Spectrum of Haemoglobinopathies in a Tertiary Care Hospital of Armed Forces

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Abstract

Background: Thalassaemia and other structural haemoglobinopathies are the major genetic disorders prevalent in certain parts of the world including India. This study presents the pattern of haemoglobinopathies amongst the referred patients of anaemia in a two-year period.

Methods: A total of 1032 patients were studied during a two-year period for anaemia investigation. Haematological indices, sickling test and haemoglobin electrophoresis with quantification of the bands was done in all cases.

Result: Out of 1032 cases, 774 (75%) were normal and 258 (25%) cases had abnormal haemoglobin pattern. Of the 258 abnormal cases, 136 (53%) were males and 122 (47%) were females. Of all cases of anaemia 370 (36%) were microcytic hypochromic, 237 (23%) macrocytic, 151 (15%) were dimorphic and the rest (26%) had normocytic normochromic picture. 82% of microcytic hypochromic anaemias had reduced serum iron and elevated total iron binding capacity (TIBC), whereas 85% had decreased serum ferritin levels. Spectrum of haemoglobinopathies prevalent were β -Thalassaemia trait (17%), followed by sickle cell trait (2.3%). Other haemoglobinopathies in descending order of frequency were sickle cell disease (1.7%), Hb D trait (1%), Hb E trait (0.8%), sickle cell - β thalassaemia, Hb E disease, E - β thalassaemia (0.6% each) and thalassaemia major (0.4%).

Conclusion: This study provides a comprehensive database on the spectrum of haemoglobinopathies in the Armed Forces. It is suggested that detection of HbA₂ should be carried out in all the high-risk groups with anaemia.

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Key Words : Haemoglobinopathies; Anaemia; Thalassaemia

Introduction

Thalassaemia and other structural haemoglobinopathies are the major genetic disorders prevalent in certain parts of the world including India. The general incidence of thalassaemia trait and sickle cell haemoglobinopathy in India varies between 3-17% and 1-44% respectively [1] but, because of consanguinity, caste and area endogamy, some communities show a very high incidence, making the disease a major public health problem in our country [1,2]. Inherited disorders of haemoglobin synthesis are an important cause of morbidity and mortality worldwide. They place a large burden on the patients, their families and even the community. They can be managed by expensive bone marrow transplantation, which is always not possible in a developing country like ours. Population screening, genetic counselling and prenatal diagnosis can prevent these genetic disorders; as it has been a success in countries like Greece, Cyprus and Italy.

All cases of anaemia referred to Department of

Laboratory Sciences, Army Hospital (R&R), New Delhi were subjected to haemoglobin electrophoresis followed by semi -quantitative analysis of the Hb bands by densitometry. This provided a definitive diagnosis in 25% of all anaemia patients. Affected persons and their families with haemoglobinopathies were offered genetic counselling. This study presents the pattern of haemoglobinopathies amongst the referred patients of anaemia in a two year period.

Material and Methods

A total of 1032 patients were referred during a two year period (2005-06) for anaemia investigation. Two ml of intravenous blood sample was collected from all cases after obtaining informed consent using ethylenediaminetetraacetic acid (EDTA) as anticoagulant in a vacutainer. If any blood transfusion was administered to the patient, the date of last transfusion was 3-4 weeks before the blood sample was taken for analysis. Haemolysate with a concentration of 1.6 g/dl to 2.2 g/dl was prepared for haemoglobin electrophoresis [3]

Haematological indices were measured using Sysmex

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KX- 21 fully automated blood cell counter, which was calibrated with commercially available controls. The sickling test was performed using freshly prepared sodium metabisulphite solution as a reducing agent [4]. Haemoglobin electrophoresis was performed using commercially available Paragon Hb electrophoresis kit (Beckman Coulter, USA) at pH 8.6. This kit provides for the electrophoretic separation of haemoglobins in an alkaline-buffered agarose gel. After electrophoresis, the haemoglobins in the gel are immobilized in a fixative solution and the gel is dried to a film. The electrophoresis pattern is visualized by staining the film with a protein-specific stain. This pattern was then quantified using a densitometer (Beckman Coulter) at 600 nm wavelength [3,5].

A value more than 3.5% of A₂ fraction of haemoglobin was taken as cut off point for determining the β thalassemia trait and more than 10% was assumed to be HbE [6]. Electrophoresis in acidic medium (pH 6.0) was also carried out by using Paragon acid Hb electrophoresis kit (Beckman Coulter) to identify and confirms the presence of HbD, HbS or HbE bands [5]. Family studies were carried out to confirm the diagnosis and carrier status, wherever it was necessary, and the relatives were also included in the analysis.

Results

Out of 1032 cases, 774 (75%) were normal and 258 (25%) cases had abnormal haemoglobin pattern. Of the 258 abnormal cases, 136 (53%) were males and 122 (47%) were females, thus giving slight male preponderance (Table 1). This may be due to the prevalent socio - cultural factors in our society, that more male patients seek medical attention.

The clinical profile of the patients is given in Table 2. Pallor was the most common manifestation and was present in 87% of the patients. Of the 1032 cases of anaemia 370

(36%) were microcytic hypochromic, 237 (23%) macrocytic, 151 (15%) were dimorphic and 261 (26%) had normocytic normochromic picture on blood film study. Haemolytic evidence was seen in 26 cases of microcytic hypochromic anaemia, 14 of macrocytic and 18 of dimorphic anaemias. Of these, 36 were direct Coomb's test positive, suggestive of auto-immune haemolytic anaemia. 82% of microcytic hypochromic anaemias had reduced serum iron and elevated total iron binding capacity (TIBC) (reduced transferritin saturation), whereas 85% had decreased serum ferritin levels (Table 3).

Bone marrow examination was done in 157 cases of macrocytic anaemias. Of these 123 had features of megaloblastic erythropoiesis and the rest had features of hypoplastic anaemia. 34 patients of macrocytic anaemia showed HbA₂ levels marginally higher than 3.5% (range 3.6-4.3%). 27 patients normalized for HbA₂ levels following treatment for megaloblastic anaemia. 56 patients who had reduced ferritin levels and showed borderline HbA₂ levels (3.1-3.5%) were treated with iron and folic acid for three months and then HbA₂ estimation was repeated. 35 of these 56 patients showed HbA₂ levels conforming to β thalassaemia traits, thereby highlighting the importance of concomitant iron deficiency in β thalassaemia trait individuals.

Table 4 gives the spectrum of haemoglobinopathies prevalent in the Armed Forces in the two years period studied. β thalassemia trait is the most common haemoglobinopathy (17%), followed by sickle cell trait (2.3%). Other haemoglobinopathies in descending order of frequency were sickle cell disease (1.7%), Hb D trait (1%), Hb E trait (0.8%), sickle cell β thalassemia (0.6%), Hb E disease, E- β thalassemia (0.6% each) and thalassemia major (0.4%). Haemoglobin abnormalities were detected in both the male and female population. Fig.1 and Fig.2 depicts bands seen on alkaline gel electrophoresis, acid gel electrophoresis and Fig. 3 shows the densitometry scan of the bands in alkaline gel electrophoresis respectively.

Table 1

Age and sex distribution of cases of different haemoglobinopathies

Age groups	Males		Females		Total	
	number	%	number	%	number	%
0-15	50	37	41	34	91	35
16-45	79	58	75	62	154	59
46 years and above	05	05	05	04	13	06
Total	136	100	122	100	258	100

Table 2

Clinical profile (n=1032)

Features	number (%)
Pallor	894 (87)
Koilonychia	12 (01)
Splenomegaly	48 (05)
Hepatomegaly	26 (03)
Angular stomatitis	18 (02)
Glossitis	56 (05)
Haemolytic facies	12 (01)
Bone pain	08 (01)
Leg ulcer	02 (00)

Table 3

Laboratory features (n=1032)

Features	number (%)
1. Peripheral blood smear	
a. Microcytic hypochromic	370 (36)
b. Macrocytic	237 (23)
c. Dimorphic	151 (15)
d. Haemolytic picture	032 (03)
e. Normocytic normochromic	261 (26)
2. Coomb's test	
a. Direct positive	36
b. Indirect positive	54
3. Serum ferritin (n=370)	
a. Low levels	370
4. Serum iron studies (n=370)	
a. Low serum iron and increased TIBC	314 (85)
5. Bone marrow studies (n=157)	
a. Megaloblastic erythropoiesis	123 (78)

Fig 1: Bands seen on alkaline gel electrophoresis. Lane 1, 2, 5 and 6 shows band in the HbA₂, HbC, HbE, HbO region, lane 3 shows band in the Hb S, HbD, HbG region and lanes 4, 7 and 8 shows normal electrophoresis pattern. Lanes 9 (bands seen in HbA₂, C, E, O region, band seen in Hb SDG region and Hb A region) and lane 10 (bands in the Hb F & Hb A region) are controls

Fig 2: Bands seen on acid gel electrophoresis. Lane 1 to 4 and lanes 6 to 8 shows HbA bands. Lane 5 shows HbA, HbS and HbF bands. Lane 9 and 10 are controls

Table 4

Spectrum of haemoglobinopathies (n = 1032)

	Male	Female	Total (n)	Percentage
Normal	430	344	774	75
Sickle cell trait	16	08	24	2.3
Sickle cell disease	12	06	18	1.7
Sickle cell- β thalassaemia	03	03	06	0.6
β Thalassaemia trait	86	90	176	17.0
Thalassaemia major	03	01	04	0.4
Hemoglobin E trait	05	03	08	0.8
Hemoglobin E disease	02	04	06	0.6
Hemoglobin D Trait	06	04	10	1
E- β Thalassaemia	03	03	06	0.6
Total	566	466	1032	100

Discussion

A large number of haemoglobin variants prevalent in the population indicate that haemoglobinopathies are not uncommon amongst our population. The inherited disorders of haemoglobin synthesis are one of the important public health problems in India.

Haemoglobinopathies are the most common disorders of erythrocytes. India is the home of several haemoglobin variants. The most common

Magnification : 110%

Comments:

Hb S+D+G = 79.6% present,

Reference Ranges:

Fraction	Rel%
1	1.5 - 3.5
2	0 - 0
3	0.2 - 2.0
4	95.0 - 98.0

Fig 3: Shows densitometry scan of lane 3 (elevated HbA₂/S/D/G levels) of Fig 1.

haemoglobinopathy in this study was β thalassemia trait followed by sickle cell trait. The patients having β thalassemia trait were from all parts of the country and the patients having sickle cell trait and sickle cell disease were mostly from Central - Eastern India where this disease is prevalent [6,7]. The patients having Hb E trait, Hb E disease and Hb E- β thalassemia were mainly from Eastern - Coastal region and North-East India (West Bengal, Assam, Manipur and Nagaland) were this abnormal Hb E and variants are highly prevalent [6,8,9]. Hb D trait (1%) was seen in patients from Northern part of the country [10]. The incidence of thalassemia major was low (0.4%) and all their families were given genetic counseling. Our data was compared with a study done by Balgir et al [2] (Table 5). The prevalence of sickle cell trait, sickle cell disease and sickle cell - β -thalassaemia are higher in their study, as the study was conducted in Central East India, where the incidence of sickle cell disease is high.

This study provides for the first time a comprehensive database on the spectrum of haemoglobinopathies in the

Table 5

Comparison of prevalence of haemoglobinopathies with existing Indian data

	Our data (%)	Balgir [2] (%)
Sickle cell trait	2.3	29.8
Sickle cell disease	1.7	7.6
Sickle cell- β Thalassemia	0.6	1.7
β Thalassemia trait	17.0	18.2
Thalassemia Major	0.4	5.3
Hemoglobin E trait	0.8	0.9
Hemoglobin E disease	0.6	0.3
Hemoglobin D trait	1	0.2
E- β Thalassemia	0.6	0.7

Armed Forces. The prevention and control of haemoglobinopathies is an uphill task for the planners, policy makers and medical and health care machinery.

It is suggested that detection of HbA₂ should be carried out in all the high-risk groups with anaemia in the Armed Forces. It is also worthwhile to sensitize the troops about the common haemoglobinopathies prevalent in the country and how to prevent them in their children by premarital HbA₂ estimation.

Conflicts of Interest

None identified

Intellectual Contribution of Authors

Study Concept : Brig GS Chopra, SM,VSM, Col V Nair,VSM

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Book Review

Sanjiv Haribhakti, Editor. Clinical G I Surgery. India Ahmedabad: Haribhakti Educational Foundation. 1st Edition. Hard bound. Two Volumes, Pages 1126. Price Rs 2000/-,ISBN No-978-81905797-0-4.

This book fulfils the need for a comprehensive text on surgical gastroenterology. It covers all the important subjects in a clinically relevant manner. It is not only an operative guide but also helps the clinician to understand the disease process, to make correct clinical decisions at relevant times to achieve an optimum outcome.

This book is published in two volumes. It covers all the clinical aspects, with a special section on practice management in GI surgery. This section includes chapters on data management, medicolegal aspects of surgery, health insurance, telemedicine for the surgeon

etc. Moreover this book has elaborately covered the diseases peculiar to the Indian subcontinent i.e. non cirrhotic portal fibrosis, extra hepatic portal venous obstruction, hydatid cysts, liver abscess etc.

The major flaw in the book is the lack of an index which makes navigation through the book a tedious task. However the brilliant compilation of text, along with judicious use of colored photographs and illustrations makes this book a must have for both surgery residents and practicing surgeons alike.

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