International Journal of Research in Medical Sciences
Chowdhury AR et al. Int J Res Med Sci. 2018 Mar;6(3):928-931
www.msjonline.org
pISSN 2320-6071 | eISSN 2320-6012
DOI: http://dx.doi.org/10.18203/2320-6012.ijrms20180617

Original Research Article

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

Anadi Roy Chowdhury, Manas Talukdar*

Department of Pathology, R. G. Kar Medical College, Kolkata, West Bengal, India

Received: 02 January 2018
Accepted: 03 February 2018

***Correspondence:**
Dr. Manas Talukdar,
E-mail: talukdarmanas09@gmail.com

ABSTRACT

Background: Iron deficiency anemia (IDA) and Beta thalassemia (BT) are two most common causes of microcytic hypochromic anemia in our country affecting the reproductive age group. It is important to discriminate between these two entities to prevent treatment with iron of individuals with thalassemia trait as well as prevent homozygous transmission of B thalassemia trait (BTT). Aim of the study was to investigate causes of microcytic anemia in antenatal mothers and to find out the role of Cell Counter and High Performance Liquid Chromatography (HPLC) so as to screen BTT and other hemoglobinopathies.
Methods: This study was done over a period of six months (May 2017 to October 2017) in the Department of Pathology in R. G. Kar Medical College. We analyzed the blood samples of all antenatal mothers attending Department of Pathology for blood tests and a complete hemogram and hemoglobin A2 (Hb A2) quantitation was done.
Results: Total cases evaluated were 2200 of which 442 patients were found to have microcytic hypochromic anemia (MCV<80%, MCH<27). Rest that is 1758 was normal. Of 442 cases of microcytic hypochromic anemia, 205 were found to have IDA, 115 BTT, 112 E trait, 1 case each of Hemoglobin E disease, E-Beta thalassemia and hereditary persistence of fetal hemoglobin (HPFH). Hemoglobinopathies like S trait and Hemoglobin J (Hb J) was found in 4 and 3 cases respectively.
Conclusions: In India, Microcytic hypochromic anemia is common and may be due to IDA, BTT or other hemoglobinopathies Cell counter-based parameters and formulas, along with HPLC can be an effective method of thalassemia screening in a society.

Keywords: Antenatal mother, Beta thalassemia trait, Iron deficiency anemia, Other hemoglobinopathies

INTRODUCTION

Iron deficiency anemia (IDA) and Beta thalassemia (BT) are two most common causes of microcytic hypochromic anemia in our country affecting the reproductive age group in large numbers. It is important to discriminate between these two entities to prevent treatment with iron of individuals with thalassemia trait as well as prevent homozygous transmission of B thalassemia trait (BTT). Thalassemia is a genetic disorder affecting synthesis of the globin chain of the hemoglobin molecule. BT is prevalent in a broad belt extending from Mediterranean basin to Southeast Asia. The south East Asian region including Indian subcontinent accounts for about 50% of the world's carriers. The prevalence of BTT is about 3.3% in India which varies in different parts of the country: 6.5% in Punjab, 8.4% in Tamil Nadu, 4.3% in south India, and 3.5% in Bengal.[1] In Bengal, general population has a prevalence of 3.6% (males) and 5.95% in antenatal mothers.

The appropriate measure to screen for alpha and beta thalassemia remains mean cell hemoglobin (<27pg) or mean corpuscular volume (<80fl). Pregnancy however induces macrocytosis which makes screening with the mean cell volume (MCV) difficult in antenatal patients. A hemoglobin pattern and iron profile should follow if the red cell indices are low. In a population where alpha thalassemia is prevalent, it is advisable to check the partner's mean cell hemoglobin (MCH) or mean corpuscular volume (MCV) as well. Further cascades of investigations will depend on these results and the prevalence of other hemoglobinopathies in that population. Invasive prenatal diagnosis remains the gold standard for diagnosis in high-risk couples. It is important to implement universal antenatal screening for thalassemia carriers in populations with a high prevalence of this condition. The only possible way to stop transmission of BTT is by proper counseling and screening the target population that is antenatal mothers.

This study aims to do so by studying automated analyzer-based blood parameters and measurement of HbA2 in antenatal mothers in our hospital.

METHODS

This study was done over a period of six months (May, 2017 to October, 2017) in the department of Pathology in R. G. Kar Medical College, one of the major tertiary care hospitals in West Bengal. We analyzed the blood samples of all antenatal mothers attending Department of Pathology for blood examination. About 3ml blood was collected in Tri potassium EDTA (K3-EDTA) vials and a complete hemogram was obtained for all samples using three-part automated cell counter (SYSMEX KX-21, Sysmex Corporation, Kobe, Japan). Diagnosis of anemia was made if Hb <11g/dl and microcytosis was considered by measuring MCV (less than 80fl).[2] Samples were run in automated cell counter within four hours of collection to avoid any changes in MCV that may occur on keeping the samples for long in K3-EDTA. The degree of change in MCV observed from second day onward may be considered less than desirable, particularly when the results are borderline normal or abnormal.[3]

Hemoglobin A2 (HbA2) quantitation was done by High Performance Liquid Chromatography (HPLC) on an automated system using beta thal short (BTS) programme (Bio Rad Variant, Bio-Rad Laboratories, CA). Diagnosis of BTT was made based on HbA2 levels more than 3.7%. Serum ferritin was estimated when necessary by chemiluminiscence method. Diagnosis of IDA was made based on plasma ferritin values lower than 15ng/ml.

RESULTS

Total cases evaluated were 2200 of which 442 patients were found to have microcytic hypochromic anemia (MCV<80%, MCH<27). Rest that is 1758 was normal. The mean age was 21.06 years having mean hemoglobin 10.9gm%. Of 442 cases of microcytic hypochromic anemia, 205 were found to have IDA, 115 BTT, 112(5.09%) E trait, 1 case each of Hemoglobin E disease, E-Beta thalassemia and hereditary persistence of fetal hemoglobin (HPFH). Hemoglobinopathies like S trait and Hemoglobin J (Hb J) was found in 4 and 3 cases respectively. (Table 1) Thus prevalence of BTT came out to be 5.23% while IDA was found to be 9.32%. Of 205 cases of IDA average age of presentation was 21.06 years, with mean hemoglobin level of 8 g%. The average age of presentation in case of BTT was 20.5 years, having a mean hemoglobin level of 9.36gm%.

Table 1: Distribution of different cases among study population.

Total number of case=2200 (100%)									
Non-anemic =1758 (79.91%)	**Microcytic, hypochromic anemia = 442 (20.09%)**								
	Name of condition	**IDA**	**BTT**	**E-trait**	**HbE**	**E β**	**HbS**	**Hb J**	**HPFH**
	Number of cases (%)	205 (9.32)	115 (5.23)	112 (5.1)	1 (0.04)	1 (0.04)	4 (0.18)	3 (0.14)	1 (0.04)

Mean MCV in BTT was 69.79 which in case of IDA were 72, Red Cell Distribution Width (RDW) in BTT came out to be 17.57 while in IDA it was 20.58. Average Hb A2 in BTT was 5 and Hb F level was found to be 1.25.

Average age of 112 Hb E trait cases was 20.37, mean hemoglobin 10.34g%, MCV and RDW being 78.39and 14.93 respectively. MCV and RDW was almost near cut off values, however, Hb A2 level was 27.44 which was higher than BTT level and Hb F 0.93 helped to make the diagnosis of E trait. There was 1 case each of E disease, E beta thalassemia, HPHF. lowest mean hemoglobin level recorded was 3.8 in case of E-Beta thalassemia.

Severity of anemia was maximum in case of E-Beta thalassemia than other thalassemias or any other hemoglobinopathy as denoted by MCV 56.7, MCH 16.5, MCHC 29, and RDW 28. Hb A2 came out to be 59.5 and Hb F 33.6. helped to make the diagnosis of E disease.

Other hemoglobinopathies encountered were S trait and Hb J disease.

DISCUSSION

In India, anemia remains one of the most common morbidity amongst antenatal mothers which if not managed properly can lead to maternal death also. Microcytic hypochromic anemia is common and may be due to IDA, BTT or other hemoglobinopathies. This study investigates causes of microcytic anemia in antenatal mothers so as to screen BTT and other hemoglobinopathies. This will not only help in identifying the cause of anemia but will also provide right treatment and counseling to the mothers. This goes a long way in maintaining a thalassemia free society. In several studies, red blood cells are described as being microcytic when the mean corpuscular volume is less than 80 fl.[4] MCV measurement by cell counter is direct, rapid, inexpensive, and automated. The prevalence of microcytosis in this study was 20.09% amongst antenatal mothers which was predominantly due to IDA (9.32%), followed by BTT (5.23%) and E trait (5.1%). Hb E Trait is mainly restricted to West Bengal and other North eastern states having a prevalence of 5% in Bengali population which is almost similar as what we got in this study.[5]

As Sur et al prevalence of BTT came out to be 5.22% in antenatal mothers, though we did not consider their ethnicity, religion and literacy status. Hence the need to control these hemoglobinopathies is substantial and an integrated approach is required keeping in mind the heterogeneity of our country.

Plasma ferritin has been used to confirm IDA in this study because it is independent of external contamination of blood samples, diurnal variation, and concurrent iron therapy in those cases of microcytosis which showed high RDW, low total RBC count.[6] Even though plasma ferritin is an acute phase reactant that can be elevated in various inflammatory conditions, as this study group comprised of healthy antenatal population, the probability of inflammation was negligible. Cut off for ferritin was chosen to be 15ng/ml was used in this study, as suggested by Susan F Clark (2008).[7] Chemiluminescence method for ferritin estimation was chosen because of its sensitivity.

High Performance Liquid Chromatography was used for quantization of HbA2 because of the simplicity of sample preparation, superior resolution, and accuracy, combined with complete automation of the method.[8] Diagnosis of BTT was based on levels of HbA2 greater than 3.7%. Reduction of HbA2 because of coincident iron deficiency did not preclude detection of BTT.[9] However, in India Vitamin B12 and folate deficiency being common Hb A2 level was interpreted with caution. In the present study, elevated RBC count, mild anemia, mildly raised RDW was indicative of BTT.

Based on these findings and limited infrastructure we can suggest the following algorithm- routine screening of all blood samples using automated cell counter, followed by HPLC of those samples showing microcytosis. Those samples which did not show any evidence of thalassemia or any other hemoglobinopathy were subjected to serum ferritin estimation for conclusive detection of IDA. An American study by Pearson et al used 79 fl as the initial screening tool.[10] Their algorithm similar to present study suggests doing HbA2 electrophoresis on all microcytic samples and serum iron for all non-BTT samples. However, we recommend doing plasma ferritin and HPLC for IDA and BTT diagnosis, respectively, which are newer and more accurate tests available, as compared to serum iron and electrophoresis used by Pearson et al. There are other studies who have followed a reverse order of tests: HPLC is performed only for non-IDA samples, with ferritin levels higher than 15ng/ml.[11] HPLC being done as a part of national program our suggested algorithm can be more cost effective.

There has been lot of discussion regarding the most appropriate target population. Though premarital counseling has been proposed to be effective target population, it may not be successful in many cases due to social issues. We suggest targeting antenatal mothers during their antenatal visits preferably during first trimester. Counseling of the couples is beneficial at this time as they are more receptive; however, problems of dropout, not attending antenatal clinics may hamper the process.

CONCLUSION

From the present study, we concluded that automated cell counter–based parameters and formulas are technically good, rapid, cheaper, easily available, and reliable methods for BTT detection. Cell counter–based parameters and formulas, along with HPLC can be an effective method of thalassemia screening in a society like ours where there is high prevalence of thalassemia and other hemoglobinopathies. We aimed at screening of antenatal mothers which is a cost-effective method of detection of BTT instead of mass screening of general population. By following this simple algorithm, targeting the antenatal mothers and proper information, education and counseling we can not only dream of a thalassemia free society but also decrease the economic burden.

Funding: No funding sources
Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee

REFERENCES

1. Sur D, Mukhopadhyay SP. Prevalence of thalassaemia trait in the state of West Bengal. J Ind Med Assoc. 2006;104(1):11-5.

2. Nathan DG, Orkin SH, Look AT, Ginsburg D. Nathan and Oski's Hematology of Infancy and Childhood. 6th ed. Philadelphia, Pa: Saunders;2003.
3. Killip S, Bennett JM, Chambers. Iron deficiency anemia. American Family Physician. 2008;78:914.
4. Lau YL, Chan LC, Chan YY, Ha SY, Yeung CY, Waye JS, et al. Prevalence and genotypes of α and β thalassemia carriers in hong kong-implications for population screening. N Eng J Med. 1997;336:1298-301.
5. Das MK, Dey B,Roy M ET AL. High prevalence of Hb E in 3 populations of The Malda district,West Bengal ,India. Hum Hered. 1991;41:84-8.
6. Massey AC. Microcytic anemia. Differential diagnosis and management of iron deficiency anemia. Med Clin North Am. 1992;76:549-66.
7. Clark SF. Iron Deficiency Anemia. Nutrition in Clinical Practice. 2008;23:128-41.
8. Madan N, Sikka M, Sharma S, Rusia U. Frequency of coincident iron deficiency and beta-thalssemia trait. Clin Pathol. 1996;49:1021-2.
9. Mittal R, Marwaha N, Basu S, Mohan H, Ravikumar A. Evaluation of iron stores in blood donors by serum ferritin. Ind J Med Res. 2006;124:641-6.
10. Pearson HA, O Brien RT, Mcintosh S. Screening for Thalessemia trait by electronic measurement of MCV. N Engl J Med. 1973;288:351.
11. Kiss TL, Ali MA, Levine M, Lafferty JD. An algorithm to aid in the investigation of thalassemia trait in multicultural populations. Arch Pathol Lab Med. 2000;124:1320-3.

Cite this article as: Chowdhury AR, 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 Med Sci 2018;6:928-31.