 3*: Academic Press, 2019.

[2] V. Venkatesan, S. Srinivasan, P. Babu, and S. Thangavel, "Manipulation of developmental gamma-globin gene expression: an approach for healing hemoglobinopathies," *Molecular and Cellular Biology,* vol. 41, pp. e00253-20, 2021. https://doi.org/10.1128/MCB.00253-20

[3] F. J. B. López, I. F. Centurion, F. J. P. Díaz, C. M. Pacheco, B. I. Cortés, B. M. T. Mendoza*, et al.*, "Structural Effect of Gγ and Aγ Globin Chains in Fetal Hemoglobin Tetramer," *Blood,* vol. 142, p. 2291, 2023. https://doi.org/10.1182/blood.2023188988

[4] C. Mussolino and J. Strouboulis, "Recent approaches for manipulating globin gene expression in treating hemoglobinopathies," *Frontiers in Genome Editing,* vol. 3, p. 618111, 2021. https://doi.org/10.3389/fgeed.2021.618111

[5] M. De Martino, R. Sessa, M. R. Storino, M. Giuliano, S. Trombetti, R. Catapano*, et al.*, "Transcriptional repressors of fetal globin genes as novel therapeutic targets in Beta-thalassemia," *Beta Thalassemia,* pp. 1-9, 2020. https://doi.org/10.5772/intechopen.90762

[6] J. Palis, "Erythropoiesis in the mammalian embryo," *Experimental Hematology,* p. 104283, 2024. https://doi.org/10.1016/j.exphem.2024.104283

[7] R. Molokie, J. DeSimone, and D. Lavelle, "Epigenetic regulation of hemoglobin switching in non-human primates," in *Seminars in hematology*, 2021, pp. 10-14. https://doi.org/10.1053/j.seminhematol.2020.12.001

[8] G. Barbarani, A. Labedz, S. Stucchi, A. Abbiati, and A. E. Ronchi, "Physiological and aberrant γ-globin transcription during development," *Frontiers in Cell and Developmental Biology,* vol. 9, p. 640060, 2021. https://doi.org/: 10.3389/fcell.2021.640060

[9] [D. E. Bauer and S. H. Orkin, "Hemoglobin switching's surprise: the versatile transcription factor BCL11A is a master repressor of fetal hemoglobin," *Current opinion in genetics & development,* vol. 33, pp. 62-70, 2015. https://doi.org/10.1016/j.gde.2015.08.001

[10] V. G. Sankaran and S. H. Orkin, "The switch from fetal to adult hemoglobin," *Cold Spring Harbor perspectives in medicine,* vol. 3, p. a011643, 2013.

[11] J. M. Manning, L. R. Manning, A. Dumoulin, J. C. Padovan, and B. Chait, "Embryonic and fetal human hemoglobins: structures, oxygen binding, and physiological roles," *Vertebrate and Invertebrate Respiratory Proteins, Lipoproteins and Other Body Fluid Proteins,* pp. 275-296, 2020. https://doi.org/10.1007/978-3-030-41769-7_11

[12] E. Khandros, P. Huang, S. A. Peslak, M. Sharma, O. Abdulmalik, B. M. Giardine*, et al.*, "Understanding heterogeneity of fetal hemoglobin induction through comparative analysis of F and A erythroblasts," *Blood, The Journal of the American Society of Hematology,* vol. 135, pp. 1957-1968, 2020.

[13] R. Feng, T. Mayuranathan, P. Huang, P. A. Doerfler, Y. Li, Y. Yao*, et al.*, "Regulation of fetal hemoglobin expression by the VHL-HIF1α oxygen sensing system," *Blood,* vol. 138, p. 574, 2021. https://doi.org/10.1182/blood-2021-149901

[14] T. Saga, M. Kanagawa, T. Harada, L. Lang, F. Yamawaki, and T. Ishihara, "Prognostic value of pretreatment fetal hemoglobin levels in patients with myelodysplastic syndromes and acute myeloid leukemia treated with azacitidine: a single-center retrospective study," *Internal Medicine,* vol. 63, pp. 781-790, 2024. https://doi.org/10.2169/internalmedicine.1216-22

[15] A. Sokolova, A. Mararenko, A. Rozin, A. Podrumar, and V. Gotlieb, "Hereditary persistence of hemoglobin F is protective against red cell sickling. A case report and brief review," *Hematology/oncology and Stem Cell Therapy,* vol. 12, pp. 215-219, 2019. https://doi.org/10.1016/j.hemonc.2017.09.003

[16] D. C. Sharma, S. Singhal, P. Woike, S. Rai, M. Yadav, and R. Gaur, "Hereditary persistence of fetal hemoglobin," *Asian Journal of Transfusion Science,* vol. 14, pp. 185-186, 2020. https://doi.org/ 10.4103/ajts.AJTS_71_16

[17] M. H. Steinberg, "Fetal hemoglobin in sickle hemoglobinopathies: high HbF genotypes and phenotypes," *Journal of Clinical Medicine,* vol. 9, p. 3782, 2020. https://doi.org/10.3390/jcm9113782

[18] I. Shaukat, A. Paudel, S. Yassin, N. Höti, and S. Mustafa, "Blessing in disguise; a case of Hereditary Persistence of Fetal Hemoglobin," *Journal of Community Hospital Internal Medicine Perspectives,* vol. 8, pp. 380-381, 2018. https://doi.org/10.1080/20009666.2018.1536241

[19] S. H. Orkin, "Molecular medicine: found in translation," *Med,* vol. 2, pp. 122-136, 2021. https://doi.org/10.1016/j.medj.2020.12.011

[20] S. K. Topfer, R. Feng, P. Huang, L. C. Ly, G. E. Martyn, G. A. Blobel*, et al.*, "Disrupting the adult globin promoter alleviates promoter competition and reactivates fetal globin gene expression," *Blood, The Journal of the American Society of Hematology,* vol. 139, pp. 2107-2118, 2022. https://doi.org/10.1182/blood.2021014205

[21] M. Cavazzana and F. Mavilio, "Gene therapy for hemoglobinopathies," *Human gene therapy,* vol. 29, pp. 1106-1113, 2018. https://doi.org/ 10.1089/hum.2018.122

[22] M. Azul, E. F. Vital, W. A. Lam, D. K. Wood, and J. D. Beckman, "Microfluidic methods to advance mechanistic understanding and translational research in sickle cell disease," *Translational Research,* vol. 246, pp. 1-14, 2022. https://doi.org/10.1016/j.trsl.2022.03.010

[23] N. H. AHMED, A. H. FARAJ, N. A. AL-ALLAWI, and S. D. JALAL, "PREVALENCE OF HAEMOGLOBINOPATHIES IN SULAIMANI–IRAQ," *Duhok Medical Journal,* vol. 2, 2008.

[24] G. J. Kato, F. B. Piel, C. D. Reid, M. H. Gaston, K. Ohene-Frempong, L. Krishnamurti*, et al.*, "Sickle cell disease," *Nature reviews Disease primers,* vol. 4, pp. 1-22, 2018. https://doi.org/ 10.1038/nrdp.2018.10

[25] N. Hebert, R. M. Georgine, G. Bodivit, E. Audureau, N. Oubaya, S. Pakdaman*, et al.*, "Fetal Hemoglobin Measurement per Red Blood Cell Provides Biological and Clinical Protective Thresholds," *Blood,* vol. 134, p. 1008, 2019. https://doi.org/10.1182/blood-2019-126727

[26] J. Old and S. Henderson, "Molecular diagnostics for haemoglobinopathies," *Expert opinion on medical diagnostics,* vol. 4, pp. 225-240, 2010.

[27] S. K. Roy, A. K. Shrivastava, and P. R. Choudhary, "Study of fetal hemoglobin with different gestational age and birth weight of the newborn," *Int J Contemp Pediatr,* vol. 8, pp. 2349-3291, 2021. https://doi.org/10.18203/2349-3291.ijcp20210522

[28] P. Jain, N. Marwah, N. Dalal, R. Pawar, M. Gill, and S. Kumar, "Delta beta thalassemia, a rare hemoglobin variant: An experience from nodal centre in North Indian state," *Journal of Applied Hematology,* vol. 13, pp. 1-4, 2022. https://doi.org/ 10.17511/jopm.2019.i11.15

[29] K. O. Iyevhobu, T. J. Okobi, E. R. Usoro, A. A. Ivie, B. A. Ken-Iyevhobu, and O. O. Victoria, "Overview of beta-thalassemia," in *Thalassemia Syndromes-New Insights and Transfusion Modalities*, ed: IntechOpen, 2023.

[30] C. Pereira, L. Relvas, C. Bento, A. Abade, M. L. Ribeiro, and L. Manco, "Polymorphic variations influencing fetal hemoglobin levels: association study in beta-thalassemia carriers and in normal individuals of Portuguese origin," *Blood Cells, Molecules, and Diseases,* vol. 54, pp. 315-320, 2015. https://doi.org/ 10.1016/j.bcmd.2015.02.001

[31] M. T. Islam, N. Sultana, S. K. Sarker, T. Hossain, S. Tasnim, M. Al Mahmud-Un-Nabi*, et al.*, "Association of diverse population of red blood cells with different disease manifestations in patients with beta-thalassemia," *Meta Gene,* vol. 27, p. 100846, 2021. https://doi.org/ 10.1016/j.mgene.2020.100846

[32] R. Bou-Fakhredin, S. Rivella, M. D. Cappellini, and A. T. Taher, "Pathogenic mechanisms in thalassemia I: Ineffective Erythropoiesis and Hypercoagulability," *Hematology/Oncology Clinics,* vol. 37, pp. 341-351, 2023. https://doi.org/10.1016/j.hoc.2022.12.005

[33] V. S. Hançer, T. Fışgın, and M. Büyükdoğan, "Two rare pathogenic HBB variants in a patient with β-thalassemia intermedia," *Turkish Journal of Hematology,* vol. 37, p. 135, 2020. https://doi.org/10.4274/tjh.galenos.2020.2020.0020

[34] M. Saboor, "Discrimination of iron deficiency, alpha and beta thalassemia on the basis of red cell distribution width and reticulocyte indices," *Clinical Laboratory,* 2021. https://doi.org/10.7754/Clin.Lab.2020.201008

[35] Q.-L. Song, Y.-L. Guo, Y.-J. He, C. He, T. Zhang, Y. Cai, *et al.*, "Diagnostic Cut-Off Value of RDW for Screening Thalassemia and the Combined Determination of MCV, MCH, HBA 2 and RDW," *Zhongguo shi yan xue ye xue za zhi,* vol. 29, pp. 847-852, 2021. https://doi.org/10.19746/j.cnki.issn.1009-2137.2021.03.030

[36] S. Mettananda and D. R. Higgs, "Molecular basis and genetic modifiers of thalassemia," *Hematology/Oncology Clinics,* vol. 32, pp. 177-191, 2018. https://doi.org/ 10.1016/j.hoc.2017.11.003

[37] H. Y. Lu, S. H. Orkin, and V. G. Sankaran, "Fetal hemoglobin regulation in beta-thalassemia," *Hematology/Oncology Clinics of North America,* vol. 37, pp. 301-312, 2023. https://doi.org/ 10.1016/j.hoc.2022.12.002

[38] Q. Zhuang, G. Wang, Y. Wang, J. Zhuang, Y. Jiang, H. Huang, *et al.*, "The value of combined detection of HbA2 and HbF for the screening of thalassemia among individuals of childbearing ages," *Zhonghua yi xue yi Chuan xue za zhi= Zhonghua Yixue Yichuanxue Zazhi= Chinese Journal of Medical Genetics,* vol. 39, pp. 16-20, 2022. https://doi.org/10.3760/cma.j.cn 511374-20210202-00104

[39] S. Marchesani, M. Di Mauro, G. Ceglie, G. Grassia, M. Carletti, R. C. Cristofaro, *et al.*, "The blood count as a compass to navigate in the ever-changing landscape of the carrier state of hemoglobinopathies: a single-center Italian experience," *Frontiers in pediatrics,* vol. 11, p. 1228443, 2023. https://doi.org/10.3389/fped.2023.1228443

[40] A. Amato, M. Cappabianca, M. Perri, I. Zaghis, P. Grisanti, D. Ponzini, *et al.*, "Interpreting elevated fetal hemoglobin in pathology and health at the basic laboratory level: new and known γ-gene mutations associated with hereditary persistence of fetal hemoglobin," *International journal of laboratory hematology,* vol. 36, pp. 13-19, 2014. https://doi.org/10.1111/ijlh.12094

[41] C. Curcio, "HBG2 and HBG1 Nucleotide Substitutions and Hbf Production in Thalassemia Patients," *American Journal of Biomedical Science & Research,* vol. 4, pp. 220-224, 08/01 2019. https://doi.org/ 10.34297/AJBSR.2019.04.000802

[42] P. Hariharan, M. Gorivale, P. Sawant, P. Mehta, and A. Nadkarni, "Dissecting the role of genetic modifiers of hemoglobinopathies: A futuristic approach towards precision medicine," 2021. https://doi.org/10.1038/s41598-021-00169-x

[43] C. Stephanou, S. Menzel, S. Philipsen, and P. Kountouris, "Genetic Polymorphisms Associated with Fetal Hemoglobin (HbF) Levels and F-Cell Numbers: A Systematic Review of Genome-Wide Association Studies," *International Journal of Molecular Sciences,* vol. 25, p. 11408, 2024. https://doi.org/10.3390/ijms252111408

[44] W.-P. Han, L. Huang, Y.-Y. Li, Y.-Y. Han, D. Li, B.-Q. An, *et al.*, "Reference intervals for HbA2 and HbF and cut-off value of HbA2 for β-thalassemia carrier screening in a Guizhou population of reproductive age," *Clinical biochemistry,* vol. 65, pp. 24-28, 2019. https://doi.org/10.1016/j.clinbiochem.2018.11.007

[45] A. Genc, D. Tastemir Korkmaz, S. Bayram, and E. Rencuzogullari, "The effect of five single nucleotide polymorphisms on Hb F variation of β-thalassemia traits and hematologically normal individuals in Southeast Turkey," *Hemoglobin,* vol. 44, pp. 231-239, 2020. https://doi.org/10.1080/03630269.2020.1787178

[46] Y.-J. Chen, Y.-Q. Li, Q. Liu, L.-Y. Tang, and F.-T. Lv, "A Chinese Male with normal hematological indices and high Hb A2 levels in β-thalassemia trait," *Hemoglobin,* vol. 44, pp. 131-133, 2020. https://doi.org/10.3389/fmolb.2020.00074

[47] M. Khan, A. Zeb, and T. Zulfiqar, "Clinical and Hematological Profile of β-Thalassemia Minor," *Pakistan Journal of Medical and Health Sciences,* vol. 16, pp. 340-341, 01/18 2022. https://doi.org/ 10.53350/pjmhs22161340

[48] H. F. Ibrahim, A. S. Mahdi, S. N. M. Al Fatlawi, A. O. Harrewee, and R. M. Hameed, "An Immunological and Molecular Study to Investigate the Genes (β-globin and HBA1F) in Patients with Thalassemia in Najaf Governorate," *Journal of Preventive, Diagnostic and Treatment Strategies in Medicine,* vol. 3, pp. 85-91, 2024. https://doi.org/10.4103/jpdtsm.jpdtsm_26_24

[49] N. Liu, V. V. Hargreaves, Q. Zhu, J. V. Kurland, J. Hong, W. Kim, *et al.*, "Direct promoter repression by BCL11A controls the fetal to adult hemoglobin switch," *Cell,* vol. 173, pp. 430-442. e17, 2018. https://doi.org/10.1016/j.cell.2018.03.016

[50] S. N. N. A. i. Mohammad, S. Iberahim, W. S. Wan Ab Rahman, M. N. Hassan, H. A. Edinur, M. Azlan, *et al.*, "Single nucleotide polymorphisms in XMN1-HBG2, HBS1L-MYB, and BCL11A and their relation to high fetal hemoglobin levels that alleviate anemia," *Diagnostics,* vol. 12, p. 1374, 2022. https://doi.org/10.3390/diagnostics12061374

[51] G. Parmeggiani, F. Gualandi, R. Selvatici, P. Rimessi, S. Bigoni, M. Taddei Masieri, *et al.*, "A family with γ-thalassemia and high Hb A2 levels," *Hemoglobin,* vol. 40, pp. 187-190, 2016. https://doi.org/10.3109/03630269.2016.1148613

[52] [N. A. Al-Allawi, A. A. Al-Doski, R. S. Markous, K. A. Mohamad Amin, A. A. Eissa, A. I. Badi, *et al.*, "Premarital screening for hemoglobinopathies: experience of a single center in Kurdistan, Iraq," *Public health genomics,* vol. 18, pp. 97-103, 2015. https://doi.org/:10.1159/000368960

[53] K.-S. Poon and K. M.-L. Tan, "Spectrum of pathogenic alterations identified after two decades of HBB gene sequencing for molecular diagnosis of beta-thalassaemias and haemoglobinopathies," *Pathology,* vol. 53, pp. 536-538, 2021. https://doi.org/10.1016/j.pathol.2020.08.022

[54] D. E. Sabath, "The role of molecular diagnostic testing for hemoglobinopathies and thalassemias," *International Journal of Laboratory Hematology,* vol. 45, pp. 71-78, 2023. https://doi.org/10.1111/ijlh.14089