Original Article

Abnormal Hemoglobin Identified on HPLC

Abnormal Hemoglobin Identified on High Performance Liquid Chromatography (HPLC) in a Secondary Care Hospital of Karachi

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ABSTRACT

Objective: To estimate the frequency of abnormal hemoglobin variants in local population.
Study Design: Descriptive / Cross sectional study.
Place and Duration of Study: This study was conducted at the Kutiyana General Hospital, a secondary care hospital in Karachi from January 2020 January 2021.
Materials and Methods: Samples were collected in Kutiyana hospital in EDTA anticoagulant containing tubes and sent to laboratory of a tertiary care university hospital.
Results: Total of 1083 blood samples were analyzed out of which 736(68%) had normal hemoglobin while 347(32%) had abnormal hemoglobin. The frequency of abnormal hemoglobins (Hb) were beta thal trait 18%, beta thal major 9.9%, Hb D trait 1.1%, Hb D disease 0.7 %, S-beta thal 1%, Sickle cell anemia 0.5%, Hb-E trait 0.4%, Sickle cell trait 0.2% Hb D beta thal 0.1 %, SD disease 0.1% and Hb-E disease 0.1%
Conclusion: Abnormal Hbvariants were detected in a significant number of samples. Beta thal trait was the most common abnormal Hb followed by beta thal major and Hb- D trait. Large scale screening studies are required in general population.
Key Words: Hemoglobin (Hb), Thalassemia (thal), Hb-D, Hb. E, HPLC, Sickle cell disease, Hb SD disease

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INTRODUCTION

Hemoglobin (Hb) is a very important and major component of red blood cells. Hb is made up of Haem and globin chains[(1)]. It is responsible for the transport of oxygen and involved in various biochemical reactions in the blood. Normal adult contains three types of hemoglobins in the blood. The main hemoglobinsin adultsare Hb-A which accounts for more than 95 % of entire Hb in circulation while Hb A2 and fetal Hb (Hb-F) contributes a very small fraction. There are also hemoglobins which are present during the embryonic and fetal life of an individual which appear during specific intrauterine periods and then disappear. These are Hb Portland, Gower 1 and 2 and fetal Hb or Hb-F.

All the above mentioned Hemoglobin disappears and only a small fraction of Hb-F remains in adult and persists throughout adult life[(1).] Hb A is composed of two alpha and two beta chains, Hb A2 is composed of two alpha and two delta chains while Hb-F is composed of two alpha and two gamma chains. The genes for alpha chains are present on Chromosome 16 while that of beta, gamma and delta chains are present on chromosome 11[(2).] These genes are genetically acquired and follow simple Mendel's law of transmission in autosomal recessive pattern. Many types of abnormalities can occur in the genetic control and regulation of these genes resulting in the production of abnormal number or quality of globin chains. These mutations can cause abnormalities in transcription and translation processes. Mutations causing deficient production of structurally normal globin chains produce a syndrome called thalassemia[(3)]. It can be alpha, beta, gamma or delta thalassemia or a combination, depending upon the type of globin chain production. Thalassemias are only important clinically if they involve alpha or beta globin chains. In beta thalassemia, if only one allele is involved, then it is called beta thalassemia trait or minor and had high Hb-A2, while if

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both the alleles are involved then it produces the clinical syndrome of beta thalassemia major which is characterized by severe anemia and high Hb-F. The same is the case with alpha chains, producing alpha thalassemia trait and alpha thalassemia major called Hb H disease[4].

But if the number of globin chain production is normal but there is a defect in the structure of these chain due to substitution of an amino acid by another amino acid, then it is called hemoglobinopathy. Both thalasemias and hemoglobinopathies have an autosomal mode of transmission. Another abnormality is the presence of high amount of Hb-F in adult life called hereditary persistence of fetal Hb or HPFH, which does not have any clinically significant effect.

There are many naturally occurring and genetically determined variants of Hb and more than 750 have been described uptill now [5]. Many of these variants are harmless but some may have serious clinical effects like severe anemia requiring blood transfusions, failure to thrive and severe crippling body pains (2) . But some may be beneficial for the area in which it occurs e.g., Sickle cell disease in tropical and African continent for malariaetc.[6]. The main abnormal hemoglobin producing clinical syndromes are sickle cell or Hb-S, Hb-D, E or C. These have autosomal recessive mode of transmission and can present as trait or disease if the alleles are same or in combination. The traits can be Hb-S, CD or E trait while the disease can be homozygous for the same disease like SS, DD, CC or EE or various combination of two different alleles of abnormal hemoglobins like S/D, SC , SE or C/E, D/E etc.[7]. Usually, they produce clinical syndromes when both the alleles are abnormal and in various combinations. They can also co-exist with genes of thalassemia and produces various clinical syndromes. The above mentioned abnormal hemoglobins are relatively common in Asian and African countries. One of the reasons may be due to intermarriages which are quiet common in these parts of world especially in Middle east and Indian subcontinent[8]. These Hb variants including fetal Hb can be detected by Hb electrophoresis and High-performance liquid chromatography (HPLC). HPLC has an advantage over Hb electrophoresis by a rapid, cost effective, accurate and precise identification and quantitation of the various Hbs. This technique utilizes the principle of cation exchange and gives results in retention time. Each individual Hb has a different retention time. The retention times for Hb A, A2, F, S, D, E, C and Q are 2.43,3.6,1.2,4.5,4.2 3.6, 5.18 and 4.7 minutes respectively.

MATERIALS AND METHODS

It was a descriptive cross-sectional study in the Institute of hematology for a period of one year starting from January 2020 till January 2021. The objective was to find out the frequency of Hb abnormalities in the population. Those samples were included after analysis in the study by non -probability technique who had abnormal Hb or anexcess of fetal Hbafter3-5 ml of whole blood was collected in EDTA tube for all the patients irrespective of age and sex except those with a history of blood transfusion in the past three months, were sent to Hematology laboratory of Dow university hospital, Ojha campus, Karachi for Hb HPLC and Complete blood count (CBC). CBC was done on fully automated Sapphire hematology analyzer while HPLC was done on Fully automatic Akray analyzer. A total of 1083 samples were collected to achieve the objective with a margin of error d = 0.007 and confidence level of 95%. All the data was entered in the computer and analyzed through SPSS 17. Statistical analysis was done and reported as percentages for categorical variables like gender and type and frequency of abnormal Hb variant while means with Standard deviation of =- 1SD was reported for continuous variables like age, Hb, hematocrit, red blood cell count, MCV and MCH.

RESULTS

A total of1248 samples were received from which 165 samples were excluded because of recent blood transfusion history, results were included in the study. n= 736 (68%) males n=260 (35%) females n= (476 (65%), showed normal results while n = 347 (32%), males n=177 (51%) and females n= (170 (49%) had abnormal results with variant or abnormal excess of normal Hb as shown in table 1, the percentage of various abnormalities with male and female percentage are also shown in figure 2.

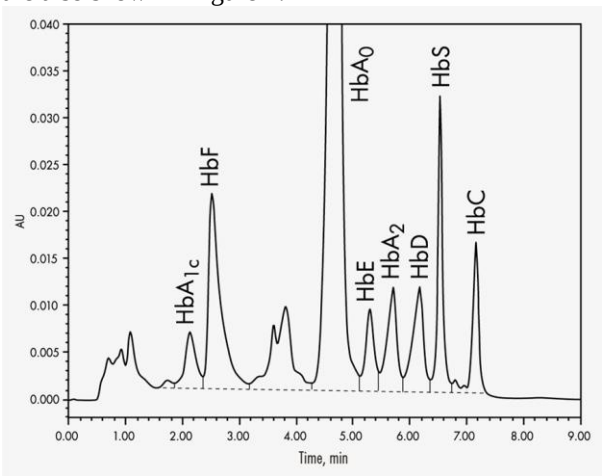

Figure No. 1. Graphic presentation of abnormal hemoglobin by retention time on HPLC

Table No.1: Split up of Frequency of different abnormal hemoglobin variants

Total HPLC =1083 **Normal results**: 736 (68%) **Abnormal results**: 347 (32%) **Split up of abnormal Hemoglobin results:**										
βThal trait	βThal Major	D trait	S/β thal	DD	SS	E trait	S trait	D/β	S/D	EE
195(18%)	107(9.9%)	12(1.1%)	11(1%)	8(7.7%)	5(0.5%)	4(.4%)	2(.2%)	1(.1%)	1(.1%)	1(%)

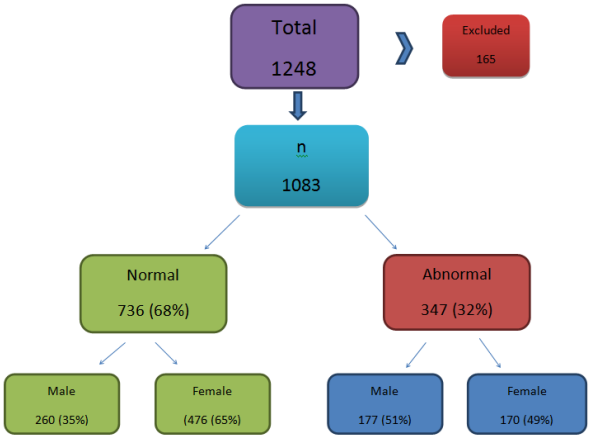

Figure No. 2: Abnormal Hemoglobins detection on routine Hb HPLC

DISCUSSION

HPLC with automation and quantitative ability appears to be a highly accurate and precise technique for direct identification and quantification of both normal and abnormal fractions [7] · Few studies have been done in this part of world and needs more studies to elaborate the importance of HPLC in hematology. In our study the overall frequency of abnormal Hb variants in Pakistan was 32% in comparison to other studies done in India which narrated a frequency of 12.7%. The reason for this difference may be due to very large sample size and a huge population of country[8].In our study beta thalasemia minor was the most common quantitative abnormality of about 18 %, characterized by high Hb A2, which is comparable to other studies done in this and nearby regions 13 % [9], 9 % , although the frequency reported by Khattak in 1992 was as low as 5.34 % [10], but the discrepancy may be due to a different sampling strategy. This trait has a gene frequency of 5-6% in Pakistan[11]) and its identification is of crucial importance in consanguinity marriages especially in this part of world with a possibility of birth of a beta thalasemia major child [12]

The second most common quantitative abnormality was beta thalasemia major 9.9 %, characterized by very high Hb-F, this was in contrast to Shaista et al[3]who reported a frequency of 24.1 % in their study in Karachi. The reason for this discrepancy may be due to the fact that their study was conducted in a hematology center dealing with hemoglobinopathies

The percentage of Hb D trait was 1.1 % of all abnormalities which is in accordance to study conducted by Usman et al showed a frequency of 1.4 % [14] but this was different from study by Shaista et al[11]and the difference again may be due to the reason that it was done in a pure hematology center. Hb D trait is an asymptomatic condition and can present with microcytosis and target cells on peripheral smear morphology[15]

Hb- D disease was detected in 0.7 % cases in our study which is nearly the same as of the study conducted in Peshawar, Pakistan by Khalid Khan[16]which shows a percentage of 01%. This disease presents with hypochromic microcytic picture of red blood cells. It is benign in trait form but presents with severe in homozygous form

Sickle cell anemia and its double heterozygous forms were1.5% of the abnormal Hb in our study which is in accordance with the study conducted by Nazish Hashmi[17]and the identification of this Hb is particularly important as it gives rise to various significant clinical syndromes, the majority of which are clinically significant having a considerable impact on family and burden on health care authorities[18].Hb E in ours study had a frequency of 0.4 % of abnormal hemoglobin which is nearly similar to a study conducted by Bushra Moiz et al which becomes significant when it combines with other abnormal hemoglobin disorders particularly beta thalassemia [19]

The limitation of our study is that not a single case of alpha thalassemia was identified although these cases are relatively common in this part of world. The reason for this that we used only HPLC for abnormal hemoglobin while a study conducted by Vijay Bhat showed that the identification can be made on sequential analyses using BioRad D10 HPLC, Alkaline gel electrophoresis, GPO α THAL-IC strips and the identification of the specific genetic lesion using an α Globin reverse dot blot hybridization assay [20].

CONCLUSION

This is one of the comprehensive studies done in this area by HPLC. Thalassemia minor was a more frequent abnormality amongst all the abnormal results, and these individuals should have genetic counseling regarding pre marriage screening of their spouses, directly or through parents or guardian as this was a non-probability sampling so general population screening needs to done to identify.

Author's Contribution:

Concept & Design of Study: Nadeem Nusrat

Drafting:	Furrukh Ali Khan, Salman Zafar
Data Analysis:	Muhammad Rizwan, Nazia Qamar, Owais Ismail Gadar
Revisiting Critically:	Nadeem Nusrat, Furrukh Ali Khan
Final Approval of version:	Nadeem Nusrat

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REFERENCES

1. A. Victor Hoffbrand. Genetic disorders of hemoglobin. Hoffbrand's Essential Haematology Wiley Blackwell Ch 7. 8th ed. 2019.p.78.
2. Kumar, Abbas, Aster. Hematopoietic and lymphoid systems. Basic pathology by Robbins. 9th ed. Saunders Elsvier; 2013.p.413-414.
3. Edourad, Kanit, Philliphe. Current and future alternative therapies for beta-thalassemia major. Biomedical J 2016;39(1):24-38.
4. Adekile A, Sukumaran J, Thomas D, et al. Alpha thalassemia genotypes in Kuwait. BMC Med Genet 2020;21.
5. Yudin J, Verhovsek M. How we diagnose and manage altered oxygen affinity hemoglobin variants. Am J Hematol 2019; 94:597.
6. Gonçalves, B.P., Gupta, Penman, Sickle haemoglobin, haemoglobin C and malaria mortality feedbacks. Malar J 2019;15:26. https://doi.org/10.1186/s12936-015-1077-5
7. R. O. Schneider. Methods for Detection of Hemoglobin Variants and Hemoglobinopathies in the Routine Clinical Laboratory. CRC Critical Reviews in Clin Lab Sci 1978; 9(3):243-271.
8. Santosh Kumar Mondal and Saikat Mandal.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):105–110.
9. Abbasi S, Qadri F, Jawed M, Ujjan ID, Abbasi S, Jawed K. Pattern of Hemoglobinopathies And Thalassemia in Children by Using HPLC At LUMHS Jamshoro Hyderabad Sindh. APMC 2019;13(2):245-8.
10. Khattak MF, Saleelm M. Prevalence of heterozygous beta thalassemia in northern areas of Pakistan. J Pak Med Assoc 1992; 42(2):32-4.
11. Ansari SH, Shamsi TS, Ashraf M. Molecular epidemiology of β-thalassemia in Pakistan: far reaching implications. Int J Mol Epidemiol Genet 2011;2(4):403-408.
12. Khalid N, Noreen K, Qureshi FM, Mahesar M. Knowledge of thalassemia and consanguinity: A multicenter hospital based retrospective cohort study from metropolitan city of Karachi, Pakistan. Professional Med J 2019; 26(9):1580-1586.
13. Shabbir S, Nadeem M, Sattar A. Type and frequency of hemoglobinopathies, diagnosed in the area of Karachi, Pakistan. Cogent Med 2016;3(1):Article: 1188875.
14. Waheed U, Satti HS, Farooq N, Zaheer HA. Frequency of haemoglobinopathies: a single-centre, cross-sectional study from Islamabad, Pakistan 2012;18(12).
15. Torres LS, Okumura JV. Hemoglobin D-Punjab: origin, distribution and laboratory diagnosis.Rev Bras Hematol Hemoter 2015;37(2):120–126.
16. Khalid Khan, Saiqa Zahor. Pattern of hemoglobinopathies on HPLC among patients referred to selected centers in Peshawar, Pakistan. Rawal Med J 2018;43(4):623-626.
17. Hashmi NK, Moiz B, Nusrat M. Chromatographic analysis of Hb S for the diagnosis of various sickle cell disorders in Pakistan. Annals of Hematol 2008;87(8):639-45.
18. Bilawal Ahmed, MD, Kelton R Clements, Jonathan. Optimizing the Economic Impact of Sickle Cell Hospitalization By Effective Care Plans. Blood 2019;134(Supplement_1):1023.
19. Bushra M, Hashmi MR, Sadaf F. Performance evaluation of ion exchange and affinity chromatography for Hb A1c estimation in diabetic patients with Hb D: A study of 129 samples. Clin Biochem 2008;41(14-15):1204-10.
20. Vijay S. Bhat, Kalyan Kumar Dewan, . Patnam. he Diagnosis of α-Thalassaemia: A Case of Hemoglobin H −α3.7 Deletion. Indian J Clin Biochem 2010;25(4):435–440.