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Original Article

Molecular and Clinical Spectrum of Hemoglobin D: An Experience at a Tertiary Care Diagnostic Center

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ABSTRACT

Objective: To determine the clinical and molecular pattern of Hemoglobin D in patients presenting at tertiary care hospital.
Study Design: Cross-sectional study.
Place and Duration of study: Department of Hematology, Armed Forces Institute of Pathology, Rawalpindi Pakistan, from Jul 2020 to Mar 2021.
Methodology: An automated cell analyzer was used to determine the whole blood count and red cell indices. D-10 BIORAD HPLC was used for quantitative assessment of Hb A, Hb F, Hb D and Hb A2. Where D-10 BIORAD HPLC showed an abnormal Hb D, capillary electrophoresis was performed using Sebia Capillary 2 Flex Piercing. Chelex DNA Extraction method was used. Molecular analyses for the confirmation of homozygosity and heterozygosity were done using Polymerase Chain Reaction (PCR) amplification. The clinical symptoms of the patients such as anemia, jaundice, pallor, weakness, hepatomegaly and splenomegaly were also noted
Results: Among 90 patients, Hemoglobin D trait was found in 78(80.4%) of the patients out of which HbD-Punjab was seen in 57(73%) and HbD-Iran was found in 21(27%). Compound Heterozygotes (HbD/β) was found in 17(17.5%) and Compound Homozygous HbD/D was found in 02 (2.1%). All HbD-Punjab and HbD-Iran traits patients were asymptomatic, and few clinical symptoms were found in Compound Heterozygotes (HbD/β) and Homozygous HbD/D.
Conclusion: Collective clinical history records, full blood count and electrophoresis and molecular results allow for the conclusive detection of the variant of Hemoglobin D. More multicenter, organized and detailed studies with larger sample size are required to draw concrete conclusions.

Keywords: Hemoglobinopathies, HbD-Punjab, HbD-Iran, Heterozygous, Homozygous.

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INTRODUCTION

Hemoglobinopathies are hereditary globin disorders, with the protein component of hemoglobin (Hb) variants typically derived from specific amino acid substitutions generated by point mutations in globin encoding genes.[1] Many hemoglobin variations may not cause symptomatic clinical symptoms but may be correlated with specific pathophysiology in some cases, such as hemoglobin S (HbS),[2] the most widespread variant of hemoglobin globally, with serious clinical consequences, such as beta thalassemia, HbD or HbC.[3] Hemoglobin D is a beta chain variant which occurs mostly in Northwest India (Punjab), Pakistan and Iran.[4] HbD was first discovered in the early 1950s, and it was given the name HbD-Los Angeles, but it was later discovered to be similar to HbD-Punjab. HbD-Punjab or Los Angeles differs from standard HbA structurally at 121 beta chain locations, where glutamine replaces glutamic acid.[5] Hemoglobin D-Iran (HbD-Iran) (HBB: c.67G>C) is an unusual form of Hb and it is caused by a codon 22 mutation in the globin gene involving a transition of GAA to CAA corresponding to glutamine replacement of glutamate.[6] There are three types of HbD: Heterozygous HbD (trait), Compound Heterozygous (HbD-thalassemia, HbSD) and Homozygous HbD, and they typically present as mild hemolytic anemia and mild to severe splenomegaly.[7] HbD is still largely unexplored, especially in Pakistan, where recent literature on its incidence, clinical attributes and diagnosis are limited. In addition, HbD has a significant geographic range and can be related to HbS, creating a compound heterozygous.[8] Heterozygous and homozygous conditions are typically asymptomatic in clinical terms and Compound Heterozygous form with another hemoglobin variant, including HbS, HbE, alpha and beta-thalassemia, are rare and associated with a chronic hemolytic disorder.[9] Though not curable, population screening, genetic therapy and prenatal diagnosis can avoid these disorders.[10] Therefore, the purpose of this study was to add knowledge to the

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current limited range of data on Hemoglobin D in order to raise attention to its significance in Pakistan.

METHODOLOGY

The study was conducted after approval of the Ethics Review Committee [Ref#FC-HEM17-35/READ-IRB/21/264] of Armed forces Institute of Pathology (AFIP), Rawalpindi, was carried out over a period of eight months, from July 2020 to March 2021. The sample size was calculated by using the WHO sample size calculator, with prevalence of HbD trait as 6.7%.[11]

Inclusion Criteria: Patients of either gender, of any age, with Hemoglobin D were included.

Exclusion Criteria: Patients with hemoglobinopathies other than Hemoglobin D and having a history of blood transfusion during the past one month before test were excluded.

After signing informed consent, a total of 97 patients of Hemoglobin D were enrolled in the study via non-probability sampling technique. An automated cell analyzer (XN-3000) was used to determine the whole blood count and red cell indices. Red cell morphology was investigated using Leishman-stained peripheral blood smears. D-10 BIORAD HPLC was used for quantitative assessment of HbA, HbF, HbD and HbA2. The cases where D-10 BIORAD HPLC showed an abnormal Hb D, capillary electrophoresis (CE) was performed using Sebia capillary electrophoresis. Genomic DNA was extracted using Chelex DNA extraction method. Molecular analyses for the confirmation of homozygosity and heterozygousity were done using Polymerase Chain Reaction (PCR) amplification. Clinical symptoms of the patients, such as, anemia, jaundice, pallor, weakness hepatomegaly and splenomegaly, were also noted.

The data was analyzed using the Statistical Package for Social Sciences (SPSS) version 21. Quantitative data was presented as mean and standard deviation, whereas qualitative variables were presented as frequency and percentage. The molecular and clinical profile of the patients was analyzed and compared using independent t-test and the *p*-value of ≤ 0.05 was considered significant.

RESULTS

A total of 97 patients with Hemoglobin D variant were found, ranging in age from 1-61 years, with mean age being 34.0±26.7 years. 54(55.6%) were male and 43(44.4%) were female. All the patients with HbD were clinically asymptomatic except 18(18.5%) patients, who were symptomatic and presented with jaundice, anemia, pallor, weakness, and hepatosplenomegaly, as shown in Table-I. Hemoglobin D trait was found in 78(80.4%) of the patients out of which HbD-Punjab was in 57(73%) and HbD-Iran was found in 21(27.5%). Compound Heterozygotes (HbD/β) were found in 17(17.5%) and Homozygous HbD/D was found in 2(2.1%) cases, as shown in Table-II. All HbD-Punjab and HbD-Iran trait patients were asymptomatic, and few clinical symptoms were found in Compound Heterozygotes (HbD/β) and Homozygous HbD/D, as shown in Table-III. The hematological profile of both symptomatic and asymptomatic patients was compared. Hb A2 was found in 18 HbD patients with low mean Hb (8.4±2.9g/dl), serum iron (35.9±3.1µg/dl) and red cell indices. HbF and Hb A2 were among their hematological characteristics. The remaining 79 individuals' levels were all within the normal range, and they were all asymptomatic. The detail hematological profile is shown in Table-IV.

Table-I: Clinical Profile of the Patients with Hemoglobin D Variant (n=97)

Clinical Profile	n(%)
Symptomatic Patients	18(18.5%)
Pallor	6(33.3%)
Hepatomegaly splenomegaly	4(22.2%)
Weakness	4(22.2%)
Anemia	2(11.1%)
Jaundice	2(11.1%)
Asymptomatic Patients	79(81.5%)

Table-II: Molecular Profile of the Patients with Hemoglobin D Variant (n=97)

Hb Electrophoresis and Molecular Analysis	n(%)
Hemoglobin D trait	
HbD-Punjab	78(80.4%)
HbD-Iran	57(73%)
Compound heterozygotes	21(27%)
HbD/β	17(17.5%)
Homozygous HbD/D	2(2.1%)

Table-III: Comparative Analysis of Clinical Profile and Hemoglobin Electrophoresis and Molecular Analysis (n=97)

Clinical Profile	Hemoglobin Electrophoresis and Molecular Analysis			
	HbD-Punjab	HbD-Iran	HbD/β	Homozygous HbD/D
Anemia	Absent	Absent	02	
Jaundice	Absent	Absent	Absent	02
Weakness	Absent	Absent	02	02
Pallor	Absent	Absent	Absent	06
Hepatosplenomegaly	Absent	Absent	02	02

Table-IV: Comparison of Hematological Profile Among Symptomatic and Asymptomatic Patients (n=97)

Hematological Profile	Patient Groups		*p*-value
	Symptomatic Patients (n=18) Mean±Standard Deviation	Asymptomatic Patients (n=79) Mean±Standard Deviation	
Hb A0	48.0±2.9	49.6±2.9	0.357
HbA2	4.4 ±1.7	2.3±1.5	0.001
HbF	2.7 ±1.9	1.3±0.7	0.334
HbD	43.±3.4	40.0±9.9	0.209
Red blood cells (million/µl)	3.0±1.9	4.8±2.0	0.001
Hemoglobin (g/ dl)	8.4±2.9	11.8±2.9	0.001
Hematocrit (%)	29.3 ±2.7	32.0±8.1	0.167
Mean Corpuscular Volume (fl)	71.9 ± 3.9	75.0±9.7	0.187
Mean Corpuscular Hemoglobin (pg)	23.0 ± 1.9	26.0+3.7	0.001
Mean Corpuscular Hemoglobin Concentration (g/dl)	24.1±2.9	29.0+3.6	0.001
Serum Iron (µg/ dl)	35.9±3.1	75.6±8.9	0.001

DISCUSSION

Hb D was initially identified on solubility test as missing Hb S as this Hb was totally soluble in 2.24 molar phosphate buffers.[11] Hb D has also been found in Iraq, the United States, Saudi Arabia, Togo, Thailand, Pakistan, Turkey and Iran.[12] In Pakistan, India and Iran, genetic disorders of Hb pose a major health problem.[13] A study from Karachi, Pakistan, found that the overall frequency of hemoglobinopathies was 34.2%, where 6.7% had Hemoglobin D trait1 but this is in contrast to a recent study in Peshawar, Pakistan, which found that the HbD trait was present in 1.6% of the population.[14] In our study, 80.4% of the patients had Hemoglobin D trait. The variation in prevalence may be due to difference in sample size, geographical location, and ethnicity. Except for a few reported case reports, no detailed data on the clinical and hematological profile of HbD exists. In our study, apart from few cases, rest of our patients of HbD were asymptomatic. Stout *et al* have documented this in an Oklahoma family,[15] which had mild anemia on stained peripheral blood smear, with several target cells and multiple spherocytes, most likely alpha-thalassemia-1 and congenital spherocytosis, in addition to having homozygous Punjabi patients. The same has been stated in other studies conducted around the world.[12,16] In our study, about 27% of the cases were HbD-Iran type and 73% HbD-Punjab type, whereas, in a study of 220 patients with HbD variants, 41.8% of the cases of HbD-Punjab and 40% of the cases of HbD-Iran were found.[12] There are few studies reporting that the incidence of HbD-Punjab varies between 1.75% to 57.8%.[12,14,17] The discrepancy in results may exist due to difference in sample size, study design, population cohorts and diagnostic tools. The high prevalence of HbD-Punjab may be due to high turnover of Punjabi patients. This has been also mentioned by some studies that Hb variants are disseminated within certain geographical and racial restrictions.[18] Furthermore,in our study, 2.1% of the HbD cases were Homozygous HbD/D and 17(17.5%) of the cases were Compound Heterozygotes HbD/β, which is in line with other recent studies.[19, 20]

CONCLUSION

In present scenario of cousin marriages, community migration, racial intermixing and changing population dynamics, Hemoglobin D pathologies can no longer be regarded as an individual disease limited to a single region. Collective clinical history records, full blood count and electrophoresis and molecular results allow for the conclusive detection of variant Hemoglobin D.

Conflict of Interest: None.

Authors' Contribution

Following authors have made substantial contributions to the manuscript as under:

AS & HMR: Data acquisition, data interpretation, critical review, approval of the final version to be published.

FA & AM: Study design, data analysis, drafting the manuscript, critical review, approval of the final version to be published.

AL & SJ: Conception, data acquisition, drafting the manuscript, 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. 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, in Pakistan. Cogent Med 2016; 3(1): 1-6. https://doi.org/10.1080/2331205X.2016.1188875
2. Ebrahimi M, Mohammadi-Asl J, Rahim F. Molecular spectrum and distribution of hemoglobinopathies in southwest of Iran: a seven-year retrospective study. J Hematopathol 2020; 13: 97–103.

https://doi.org/10.1007/s12308-019-00373-2
3. Biswas TK, Pillai A. Hemoglobin-D Punjab—rare hemolytic anemia in the elderly: a case report. MGM J Med Sci 2019; 6(3): 148-151.
4. Nasiri A, Rahimi Z, Vaisi-Raygani A. Hemoglobinopathies in Iran: an updated review. Int J Hematol Oncol Stem Cell Res 2020; 14(2): 140–150. https://doi.org/10.18502/ijhoscr.v14i2.2570
5. Warghade S, Britto J, Haryan R, Dalvi T, Bendre R, Chheda. Prevalence of hemoglobin variants and hemoglobinopathies using cation-exchange high-performance liquid chromatography in central reference laboratory of India: a report of 65779 cases. J Lab Physicians 2018; 10(1): 73–79. https://doi.org/10.4103/JLP.JLP_161_17
6. Ali AM. Haemoglobin D Iran with beta thalassemia in a primigravida with anemia: a case report. WJ Gynecol Women Health 2019; 2(4): 1-4.
7. Panyasai S, Rahad S, Pornprasert S. Coinheritance of hemoglobin D Punjab and β0-thalassemia 3.4 kb deletion in a Thai girl. Asian J Transfus Sci 2017; 11(2): 199–202. https://doi.org/10.4103/ajts.AJTS_102_16
8. Patel KD. Variants of Hemoglobin D Punjab: a retrospective study. Saudi J Pathol Microbiol 2019; 4(7): 507-511.
9. Srinivas U, Pati HP, Saxena R. Hemoglobin D-Punjab syndromes in India: a single center experience on cation-exchange high performance liquid chromatography. Hematology 2010; 15(3): 178-181. https://doi.org/10.1179/102453310X12583347009530
10. Tripathi P, Gupta A, Tyagi S. Compound heterozygote of Hb D-Punjab and Hb D-Iran: an interesting finding. Indian J Hematol Blood Transfus 2019; 35(1): 172–173. https://doi.org/10.1007/s12288-018-1050-4
11. Dass J, Gupta A, Mittal S, Saraf A, Langer S, Bhargava M, et al. Comparison of the characteristics of two hemoglobin variants, Hb D-Iran and Hb E, eluting in the Hb A2 window. Blood Res 2017; 52(2): 130-134. https://doi.org/10.5045/br.2017.52.2.130
12. Zakerinia M, Ayatollahi M, Rastegar M, Amanat S, Askarinejad AR, Amirghofran S, et al. Hemoglobin D (Hb D Punjab/Los Angeles and Hb D Iran) and co-inheritance with alpha- and beta-thalassemia in southern Iran. Iran Red Crescent Med J 2011; 13(7): 493-498.
13. Shanthala Devi AM, Rameshkumar K, Sitalakshmi S. Hb D: a not so rare hemoglobinopathy. Indian J Hematol Blood Transfus 2016; 32(1): 294–298. https://doi.org/10.1007/s12288-015-0580-3
14. Khan K, Zahoor S. Patterns of hemoglobinopathies on HPLC among patients referred to selected centers in Peshawar, Pakistan. Rawal Med J 2018; 43(4): 623-626.
15. Stout C, Holland CK, Bird RM. Hemoglobin D in an Oklahoma family. Arch Intern Med 1964; 114: 296-300.
16. Pandey S, Mishra RM, Pandey S, Shah V, Saxena R. Molecular characterization of hemoglobin D Punjab traits and clinical hematological profile of the patients. Sao Paulo Med J. 2012; 130(4): 248-251. https://doi.org/10.1590/S1516-31802012000400009
17. Patel AP, Naik MR, Shah NM, Sharma NP, Parmar PH. Prevalence of common hemoglobinopathies in Gujarat: an analysis of a large population screening programme. Natl J Community Med 2012; 3: 112–116.
18. Torres Lde S, Okumura JV, Silva DG, Bonini-Domingos CR. Hemoglobin D-Punjab: origin, distribution and laboratory diagnosis. Rev Bras Hematol Hemoter 2015; 37: 120–126. https://doi.org/10.1016/j.bjhh.2015.02.007
19. Agrawal MG, Bhanushali AA, Dedhia P, Jeswani KD, Dayanand S, Dasgupta A. Compound heterozygosity of Hb D(Iran) (beta(22) Glu→Gln) and beta(0)-thalassemia (619 bp-deletion) in India. Eur J Haematol. 2007; 79: 248-250. https://doi.org/10.1111/j.1600-0609.2007.00890.x
20. Gupta A, Saraf A, Dass J, Mehta M, Radhakrishnan N, Saxena R, et al. Compound heterozygous hemoglobin D-Punjab/hemoglobin D-Iran: a novel hemoglobinopathy. Indian J Hematol Blood Transfus 2014; 30(1): 409-412. https://doi.org/10.1007/s12288-014-0375-1