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HIGH-PERFORMANCE LIQUID CHROMATOGRAPGY(HPLC) AS A TOOL TO DIAGNOSE HEMOGLOBINOPATHIES IN MICROCYTIC HYPOCHROMIC ANAEMIA

Pathology

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ABSTRACT

Hemoglobinopathies are the most common genetically inherited disorders. Cation exchange high-performance liquid chromatography (CE-HPLC) is one of the best methods for screening, detection, and identification of various hemoglobinopathies which provides rapid, reproducible, and precise results. It has the advantage of quantifying HbF and HbA2 along with Hb variant screening in single and highly reproducible system.The study was conducted to determine the prevalence of haemoglobinopathies in patients with microcytic hypochromic anaemia and to assess the suitability of HPLC routinely for screening antenatal cases & patients with anaemia.This prospective study was conducted in the Department of Pathology, Government Medical College, Amritsar from June 2020 to May 2021 on 200 females of 15 to 45 years of age with microcytic hypochromic anaemia. Out of 200 patients, 13 (6.5%) were found to have hemoglobinopathies. The remaining 187 cases (93.5%) didn't have any hemoglobinopathy. Out of 13 cases with hemoglobinopathy, 10 (5%) cases were found to have β-thalassemia trait, 2 (1%) cases had HPFH (Hereditary Persistence of Fetal Hemoglobin) and only one (0.5%) case had HbD. It was concluded that RBC indices, HPLC finding, and family study were sufficient to detect and manage most of the haemoglobin 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.

KEYWORDS

Hemoglobinopathies, HPLC, thalassemia , RBC indices, microcytic hypochromic anaemia

INTRODUCTION

Hemoglobinopathies are the most common genetically inherited disorders. The data of World Health Organization (WHO) estimate that approximately 5% of world's population are being carriers for the genetic hemoglobin (Hb) disorders. Every year, there are over 42 million carriers and more than 12,000 infants born with a major and clinically significant hemoglobinopathy. In India, the cumulative gene frequency of hemoglobinopathies is around 4.2%. Worldwide migration of human population, relatively higher frequency of consanguineous marriages in many of the high frequency countries, has equally contributed for the increased burden of hemoglo binopathies.[1]

Cation exchange high-performance liquid chromatography (CE-HPLC) is one of the best methods for screening, detection, and identification of various hemoglobinopathies which provides rapid, reproducible, and precise results. It has the advantage of quantifying HbF and HbA2 along with Hb variant screening in single and highly reproducible system. The simplicity of the automated system with internal sample preparation, superior resolution, rapid assay time, and accurate quantification of Hb fractions establishes CE-HPLC as an ideal methodology for routine clinical laboratory. Definite identification of mutations, specifically in case of rare variants of hemoglobins, can be achieved by molecular methodologies.[2]

The basic principle of CE-HPLC involves passing the analyte of interest at a high pressure through a cylindrical column packed with small spherical particles (typically 5 µm diameter silica gel, called the stationary phase). Different hemoglobins adsorb onto the silica packing with different intensities based on their ionic interactions. T Different hemoglobins elute out with the perfusing buffer at different but characteristic time points in response to the continually changing salt gradient.[3]

The genetic disorders of hemoglobin, particularly the β-thalassemias are a considerable health problem in India and contribute significantly to morbidity and mortality. Identification of these disorders is immensely important epidemiologically and they can be prevented by population screening. In various parts of India, the prevalence of β-thalassemia is different: 6.5% in Punjab, 8.4% in Tamilnadu, 4.3% in south India, and 3.5% in Bengal. β-thalassemia has a high prevalence in some communities, such as Sindhi, Luvana, Tribes, and Rajputs. In the studies from north India, major groups of thalassemics from Uttar Pradesh (UP) are the migrant ethnic populations of Punjab and Sindh origin.[4]

The present study was conducted with the aim to detect hemoglo binopathies using High Performance Liquid Chromatography (HPLC) in microcytic hypochromic anaemia in reproductive age group females (15 years -45 years).

MATERIALS AND METHOD:

This prospective study was conducted in the Department of Pathology, Government Medical College, Amritsar from June 2020 to May 2021.

Inclusion Criteria:

- Patients not responding to conventional treatment and with a clinical suspicion of hemaoglobinopathy were included in the present study.
- **Age:** 15 yrs – 45 yrs females
- **Sex:** Females
- Anaemia with haemoglobin less than:
- Peripheral blood picture – microcytic hypochromic
- MCV less than 83 fL
- MCH less than 27 picogram

Exclusion Criteria:

- Non co-operative patients.
- Age: subject below 15 yrs and above 45 years
- Other than mirocytic hypocromic anaemia.
- Confirmed cases of iron deficiency anaemia.
- Patients with Anaemia of chronic disorders.
- Subject who received transfusion within 3 months
- Subject who received iron therapy

All anaemic patients after fulfilling inclusion and exclusion criteria were recruited for the study after taking their informed consent. Detailed history was taken.

RESULTS

- The mean age of study groups was 26.92 ± 6.30 years. There were 51 (25.5%) ante-natal cases and 149 (74.5%) were microcytic hypochromic anemia non-ANC cases.
- Out of 200 patients, 13 (6.5%) were found to have hemoglo binopathies. The remaining 187 cases (93.5%) didn't have any hemoglobinopathy.

- The mean Hb of cases was 8.79 ± 1.25 g/dl. The mean TLC was 6439 ± 2075.04 cells/cumm. The mean MCV and MCH was 74.85 ± 3.82 fl and 22.13 ± 2.80 pg respectively. The mean RDW (%) was 16.52 ± 1.64. The mean HbA2 (%) and HbF (%) was 3.24 ± 5.69 and 0.71 ± 1.81 respectively.
- There were total 51 antenatal cases in the study, out of which 48 cases did not have any hemoglobinopathy. The remaining 3 cases (1.5%) were found to have β-thalassemia trait.
- The mean Hb of pregnant cases with hemoglobinopathies was 8.83 ± 1.15 g/dl. The mean TLC was 4333.33 ± 1026.32 cells/cumm. The mean MCV and MCH was 75.80 ± 7.80 fl and 21.77 ± 4.11 pg respectively. The mean RDW (%) was 15.53 ± 3.95. The mean HbA2 (%) and HbF (%) was 5.33 ± 0.25 and 2.57 ± 2.37 respectively.
- The mean Hb of pregnant cases without hemoglobinopathies was 8.66 ± 1.37 g/dl. The mean TLC was 6183.33 ± 1963.10 cells/cumm. The mean MCV and MCH was 75.19 ± 3.94 fl and 21.16 ± 2.68 pg respectively. The mean RDW (%) was 16.52 ± 1.60. The mean HbA2 (%) and HbF (%) was 2.79 ± 0.52 and 0.47 ± 0.21 respectively.
- There were total 149 non-pregnant cases in the study, out of which 139 cases did not have any hemoglobinopathy. The remaining 10 cases comprised of 7 (3.5%) cases of β-thalassemia trait, 2 (1%) cases of HPFH and 1 (0.5%) case of HbD.
- The mean Hb of non- pregnant cases was 8.81 ± 1.25 g/dl. The mean TLC was 6563.76 ± 2103.51 cells/cumm. The mean MCV and MCH was 74.72 ± 3.72 fl and 22.45 ± 2.76 pg respectively. The mean RDW (%) was 16.54 ± 1.61. The mean HbA2 (%) and HbF (%) was 3.34 ± 6.58 and 0.75 ± 2.06 respectively.
- The mean age of patients with and without hemoglobinopathy was found to be 25.54 ± 4.79 and 27.02 ± 6.39 years respectively. The mean age of patients with β-thalassemia trait, HPFH and HbD was found to be 26.10 ± 6.39, 25.50 ± 6.36 and 20.00 respectively.
- The mean Hb of cases with hemoglobinopathies was 8.65 ± 0.99 g/dl. The mean TLC was 5130.77 ± 1360.99 cells/cumm. The mean MCV and MCH was 75.31 ± 5.24 fl and 22.48 ± 3.21 pg respectively. The mean RDW (%) was 15.80 ± 2.12. The mean HbA2 (%) and HbF (%) was 10.03 ± 21.91 and 3.98 ± 6.41 respectively.
- The mean Hb of cases without hemoglobinopathies was 8.80 ± 1.26 g/dl. The mean TLC was 6529.95 ± 2087.90 cells/cumm. The mean MCV and MCH was 74.82 ± 3.72 fl and 22.11 ± 2.78 pg respectively. The mean RDW (%) was 16.57 ± 1.60. The mean HbA2 (%) and HbF (%) was 2.77 ± 0.50 and 0.48 ± 0.24 respectively.
- The mean Hb of cases with thalassemia trait was 8.57 ± 1.07 g/dl. The mean TLC was 5210.00 ± 1544.49 cells/cumm. The mean MCV and MCH was 74.30 ± 5.21 fl and 22.18 ± 3.25 pg respectively. The mean RDW (%) was 15.76 ± 2.20. The mean HbA2 (%) and HbF (%) was 4.37 ± 1.35 and 1.47 ± 1.51 respectively.
- The mean Hb of cases with HPFH was 9.00 ± 1.13 g/dl. The mean TLC was 5150.00 ± 70.71 cells/cumm. The mean MCV and MCH was 81.25 ± 1.06 fl and 25.50 ± 0.71 pg respectively. The mean RDW (%) was 16.40 ± 2.97. The mean HbA2 (%) and HbF (%) was 1.95 ± 0.07 and 18.00 ± 2.83 respectively.
- The Hb of case with HbD was 8.70 g/dl. The TLC was 4300 cells/cumm. The mean MCV and MCH was 73.5 fl and 19.5 pg respectively. The RDW (%) was 15. The HbA2 (%) and HbF (%) was 82.8 and 1.00 respectively.
- The mean Hb of pregnant cases was 8.83 ± 1.15 g/dl. The mean TLC was 4333.33 ± 1026.32 cells/cumm. The mean MCV and MCH was 75.80 ± 7.80 fl and 21.77 ± 4.11 pg respectively. The mean RDW (%) was 15.53 ± 1.58. The mean HbA2 (%) and HbF (%) was 5.33 ± 0.25 and 2.57 ± 2.37 respectively.
- The mean Hb of non- pregnant cases was 8.59 ± 1.00 g/dl. The mean TLC was 5370.00 ± 1400.04 cells/cumm. The mean MCV and MCH was 75.16 ± 4.79 fl and 22.70 ± 3.12 pg respectively. The mean RDW (%) was 15.88 ± 1.58. The mean HbA2 (%) and HbF (%) was 11.44 ± 25.11 and 4.40 ± 7.26 respectively.

DISCUSSION

In the present study, out of 200 cases, the majority of them, i.e. 93 (46.5%) were in the age group 25-34 years, followed by 81 cases (40.5%) who belonged to age group of 15-24 years and remaining 26 cases (13%) were of age between 35-44 years with a mean age of 26.92 ± 6.30 years. In the study conducted by Chowdhury AR et al[5] to determine prevalence of hemoglobinopathies in antenatal mothers and role of HPLC in Eastern India, the mean age was found to be 21.06 years.

In the present study, there were 51 (25.5%) ante-natal cases and 149 (74.5%) were microcytic hypochromic anemia non-ANC cases. Out of 200, 13 cases were found to have hemoglobinopathies and thus present study showed a prevalence of 6.5%. Out of 13 cases with hemoglobinopathies, 10 (5%) cases were found to have β-thalassemia trait, 2 (1%) cases had HPFH (Hereditary Persistence of Foetal Hemoglobin) and only one (0.5%) case had HbD.

In the present study, the total prevalence of Beta Thalassemia Trait was found to be 5% and in antenatal cases, it was found to be 5.9%(3 out of 51 cases) which is comparable with the findings of Sur D et al.[6]

HPFH was first documented in Ghana and has also been described in non-African populations.[7] Warghade S et al[2] reported 0.15% cases of HPFH in their study. The present study reported 1% case of HPFH.

HbD Punjab is the fourth most common haemoglobin variant worldwide. In the study by Bhavani D et al[8], three cases of HbD were observed with HbA2 ranging between 2.2 to 2.7% with normal HbF.The present study showed only 0.5% cases with HbD.

The automated HPLC system is intended for separation and determination of area percentage for HbA2 and HbF and for qualitative detection of abnormal hemoglobins. With the integration of proper algorithms involving retention time, % Hb, and peak characteristics, a clinical laboratory is capable of identifying 75% of the common variants encountered without the need for confirmatory studies such as alkaline and acid electrophoresis. In the light of family study, ethnicity and clinical data, we can detect most of the frequently occurring clinically significant variants by HPLC method alone.[9]

The patients with Beta Thalassemia Trait in our study showed mean Hb of 8.57 ± 1.07 g/dl. In studies by Philip J et al[10] and Baruah MK et al[11], mean Hb was found to be 9.8±2.4 and 7.9±3.4 g/dl respectively. In both these studies value of MCV was less than 80 fl and MCH was less than 27 pg which is similar to our study. Thus along with detailed peripheral blood picture study, values of hemoglobin and RBC indices also help in timely detection of beta thalassemia trait.

About 1.1% of couples around the world are at risk for having children with a haemoglobin disorder, of which 2.7 per 1000 conceptions are actually affected. Hemoglobin disorders contribute to 3.4% of mortality in children aged less than five years globally.[10] Detection of asymptomatic carriers by reliable laboratory methods is the cornerstone of prevention of this serious health problem. CE-HPLC has become the preferred technique suitable in Indian scenario, as it can detect most of the clinically significant variants. With the timely screening of antenatal mothers and proper information, education and counselling, the burden of hemoglobinopathies can be reduces in our country and worldwide.

CONCLUSION

The present study highlights the prevalence of hemoglobinopathies in patients with microcytic hypochromic anemia. This 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. RBC indices, HPLC finding, and family study are sufficient to detect and manage most of the haemoglobin 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 thalassemia and rare variants in routine practice.

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