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Research Article

EVALUATION OF MICROCYTIC HYPOCHROMIC ANEMIA BY ELECTROPHORESIS FOR HEMOGLOBINOPATHIES IN YOUNG POPULATION

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ABSTRACT

Objectives: Hemoglobinopathies are serious genetic blood disorders requiring lifelong blood transfusions and treatment in its most severe and chronic form. If undiagnosed or untreated, these disorders ultimately lead to death. South Asia especially India, Pakistan, and Bangladesh has high populations of hemoglobinopathies. In India, there are an estimated 100,000 thalassemia majors patients and nearly 3.5 to 4 million carriers of this genetic disorder. Tribal populations in India have a range of 5–40% sickle cell anemia (SCA) sufferers. The aim of the study was to detect hemoglobinopathies by electrophoresis in microcytic hypochromic anemia in young population in the area covered by tertiary care center at Index Medical College and Hospital.

Methods: A cross-sectional study was carried out in a hospital in rural area of Indore on young patients (10–30 years age group) who attended outpatient department from June 2018 to March 2020. Total 517 cases of moderate to severe anemia were included in the study. All cases were subjected to a series of hematological investigations, which include complete hemogram, peripheral blood smear, and some standard procedures. Sysmex XS800i fully automated Cell counter was used for complete blood count. It is the 5-part differential instrument. It reports on all the five subpopulations, namely, neutrophils, eosinophils, basophils, monocytes, and lymphocytes. A blood smear is a blood test used to look for abnormalities in blood cells.

Results: In the study, out of 517 cases 34.4% cases were positive on Hb electrophoresis and 65.6% were negative. On electrophoresis, the number of sickle cell anemia cases was 34.3%, sickle cell trait – 20.8%, sickle beta-thalassemia – 29.8% and beta-thalassemia major – 29.8% was observed. In hemoglobinopathies, of the 73 females, 28.8% were having beta-thalassemia trait, 27.4% were having SCA 23.3% were having sickle beta-thalassemia, and 20.5% were having sickle cell trait. Of the 105 males, 30.5% were having beta-thalassemia trait, 9.5% were having sickle beta-thalassemia, 39.0% were having SCA, and 21.0% were having sickle cell trait.

Conclusion: The ANMs, ASHA, and Anganwadis should be taught about the importance of screening of hemoglobinopathies. So that they can explain the purpose of screening to the female during pregnancy and to the families in the rural areas. At primary level, the CHCs and PHCs should be equipped with instruments for basic testing of hemoglobinopathies.

Keywords: Microcytic, Hypochromic, Anemia, Hemoglobinopathies, Electrophoresis.

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INTRODUCTION

Hemoglobinopathies are serious genetic blood disorders requiring lifelong blood transfusions and treatment in its most severe and chronic form. If undiagnosed or untreated, these disorders ultimately lead to death. South Asia especially India, Pakistan, and Bangladesh has high populations of hemoglobinopathies. In India, there are an estimated 100,000 thalassemia majors patients and nearly 3.5–4 million carriers of this genetic disorder. Tribal populations in India have a range of 5–40% sickle cell anemia (SCA) sufferers. In Eastern India, variant hemoglobinopathies like HBE are as common as 3–50% of the population.

In India, beta-thalassemia is prevalent across the country, with an average frequency of carriers being 3–4% [1-3]. A higher frequency has been observed in certain communities, such as Sindhis, Punjabis, Gujaratis, Bengalis, Mahars, Kolis, Saraswats, Lohanas, and Gaurs [4]. HbS is highly prevalent in the tribal populations of Southern, Central, and Western states reaching as high as 48% in some communities. HbE is common in the North-eastern states and has a carrier frequency as high as 50%, in some areas. It is found in lower frequencies in the Eastern states of West Bengal, Bihar, and Uttar Pradesh, while HbD is present in about 2% of people in Punjab. The first case of β-thalassemia/Hb E disease in India was reported by Chatterjea *et al.* [5] and that of β-thalassemia/sickle cell disease by Nail *et al.* [6] Subsequently, other variants hemoglobins were reported-C, D, F, G, H, J, K, L, M, Q (India), G, etc. [7-11] Hemoglobins S, D, and E were observed to be quite common: Hb S has been found mostly in tribal communities, Hb D in Gujaratis and Punjabis and Hb E in Bengalis, Assamese and Nepalese. Sickle Cell Disease (SCD) is another hemoglobin disorder that requires lifelong management and contributes to infant and childhood morbidity and mortality. SCD is caused by inheritance of two abnormal HbS genes, one from each parent or Hb S gene from one parent and HbE or ù thalassemia gene from the other. Sickle cell syndromes include (SCD, HbSS), also called SCA, as well as disorders due to sickle cell gene combined with another hemoglobinopathy such as Hb C, E, or beta-thalassemia. In India – First described in the Nilgiri Hills of Northern Tamil Nadu in 1952 [12], the sickle cell gene is now known to be widespread among people of the Deccan plateau of central India with a smaller focus in the north of Kerala and Tamil Nadu [13]. Extensive studies performed by the Anthropological Survey of India [14] have documented the distribution and frequency of the sickle cell trait which reaches levels as high as 35% in some communities.

Objectives

The aim of the study was to detect hemoglobinopathies by electrophoresis in microcytic hypochromic anemia in young population in the area covered by tertiary care center at Index Medical College and Hospital.

METHODS

A cross-sectional study was carried out in a hospital in rural area of Indore on young patients who attended outpatient department (OPDs)

from June 2018 to March 2020. Total 517 cases of moderate-to-severe anemia were included in the study. All young patients (10–30 years) who presented with pallor and were detected to have microcytic hypochromic picture on peripheral smear examination were included in the study.

Exclusion criteria

The following criteria were excluded from the study:

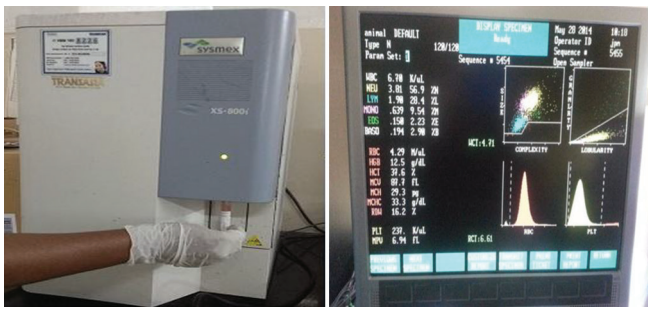
1. Peripheral picture other than microcytic hypochromic anemia.
2. Those who are not willing.

All patients underwent following investigations:

1. Complete blood count (CBC).
2. Peripheral blood smear study.
3. Sickling Test.
4. Hemoglobin Electrophoresis.

Sysmex XS800i fully automated Cell counter was used for CBC. It is the 5-part differential instrument. It reports on all the five subpopulations, namely, neutrophils, eosinophils, basophils, monocytes, and lymphocytes. MCV is a key diagnostic indicator. An important factor, namely, iron deficiency anemia (IDA) coexisting with beta-thalassemia trait can have very low value for MCV. For all purposes, MCV of <=65 fl indicates possibility of coexistence of beta-thalassemia trait and iron deficiency.

Automated cell counter Sysmex XS 800i



A CBC report provides:-

1. Leukocyte count with differential – total white blood cell count and a five part differential (lymphocytes, monocytes, neutrophils, basophils, and eosinophils).
2. Red blood cell count (also known as corpuscles).
3. Platelet count (also known as thrombocytes) – critical for blood clotting.
4. Platelet volume (measured in femtoliters).
5. Hemoglobin concentration (measured in grams per deciliter).
6. Hematocrit (also known as packed cell volume, expressed as a percentage measuring the total amount).

Peripheral Blood Smear:

A blood smear is a blood test used to look for abnormalities in blood cells. The three main blood cells that the test focuses on are:

- >Red cells,
- >White cells,

Blood films are made by placing a drop of blood on one end of a slide and using a spreader slide to disperse the blood over the slide's length. The aim is to get a region, called a monolayer, where the cells are spaced far enough apart to be counted and differentiated. Microscopic examination of the shape, size, and coloration of red blood cells is useful for determining the cause of anemia. Disorders such as IDA, SCA, megaloblastic anemia, and microangiopathic hemolytic anemia result in characteristic abnormalities on the blood film [15].

Reticulocyte count

Principle: Brilliant cresyl blue is an isotonic stain selectively stains nucleic material of erythrocytes called reticulocyte, which can be seen under a microscope directly or with a counter stain [16-18].

Sickling test

When red cells containing Hb S are subjected to deoxygenation, they become sickle-shaped while cells that do not contain Hb S remain normal. Certain reducing chemical agents such as 2% sodium metabisulfite or sodium dithionate can deprive red cells of oxygen.

Electrophoresis

Cellulose acetate electrophoresis at alkaline pH:

Hemoglobin electrophoresis at pH 8.4–8.6 using cellulose acetate membrane is simple, reliable and rapid. It is satisfactory for the detection of most common and clinically important hemoglobin variants [19-21].

OBSERVATION AND RESULTS

The above table shows the distribution of patients according to group. Out of 517 cases, there were 339 (65.6%) which were negative on electrophoresis (Group I) and 178 (34.4%) patients were positive on electrophoresis (Group II). Majority of the patients were negative on electrophoresis.

The above table shows the distribution according to hemoglobinopathies in relation to gender positive on electrophoresis. Of the 73 females, 21 (28.8%) were having beta-thalassemia trait, 17 (23.3%) were having sickle beta-thalassemia, 20 (27.4%) were having SCA, and 15 (20.5%) were having sickle cell trait. Of the 105 males, 32 (30.5%) were having beta-thalassemia trait, 10 (9.5%) were having sickle beta-thalassemia, 41 (39.0%) were having SCA and 22 (21.0%) were having sickle cell trait. The association between hemoglobinopathies and the gender was found to be statistically not significant (p=0.068), showing that hemoglobinopathies are independent of the gender.

The above table shows the distribution according to final diagnosis in relation to age in both the groups.

In the study, 73 (41.1%) patients having hemoglobinopathy presented with moderate type of anemia, 70 (39.3%) presented with severe type of anemia, 30 (16.8%) have very severe type of anemia, and the least percentage of mild anemia 5 (2.8%). The patients of beta-thalassemia presented with equal percentage 19 (35.9%) of moderate and severe anemia, 129 22.6 %) cases of very severe anemia, and 3 (5.6%) mild anemia. In sickle beta-thalassemia, moderate type of anemia was seen in maximum people 11(40.8%), severe anemia in 10 (37%), and very severe in 06 (22.2%) patients. The maximum number of cases of SCA presented with moderate type of anemia 29(47.5%) followed by severe anemia 24 (39.3%), 07 (11.5%) very severe, and 01 (1.7%) mild anemia. The sickle cell trait cases presented with 17 (46%) severe anemia, 14 (37.8%) in moderate anemia, 05 (13.5%) very severe anemia, and 01 (2.7%) with mild anemia.

Table 1 : Distribution of patients according to group

Group	n (%)
Cases negative on electrophoresis (Group-I)	339 (65.6)
Cases positive on electrophoresis (Group-II)	178 (34.4)
Total	517 (100.0)

Table 2: Distribution according to hemoglobinopathies in relation to gender

Type of Hemoglobinopathies	Female, n (%)	Male, n (%)
Beta-thalassemia trait	21 (28.8)	32 (30.5)
Sickle beta-thalassemia	17 (23.3)	10 (9.5)
SCA	20 (27.40	41 (39.0)
Sickle cell trait	15 9 (20.50)	22 (21.0)
Total	73 (100.00)	105 (100.0)

Pearson Chi-square test=7.129, df=3, p=0.068, not significant. SCA: Sickle cell anemia

DISCUSSION

The study was conducted on 517 individuals attending the OPD of Index Medical College (tertiary care center) between June 2018 and March 2020. The initial diagnosis was made on hemogram and peripheral smear which showed reduced hemoglobin and MCV with microcytic hypochromic cells, respectively. Subsequently, all of these cases were subjected to electrophoretic study. One hundred and seventy-eight out of the total of 517 (34.4%) cases were found to have one or the other form of hemoglobinopathy. Rest, that is, 339 (65.6%) were subjected to further investigations like iron studies and revealed a mix of IDA (80.5%) and anemia of chronic disease (19.5%). Out of 178 cases, 34.3% cases were of SCA, 29.8% cases of beta-thalassemia, 20.8% cases of sickle cell trait and 15.2% cases of sickle beta-thalassemia. Other studies conducted from time to time showing burden of hemoglobinopathies in various parts of India are in consistence with our study. In a study by Narang *et al*., 78 (39%) cases of one or the other form of hemoglobinopathies were documented [22]. A study conducted in Gujarat from 2010 to 2011, 2022 individuals were screened out of which 788 (38.97%) were having hemoglobinopathies (Patel *et al*., 2012) [23]. In the present study, the number of female patients 238 (46.1%) was less than the male patients 279 (53.9%). A study conducted by Chopra and coworkers (2008), out of 258 abnormal cases, 136 (53%) were males and 122 (47%) were females, thus giving slight male preponderance.This may be due to the prevalent sociocultural factors in our society that more male patients seek medical attention [24].

In our study, out of 178 cases positive on electrophoresis 73 (41.0%) were females showing high incidence of beta-thalassemia trait and 105 (59%) were males showing high incidence of SCA. The variation of the hemoglobinopathy in the gender is may be due to the consanguinity in marriages which is a well-accepted social norm in some communities irrespective of religion, cast, educational status, and economical background. However, the result was not in agreement with studies done by Nema *et al*. (2013), Narang *et al*. (2017), and Gupta *et al*. (2018) which show high incidence of sickle beta-thalassemia in both females and males [22,25,26].

Hemoglobinopathies are a diverse group of inherited disorders of hemoglobin production and synthesis. SCA and thalassemia are inherited abnormalities of hemoglobin synthesis and are the most serious public health problems in central India, in particular, and India, in general, reflecting the genetic heterogeneity of the population [27]. The geographical distribution of HBS and beta-thalassemia in India is not uniform as the prevalence varies from 2% to 22% and 1% to 15%, respectively, in different regions of the country [27]. Our study which was carried out in the rural area in Malwa region of Madhya Pradesh showed 61 (34.3%) out of 178 cases of SCA followed by 53 (29.8%) cases of beta-thalassemia trait, 37 (20.8%) cases of sickle cell trait and

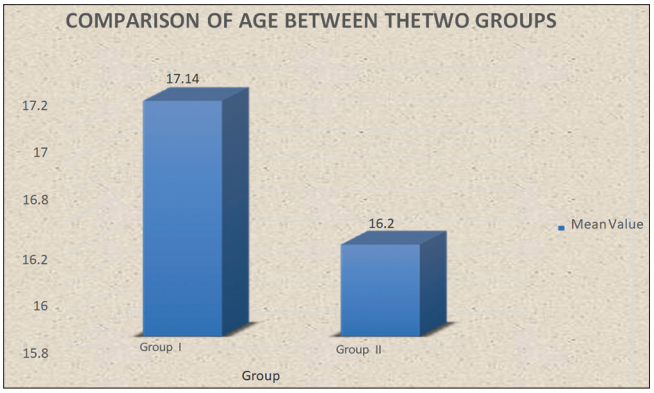


Fig. 1: Bar diagram showing comparison of mean age between the two groups

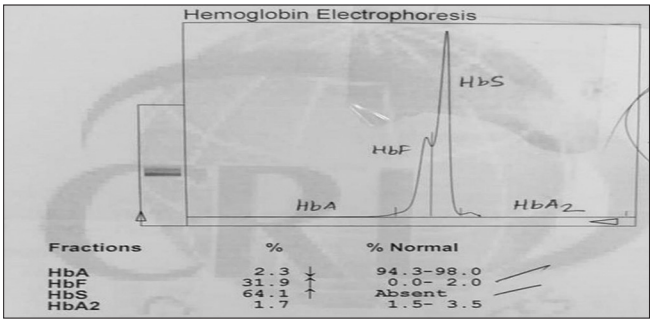


Fig. 2: Hb Electrophoresis showing sickle cell anaemia pattern

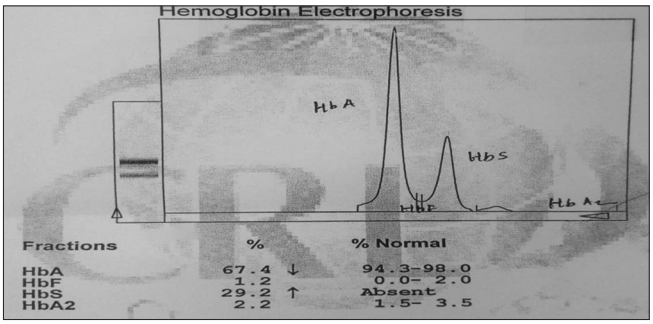


Fig. 3: Hb Electrophoresis showing Sickle Cell Trait pattern

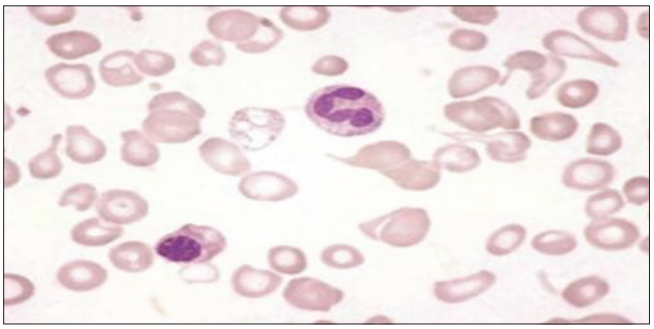


Fig. 4: Peripheral blood Smear showing nucleated RBC, tear drop cells, target cell, and anisopoikilocytosis

27 (15.2%) cases of sickle beta-thalassemia. In India, average frequency of sickle cell gene is around 5% with highest reported from Orissa 9%, Assam with 8.3%, and Madhya Pradesh 7.4% [28]. The highest frequency of beta-thalassemia trait is reported in Gujarat (10–15%) followed by Sindh (10%), Punjab (6.5%), and Tamilnadu (8.4%) [28].

Table 3: Distribution of final diagnosis in relation to age in both the groups

Diagnosis	≤13 years				>13 years			
	Group I, n (%)		Group II, n (%)		Group I, n (%)		Group II, n (%)	
Beta-thalassemia trait	-	-	20	27.8	-	-	33	31.1
Sickle beta-thalassemia	-	-	12	16.7	-	-	15	14.2
SCA	-	-	30	41.7	-	-	31	29.2
Sickle cell trait	-	-	10	13.9	-	-	27	25.5
Anemia of chronic disease and other causes	26	22.0	-	-	40	18.1	-	-
Iron deficiency	92	78.0	-	-	181	81.9	-	-
Total	118	100.0	72	100.0	221	100.0	106	100.0

SCA: Sickle cell anemia

Table 4: Severity of anemia in various hemoglobinopathies

Type of Hemoglobinopathies	Mild anemia (10–11 g/dL), n (%)	Moderate anemia (7–10 g/dL), n (%)	Severe anemia (4–7 g/dL), n (%)	Very severe anemia (<4 g/dL), n (%)
Beta-thalassemia trait	3 (5.6)	19 (35.9)	19 (35.9)	12 (22.6)
Sickle beta-thalassemia	0	11 (40.8)	10 (37)	6 (22.2)
SCA	1 (1.7)	29 (47.5)	24 (39.3)	7 (11.5)
Sickle cell trait	1 (2.7)	14 (37.8)	17 (46)	5 (13.5)
Total	5 (2.8)	73 (41.1)	70 (39.3)	30 (16.8)

SCA: Sickle cell anemia

However, the studies conducted by Nema *et al*. (2013), Narang *et al*. (2107), and Gupta *et al*. (2018) are not in the agreement with our study. They showed the preponderance of sickle beta-thalassemia in the same area this difference may be due to migration of different ethnic groups, literacy rates, and rate of consanguineous marriage [22,25,26].

CONCLUSION

Many times complete blood picture may not reveal the exact morphology needed to identify the hemoglobinopathy; hence, in such type of cases, Hb electrophoresis is done. Hemoglobin electrophoresis is a rapid, reliable, and cost-effective method to detect hemoglobinopathies. To reduce the burden of hemoglobinopathies, screening of all anemic patients should be done in all areas and in all communities where the socioeconomic condition indicate frequent occurrence of genetic mutations.

RECOMMENDATIONS

General counseling and screening of the adolescents should be done to avoid marriage between carriers. Mandatory rules for neonatal screening for hemoglobinopathies after 6 months should be taken by Government. The ANMs, ASHA and Anganwadis should be taught about the importance of screening of hemoglobinopathies. So that they can explain the purpose of screening to the female during pregnancy and to the families in the rural areas. At primary level, the CHCs and PHCs should be equipped with instruments for basic testing of hemoglobinopathies.

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CONFLICTS OF INTEREST

None declared.

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Nil.

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