Original Article

Measurement of Hemoglobin Variants in Hemoglobinopathies

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Abstract

Background: Hemoglobinopathies are a group of inherited disorders triggered by defects in globin genes which lead to abnormal hemoglobin (Hb) level and structure with reduced oxygen-carrying capacity. Qualitative and quantitative laboratory measurements are routinely used to diagnose and differentiate various types of hemoglobinopathies. However, these measurements vary across diseases and populations. **Aims:** To report the measurement of Hb variants in a Saudi cohort of patients with hemoglobinopathies and hemoglobinopathy traits (sickle-cell and thalassemia traits). **Methods:** This was a retrospective study on patients with hemoglobinopathies who attended King Abdulaziz Medical City (Jeddah city, Western Saudi Arabia) in 2019. The data comprised patient diagnosis, age, gender, complete blood count and Hb electrophoresis. **Results:** The study included 184 patients (52% females, 48% males) of which 70% were aged >12 years, 28% aged >1-12 years and 2% aged <1 year. Maximum patients were diagnosed with sickle cell disease (61%) followed by hemoglobinopathy trait (19%). Hb concentration, red blood cell (RBC) count and packed cell volume (PCV) were significantly reduced in children and adolescent and above age group patients whereas in infants, no significant difference was found in RBC count and PCV. The mean HbA% was highest in alpha-thalassemia trait (97%) followed by beta-thalassemia minor (92.8%). Moreover, HbD disease was found in 82.8%, HbC disease in 67.6%, beta-thalassemia intermediate in 51.8%, sickle cell trait in 47.5% and HbE disease in only 12%. In addition, HbF and HbA2 were present in all detected hemoglobinopathies and traits at varying levels. **Conclusion:** Hemoglobinopathies and hemoglobinopathy traits require comprehensive laboratory examination using CBC, blood cell morphology, qualitative and quantitative electrophoresis. The results indicate the importance of quantifying HbA, HbF and HbA2 to aid in the differential diagnosis of different types of hemoglobinopathies.

Keywords: Hemoglobinopathies, sickle cell, hemoglobin patterns, electrophoresis, complete blood count.

Introduction

Hemoglobinopathies are a group of blood disorders triggered by defects in α-globin or β-globin gene leading to the production of an abnormal amount or structure of the oxygen-carrying molecule i.e. hemoglobin (Hb). These disorders are prevalent in low to middle income countries and it is estimated that 300,000 – 400,000 babies are born annually with severe form of hemoglobinopathies.[1] Adult Hb profiles are normally composed of 95-98% of HbA, 2-3% HbA2 and 0.8-2% HbF. The latter type i.e. HbF is higher in newborns (50-80%) and drops to <8% before six months of age. It further decreases to 1-2% six months after birth with rise in HbA.[2,3] Hemoglobinopathies can be diagnosed by examining different Hb variants i.e. HbA, HbA2, HbF, HbS, HbE, HbC and HbD) and their concentrations are measured to predict the disease severity.[4-6]

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Although both genders are equally affected yet males with sickle cell hemoglobinopathy tend to show higher morbidity and mortality, primarily due to hormonal variations.[7]

The prevalence of different types of hemoglobinopathies varies across regions. For instance, various cohort studies have shown that beta-thalassemia is the most frequently encountered hemoglobin disorder in Iraq, Pakistan and India[8-10], whereas, alpha thalassemia and sickle cell traits are prevalent in Brazil[11] and Saudi Arabia respectively.[12] Due to the high rate of consanguinity[12,13], the prevalence of these traits in Saudi Arabia is considered one of the highest as compared to its neighboring countries.[14] This emphasizes the importance of accurate detection and stratification of patients using different qualitative and quantitative laboratory measurements such as electrophoresis, complete blood count (CBC), smear and clinical and family history examination.[15]

This study aimed to measure the variants of Hb in a cohort of patients from western region of the Saudi Arabia, with hemoglobinopathies and their traits (sickle-cell and thalassemia).

Methods

This study was conducted in King Abdulaziz Mdical City tertiary care center, Jeddah, Saudi Arabia, from 1/1/2019 to 31/12/2019. After getting ethical clearance from the institutional review board of King Abdullah International Medical Research Center (IRB# SP20/278/J), medical records of patients with hemoglobinopathies were collected retrospectively . Any data disclosing the subject's identity was not collected or used in any research document to protect patients' confidentiality and anonymity. After that, diagnosis and results of pre-treatment CBC and Hb electrophoresis were extracted. The main CBC parameters investigated were: red blood cell (RBC) count, Hb concentration, mean cell volume, mean cell Hb, mean cell Hb concentration and the packed cell volume. On the other hand, Hb electrophoresis results were generated by CAPILLARYS™ 2 system (Sebia, UK) and represented in percentage. The patient's data was divided into three different groups with respect to their age i.e. infants (1 day - 1 year old), children (>1-12 years old) and adolescents and above (>12 years old). In the end, data was analyzed using the JMP statistical analysis software (Version 13. SAS Institute Inc., Cary, NC, 1989-2021).

Results

A total of 184 patients with hemoglobinopathies were recruited in the study including 52% (n=96) females and 48% (n=88) males. Of all these patients, 70% were aged >12 years, 28% aged >1-12 years and 2% aged <1 year. The median age of study subjects was 21 yearswith interquartile age range of 11-34. The most frequent hemoglobinopathy disease found was sickle cell disease (61%) followed by beta-thalassemia minor (6.5%) and hemoglobin SC disease (3.3%). With respect to the hemoglobinopathy traits, sickle cell trait was present in 19% and alpha thalassemia trait was present in 7.1% of the patients.. There were other hemoglobinopathies also which were found in only 2.7% of the patients. The statistical analysis revealed no significant association between hemoglobinopathies and gender ($p > 0.518$). The results of ANOVA test also revealed no significant association between hemoglobinopathies with different age groups. In addition, an independent t-test was conducted to compare patients' CBC results (PCV, Hb concentration and RBC count) with age- and sex- matched hospital reference values. Significant differences were found in all age groups except the RBC and PCV values in the infant age group (Table 1).

Table 1. Mean of PCV, Hb and RBC in different age groups.

Variables	Age group	Hematological parameter result		p value
		Patients Mean (±SD)	Reference values	
PCV	Infants (1 day – 1 year)	33.5 (±4.04)	38.0	0.1123
	Children (>1-12) years	29 (±4.46)	39.0	<0.0001*
	Adolescent and above (>12) years	28.3 (±5.08)	42.5	<0.0001*
Hb	Infants (1 day – 1 year)	11.4 (±1.33)	13.1	0.0433
	Children (>1-12)years	9.3 (±1.28)	13.4	<0.0001*
	Adolescent and above (>12) years	9.3 (±1.5)	14.3	<0.0001*
RBC	Infants (1 day – 1 year)	4.97 (±0.9)	4.8	0.7263
	Children (>1-12)years	3.65 (±1.09)	4.8	<0.0001*
	Adolescent and above (>12) years	3.45 (±0.98)	4.8	<0.0001*

CBC= Complete blood count, PCV= Packed cell volume, Hb= Hemoglobin, RBC= Red blood cell.
*significant p value <0.05

Analysis of hemoglobin variants and their concentration means are summarized in Table 2. An independent t-test was performed on each Hb variant and revealed that HbA was a significant diagnosing factor for beta-thalassemia minor, sickle cell trait and sickle cell disease (P <0.01). Moreover, HbA2 was found to be significant for differentiating beta-thalassemia minor, sickle cell trait and sickle cell disease (P <0.01). On the other hand, the presence of HbA and HbA2 was not found statistically significant for the differential diagnosis of alpha thalassemia trait(P > 0.7). However, HbF was found as a significant diagnosing factor for both sickle cell trait (P= 0.013) and sickle cell disease (P <0.01). Conversely, it was not found significant for differentiating beta-thalassemia minor and alpha thalassemia traits (P > 0.2).

Table 2. Hemoglobin variants (mean ±SD).

Hemoglobinopathies and traits	No. of patients (%)	HbA%	HbF%	HbA2%	HbS%	HbC%	HbD%	HbE%
Sickle cell disease	113 (61.4%)		15.7 ±4.2	3.5 ±0.7	80.8 ±9.3			
Sickle cell trait	35 (19%)	47.5 ±8.2	3.69 ±1.7	3.01 ±0.45	45.8 ±6.9			
Alpha thalassemia trait	13 (7.1%)	97 ±1.03	0.43 ±0.58	2.57 ±0.93				
Beta thalassemia minor	12 (6.5%)	92.8 ±1.68	1.8 ±1.43	5.4 ±2.4				
Hb SC disease	6 (3.3%)	5.38 ±0.8	5.78 ±0.94	3.04 ±1.84	47.2 ±5.3	38.6 ±6.31		
Hb E disease	2 (1.1%)	12 ±0.3	9.3 ±0.9	7.6 ±2.3			0.3 ±0.01	70.8 ±7.9
Beta thalassemia intermedia	1 (0.5%)	51.8	38.5	9.7				
Hb C disease	1 (0.5%)	67.6	0.2	1		31.2		
Hb D disease	1 (0.5%)	82.8	1.5	2.2		0.5	13	

Discussion

Hb variants are widely distributed in different diseases of hemoglobin. This study emphasizes the significance of qualitative and quantitative measurement of these variants in diagnosing and differentiating patients with hemoglobinopathies. The study revealed no significant association of hemoglobinopathies with both gender and age. In CBC results of patients with hemoglobinopathies, Hb was found significantly lower in adults and children than in infantsindicating disease progression and anemia development. Analysis of Hb patterns revealed that HbA and HbA2 are the two most significant normal Hb in diagnosis. In contrast, HbF was not found significant in the diagnosis of hemoglobinopathies except in patients with thalassemia.

The Hb in RBCs of normal adult typically constitutes 95-98% HbA, 2-3% HbA2 and up to 2% HbF.[2] Quantifying these variants is critical for differentiating different types of hemoglobinopathies. This study showed that quantification of HbA, HbF and HbA2 is important for distinguishing between subtypes of thalassemia i.e. beta-thalassemia minor, beta-thalassemia intermedia and alpha thalassemia trait. The results depicted that HbA and HbF assisted the differentiation between beta-thalassemia minor and beta-thalassemia intermedia. HbA was reduced in beta-thalassemia intermedia (51.8%) and beta-thalassemia minor (92.8%), while HbF was increased in beta-thalassemia intermedia (38.5%) as compared to beta-thalassemia minor (1.8%). Conversely, HbA2 seemed to have no diagnostic value in differentiating beta-thalassemia intermediate and beta-thalassemia minor. This is in accordance with other studies which demonstrated the quantities of Hb variants in beta-thalassemia minor (high HbA, 0.5-6% HbF, variable HbA2) and beta-thalassemia intermedia (≈ HbA, up to 100% HbF, variable HbA2).[16] In another study, HbA2 were found elevated in HbE disease (>5%) and reduced in alpha thalassemia trait (<3%).[17] However, likewise other studies, the profile of Hb variants in alpha thalassemia trait was found close to normal i.e. HbA (97%), HbF (0.43%) and HbA2 (2.57%).[16] Unlike other studies which have shown an increase in HbF in thalassemia patients[18,19], this study showed no significant role of HbF in differntating alpha thalassemia trait and beta-thalassemia minor, however, it was found elevated in beta thalassemia intermedia. This study did not include beta-thalassemia major which can be diagnosed by the absence of HbA, the presence of HbF (70-95%) and variable levels of HbA2.[16,18] This study also reported the absence of HbA in sickle cell disease and its presence in sickle cell trait (47.5% ± 8.2). Moreover, HbS was significantly higher in sickle cell disease (80.8

±9.3) as compared to sickle cell trait (45.8 ± 6.9). HbS is an abnormal form with high diagnostic importance as a qualitative marker for sickle cell disease.[19] Both sickle cell disease and trait are diagnosed by the presence of HbS; therefore, quantitative measurements are important for differential diagnosis. Although HbF and HbA2 variants were also present at variable levels, yet they have no significance in differentiating sickle cell disease and trait. In addition, the presence of HbS and HbC in study subjects revealed HbSC disease regardless of the other Hb variants. Additionally, 2% of our cohort were diagnosed with the rare HbC, HbD and HbE diseases. Limitations of this study include its generalizability to patients from other regions and the low number of rare Hb variantsi.e. HbC, HbD and HbE.

Conclusion

This study highlights the importance of using qualitative and quantitative tests for the measurement of Hb variants to facilitate diagnosis of hemoglobinopathies and hemoglobinopathy traits. The results revealed that HbA, HbA2 and HbF are more of quantitative importance while HbS, HbC, HbE and HbD are more of qualitative importance as they can be diagnosed irrespective of their quantities.

Abbreviations

CBC: complete blood count
Hb: hemoglobin
HbC: hemoglobin C
HbD: hemoglobin D
HbE: hemoglobin E
HbS: hemoglobin S
PCV: packed cell volume
RBC: red blood cells

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References

1. Weatherall DJ, Clegg JB. Inherited haemoglobin disorders: an increasing global health problem. Bull World Health Organ. 2001; 79(8): 704-12. Available from: http://www.ncbi.nlm.nih.gov/pmc/articles/pmc2566499/.
2. Pagana KD, Pagana TJ. Mosby's diagnostic and laboratory test reference. 12th ed. Elsevier Inc.; 2015. Available from: http://www.worldcat.org/oclc/1198437099.
3. Stamatoyannopoulos G. Control of globin gene expression during development and erythroid differentiation. Exp Hematol. 2005; 33(3): 259-71. doi: https://doi.org/10.1016/j.exphem.2004.11.007.
4. Weatherall DJ. Hemoglobinopathies worldwide: present and future. Curr Mol Med. 2008; 8(7): 592-99. doi: https://doi.org/10.2174/156652408786241375.
5. Sonati Mde F, Costa FF. The genetics of blood disorders: hereditary hemoglobinopathies. J Pediatr (Rio J). 2008; 84(4 Suppl): S40-51. doi: https://doi.org/10.2223/jped.1802.
6. CDC. Hemoglobinopathies: Current Practices for Screening, Confirmation and Follow-up. Association of Public Health Laboratories; 2015. Available from: https://www.cdc.gov/ncbddd/sicklecell/documents/nbs_hemoglobinopathy-testing_122015.pdf.
7. Ceglie G, Di Mauro M, Tarissi De Jacobis I, et al. Gender-Related Differences in Sickle Cell Disease in a Pediatric Cohort: A Single-Center Retrospective Study. Front Mol Biosci. 2019; 6: 140. doi: https://doi.org/10.3389/fmolb.2019.00140.
8. Ali TH, Alamiry AAN, Majeed MN. Detection of hemoglobinopathies in hypochromic, microcytic and sickeled cell blood films by hemoglobin electrophoresis. Thi-Qar Med J. 2011; 5(1): 139-48. Available from: https://jmed.utq.edu.iq/index.php/main/article/view/243.
9. Khan K, Zahoor S. Pattern of Hemoglobinopathies on HPLC among patients referred to selected centers in Peshawar, Pakistan. Rawal Med J. 2018; 43(4): 623-26. Available from: http://rmj.org.pk/fulltext/27-1510651859.pdf.
10. Mondal SK, Mandal S. Prevalence of thalassemia and hemoglobinopathy in eastern India: A 10-year high-performance liquid chromatography study of 119,336 cases. Asian J Transfus Sci. 2016; 10(1): 105-10. doi: https://doi.org/10.4103/0973-6247.175424.
11. Carlos AM, Souza RA, Souza BM, et al. Hemoglobinopathies in newborns in the southern region of the Triângulo Mineiro, Brazil. Cross-sectional study. Sao Paulo Med J. 2015; 133(5): 439-44. doi: https://doi.org/10.1590/1516-3180.2015.00042302.
12. el-Hazmi MA, al-Swailem AR, Warsy AS, al-Swailem AM, Sulaimani R, al-Meshari AA. Consanguinity among the Saudi Arabian population. J Med Genet. 1995; 32(8): 623-26. doi: https://doi.org/10.1136/jmg.32.8.623.
13. Memish ZA, Owaidah TM, Saeedi MY. Marked regional variations in the prevalence of sickle cell disease and β-thalassemia in Saudi Arabia: findings from the premarital screening and genetic counseling program. J Epidemiol Glob Health. 2011; 1(1): 61-8. doi: https://doi.org/10.1016/j.jegh.2011.06.002.
14. Memish ZA, Saeedi MY. Six-year outcome of the national premarital screening and genetic counseling program for sickle cell disease and β-thalassemia in Saudi Arabia. Ann Saudi Med. 2011; 31(3): 229-35. doi: https://doi.org/10.4103/0256-4947.81527.
15. An R, Huang Y, Man Y, et al. Emerging point-of-care technologies for anemia detection. Lab Chip. 2021; 21(10): 1843-65. doi: https://doi.org/10.1039/d0lc01235a.

16. Kohne E. Hemoglobinopathies: clinical manifestations, diagnosis, and treatment. Dtsch Arztebl Int. 2011; 108(31-32): 532-40. doi: https://doi.org/10.3238/arztebl.2011.0532.
17. Denic S, Agarwal MM, Al Dabbagh B, et al. Hemoglobin A2 Lowered by Iron Deficiency and α -Thalassemia: Should Screening Recommendation for β -Thalassemia Change? ISRN Hematol. 2013; 2013: 858294. doi: https://doi.org/10.1155/2013/858294.
18. Brancaleoni V, Di Pierro E, Motta I, Cappellini MD. Laboratory diagnosis of thalassemia. Int J Lab Hematol. 2016; 38(Suppl 1): 32-40. doi: https://doi.org/10.1111/ijlh.12527.
19. Clarke GM, Higgins TN. Laboratory investigation of hemoglobinopathies and thalassemias: review and update. Clin Chem. 2000; 46(8): 1284-90. doi: https://doi.org/10.1093/clinchem/46.8.1284.