Original Article

Analysis of role of red blood cell indices and high performance liquid chromatography in early diagnosis of hemoglobinopathies in pregnant women

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Hemoglobin consists of a globin protein and iron porphyrin complex ham. Oxygen carrying is the lead function of hemoglobin. Hemoglobinopathies are inherited as autosomal recessive disorder. Hemoglobinopathies impose a potentially serious threat to mother as well as fetus in antenatal cases. Life threatening complications such as pyelonephritis, haematuria and thrombophlebitis are seen throughout pregnancy. Hemoglobin level estimation, General blood picture examination, Red cell indices calculation using electronic cell counter High Performance Liquid Chromatography, Immunological assays, Structural analysis, Cellulose acetate electrophoresis are various investigations to diagnose hemlobinopathies. Among these HPLC (High Performance Liquid Chromatography) is the gold standard to estimate hemoglobin variant. This study is intended to analyse the role of BC indices in early screening of hemoglobinopathies and their early confirmation with HPLC so as to avoid and manage poor outcome as early as possible.

KEY WORDS: Red Cell indices, HPLC, Hemoglobinopathies

INTRODUCTION

Hemoglobin is made up of a protein: Globin and an iron porphyrin complex: Haem. There are two pairs of globin chains attached with haem group. Oxygen carrying is the leading function of hemoglobin. There are more than a thousand variants of hemoglobin. Alterations in the structure of hemoglobin is brought about by point mutation and less commonly by shortening or lengthening of globin chain. Hemoglobinopathies are a group of recessively inherited blood disorders characterized by reduced synthesis of normal globin chain or synthesis of structurally abnormal globin chain.[1] They are inherited as autosomal recessive disorder. The common ones are sickle cell anemia and thalassemia. Other examples include sickle cell trait, HbE trait, and HbC trait. Hemoglobinopathies are the most common single gene disorder worldwide.[2,3] Across the world, hemoglobinopathies are more common in Europe, Middle East, Africa, and Asia. Global estimates suggest that hemoglobinopathies run in 7% of the world population.[4] Beta-thalassemia is more common in Punjabis, Sindhis, Bengalis, Gujratis, Bhanushalis, and Jains; alpha-thalassemia is reported to be higher among tribal groups. The sickle cell anemia is common in certain tribes of Madhya Pradesh, Maharashtra, Orissa, Gujarat, and Kerala.[5-11] Hemoglobinopathies impose a potentially serious

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threat to mother as well as fetus in antenatal condition.[12] About 1.1% of couples are at risk for having children with hemoglobin disorder.[13] These disorders lead to complications such as pain, infection, multiorgan failure, stroke, pulmonary, and renal hypertension. This study is conducted to screen the cases and type of hemoglobinopathies in pregnant women using red blood cell (RBC) indices and high-performance liquid chromatography (HPLC) technique.

MATERIALS AND METHODS

A total of 120 pregnant women attending the obstetrics and gynaecology outpatient department in Rohilkhand medical college, Bareilly, were screened during the period of November 1st– October 31st, 2021, after taking Institutional Ethical Committee clearance. The cases to be studied included all the primi gravida antenatal cases, females who recently had miscarriage/still birth, females for fertility testing, and females referred by any obstetrician/midwife to rule out hemoglobinopathies. After taking informed consent, blood sample was collected in EDTA vial using disposable needles and syringes from each individual. Complete blood count and high performance liquid chromatography were carried out using Sysmex transasia xs 1000i and Biorad D 10 analyser, respectively. A sample report and chromatogram were generated for each sample. Interpretations were made. Normal range for HbA_2 was taken as ≤3.5% and HbF ≤0.8%. All samples with HbA_2 level between 4 and 9% and HbF between 1 and 5% were labeled as beta-thalassemia trait whereas those with HbA_2 level 20–40% and normal HbF level were considered to be HbE trait. Cases with Mentzer Index <13 were considered to be suggestive of beta-thalassemia were as those with more than 13 were suggestive to be cases of iron deficiency anemia.

Statistical Analysis

The data were imported in Statistical Package for the Social Science version 23.0 software where means and standard deviation were calculated. $P \leq 0.005$ was considered statistically significant.

RESULTS

Out of 120 study, participants 19 (15.8%) were found to have hemoglobinopathies whereas rest 101 were found to have normal hemoglobin. Out of the 19 cases, which comprised 15.8% of the total participants in our study, 18 (94.73%) were those of beta-thalassemia trait whereas 1 case (0.05%) was that of HbE trait. This study showed a prevalence of 15.8% of hemoglobinopathies in the study population. The most common age group of cases in the study was 28–30 years. This indexed study depicted that mean HbA_1 in hemoglobinopathies was 87.5 which was slightly lower than those in normal subjects, that is, 92.3. All the cases had a HbA_2 level more than 4. Those ones with beta-thalassemia trait had HbA_2 between the range of 4–9% while the only case with HbE trait was having HbA_2 more than 9%. The HbF level in maximum number of study participants (72.5%) was <0.8%. Moreover, out of the total 19 cases of hemoglobinopathies, 13 had HbF level more than 0.8%. In this indexed study, it was also concluded that the mean corpuscular volume (MCV) value among cases was 65.1 fL whereas the mean MCV in negative cases was much higher, that is, 83.0 fL. Similarly, the mean corpuscular hemoglobin (MCH) value among cases was <21.5 pg which was less than that in negative cases where the mean MCH was 26.2 pg. In this indexed study, the mean RBC count among the cases was 3.58 million per mm^3 which was almost equal to that in negative cases, that is, 3.59 million per mm^3. In this indexed study, the mean red cell distribution width (RDW) value among cases was 18.46 which was slightly greater than that in negative cases which showed mean RDW value 17.11.

DISCUSSION

Hemoglobinopathies are one of the most severe form of genetic disorder and hence are of great importance to be dealt with from the public health point of view. The study conducted by Swaroop *et al*.[14] in 2019 concluded that the prevalence of hemoglobinopathies in their study population was 4.1% and the most common cases were those of beta-thalassemia, that is, 92.85% of the total cases found to have hemoglobinopathies. The study conducted by Sawaimul *et al*.[15] in 2018 concluded 4% of their participants to exhibit hemoglobinopathies and maximum number of cases were of beta-thalassemia. Our study showed that the prevalence of hemoglobinopathies was much higher, that is, 15.8% but the most common cases were those of beta-thalassemia. George *et al*.[16] in 2000 reported that subjects with hemoglobinopathies showed HbA_2 level in females between 4.5 and 7.3%. Our study also showed HbA_2 between the range of 4–9%. Sawaimul *et al*.[15] in a study in 2018 framed that HbF levels were more than 1% in cases of beta-thalassemia. Similarly, in our study, 13 out of 19 positive cases had HbF level more than 0.8%. This study showed a strong association of HbA_1, HbA_2, HbF and Mentzer Index with hemoglobinopathies in pregnant women as the *P* value in all these parameters was <0.005. In a study conducted by Chowdhury and Talukdar[17] et at in 2018, it was found out that beta-thalassemia cases had a mean MCV of 69.79. The study conducted by George *et al*.[16] in a study in the year 2000 reported MCH value <27 pg in beta-thalassemia cases. In general practice, Mentzer index is very oftenly used to differentiate between iron deficiency anemia and thalassemia; but in our study, only five cases showed Mentzer index value <13. Findings revealed in a study conducted by Mediratta *et al*.[18] in 2015 reported RBC value ranging between 3.9 and 5.32 million/mm^3 in the subjects having this disorder. The study conducted by Chowdhury and Talukdar[17] in 2018 showed an average RDW of 14.93. This study showed a strong association of MCV, MCH, and RBC count with hemoglobinopathies in pregnant women as the *P*-value in these parameters was <0.005.

In our study, we aimed pregnant mothers as our target study population for early screening of this disorder and hence aid in proper management in diagnosed cases. Hemoglobin level estimation, red blood cell indices evaluation, and HPLC can help in screening of the population at risk by this disorder and hence,

directing toward further management of the diagnosed subjects. The approach is safe, cost-effective, and easily available.

CONCLUSION

This present study concluded that beta-thalassemia trait and HbE are the most prevalent hemoglobinopathies in this region. Complete blood count and HPLC test can easily tell about this disorder. The present study showed the significant differences in RBC indices between beta-thalassemia trait and normal subjects can help in picking out positive cases from the normal population. However, late registration and refusal for the prenatal diagnosis are the main hurdles in the screening of hemoglobinopathies.

REFERENCES

1. Bain BJ. Haemoglobinopathy diagnosis: Algorithms, lessons and pitfalls. Blood Rev 2011;25:205-13.
2. Henderson S, Timbs A, McCarthy J, Gallienne A, Van Mourik M, Masters G, *et al*. Incidence of haemoglobinopathies in various populations-the impact of immigration. Clin Biochem 2009;42:1745-56.
3. Modell B, Boulyzhenkov V. Distribution and control of some genetic disorders. World Health Stat Q 1988;41:209-18.
4. Tan YL, Kidson-Gerber G. Antenatal haemoglobinopathy screening in Australia. Med J Aust 2016;204:226-30.
5. Colah RB, Mukherjee MB, Martin S, Ghosh K. Sickle cell disease in tribal populations in India. Indian J Med Res 2015;141:509-15.
6. Kaur M, Das GP, Verma IC. Sickle cell trait & disease among tribal communities in Orissa, Madhya Pradesh & Kerala. Indian J Med Res 1997;105:111-6.
7. Balgir RS. The burden of haemoglobinopathies in India and challenges ahead. Curr Sci 2000;79:1536-47.
8. Balgir RS. The genetic burden of haemoglobinopathies with special reference to community health in India and the challenges ahead. Indian J Hematol Blood Transfus 2002;20:2-7.
9. Balgir RS. Spectrum of hemoglobinopathies in the state of Orissa, India: A ten years cohort study. J Assoc Physicians India 2005;53:1021-6.
10. Verma IC, Saxena R, Thomas E, Jain PK. Regional distribution of β-thalassemia mutations in India. Hum Genet 1997;100:109-13.
11. Choudhury V, Kotwal J, Saxena R. Thalassemia screening and control programme. Pediatr Today. 1998;1:283-9.
12. Greer JP, Arber DA, Glader B, List AF, Means RT, Paraskevas F, *et al*. Wintrobes's Clinical Hematol. 12th ed., Vol. 37. PA, USA: Lippincott Williams and Wilkins; 2012. p. 1058.
13. Modell B, Darlison M. Global epidemiology of haemoglobin disorders and derived service indicators. Bull World Health Organ 2008;86:480-7.
14. Swaroop N, Laspal P, Seth S, Verma V. Prevalence of thalassemia in antenatal patients in a rural tertiary care centre of western Uttar Pradesh. Indian J Obstet Gynecol Res 2019;6:469-71.
15. Sawaimul K D, Iqbal M B, Sawaimul V D, Kambale T, Hanmante R. Study to identify the role of high performance liquid chromatography in detecting haemoglobinopathies in antenatal patients. Indian J Pathol Oncol 2018;5:6-11.
16. George E, Jamal AR, Khalid F, Osman KA. High performance liquid chromatography (HPLC) as a screening tool for classical Beta-thalassaemia trait in Malaysia. Malays J Med Sci 2021;8:40-6.
17. Chowdhury A, Talukdar M. Prevalence of thalassemia and hemoglobinopathy in antenatal mothers with relation to complete hemogram and high performance liquid chromatography-a hospital based study of Eastern India. Int J Res in Med Sci 2018;6:928-30.
18. Mendiratta SL, Bajaj S, Popli S, Singh S. Screening of women in the antenatal period for thalassemia carrier status: Comparison of NESTROFT, red cell indices and HPLC analysis. J Fetal Med 2015;2:21-5.