Section: Pathology

Original Research Article

RANGE OF FETAL HEMOGLOBIN IN VARIOUS HEMOGLOBINOPATHIES IN A TERTIARY CARE CENTER: A COMPREHENSIVE ANALYSIS

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Abstract

Background: Fetal hemoglobin (HbF) serves as a crucial marker in understanding hemoglobin disorders, with its persistent expression significantly influencing disease manifestations and severity across various hemoglobinopathies. However, the precise distribution and range of HbF levels across different hemoglobin disorders remain incompletely characterized in clinical settings. This study aimed to conduct a detailed characterization of fetal hemoglobin (HbF) levels across a spectrum of hemoglobinopathies in a tertiary healthcare setting, while analyzing associated hematological parameters to establish comprehensive reference ranges for different conditions. **Materials and Methods:** A retrospective, cross-sectional analysis was performed, incorporating detailed hematological profiling of patients with various hemoglobin disorders. The study utilized standardized laboratory techniques to measure multiple parameters, including fetal hemoglobin percentage, complete blood count metrics (RBC count, hemoglobin concentration, hematocrit), and red cell indices (MCV, MCH, MCHC). **Result:** The investigation revealed distinct patterns of HbF expression across different hemoglobinopathies. The highest levels were observed in delta beta homozygous conditions (95.3 ± 6.08%), HPFH Homozygous (90.3 ± 6.86%), and beta thalassemia major (85.7 ± 7.61%). Moderate elevations were found in beta thalassemia intermedia (43.1 ± 24.2%) and HbF high for age (38.3 ± 41.5%). Lower ranges were documented in sickle cell disorders (15.9 ± 6.55% in sickle cell anemia) and beta thalassemia trait (8.34 ± 5.78%), with the lowest levels observed in sickle cell trait (1.55%). **Conclusion:** This comprehensive analysis establishes distinct ranges of fetal hemoglobin across various hemoglobinopathies, demonstrating significant variability in HbF expression patterns. These findings suggest underlying genetic mechanisms and potential compensatory responses specific to each condition. While the study was limited by its single-center nature and varied sample sizes, it provides valuable reference data for clinical assessment and management of hemoglobin disorders. Future multi-center studies with larger cohorts are recommended to validate these findings and explore the genetic factors contributing to HbF variations.

INTRODUCTION

Fetal hemoglobin (HbF) is the primary oxygen-carrying protein during fetal development, characterized by its enhanced oxygen affinity crucial for fetal survival. While HbF typically comprises the majority of hemoglobin during gestation, it is progressively replaced by adult hemoglobin (HbA)

through a precisely regulated hemoglobin switching process that begins shortly before birth and continues through the first year of life.[1] However, in various hemoglobinopathies, this switching process may be altered, leading to persistent or elevated HbF levels that can significantly influence disease manifestation and severity.[2]

The clinical significance of HbF extends beyond its developmental role, as its persistence or elevation in adults has emerged as a critical modifier of disease severity in various hemoglobin disorders.[3] This phenomenon has been particularly well-documented in conditions such as sickle cell disease, beta-thalassemia, and certain rare hemoglobin variants, where elevated HbF levels often correlate with milder clinical presentations and better outcomes.[4]

Recent advances in our understanding of hemoglobin disorders have highlighted the complex interplay between genetic modifiers, environmental factors, and HbF expression.[5] The regulation of γ-globin gene expression, which determines HbF levels, involves multiple genetic loci and molecular pathways, including well-characterized elements such as BCL11A, SOX6, and the β-globin locus control region. These insights have not only enhanced our understanding of hemoglobin switching but have also opened new therapeutic avenues targeting HbF induction.[6]

In the Indian context, where hemoglobinopathies represent a significant public health challenge, understanding the distribution and clinical implications of HbF levels becomes particularly relevant.[7] The diverse genetic background of the Indian population, combined with high rates of consanguinity in certain communities, has led to a complex spectrum of hemoglobin disorders with varying clinical presentations and HbF patterns.[8]

Despite the recognized importance of HbF levels in hemoglobinopathies, there remains a need for comprehensive data characterizing HbF distribution patterns across different hemoglobin disorders, particularly in the Indian population.[9] Current diagnostic approaches often rely on multiple parameters, including complete blood counts, hemoglobin electrophoresis, and molecular studies. However, the specific contribution of HbF patterns to diagnosis and prognosis requires further elucidation.[10]

This study aims to address this knowledge gap by systematically characterizing the distribution of HbF percentages across different hemoglobinopathies, alongside other key hematological parameters.[11] Our objectives include: Establishing characteristic HbF patterns associated with specific hemoglobin disorders in our population, Analyzing the correlation between HbF levels and other hematological parameters, Evaluating the diagnostic utility of HbF patterns in differentiating various hemoglobinopathies, Investigating potential associations between HbF levels and clinical severity in different disorders.[12]

Understanding these patterns and relationships could significantly enhance our diagnostic accuracy and prognostic capabilities, potentially influencing treatment decisions and patient outcomes. Furthermore, this knowledge could contribute to the development of more targeted therapeutic approaches, particularly in conditions where HbF induction represents a viable therapeutic strategy.

MATERIALS AND METHODS

Study Design and Setting: This cross-sectional study was conducted at [Insert tertiary care center name] between [23/01/2023]and [22/01/2024]. The study aimed to evaluate the range of Fetal Hemoglobin (HbF) levels across various hemoglobinopathies and analyze associated hematological parameters.

Study Population: Patients diagnosed with various hemoglobinopathies attending the tertiary care center during the study period were included. The hemoglobinopathies studied comprised:

- Beta Thalassemia (Major, Intermedia, and Trait)
- Sickle Cell Disease, Anemia, and Trait
- Hereditary Persistence of Fetal Hemoglobin (HPFH and HPFH Homozygous)
- Delta Beta Thalassemia (Homozygous and Trait)
- HbE Trait
- Hb D Heterozygous
- Cases with High HbF for age

Laboratory Methods

Hemoglobin Analysis

- HbF quantification was performed using High-Performance Liquid Chromatography (HPLC)
- The analysis was conducted following standard operating procedures and manufacturer guidelines
- Quality control measures were implemented as per laboratory protocols

Hematological Parameters

The following parameters were analyzed for each patient:

- Complete Blood Count (CBC) including:
  - Red Blood Cell (RBC) count ($\times 10^6/\mu L$)
  - Hemoglobin (Hb) concentration (g/dL)
  - Hematocrit (HCT) percentage
  - Red cell indices:
    - Mean Corpuscular Volume (MCV) in fL
    - Mean Corpuscular Hemoglobin (MCH) in pg
    - Mean Corpuscular Hemoglobin Concentration (MCHC) in g/dL

Data Collection and Analysis

- Demographic and clinical data were collected from patient records
- Laboratory results were retrieved from the hospital's laboratory information system
- Data were organized and analyzed using appropriate statistical software

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- Results were expressed as mean ± standard deviation for parameters with multiple observations
- For conditions with single observations, individual values were reported

Statistical Analysis

- Descriptive statistics were calculated for all parameters
- Mean and standard deviation were computed for each hematological parameter across different hemoglobinopathies
- For conditions with single cases (HbE trait, Delta beta thalassemia trait, and Hb D Heterozygous), only absolute values were reported

Ethical Considerations: The study protocol was approved by the Institutional Ethics Committee of [Shri M P Shah Medical College, GG Hospital] (Approval number: [199/05/2022]). As this was a retrospective study using anonymized data, the requirement for individual patient consent was waived.

RESULTS

[Table 1] compares different blood measurements across various conditions, with the following key parameters:

1. HbF (%) - Fetal Hemoglobin percentage
2. RBC (×10⁶/µL) - Red Blood Cell Count
3. Hb (g/dL) - Hemoglobin level
4. HCT (%) - Hematocrit (percentage of blood volume occupied by red blood cells)
5. MCV (fL) - Mean Corpuscular Volume (average size of red blood cells)
6. MCH (pg) - Mean Corpuscular Hemoglobin (average amount of hemoglobin per red blood cell)
7. MCHC (g/dL) - Mean Corpuscular Hemoglobin Concentration

Some key observations from the data

1. Highest HbF levels:
- Delta beta homozygous (95.3%)
- HPFH Homozygous (90.3%)
- Beta thalassemia Major (85.7%)

2. Lowest HbF levels:
- Hb D Heterozygous (2.20%)
- Sickle cell trait (1.55%)
- HbE trait (5.30%)

3. Notable patterns:
- Sickle cell conditions tend to have higher RBC counts
- Beta thalassemia conditions show varying degrees of anemia (low Hb)
- Some conditions marked with asterisk (*) only had one case, so no standard deviation is available

Table 1: Range of HbF observed in various hemoglobinopathies in a tertiary health care centre.

Diagnosis	HbF (%)	RBC (×10⁶/µL)	Hb (g/dL)	HCT (%)	MCV (fL)	MCH (pg)	MCHC (g/dL)
HbF High for age	38.3 ± 41.5	4.30 ± 2.14	8.31 ± 4.27	26.9 ± 13.5	64.4 ± 10.6	20.3 ± 4.90	31.3 ± 2.86
Beta Thal Trait	8.34 ± 5.78	4.45 ± 1.25	8.32 ± 1.32	27.3 ± 4.54	63.6 ± 9.95	20.9 ± 4.65	30.6 ± 1.69
HPFH	18.9 ± 3.04	4.79 ± 1.20	11.0 ± 2.46	33.9 ± 8.03	71.8 ± 8.99	22.8 ± 2.92	31.8 ± 0.73
Delta beta homozygous	95.3 ± 6.08	4.93 ± 1.68	10.7 ± 4.10	32.6 ± 11.7	66.3 ± 1.06	21.6 ± 1.06	32.6 ± 0.99
Beta thalassemia intermedia	43.1 ± 24.2	4.55 ± 3.05	12.4 ± 10.3	36.2 ± 25.1	52.3 ± 24.0	26.0 ± 5.62	30.4 ± 3.61
HPFH Homozygous	90.3 ± 6.86	2.40 ± 0.72	5.95 ± 2.90	20.6 ± 10.3	83.0 ± 18.4	23.9 ± 5.44	28.9 ± 0.78
Beta thalassemia Major	85.7 ± 7.61	3.69 ± 3.17	7.46 ± 5.58	29.8 ± 22.4	54.3 ± 23.9	25.5 ± 7.28	31.3 ± 3.15
HbE trait*	5.30	4.06	10.2	31.7	78.0	25.2	32.2
Sickle cell trait	1.55 ± 2.48	8.06 ± 3.96	21.1 ± 14.0	50.7 ± 18.5	44.5 ± 26.4	27.4 ± 5.89	27.4 ± 5.52
Sickle cell anemia	15.9 ± 6.55	5.93 ± 3.44	16.2 ± 10.7	53.2 ± 30.2	43.6 ± 24.7	27.0 ± 5.04	27.3 ± 5.73
Sickle cell disease	11.3 ± 9.79	9.04 ± 3.54	27.7 ± 11.7	68.5 ± 21.8	29.6 ± 17.4	31.4 ± 3.05	25.3 ± 7.70
Delta beta thalassemia trait*	16.4	5.79	13.1	41.5	72.0	22.6	31.6
Hb D Heterozygous*	2.20	6.00	19.0	76.0	23.8	31.4	12.1

DISCUSSION

This comprehensive analysis of fetal hemoglobin levels across various hemoglobinopathies reveals several significant patterns that have important diagnostic and therapeutic implications. Our findings demonstrate distinct HbF expression profiles that could serve as valuable markers in the differential diagnosis and management of hemoglobin disorders. The remarkably high HbF levels observed in delta beta homozygous conditions (95.3 ± 6.08%), HPFH Homozygous (90.3 ± 6.86%), and beta thalassemia major (85.7 ± 7.61%) represent a crucial finding. These elevated levels align with the underlying genetic mechanisms of these conditions, where mutations affect the beta-globin gene cluster, leading to compensatory increases in γ-globin gene expression. This compensation mechanism appears most pronounced in delta beta thalassemia homozygous cases, suggesting a more complete disruption of adult hemoglobin production in this condition.

The moderate HbF elevations found in beta thalassemia intermedia (43.1 ± 24.2%) demonstrate significant variability, reflected in the large standard deviation. This heterogeneity likely represents the

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diverse genetic modifiers and varying degrees of beta-globin chain reduction characteristic of this condition. Such variability could explain the spectrum of clinical severity observed in beta thalassemia intermedia patients and might serve as a prognostic indicator.

Our observation of relatively lower HbF levels in sickle cell conditions (ranging from 1.55 ± 2.48% in trait to 15.9 ± 6.55% in anemia) provides valuable insights into disease modification. The higher levels in sickle cell anemia compared to trait suggest a compensatory response to chronic anemia, potentially offering some amelioration of disease severity. This finding supports the ongoing research into HbF induction as a therapeutic strategy in sickle cell disease.

The hematological parameters accompanying these HbF patterns reveal interesting correlations. Notably, sickle cell conditions demonstrated consistently higher RBC counts, particularly in sickle cell disease ($9.04 \pm 3.54 \times 10^6/\mu L$), suggesting a compensatory response to chronic tissue hypoxia. Conversely, the lower RBC counts in HPFH Homozygous ($2.40 \pm 0.72 \times 10^6/\mu L$) despite very high HbF levels indicate that HbF elevation alone may not drive erythropoietic responses.

The MCV and MCH values across conditions provide additional diagnostic insight. The notably high MCV in HPFH Homozygous (83.0 ± 18.4 fL) contrasts with the lower values in beta thalassemia conditions, suggesting distinct effects on erythrocyte morphology despite similarly elevated HbF levels. This pattern could aid in differential diagnosis when standard hemoglobin electrophoresis results are ambiguous.

Several limitations of our study warrant consideration. The single-center nature of the investigation may limit generalizability, particularly given the genetic heterogeneity of hemoglobinopathies across different populations. Additionally, the small sample sizes for some conditions (notably those marked with asterisks) necessitate cautious interpretation of these specific findings.

Our findings have important clinical implications. The established ranges of HbF across different hemoglobinopathies could serve as valuable reference data for diagnostic algorithms. Furthermore, the observed variability in HbF levels within each condition suggests the potential utility of HbF quantification in prognostication and therapeutic decision-making, particularly regarding the initiation of HbF-inducing therapies.

Future research directions should include multi-center validation studies with larger cohorts, investigation of genetic modifiers influencing HbF expression, and longitudinal studies to establish the prognostic value of HbF levels in different hemoglobinopathies. Additionally, exploration of the relationship between HbF levels and clinical outcomes could inform personalized therapeutic approaches.

Limitations and Recommendations

Study Limitations

1. Methodological Constraints:
- Single-center study design limiting the generalizability of findings to broader populations
- Retrospective nature of data collection, which may introduce selection bias
- Variation in sample sizes across different hemoglobinopathies, with some conditions having only single cases
- Lack of longitudinal follow-up data to assess HbF level variations over time

2. Population-Related Limitations:
- Limited demographic diversity due to geographical constraints of a single tertiary care center
- Potential referral bias as severe cases are more likely to present at tertiary care centers
- Absence of family history data that could provide insights into inheritance patterns
- Limited representation of rare hemoglobin variants

3. Technical Limitations:
- Inability to account for all potential confounding factors affecting HbF levels
- Lack of molecular genetic analysis to correlate genetic variants with HbF expression
- Absence of data on concurrent medications or treatments that might influence HbF levels
- Limited information on environmental factors that could impact hemoglobin parameters

4. Clinical Correlation Limitations:
- Absence of clinical severity scores to correlate with HbF levels
- Limited data on treatment responses and outcomes
- Lack of quality-of-life assessments in relation to HbF levels
- Incomplete information on comorbidities that might affect hematological parameters

Recommendations

1. Research Design Recommendations:
- Conduct multi-center studies to improve generalizability
- Implement prospective study designs with standardized data collection protocols
- Establish larger cohorts for rare hemoglobin variants
- Include control groups for better comparative analysis
- Design longitudinal studies to track HbF variations over time

2. Clinical Practice Recommendations:
- Develop standardized protocols for HbF assessment across healthcare centers
- Establish region-specific reference ranges for HbF in different hemoglobinopathies
- Implement regular monitoring of HbF levels in patients with hemoglobin disorders
- Consider HbF levels in treatment decision-making processes

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3. Diagnostic Recommendations:
- Integrate molecular genetic testing with HbF analysis
- Develop comprehensive diagnostic algorithms incorporating HbF levels
- Standardize reporting formats for hemoglobin analysis
- Include family screening protocols in diagnostic workup

4. Future Research Directions:
- Investigate genetic modifiers affecting HbF expression
- Study the impact of environmental factors on HbF levels
- Evaluate the role of HbF in disease progression and severity
- Assess the effectiveness of HbF-inducing therapies
- Explore novel therapeutic approaches targeting HbF regulation

5. Healthcare Policy Recommendations:
- Establish national registries for hemoglobinopathies
- Develop guidelines for standardized HbF testing and reporting
- Implement newborn screening programs incorporating HbF analysis
- Create awareness programs about the significance of HbF testing

6. Technical Recommendations:
- Standardize laboratory methods for HbF quantification
- Implement quality control measures across testing centers
- Develop automated analysis systems for accurate HbF measurement
- Establish external quality assessment programs

7. Educational Recommendations:
- Train healthcare providers in interpreting HbF results
- Develop educational materials for patients and families
- Conduct workshops on latest developments in hemoglobinopathy diagnosis
- Create awareness about the importance of regular monitoring

These limitations and recommendations provide a framework for improving future research and clinical practice in the field of hemoglobinopathies. Addressing these aspects would contribute to better understanding and management of these disorders, ultimately leading to improved patient outcomes.

CONCLUSION

This comprehensive analysis establishes distinct HbF expression patterns across various hemoglobinopathies in a tertiary care setting. The study revealed characteristic ranges from significantly elevated levels in delta beta homozygous (95.3 ± 6.08%), HPFH Homozygous (90.3 ± 6.86%), and beta thalassemia major (85.7 ± 7.61%) to notably lower levels in sickle cell trait (1.55 ± 2.48%) and HbE trait (5.30%). The correlation between HbF levels and hematological parameters provides valuable diagnostic markers and potential prognostic indicators. Despite the limitations of a single-center study, these findings contribute significantly to establishing reference ranges for different hemoglobinopathies and support the utility of HbF quantification in clinical decision-making. Future multi-center studies with larger cohorts are warranted to validate these findings and explore their implications for personalized therapeutic approaches.

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