Hemoglobin Electrophoresis as a Diagnostic Tool in Anaemias

Santhosh SR*, Sampath S+

ABSTRACT

Inherited abnormalities of hemoglobin synthesis include the disorders ranging from thalassemia syndromes to structurally abnormal hemoglobin variants. Identification of these disorders is immensely important during armed force recruitment, screening of families with history of hemoglobinopathies and for patient management. Hemoglobin electrophoresis is a test that is performed to quantify the different types of hemoglobins present. The automated hemoglobin electrophoresis system was acquired and installed at IAM under the ISRO Human Space Programme in July 2010. We have analyzed all the Hb electrophoresis runs done in our Dept since installation. A total of 183 cases were analyzed in the present study and 31 fresh cases of Beta-thalassemia trait (βTT) were detected.The details of the categories of samples analyzed and the results of electrophoresis are discussed in the paper. The aero medical importance of detection of hemoglobinopathies in flyers/ candidates is discussed.

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Key words: Thalassemia syndromes, Hemoglobinopathies, Hemoglobin electrophoresis, Beta-thalassemia trait.

Introduction

Hemoglobin electrophoresis is a technique which can differentiate and quantitate different types of hemoglobin. It uses the principles of gel electrophoresis to separate out the various types of hemoglobin. The test can detect abnormal levels of HbS, the form associated with sickle-cell disease as well as other abnormal hemoglobin-related blood disorders, such as hemoglobin C. It can also be used to determine whether there is a deficiency of any normal form of hemoglobin, as in thalassemias. Different hemoglobins have different charges, and according to those charges and the amount, hemoglobins move at different speeds in the gel whether in alkaline gel or acid gel.

India is an ethnically diverse country and all the ethnic population are well represented in our armed forces personnel and their families. There is a variation in the prevalence of hemoglobinopathies in different regions and population groups in the country. The frequency of beta-thalassemia trait (βTT) has variously been reported from <1% to 17% and an overall of 3.3% is stated for India (1). A high frequency of Hb D has been reported from the North in the Punjabi population, Hb E in the eastern region of India and Hb S mainly reported from population of tribal origin from different parts of the country(2). Antenatal screening of pregnant ladies with anaemia helps in timely detection of disease or helps in counseling in carrier state. The technique also has extended role in screening families with positive cases.

The automated hemoglobin electrophoresis system was acquired and installed under the ISRO Human Space Programme in July 2010. The present study was taken up with the objective to analyze all the samples coming for Hb electrophoresis in our Dept since installation.

Materials and Methods

The Hb electrophoresis was done on the

* *Sc C, Dept of Aviation Pathology, IAM, IAF, Bangalore*

\+ *Classified Specialist Path & Bio-chem, Dept of Aviation Pathology, IAM, IAF, Bangalore*

SEMI-automated electrophoresis system from Alere (Helena Biosciences, Europe). Samples were received from CHAFB (Obs & Gyne, Medical and Pediatrics wards and OPDs) and from MEC, IAM. Samples were sent in EDTA along with clinical details. As soon as the samples were received, basic counts- Hb TLC, DLC, MCV, MCH, MCHC, and PBS were done. Hemolysate of the sample was prepared and the Hb of the hemolysate was adjusted to a Hb between 2-3 gm/dl. Very small quantity (35 ul) of samples of hemolysates prepared from whole blood are applied to sample tray, the applicator automatically applies the samples to the ready to use Alkaline Hb Gel (Agarose in a Tris/ EDTA/ Glycine buffer with Sodium Azide). The hemoglobins in the sample were separated by electrophoresis (200 volts, 30 minutes) using an alkaline buffer (pH 8.2-8.6), and were stained with Acid Blue Stain. The patterns were scanned on a scanning densitometer, and the relative percent of each band was determined and interpreted as per the manufactures instructions.

All the results of Hb electrophoresis done at IAM from Jul10 to Aug 2011 were analysed for this study.

Results

A total of 183 samples were processed in the span of 13 months. The details of the samples processed are tabulated in table 1. 110 cases of anaemias included 65 males and 45 females. The mean Hb levels in males and females were 11.2 and 9.15 g/dl respectively. Most of the cases referred had a PBS of a microcytic hypochromic (92%), followed by normocytic normochromic picture. The mean MCV in the microcytic hypochromic cases was 68 fL. All the above cases were screened for iron deficiency diseases (Serum iron, TIBC and serum ferretin levels).

Table 1. The details of the categories of sample processed in the study *

Sl No	Categories of sample	Number of samples
1	Anaemias (Other than ANC)	110
2	Antenatal screening of anaemia in pregnancy	43
3	Screening of families	14
4	Old diagnosed cases of Hemo-globinopathies from MEC	16
	Total	183

Of the 110 cases of anaemias screened, 37 cases were referred from MEC, IAM for medicals with low Hb levels (<13gm% in males and <11.5gm% in female). This included both existing flyers as well as candidates for recruitment into both civil and military population. These 37 cases consisted of 23 cases (15 candidates for recruitment into armed forces and 8 trained aircrew) from service and 14 from civil aircrew. There were 4 fresh cases of thallasemia traits (3 male & 1 female) and 1 case of elevated Hb S/D/G. All positive cases were all found in candidates for recruitment into Air Force. The 4 thallasemia traits diagnosed had a mean Hb of 10.8 gm/dl.

The results of different types of hemoglobinopathies diagnosed in each category are tabulated in table 2-4. The results of Hb electrophoresis in the 110 cases of anaemias are as given in table 2.

Table 2. Hemoglobin electrophoresis of anaemia cases

Hb Electrophoresis Result	No of cases
Normal	86
Beta-thalassemia trait (âTT)	22(4 candidates ,18 from hospital referrals)
Elevated Hb S/D/G	2 (1 candidate,1 from hospital referral)
Elevated Hb E/C	0

Among the two cases of elevated Hb S/D/G levels, one was 45 year old male Gp Capt with Hb of 12.2% showed decreased HbA1 levels (63.78%) and a band at S/D/G region (31.35%). This was further confirmed by HPLC as HbD. Another was a 21 year old male candidate who came to MEC for recruitment with Hb12.4% shows decreased HbA1 levels (61.34%) and a band at S/D/G region (36.35%).

There were 43 cases of pregnant ladies with anaemias referred from gynecology OPD and ward of CHAFB (Table 3). The mean Hb was 9.14 g/dl and the PBS picture was microcytic hypochromic (75%), followed by normocytic normochromic type (25%). The mean MCV in the microcytic hypochromic cases was 69fL.

Table 3: The results of Hb electrophoresis in ANC cases

Hb Electrophoresis Result	No of cases
Normal	36
Beta-thalassemia trait (âTT)	4
Elevated Hb S/D/G	1
Elevated Hb E/C	2

Around 14 cases with a family history of hemoglobinopathies were also screened in the present studies, the results of Hb electrophoresis is shown in table 4.

Table 4: The results of Hb electrophoresis in cases with a family history of hemoglobinopathies

Hb Electrophoresis result	No of cases
Normal	6
Beta-thalassemia trait (βTT)	5
Elevated Hb S/D/G	1
Elevated Hb E/C	2

Among the 2 cases of elevated Hb E/C levels, one was 26 year old (ANC with jaundice) with Hb-10.8% showed a band at A2 region (72%). The other was 23 year old case with Hb- 11.4 shows a band at A2 region (95.8%). Both these ladies belonged to Assam. Both the cases were referred for HPLC confirmation. The elevated S/D/G case was 18 year old (ANC with anemia) with Hb-9.7% and showed decreased HbA1 levels (51.64%) and a band at S/D/G region (39.58%). This case was referred for HPLC confirmation and screening for sickle cell trait.

The cases with elevated Hb S/D/G or Hb E/C were referred for HPLC confirmation

The representative electrophoresis gels of normal & each type of hemoglobinopathies and their interpretation are depicted in figures as Figure A to D.

16 old thallasemias cases, who came to MEC for routine medicals, were also included in the present study. These were run as control samples for validating our equipment, the mean HbA2 levels in the old thallasemia cases was 6.3%.

There were a total of 31 fresh cases of thalassemias diagnosed at our centre. The mean Hb was 10.85gm/dl and the mean HbA2 levels in all of them was 6.2%. Confirmation of these cases by HPLC was done in the initial 4 cases and A2 levels correlated with ours.

Discussion

Thallasemia is a quantitative hemoglobinopathy in which there is reduced synthesis of hemoglobin causing microcytic hypochromic anaemia. Depending on which globin chain is deficient, there are four recognized forms, alpha and beta thalassemia, each with a homozygous and heterozygous expression. The clinical manifestations of both vary. The symptoms of both homozygous forms are usually so severe that usually few patients survive into adulthood because of severe anaemia and cardiopulmonary complications. On the other hand heterozygous thallasemia, also

known as thallasemia minor or trait causes only slight anaemia with hemoglobin levels never going below 9g/dL. Furthermore these patients have no symptoms and live normal life spans.

Both á thalassemia trait and â thalassemia trait should be differentiated from iron deficiency anemia, which is the most common cause of hypochromasia and microcytosis. In thalassemia trait, the RBC indices, including the mean corpuscular volume (MCV) and mean corpuscular Hb (MCH) are both significantly low for an Hb level that is either normal or only slightly low. In addition, the RBC distribution width (RDW) is usually normal, reflecting the homogenous population of the RBCs in thallassemia. On the other hand iron deficiency anemia is known to be associated with anisocytosis. The Mentzers index is another useful tool to differentiate the iron deficiency anemia and thalassemia in those setups where electrophoresis is not available .

Hb electrophoresis is a widely used rapid method for investigating anaemias. It identifies some variants of hemoglobins which are well characterized. Some Hb variants can not be separated & quantitation is not very accurate with Hb electrophoresis. High Performance Liquid Chromatography is the gold standard for confirmation & more sensitive method used to characterize rare hemoglobinopathies not well detected with other methods

In β thalassemia trait, elevated levels of Hb A_2 and F (the beta chains being decreased, the alpha chains combine with gamma and delta chains to make Hb F and A2) are usually helpful in confirming the diagnosis. A2 levels however may decrease in iron deficiency anaemia and increased levels may be seen in megaloblastic anaemia. However, in á thallassemia, the Hb electrophoresis results are usually normal; in this case, and in cases in which iron study results are also nondiagnostic, nonspecific tests may help to differentiate iron deficiency anemia or anemia of chronic inflammation from thallassemia. Free erythrocyte protoporphyrin (FEP) levels are usually elevated in patients with iron deficiency or anemia of chronic inflammation but not with thallassemia. The soluble transferrin receptors (sTfR) levels are high in patients with iron deficiency but not in those with anemia of chronic infection or thalassemia. In such cases globin chain electrophoresis and DNA analysis is confirmatory (3).

As most patients with thalassemia trait may never have any symptoms during their life span, treatment is usually not required for this benign hematological disorder. Medical waivers for pilots with minor form of the disorder can be considered favorably as long as the anaemia is minimal and the patient is symptom free (4). U.S. Navy Aeromedical Reference and Waiver Guide section 8.5 also allows flying provided there are no other hemoglobinopathies present and the acceptable values for hematocrit of 40-52% in males and 37-47% in females are maintained. However patients who have required splenectomy because of their thalassemia are permanently disqualified from military flying (5).

The other two hemoglobinopathies which we came across in the present study were elevated Hb S/D/G and elevated Hb E/C.

As per Indian Air Force Publications 4303 [6], cases of thalassemia trait detected first time in aircrew need to be evaluated in detail and observed in Low Medical Category for 12-24 weeks. Aircrew with Hb levels of > 11.5 g/dl (males) and 10.5 g/ dl (females) can be considered for upgradation to medical category A2G2 (P), after a period of observation of 24-48 weeks. Ground duty officers can be upgraded to medical category A4G2 (P) after

a similar observation. However as per IAP 4303 Para 3.13.4 , all candidates with evidence of hereditary haemolytic anemias (due to red cell membrane defects or due to red cell enzyme deficiencies) and hemoglobinopathies (Sickle cell disease, Beta-thalassemia : Major, intermedia, minor, trait, and Alpha thalassemia etc) are to be considered unfit for service. We had four candidates dignosed as thalassemia trait and one candidate with elevated Hb S/D/G level. All candidates were made unfit for recruitment in to flying.

In the present study we had about 31 new cases of Beta-thalassemia trait (âTT) from different population, 4 of them being candidates for aircrew duty. Two antenatal cases with elevated Hb C/E levels were detected in the present study and both of them were residents of Assam where Hb E is common. As per an Indian study gene frequency of Hb D was greater in school children from Delhi (1.1%) than in Mumbai (0.7%). Hb S trait (0.2%) was observed exclusively in children from Mumbai. A low incidence of Hb E trait (0.04%) was seen in children in Mumbai. A higher incidence is reported from the East. (1)

Conclusion

Hb electrophoresis is a versatile tool for investigating anaemias. In our setup we have done only alkaline Hb electrophoresis and therefore cases of Hb C/E and Hb D/G/S were not differentiated. These can be differentiated on Acid Hb electrophoresis or by HPLC. The exact prevalence of hemoglobinopathies in armed forces population is not known. A long term study is recommended to find out the prevalence of hemoglobinopathies in Indian Armed Forces.

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