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Dr. Samir Bhagora
Postgraduate Resident Doctor, Department of Pathology, B.J. Medical College, Ahmedabad, Gujarat, India

Dr. Vaishali Anand
Postgraduate Resident Doctor, Department of Pathology, B.J. Medical College, Ahmedabad, Gujarat, India

Dr. Hansa Goswami
Postgraduate Resident Doctor, Department of Pathology, B.J. Medical College, Ahmedabad, Gujarat, India

Corresponding Author:
Dr. Samir Bhagora
Postgraduate Resident Doctor, Department of Pathology, B.J. Medical College, Ahmedabad, Gujarat, India

Evaluation of hemoglobinopathies in microcytic hypochromic anemia cases

Dr. Samir Bhagora, Dr. Vaishali Anand and Dr. Hansa Goswami

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Abstract
Introduction: Hemoglobinopathies are the group of genetic disorders of hemoglobin in which there is a quantitative or qualitative abnormal production hemoglobin molecule. In India, beta-thalassemia is the most common autosomal recessively inherited monogenic disorder with approximately 30 million carrying the defective gene, with carrier frequency ranging from 3% to 17%.
Aim and Objective
1) Analyze laboratory aspects, namely, hematological profile and HPLC findings of the hemoglobin variants detected in Microcytic hypochromic anemia cases.
2) Discuss problems that faced in diagnosis of hemoglobinopathies in HPLC.
Method Material: 200 samples of microcytic hypochromic anemia patients were collected and analyzed on the Bio-Rad Variant II HPLC system with use of the Variant II β -Thalassemia Short Program Reorder Pack (Bio-Rad Laboratories). An Hb A2/F calibrator and two levels of controls (BIO-RAD) were analyzed at the beginning of each ru The software delivers a printed report showing the chromatogram, with all the hemoglobin fractions eluted. The integrated peaks are assigned to manufacturer-defined "windows" derived from specific retention time (RT).
Observations & Result: Out of 200, 34 cases displayed abnormal hemoglobin fractions on HPLC Majority case (16) of Beta thalassemia and secondly Sickle cell trait are diagnosed. Other Variant of Hemoglobinopathies like Sickle cell disease, Beta Thalassemia major, HbD Punjab trait, HbS/β-thal and HbE/β-thal also diagnosed.
Discussion: Thalassemia being the major concern in this study with 16 case of β-thalassemia trait and 4 cases of β- thalassemia major in our study. Early detection is very important to preventing birth of homozygous thalassemia major child by genetic counseling. In our present study 7 cases of sickle cell trait detected in anemic patients. Detection of sickle cell trait can be helpful in patients of the possible complications and the preventive measures to be taken.

Keywords: Hemoglobinopathies, Microcytic Hypocromic Anemia**,** β-thalassemia trait, β- thalassemia major, sickle cell trait

Introduction
Hemoglobinopathies are the group of genetic disorders of hemoglobin in which there is a quantitative or qualitative abnormal production hemoglobin molecule [1, 2].
The carriers of Hb disorders in the world are estimated to be 269 million. Hemoglobin (Hb) abnormalities are the most frequent genetic disease, affecting approximately 7 per cent of the world population. About 3% of the world's population (150 million people) carry beta-thalassemia genes. In India, beta-thalassemia is the most common autosomal recessively inherited monogenic disorder with approximately 30 million carrying the defective gene, with carrier frequency ranging from 3% to 17% [3].
Sickling syndrome characterized by the presence of HbS which imparts sickle shape to RBCs and decrease O_2 carrying capacity. Homozygous state is most severe form of disease. These disorders, which were mainly confined to certain areas, religions, castes, and tribes, particularly with endogamous norms of marriages [2, 4].

Aim and Objective
1. Analyze laboratory aspects, namely, hematological profile and HPLC findings of the hemoglobin variants detected in Microcytic hypochromic anemia cases.
2. Discuss problems that faced in diagnosis of hemoglobinopathies in HPLC.

Method and Material

In this study, target group is, Anemic patients in Civil hospital, Ahmedabad.2ml EDTA blood sample were collected. All blood samples were run on Sysmex KX 21 cell counter before performing HPLC to obtain the Hb values and red blood cell (RBC) indices the results of Hb, MCV, MCH, MCHC, RBC count RDW was correlated. Then all samples were analyzed on the Bio-Rad Variant II HPLC system with use of the Variant II β -Thalassemia Short Program Reorder Pack (Bio-Rad Laboratories), All samples are mixed by the Variant II sampling station, diluted with the specific hemolyzing/wash buffer, and injected into an assay- 30 specific analytic cartridge. The Variant II dual pumps deliver a programmed buffer gradient of increasing ionic strength to the cartridge, where the hemoglobin fractions are separated based on their ionic interaction with the cartridge material.

An Hb A2/F calibrator and two levels of controls (BIO-RAD) were analyzed at the beginning of each run. The total area acceptable was between 1 and 3 million µ Volt-seconds. Blood transfusion history, Treatment history, and family history was taken. The software delivers a printed report showing the chromatogram, with all the hemoglobin fractions eluted.

The integrated peaks are assigned to manufacturer-defined "windows" derived from specific retention time (RT). This RT is the time that elapses from the sample injection to the apex of the elution peak, of normal hemoglobin fraction and common variants. The "windows" are established ranges in which common variants have been observed to elute using the variant beta-thalassemia short program [5].

Observations & Result

Total of 200 cases of Microcytic hypochromic anemia were studied. Out of these, 34 cases displayed abnormal hemoglobin fractions on HPLC show in Table 2. And its values of hematological parameters in each of these cases is given in Table 3.

Table 1: Hemoglobin pattern in study object.[1]

Hemoglobin pattern	No. of cases(%)n=200
Normal Hb	166 (83%)
β-Thal trait	16 (8%)
β-Thal major	4 (2%)
HbS/β-thal	1 (0.5%)
HbE/β-thal	1 (0.5%)
HbD-Punjab trait	1 (0.5%)
HbS trait	7 (3.5%)
Homo Sickle (HbSS)	4 (2%)

Table 2: Abnormal hemoglobin fractions on HPLC.

Presumptive value of HPLC	Hb F%	Hb A2%	Variant %
β-Thal trait	0.8-1.3	4.6-5.5	
β-Thal major	4.8-5.6	48-88	
HbS/β-thal	15.2	5.1	62.6
HbE/β-thal	69.7	24.4	
HbD-Punjab trait	0.9	0.8	37
HbS trait	1.4-5.2	2.1-3.2	26.5-39.2
Homo Sickle (HbSS)	14.2-20.5	1.2-4.2	66.2-75.9

Table 3: Hematological parameters

	Hb (g/dl)	MCV (fl)	MCH (pg)	MCHC (%)	RDW-CV (%)
β-Thal trait	8.2-11.2	60-79	18.1-26.4	29.1-30.5	14.1-18.2
β-Thal major	5.7-6.1	64-67	18.3-20.7	28.3-32.3	28.9-29.3
HbS/β-thal	8.5	64.6	21.9	33.9	18.9
HbE/ β-thal	4.8	52	15.5	30	24.4
HbD-Punjab trait	11.3	73	23.9	32	15.4
HbS trait	7.1-11.9	62-78	22.7-25.6	32.2	16.8-17.2
Homo Sickle (HbSS)	7.6-11.9	71-80	23.2-26.2	31.6-32.4	14.2-17.9

Normal sample shows Hb A > 82%, Hb F is <2 %, Hb A2 is 2-4%, P2 and P3 acceptable up to 6%. The major abnormality observed in thalassemia cases was high Hb A2%. A cut of over 3.9% was taken for diagnosis of β Thalassemia Trait.[6] A total of 16 (8%) of β Thalassemia trait were diagnosed with HbA2 is > 3.9% and HbF is < 2% it shows Hb between 8.2-11.2mg% and MCV between 60-79 fl with majority cases show hypochromic microcytic picture with high RBC count. 4 cases (2%) of β-Thalassemia major shows Hb A2% between 48-88 and show microcytic hypochromic anemia in majority cases with Hb ranging from 5.7-6.1. High RDW range show marked anis poikilocytosis. There is 2 type of double heterozygous variant in one case (0.5%) of Hb S and β- Thalassemia shows 62.6% variant Hb s window, 15.2% Hb F with Hb A2 5.1% and Another case (0.5%) of Hb E and β- Thalassemia shows 69.5% Hb F,24.4% Hb A2 and shows more anemic with Hb 4.2 and more RDW 24.4%.

Hb D Punjab trait showing almost no clinical significance and showing 37% variant D window. 7 case (3.5%) of Sickle cell trait and 4 case (2%) of homozygous sickle noted and both types have almost similar hemogram indices and differentiated by HPLC. Here Sickle cell trait cases 33.8-39.2% variant Hb s window. Sickle cell trait cases 33.8-39.2% variant Hb s window. Homozygous HbS 66.2-72.1% variant Hb s window.

Discussion

Microcytic hypochromic anemia can be occur due to multiple factors like Nutritional deficiency, Hemorrhagic condition or Hereditary factors such as hemoglobinopathies. [7, 8]. We attempted this study to evaluate hemoglobinopathies using HPLC and hemogram indices in Anemia Patients. The identification of Hb variants by conventional techniques are often presumptive [9].

HPLC offers the distinct advantage over classic Hb electrophoresis as it can more accurately identify and quantitate abnormal Hb variants. HPLC has been shown to be rapid, sensitive, specific, and reproducible alternative to conventional Hb electrophoresis [10].

Table 4: Prevalence comparison with various study.

Prevalence	Our study	Sachdev *et al.* [10].	Mondal *et al.* [11].	Philip *et al.* [4].
Hb variants	17%	12.5%	11.43%	15.8%
β -Thal trait	8%	4.05%	4.29%	4.8%
HbS trait	3.5%	0.48%	0.46%	1.24%

In Table no 4 shows prevalence comparison of Hb variant, β- thalassemia trait, HbS trait with various study. Our target population is anemic patients so prevalence is slightly higher in our study.

Thalassemia being the major concern in this study with 16 case of β-thalassemia trait and 4 cases of β- thalassemia major in our study. In the screening for classical β - thalassemia trait, the hallmark is the presence of an elevated level of HbA_2 [12]. Early detection is very important to preventing birth of homozygous thalassemia major child by genetic counseling. There are several studies regarding the impact of iron deficiency on HbA2 level and controversy over its significance in screening of beta thalassemia trait [9, 13, 14]. In such an iron deficiency case of they may be silent carrier so we do parental study in high HbA2 cases.

In our present study 7 cases of sickle cell trait detected in anemic patients. Detection of sickle cell trait can be helpful in patients of the possible complications and the preventive measures to be taken. Prenatal or early post-natal diagnosis of sickle cell disease helps in institution of prompt therapy before the onset of serious complications of the disease and also prevent birth of sickle cell disease child [2, 4].

Double heterozygous state of Hb E and Beta thalassemia presented as thalassemia major. In our study one such patient was detected with severe anemia (Hb-4.2 gm/dl).Another Double heterozygous state of Hb S and Beta thalassemia presented moderate anemia with some signs of sickle cell disease, which are usually less frequent and less severe than those of pure sickle cell disease.

1 case of HbD Punjab heterozygous presented without significant clinical symptoms. But association with HbS and Thalassemia causes significant clinical symptoms.

It is a common practice among clinicians that to give iron therapy in all anaemic patients. Identification of Hb variants is very important so we can prevent unnecessary iron overload in this patient. Premarital screening is still not always done in India so best approach is done the screening of patient presented in hospital OPD and antenatal population. Counselling of positive cases about their nature of disease and can affects their children is mandatory to prevent the birth of homozygous inheritance.

Conclusion

To conclude, RBC indices, HPLC finding, and family study are sufficient to detect and manage most of the hemoglobin variants prevalent in this country. However, one has to be aware of the limitations and problems associated with the diagnostic methods to avoid false negative diagnosis in day-to-day practice. Genetic studies are indicated to confirm borderline cases and to detect silent carriers of beta thalassemia, alpha thalassemia, and rare and novel variants in routine practice. The present study conducted using HPLC reflects the magnitude of thalassemia and hemoglobinopathies in a small hospital-based population which may be in fact the tip of an iceberg, but this type of study can definitely help to increase awareness among both health care givers and general population.

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