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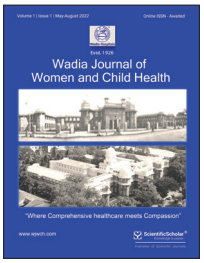

Original Article

Is complete blood count sufficient to rule out hemoglobinopathy carrier state in females visiting antenatal care clinic: A study at one center

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ABSTRACT

Objectives: Anemia due to hemoglobinopathies is one of the most common inherited disorders in the world, which is caused by the dysfunction of hemoglobin (Hb) molecules and can cause severe morbidity and mortality. It is necessary to screen all pregnant women for carrier state during routine antenatal care (ANC) check-up.

Material and Methods: This prospective observational study conducted over a period of 6 months, from February to August 2024, on pregnant women visiting our ANC clinic. Forty females were diagnosed abnormal hemoglobins on high-performance liquid chromatography (HPLC) during the study period were included.

Results: Twenty five (62%) women had the beta-thalassemia trait, whereas the remaining 18%, 7.5%, 7.5%, and 5% were heterozygous for HbE, HbS, delta-beta-thalassemia, or hereditary persistence of fetal hemoglobin, and HbD Punjab. Most of these females (57.5%), in their carrier state, had normal complete blood count (CBC) and peripheral smear (PS) findings.

Conclusion: This study contributes to our understanding of the prevalence of several heterozygous hemoglobinopathies in females of reproductive age, many of them showed normal CBC and PS findings. This, in turn, helps us to find out prevalence of as carrier state in ANC centers by routine HPLC testing done on women all irrespective of CBC and PS findings. After diagnosing carrier state, further workup to prevent hemoglobinopathies in the next generation was planned.

Keywords: High-performance liquid chromatography, Peripheral smear examination, Complete blood count

INTRODUCTION

In India, anemia is seen as a severe public health concern that can affect people of any age. Hemoglobinopathies are a prevalent category of hereditary illnesses worldwide. A class of diseases known as hemoglobinopathies alters the hemoglobin (Hb) molecule's structure, which, in turn, impacts its functionality.[1]

A heme molecule is attached to each of the two polypeptide chains that make up the tetrameric protein known as Hb. Mutations or deletions in the genes encoding the alpha (α) and beta

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(β) globin chains of the human Hb molecule result in hemoglobinopathies, which are monogenic disorders.[2,3]

Thalassemias fall under the category of autosomal recessive disorder and various gene mutations are frequently seen in a wide geographic span around the world. Numerous research conducted in India have revealed that certain communities, such as the Sindhis, Kutchhi Bhanushalis, and Punjabis, have higher rates of β-thalassemia.[4]

The α gene cluster consists of α and tau and is located on chromosome 16. Mutations cause serious variants such as HbH illness. Likewise, the β gene clusters consist of β, delta, gamma, and epsilon and are located on chromosome 11. When combined with HbE or HbD-Punjab, β thalassemia can be heterozygous or homozygous due to deletions in the β globin gene. The outcome of point mutations in sickle cell disease is the same. A fatal Hb Bart's hydrops fetalis occurs due to deletion of all four genes.[5,6]

Herrick first described a few peculiar, elongated, and sickle-shaped red corpuscles in a case of severe anemia, in 1910. In 1949, Pauling *et al*. have the first to identify sickle cell disease molecularly and demonstrate it electrophoretically.[6,7]

Thalassemia was first identified by Cooley and Lee in 1925, as a syndrome with chronic, progressive anemia, peripheral blood erythroblastosis, splenomegaly, and presence of affected family members.[8]

Peripheral smears (PSs) are performed when low Hb and aberrant red blood cell (RBC) indices are observed, in addition to routine complete blood count (CBC) testing. However, other confirmatory tests such as electrophoresis and liquid chromatography are used when these tests are not conclusive in determining the cause of anemia. PSs, RBC indices, and CBC all support the need for further evaluation for abnormal Hbs.

The basic, extremely sensitive, and precise method known as high-performance liquid chromatography (HPLC) aids in the quick identification of different forms of Hb.[8]

These aforementioned techniques help in identifying and taking appropriate preventable measures to decrease transfusion dependent anemia in next generation.

MATERIAL AND METHODS

We conducted a prospective study for a period of 6 months, from February 2024 to August 2024, on females who came to our hospital for antenatal care and had abnormal Hbs diagnosed on HPLC. A blood sample was collected in a vacutainer containing ethylenediaminetetraacetic acid and processed within 24 h of collection. This sample was first processed for CBC and PSs and then analyzed in the Bio-Rad D-10 Dual Program. The values of RBCs and their various parameters were then analyzed, and a diagnosis was made for hemoglobinopathy.

PSs were then made, stained, and examined. Cases in which sickle cells were seen, a manual sickling test was performed, which showed the presence of sickle cells, on incubating the sample mixed with sodium meta-bi-sulfate.

Mindray BC 6200, the machine that is used in our laboratory for the calculation of various cellular elements in blood is a 7-part fully automated hematology analyzer. It works on the principle of electrical impedance, wherein the electrical resistance is calculated and is produced by a particle suspended in a conductive diluent, as it passes through an aperture of known dimensions. Hb is measured using the principle of the colorimetric/ sodium lauryl sulphate (SLS) method.

The Bio-Rad D-10 Dual Program machine is based on the chromatographic separation of analytes by the ion exchange HPLC. The samples are automatically diluted on the D-10 and injected into the analytical cartridge. The D-10 delivers a programmed buffer gradient of increasing ionic strength to the cartridge, where various Hbs are separated based on their ionic interaction with cartridge material. The separated Hb then passes through the flow cell of the filter photometer, where absorbance at 415 nm is measured. The data collected are then transferred using the software, and graphs are formed. The retention time varies for different Hbs.

RESULTS

The abnormal Hbs in our study include, cases where the possibility of α thalassemia could not be ruled out, β-thalassemia trait, delta-β-thalassemia heterozygous, hereditary persistence of fetal hemoglobin (HPFH) heterozygous, HbS heterozygous, HbE heterozygous, and HbD Punjab heterozygous [Figure 1].

The hemoglobinopathies that were least prevalent in this study were HbD Punjab, of which we encountered only two cases.

Out of 40 cases, 62% (25 cases) had Beta thalassemia trait, while 18% (7 cases), 7.5% (3 cases), 7.5% (3 cases) and 5% (2 cases) of females had HbE heterozygous, HbS heterozygous,

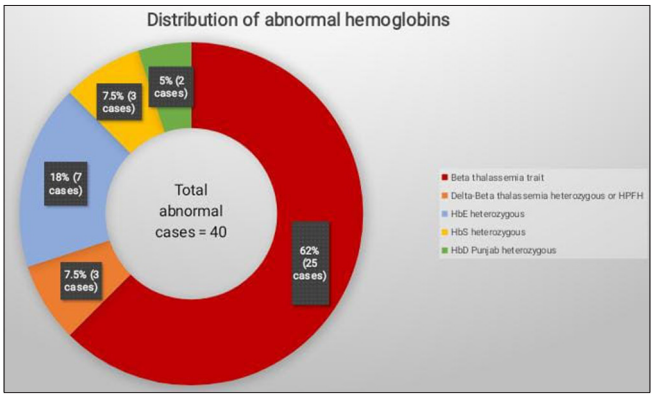

Figure 1: Distribution of abnormal hemoglobins.

Delta-Beta thalassemia heterozygous or HPFH heterozygous and HbD Punjab heterozygous respectively [Figure 1].

The cases with S-window were confirmed with a manual sickling test.

The PSs of various abnormal Hbs showed polychromasia and anisopoikilocytosis of RBCs, most common being target cells and schistocytes.

Not all abnormal hemoglobins had a microcytic hypochromic morphology in the PS; these include HbD Punjab heterozygous, HbE heterozygous, and HbS heterozygous, as only a few among these showed a microcytic hypochromic pattern, while delta-β-thalassemia heterozygous and HPFH had normocytic normochromic cells as seen on CBC and PS.

Among the 40 cases with abnormal Hbs, only 17 cases (42.5%) were indicated for hemoglobinopathy analysis, based on RBC indices in CBC, as opposed to the remaining 23 cases (57.5%), in which the RBC indices and CBC were within normal range. The Mentzer index, which is usually used to diagnose Beta thalassemia, based on RBC parameters in CBC, such as mean corpuscular volume and total RBC count, was only indicative in 16 cases out of the 25 cases (64%) [Figure 2]. Thus, indicating that females with normal CBC and PS findings, having abnormal Hb, could be missed, and further workup should be done to prevent major hemoglobinopathies in newborns.

Husband's screening was advised in all the cases with abnormal Hbs.

DISCUSSION

Inherited hemoglobinopathies, which range from near-normal Hb to severe transfusion-dependent hemoglobinopathies, are common in the Indian population. Common Hb disorders in India include sickle cell anemia, HbE, HbD Punjab, and β-thalassemia.[8]

Hemoglobinopathy carriers may present unique challenges for women who are or plan to become pregnant. This has a major effect on the fetus and the mother's pregnancy outcomes. To avoid inheritance in the future offspring, practitioners must successfully identify and manage these illnesses. If the approach relies on RBC indices and is followed, if necessary, by HPLC testing, some carrier states cases may go unnoticed.[6]

Few studies in other parts of the world have been done that are comparable to ours [Table 1].[8-11]

In a related study, 423 expectant females were evaluated in Thailand in 2003 by Sanchaisuriya *et al.* HbE thalassemia heterozygous was the most prevalent kind of defective Hb, seen in 62.4% of the female participants in this study. Two modified tests were used on the females: the modified dichlorophenol-indophenol test and the modified osmotic fragility test. The Hb patterns in the females with probable hemoglobinopathies were then identified using an automated high-performance liquid chromatography analyzer in conjunction with α- and β-globin gene polymerase chain reaction analysis.[9]

In 2007, a comparable study was carried out in Lao. A modified dichlorophenol-indophenol test, the osmotic fragility test, and HPLC were used to screen 307 pregnant women for hemoglobinopathies and thalassemia. The results of this study were likewise in line with the earlier study carried out in Thailand, which indicated that the most prevalent hemoglobinopathy was heterozygous HbE thalassemia.[10]

In 2008, Sachdev *et al.* conducted an 8-month study in North India. Hemoglobinopathies were investigated in

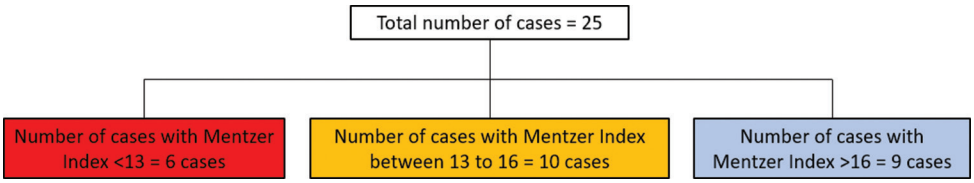

Figure 2: Beta-thalassemia trait distribution based on Mentzer index.

Table 1: Studies done globally on various hemoglobinopathies.

Sr No.	Studies	No. of cases	Normal cases (%)	Abnormal cases (%)	Most common hemoglobinopathy
1	Sanchaisuriya K, *et al.* Thailand[9]	423	37.6	62.4	HbE Thalassemia heterozygous (22.2%)
2	Savongsy O, *et al.* Lao[10]	307	50.2	49.8	HbE Thalassemia heterozygous (30.2%)
3	Sachdev R, *et al.* New Delhi, India[11]	2600	91.1	8.9	Beta thalassemia intermedia and major (1%)
4	Shankar R, *et al.* Jharkhand, India[8]	200	40	60	Sickle cell disease (17%)
5	Our study, Mumbai, India	600	78	22	Beta thalassemia trait (4%)

these 2600 patients. Three hundred and twenty-seven of the patients in total had aberrant Hbs on HPLC. With 15 cases of β thalassemia major and 16 cases of thalassemia intermedia, β-thalassemia was the most prevalent abnormality. HbD-Punjab heterozygous, HbE variation (HbE homozygous, HbE heterozygous, and HbE – β-thalassemia trait double heterozygous), double heterozygous for Hb Q India and β-thalassemia trait, HbS homozygous, HbD-Iran, and HbJ are among the various abnormal Hbs.[11]

A study was carried out in Jharkhand, India, between 2013 and 2014 by Shankar *et al*. involved 200 participants who underwent HPLC testing for hemoglobinopathies. Both men and women were involved in the study. The majority of the participants in our study had normal Hb levels. The results showed that sickle cell disease and sickle-β-thalassemia were the most common hemoglobinopathies, respectively.[8]

CONCLUSION

This study shows that many times abnormal Hb carrier individuals do not always manifest with abnormal parameters of RBC indices on CBC. Majority of cases in our study had normal RBC indices and PS findings, thus not requiring any further investigations for detection of abnormal Hbs. This may in turn result in many individuals of the next generation being affected with either major or minor hemoglobinopathies. Hence, CBC, PS findings, and HPLC together can prevent and reduce the load of abnormal hemoglobinopathies in the next generation.

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Ethical approval

Since no patient information has been shared, Ethics Committee approval is not required.

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent.

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Nil.

Conflicts of interest

There are no conflicts of interest.

Use of artificial intelligence (AI)-assisted technology for manuscript preparation

The authors confirm that there was no use of artificial intelligence (AI)-assisted technology for assisting in the writing or editing of the manuscript and no images were manipulated using AI.

REFERENCES

1. Chauhan A, Prasad M. Outcome of pregnancy with hemoglobinopathy in a tertiary care center. J Obstet Gynecol India 2018;68:394-9.
2. Rappaport VJ, Velazquez M, Williams K. Hemoglobinopathies in pregnancy. Obstet Gynecol Clin North Am 2004;31: 287-317.
3. Salazar EA, Ivo ML, Félix de Freitas SL, Figueiredo MS, de Souza Gerk MA, Nunes CB, *et al*. Pregnancy complications and perinatal outcomes of women with hemoglobinopathies. Int Arch Med Sect Hematol 2016;9(348). doi: 10.3823/2219.
4. Greer JP, Rodgers GM, Glader B, Arber DA, Means RT, List AF, *et al*. Wintrobe's clinical hematology. 14th ed. China: Wolter Kluwer; 2019.
5. Hoppe CC. Prenatal and newborn screening for hemoglobinopathies. Int J Lab Hematol 2013;35:297-305.
6. Dayyabu AL. Hemoglobinopathies in perinatal medicine: Challenges in management. Donald Sch J Ultrasound Obstet Gynecol 2022;16:222-37.
7. Pauling L, Itano HA, Singer SJ, Wells IC. Sickle cell anemia, a molecular disease. Science 1949;110:543-548.
8. Shankar R, Prasad KN, Banerjee BC. Role of HPLC (High Performance Liquid Chromatography) as a tool for detection of hemoglobinopathies. IOSR J Dent Med Sci 2019;18:1-6.
9. Sanchaisuriya K, Fucharoen S, Fucharoen G, Ratanasiri T, Sanchaisuriya P, Changtrakul Y, *et al*. A reliable screening protocol for thalassemia and hemoglobinopathies in pregnancy. Am J Clin Pathol 2005;123:113-8.
10. Savongsy O, Fucharoen S, Fucharoen G, Sanchaisuriya K, Sae-Ung N. Thalassemia and hemoglobinopathies in pregnant Lao women: Carrier screening, prevalence and molecular basis. Ann Hematol 2008;87:647-54.
11. Sachdev R, Dam AR, Tyagi G. Detection of Hb variants and hemoglobinopathies in Indian population using HPLC: Report of 2600 cases. Indian J Pathol Microbiol 2010;53:57-62.