Open Access

Original Article

Clinical and Hematological Characteristics of Patients with Fast Moving Hemoglobins Diagnosed in a Tertiary Level Laboratory

Syeda Samia Shafaat, Maymoona Suhail Qasmi, Muhammad Iqbal, Manzar Bozdar, Saima Saad

Department of Hematology, Armed Forces Institute of Pathology/National University of Medical Sciences (NUMS) Rawalpindi Pakistan

ABSTRACT

Objective: To analyze hematological and clinical characteristics of fast-moving hemoglobin variants.
Study Design: Descriptive cross-sectional.
Place and Duration of Study: Department of Hematology, Armed Forces Institute of Pathology, Rawalpindi Pakistan, from March 2021 to March 2022.
Methodology: A total of 637 patients, enrolled over a period of two years, were included in this cross-sectional study. Inclusion criteria comprised of all patients reporting for hemoglobin studies for screening hemoglobin disorders. Sysmex XN 3000 automated hematology analyzer was used to perform complete blood counts, peripheral blood smears were assessed for red blood cell morphology and hemoglobin electrophoresis was done using three parallel techniques.
Results: Fast moving hemoglobins were diagnosed in 12(1.8%) patients, with a male to female ratio of 0.8:1 and mean age of 25.6±15.1 years. Clinical presentations of the patients included anemia in 7(58%) and transfusion history in 3(25%) patients. Peaks of Hb H and J were seen on Sebia capillary zone electrophoresis, in zones of Z15 (20-40 seconds) for Hb H and Z12 (80-100 seconds) for Hb J respectively. Retention times at High Performance Liquid Chromatography of Hb H and Hb J were 0.39 and 0.6 mins respectively. Cellulose acetate membrane revealed fast moving bands in all patients.
Conclusion: Fast moving hemoglobins remain an underdiagnosed entity, needing urgent allocation of resources for assisting patients in diagnosis and management.

Keywords: Alpha-Thalassemia, Hemoglobin H disease (Hb H), Hemoglobin J (Hb J).

How to Cite This Article: *Shafaat SS, Qasmi MS, Iqbal M, Bozdar M, Saad S. Clinical and Hematological Characteristics of Patients with Fast Moving Hemoglobins Diagnosed in a Tertiary Level Laboratory. Pak Armed Forces Med J 2024; 74(6): 1540-1543. DOI: https://doi.org/10.51253/pafmj.v74i6.9331*

INTRODUCTION

Alpha and beta thalassaemias are two common inherited haemoglobin (Hb) disorders with fast-moving hemoglobins (FMHs) having a higher electrophoretic mobility towards anode, due to substitution of amino acids with a negative charge in either alpha, beta or gamma globin genes, subsequently leading to a gain of two negative charges. Multiple variants of these Hb are identified worldwide, a few of which include Hb H, Hb Barts, N-Baltimore, J-Baltimore, J-Toronto, Detroit, Tacoma, K-Ibadan and Hofu. Out of these only Hb H and Hb J have been identified in Asian population.[1] Abnormalities of beta or alpha chains produce most of the clinically significant haemoglobinopathies where alpha-thalassaemias occur as a result of α-globin genes mutations or deletions (or combinations of both) present on short arm of chromosome 16.[2] Four α-globin genes are present in healthy genome (each chromosome having two) and genetic abnormalities may present clinically with variations ranging from silent carrier state (single α-globin gene deleted) to Hydrops fetalis (all four α-globin genes deleted).[3] Beta-thalassaemia is highly prevalent in Pakistan, with a carrier rate of 5-7% while three α-globin gene deletions and/or mutations result in Hb H disease, which is of intermediate clinical severity and presents clinically as thalassaemia intermedia.[4] Alpha-globin gene variant results from negatively charged amino acids replacement in either alpha, beta or gamma globin chains.[5] Standard screening test for Hb variants detection is electro-phoresis while distinctive retention times on High Performance Liquid Chromatography (HPLC) are used to detect these Hb, however, alkaline gel electrophoresis (at pH 8.6) exhibits their fast-moving nature, thus, HPLC and alkaline gel electrophoresis are both helpful in detection and quantitation of Hb variants.[6]

METHODOLOGY

This descriptive cross-sectional study was conducted at the Department of Hematology, Armed Forces Institute of Pathology (AFIP), Rawalpindi, Pakistan. Permission was sought from the institutional Ethics Committee prior to commencement of project (write ERC Cons-HEM-5/READ-IRB /22/1077). The

Correspondence: Dr Syeda Samia Shafaat, Department of Hematology, Armed Forces Institute of Pathology, Rawalpindi Pakistan
Received: 27 Oct 2022; revision received: 29 Jan 2024; accepted: 15 Feb 2024

study was conducted over a period of two years from March 2021 to March 2022. Sample size was calculated using World Health Organisation (WHO) sample size calculator, taking confidence interval at 95%, margin of error at 5% and reported prevalence of fast moving hemoglobin as 1.7%.[3] All participants were selected by non-probability consecutive sampling.

Inclusion criteria: All patients, of either gender, who presented for evaluation of anemia and suspected haemoglobinopathy, were included.

Exclusion criteria: Patients who had no history of anemia or any hemoglobinopathy were excluded.

Detailed history was taken along with general physical examination and findings were noted, particularly age, gender, signs and symptoms, and transfusion history.[7] Under aseptic technique, antecubital vein was accessed to obtain venous blood of 5 ml volume and collected in ethylene diamine triacetate anticoagulant. Sysmex XN 3000 automated hematology analyzer was used to perform complete blood counts within 1 to 3 hours of sample collection. Total red blood cells count (TRBC), Hb, mean cell volume (MCV), mean corpuscular haemoglobin (MCH) and red cell distribution width standard deviation (RDW-SD) were analyzed. Washed red blood cells were used to prepare the hemolysate in Tris EDTA borate buffer which has pH 8.9 and cellulose acetate membrane was used to perform Hb electrophoresis. Fast moving bands of Hb variants of the patients were assessed visually by comparison with normal control. Sebia capillary zone electrophoresis automated analyzer and HPLC were also used for Hb variants separation, which gave percentages of Hb A2, Hb A, Hb F, Hb J or Hb H and retention time respectively of each Hb variant.[8] Microscopic visualization of Hb H inclusions was done on smears stained by supra vital staining (new methylene blue) stain at 37° for 1 to 2 hours 9, as shown in Figure-1.

RESULTS

Data for a total of 637 patients was analyzed, of which, 12(1.8%) patients were diagnosed with having fast moving hemoglobins, 5(41.6 %) were males while 7(58.3%) were females. The age of patients was between 10 and 63 years with a mean age of 25.6±15.1 years. Anemia was diagnosed in 7(58%) patients, pallor in 7(58%) and splenomegaly in 2(16%) patients. Transfusion history was found in 3(25%) patients although they were not very frequently transfused. Analyzer's mean hematological parameters are tabulated in Table-I. Anisopoikilocytosis with hypochromia and microcytosis was present on RBC morphology on peripheral film of patients with anemia. Mean reticulocyte count was 3.4±4.3%. Supra vital staining of Hb H by new methylene blue stain showed Hb H inclusions in 100% cases. Cellulose acetate membrane was used for fast moving band of Hb H and J visualization in all patients.

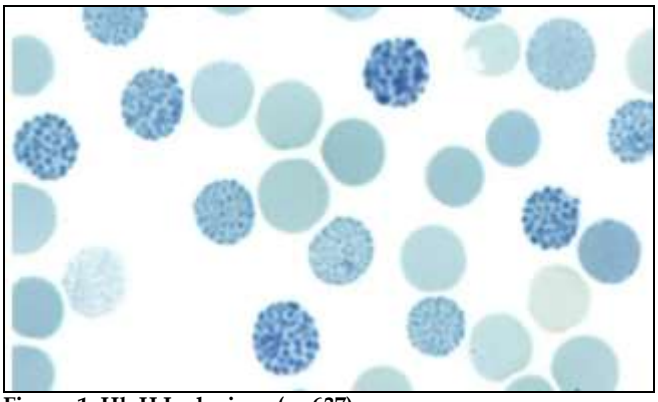

Figure-1: Hb H Inclusions (n=637)

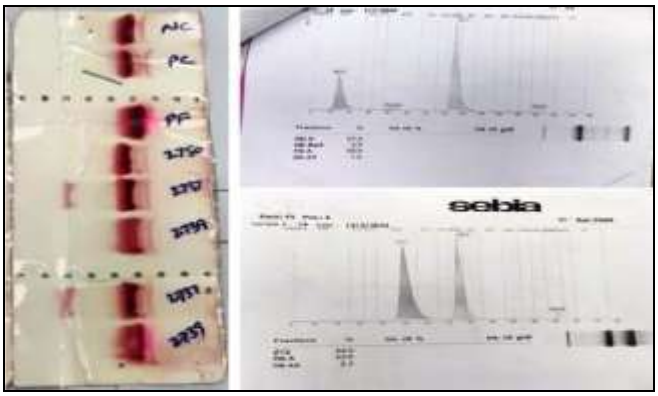

Figure-2: Peaks and Bands of Fast-moving Hemoglobins (n=637)

DISCUSSION

Alpha-thalassemia is one of the most common single gene disorders, affecting 5% of the population of the world while the prevalence of α+-thalassemia in Pakistan is 15-20%, but that of α⁰-thalassemia has not been documented but the high prevalence of α+-thalassemia trait in our region is likely the reason for the low frequency of Hemoglobin H disease, which is reported 1.7% in literature.[3] Identification and quantification of normal and abnormal hemoglobin is widely done by Hb electrophoresis, which is also used as a screening tool.[10] Interpretation of Hb electrophoresis results should be clinically correlated with family history and results of red cell morphology,

Table-I: Hematological Data of Patients with Fast Moving Haemoglobins (n=637)

Age	Gender	Hb (g/dL)	Retic count	MCV (fL)	MCH (pg)	RDW-SD	RBC (x 1012 /L)	HB-A (%)	HB-A2 (%)	HB-F (%)	HB-H (%)	HB-J (%)	Transfusion history	Spleen palpable
13	F	8.7	0.7	65.2	18.7	41.7	4.65	42.9	1.6	-	-	55.6	No	No
12	F	11.7	1.0	80.7	26.0	45.3	4.50	43.6	2.4	-	-	54.0	No	No
41	M	14.8	1.0	81.5	27.2	41.1	5.45	42.7	2.3	-	-	55.0	No	No
31	F	8.6	0.9	53.7	16.3	37.4	5.20	96.1	1.4	-	2.5	-	No	No
10	F	8.3	10.0	81.4	22.4	73.1	3.71	68.9	1.4	-	27.2	-	Yes	No
29	F	7.5	1.2	57.1	17.2	41.8	4.36	93.5	1.2	-	5.3	-	Yes	Yes
27	M	12.4	1.6	54.3	16.6	41.3	7.46	94.2	1.1	-	4.7	-	No	No
63	M	13.0	1.9	76.0	24.0	44.2	5.42	59.0	1.8	1.4	-	39.7	No	No
30	F	12.1	1.8	79.6	26.6	47.1	4.55	52.5	2.1	-	-	17.6	No	No
12	M	5.6	5.2	89.2	27.5	50.8	2.04	90.3	3.0	0.5	6.2	-	Yes	Yes
21	M	10.0	1.4	72.6	19.7	56.9	5.08	67.5	0.9	-	31.3	-	No	No
19	F	10.1	1.7	73.1	20.2	32.4	4.29	88.5	2.4	-	9.1	-	No	No

F=Female, M=Male

Hb, haematocrit, red cell indices and serum iron studies.[11] Of the hemoglobin normally present in adults, Hb A migrates the furthest when tested on cellulose acetate membrane, followed by Hb F, while Fast moving Hb is located near the anode past Hb A, however, Hb H and Hb J move almost 20-30 mm and 5-10 mm away from Hb A respectively.[12] Three α-globin gene deletions and/or mutations result in Hb H disease which is of intermediate clinical severity and may present clinically as thalassemia intermedia.[13] Hb H phenotypic variability depends on the type of mutation present in the patient, as it has been noted that non-deletional Hb H disease is more severe than deletional while some single or multiple changes in bases in Hb alpha or beta chains correspond with Hb J variants 5 but marked clinical variability may be present, such as repeated blood transfusions in some patients, or because of anemia and splenomegaly in others,[13] and Iron Overload may also develop in these patients with age or due to frequent blood transfusions.[14,15] More than fifty variants of Hb J have been identified,[16] but mostly it is accidentally diagnosed and clinically silent.[17,18] In heterozygous variants ,the normal allelic gene produces normal chains which compensates for the abnormal gene, whereas in homozygous disease both genes are affected.[19] Our findings were similar to another study, particularly with frequency of occurrence and gender distribution.[20]

LIMITATIONS OF STUDY

A potential limitation of this study was the underrepresentation of fast-moving hemoglobins due to their underdiagnosed nature. This may have led to a smaller sample size or biased data, potentially impacting the generalizability of the findings. Additionally, limited access to advanced diagnostic tools or specialized laboratories could have influenced the accuracy and comprehensiveness of the analyses. Future research should focus on increasing awareness, improving diagnostic resources, and broadening the scope of investigations to address these limitations.

CONCLUSION

Fast-moving hemoglobins remain an underdiagnosed and often overlooked entity, highlighting the critical need for increased awareness and resource allocation to support timely diagnosis and effective management. Addressing this gap requires the development of advanced diagnostic tools, improved access to specialized care, and targeted education for healthcare professionals to enhance recognition of these conditions.

Conflict of Interest: None.

Funding Source: None.

Authors' Contributions:

The following authors have made substantial contributions to the manuscript as under:

SSS & MSQ: Study design, data interpretation, drafting the manuscript, critical review, approval of the final version to be published.

MI & MB: Conception, data analysis, drafting the manuscript, approval of the final version to be published.

SS: Data acquisition, critical review, approval of the final version to be published.

Authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

REFERENCES

1. Ünal S, Oktay G, Acıpayam C, İlhan G, Gali E, Celkan T, et al. Hemoglobin H disease in Turkey: experience from eight centers. Turk J Hematol 2016; 33(1): 56. https://doi.org/10.4274/tjh.2015.0228

2. Vichinsky EP. Alpha thalassemia major—new mutations, intrauterine management, and outcomes. Hematology Am Soc Hematol Educ Program 2009: 35–41. https://doi.org/10.1182/asheducation-2009.1.35
3. Rehman A, Ahmed S, Ansari U. Clinical and haematological characteristics of haemoglobin H. Pak J Med Health Sci 2015; 9(3): 1096–1098.
4. Laosombat V, Viprakasit V, Chotsampancharoen T, Wongchanchailert M, Khodchawan S, Chinchang W, et al. Clinical features and molecular analysis in Thai patients with HbH disease. Ann Hematol 2009; 88(12): 1185–1192. https://doi.org/10.1007/s00277-009-0764-1
5. Srinivas U, Mahapatra M, Pati HP. Hb J Meerut, a fast-moving hemoglobin: a study of seven cases from India and a review of literature. Am J Hematol 2007; 82(7): 666–667. https://doi.org/10.1002/ajh.20958
6. Malik SA, Malik MAS, Malik SA. Detection of alpha thalassemia in cord blood in Bahawalpur region. Pak J Med Health Sci 2016; 10(3): 866–869.
7. Aksu T, Coşkun C, Kuskonmaz B, Ünal S, Aytac S, Gümrük F, et al. Hb H disease diagnosed during adolescent pregnancy. Hemoglobin 2020; 44(2): 137–138. https://doi.org/10.1080/03630269.2020.1747318
8. Baruah MK, Saikia M, Baruah A. Pattern of hemoglobinopathies and thalassemias in upper Assam region of North Eastern India: high-performance liquid chromatography studies in 9000 patients. Indian J Pathol Microbiol 2014; 57(2): 236–243. https://doi.org/10.4103/0377-4929.134656
9. Kulaç İ, Ünal Ş, Gümrük F. Brilliant cresyl blue staining for screening hemoglobin H disease: reticulocyte smear. Turk J Haematol 2009; 26(1): 45. https://doi.org/10.5152/tjh.2009.02
10. Farashi S, Najmabadi H. Diagnostic pitfalls of less well-recognized HbH disease. Blood Cells Mol Dis 2015; 55(4): 387–395. https://doi.org/10.1016/j.bcmd.2015.08.010
11. Basu S, Maity S, Mitra M, Ray J. HbH disease: a case study. Ann Inst Child Health Calcutta 2014; 14(1): 55–58.
12. Bain BJ. The alpha, beta, delta, and gamma thalassaemias and related conditions. In: Haemoglobinopathy Diagnosis. 2nd ed. Oxford: Wiley-Blackwell; 2006: 63–138.
13. Raman S, Sahu N, Senapati U. A study of haemoglobinopathies and haemoglobin variants using high-performance liquid chromatography (HPLC) in a teaching hospital of Odisha. J Evol Med Dent Sci 2017; 6: 842–850. https://doi.org/10.14260/jemds/2017/181
14. Shabbir S, Nadeem M, Sattar A, Ara I, Ansari S, Farzana T, et al. Type and frequency of hemoglobinopathies diagnosed in the area of Karachi, Pakistan. Cogent Med 2016; 3(1): 1188875. https://doi.org/10.1080/2331205X.2016.1188875
15. Chao YH, Wu KH, Wu HP, Liu SC, Peng CT, Lee MS, et al. Clinical features and molecular analysis of HbH disease in Taiwan. Biomed Res Int 2014; 2014: 1–5. https://doi.org/10.1155/2014/271065
16. Mahdavi MR, Bayat N, Hadavi V, Karami H, Roshan P, Najmabadi H, et al. Report of haemoglobin J-Toronto and alpha thalassemia in a family from North of Iran. J Pak Med Assoc 2012; 62(4): 396.
17. Celik MM, Gunesacar R, Oktay G, Duran GG, Kaya H. Spectrum of α-thalassemia mutations including first observation of -FIL deletion in Hatay Province, Turkey. Blood Cells Mol Dis 2013; 51(1): 27–30. https://doi.org/10.1016/j.bcmd.2013.03.010
18. Zhang J, Li P, Yang Y, Yan Y, Zeng X, Li D, et al. Molecular epidemiology, pathogenicity, and structural analysis of haemoglobin variants in the Yunnan province population of Southwestern China. Sci Rep 2019; 9(1): 1–8. https://doi.org/10.1038/s41598-019-44711-3
19. Karakaş Z, Koç B, Temurhan S, Elgün T, Karaman S, Asker G, et al. Evaluation of alpha-thalassemia mutations in cases with hypochromic microcytic anemia: the Istanbul perspective. Turk J Hematol 2015; 32(4): 344. https://doi.org/10.4274/tjh.2015.0197
20. Tariq M, Zafar Q, Waheed W, Shuaib A, Qaiser J, Malik MN. Haemoglobinopathies in referred cases for haemoglobin electrophoresis in a tertiary care teaching hospital. J Rawalpindi Med Coll 2019; 23(S-2).