Hematological Findings In Various Hemoglobinopathies : A 3 Years Study At Tertiary Care Centre

Dr. Yagnik Prafulchandra Tank[1], Dr. Bansari Dilipkumar Thakkar[2], Dr. Alpeshkumar Maheshbhai Maru[3], Dr. Anandkumar Aravindbhai Kanasagara[4*]

1. Assistant Professor, Pathology Department, Dr. N.D. Desai Faculty of Medical Science & Research, Nadiad, Gujarat
2. Assistant Professor, Microbiology Department, Dr. N.D. Desai Faculty of Medical Science & Research, Nadiad, Gujarat
3. Associate Professor, Pathology Department, Dr. N.D. Desai Faculty of Medical Science & Research, Nadiad, Gujarat
4. Assistant Professor, Pathology Department, Dr. N.D. Desai Faculty of Medical Science & Research, Nadiad, Gujarat

Corresponding Author: Dr. Anandkumar Aravindbhai Kanasagara

Email: spl.naroda@yahoo.com

Abstract

Introduction: Hemoglobinopathies constitute a very important causative factor for anaemias of childhood. Two common hemoglobinopathies in India are Beta Thalassemia and Sickling disorders. **Objective**: The objective of this study is to know the hematological findings in various types of hemoglobinopathies & their distribution in various age and sex groups & communities, so that a solid conclusion can be made about screening of these hemoglobinopathies on high risk basis. **Material and Methods**: This is an observational study in 100 known patients of hemoglobinopathies who came to a tertiary care hospital from September 2015 to September 2018. Screening hematological tests for hemoglobinopathies such as complete blood count indices with Mentzer's Index, Sickling test & peripheral blood smears were performed on all the patients and analyzed. **Results**: All the patients were having characteristic hematological findings. Among the 100 patients there were 56 males and 44 females with age group ranging from 1 year to 32 years. 54% patients were having Beta Thalassemia, 40% patients were having Sickle cell disorder & 6% were having combination of both. Beta Thalassemia was most common in Bhanushali community and Sickle cell disorder was most common in Lohana community. **Conclusion**: Hemoglobinopathies can be diagnosed earlier with meticulous use of simple & cost effective hematological screening tests in high risk age groups and communities in developing countries like India.

Keywords: Beta Thalassemia, Mentzer's Index, Peripheral blood smear, Sickling Disorder, Sickling test.

Introduction

Hemoglobinopathies constitute a very important causative factor for anemias of childhood. They mimic nutritional anemias and then prove deceptive and refractory to the usual corrective measures. The two common Hemoglobinopathies widely distributed across India are Beta Thalassemia and sickling disorders [1]. The former is more common in certain non-tribal ethnic groups and later amongst the tribal population.

Beta Thalassemia is considered the most common inherited autosomal recessive genetic disorder worldwide. It is characterized by partial or complete failure to synthesize the polypeptide chains of hemoglobin molecule. Different types of thalassemia's are associated with defects in any of the polypeptide chains (alpha, beta, gamma or delta). Beta Thalassemia is so common and produce severe anemia in their homozygous and

compound heterozygous state [2].

Clinically thalassemia's are classified according to their severity as major, intermedia and minor forms. Beta Thalassemia major, the homozygous state & it is transfusion dependent disorder. Beta Thalassemia intermedia, the double heterozygous state, is characterized by anemia and splenomegaly and require infrequent transfusion. Beta Thalassemia minor / Beta Thalassemia trait, the heterozygous state, is often asymptomatic. It is milder form of anemias but most of them are healthy and requires no special treatment [3].

Regarding hematological findings in Beta Thalassemia, majority patients had mentzer's index below 13 and having microcytic hypochromic RBCs with predominant target cells.

Sickling disorders are associated with defect in the globin chain of hemoglobin molecule resulting in several complications due to reduced and/or de-oxygenated form of HbS (Pathological form of Hemoglobin in sickling disorders). Cases of homozygous state are termed as 'Sickle cell anemia' and manifest as the most severe form of disease while heterozygous state are termed as 'Sickle cell trait. [3]

Hematological findings in sickling disorders includes hemolytic peripheral smear picture with sickle cells which is get confirmed by positive sickling test.

Sometimes, there is double heterozygous state of sickling disorders and β Beta Thalassemia. This is called HbS- β Beta Thalassemia syndrome. In this disorder, prognosis is better than that of Beta Thalassemia major or sickle cell anemia.

The present study is carried out with a view to know the Hematological findings in various types of Hemoglobinopathies & their distribution in various age and sex groups & communities.

Materials And Methods

Hundred patients are included in this study having Hemoglobinopathies who came to a tertiary care hospital from September 2015 to September 2018. The information recorded includes age, sex, demographic features, family history, past history, hematological parameters and hemoglobin electrophoresis report. The samples of blood were collected in the EDTA anticoagulant vacutainers. The samples were processed in Horiba Five-part hematology analyzer. Hemoglobin (HB), Hematocrit (HCT), Total red blood cell count (RBC), Mean corpuscular volume (MCV), Mean corpuscular hemoglobin (MCH), Mean corpuscular hemoglobin concentration (MCHC), Red cell distribution width (RDW), Total white blood cell count, Differential WBC count and Platelet count were noted. Mentzer's Index calculation, Sickling test & peripheral blood smear examination were also performed and analyzed for each patients and correlated with blood indices.

Mentzer's Index

It is calculated by dividing MCV (in fl) with RBC count (in millions/cumm). It is helpful in differentiating iron deficiency anemia from Beta Thalassemia trait in a case of microcytic anaemia. If Mentzer's Index value is less than 13, beta Thalassemia trait is more likely while if Mentzer's Index value is more than or equal to 13, iron deficiency anaemia is more likely. The principle involved is as follows: In iron deficiency, the marrow cannot produce required amount of RBCs and they are small (microcytic), so the RBC count and the MCV will both be low, and as a result, the index will be greater than 13. Conversely, in Beta Thalassemia, which is a disorder of globin chain synthesis, the number of RBCs produced is normal, but the cells are smaller and more fragile. Therefore, the RBC count is normal, but the MCV is low, so the index will be less than 13[4].

Peripheral Blood Smear

Blood smears of every patients were prepared on glass slides and stained by Leishman's stain and observed for changes in RBC series. In Beta Thalassemia trait patients peripheral blood smear shows target cells, while in Beta Thalassemia major & intermedia smear shows target cells as well as hemolytic changes (fragmented and few nucleated RBCs). In Sickle cell anemia patients sickle cells and hemolytic changes are

seen in peripheral smear. Sickle cell trait patients show hemolytic changes, cigar shaped cells and occasional Sickle shaped cells which can be confirmed by sickling test[5].

SICKLING TEST

The principle of sickling test is based on microscopical observation of sickling of red blood cells when exposed to a low oxygen tension. 20 micro liters of EDTA blood sample is mixed with 100 micro liters of 2% sodium metabisulphite on a glass slide, put a coverslip over it and then sealed with paraffin wax. Slide is examined for sickle cells at 30 minutes, 1 hour, 4 hours and 24 hours. In sickle cell anemia RBCs are converted into sickle shape immediately or within 30 minutes while in sickle cell trait it may take 4 to 24 hours[5].

Results

Table-1 Distribution of Hemoglobinopathies

Hemoglobinopathy	No. of Patients
Beta Thalassemia Major	04
Beta Thalassemia Intermedia	02
Beta Thalassemia Minor	48
Sickle cell Anemia	04
Sickle cell trait	36
HbS/Beta Thalassemia	06
Total	100

As shown in above table, most common hemoglobinopathy in our study was Beta Thalassemia minor and second most common was sickle cell trait.

Table-2- Age distribution

Age group (In years)	Beta Thalassemia Major	Beta Thalassemia Intermedia	Beta Thalassemia Trait	Sickle cell anemia	Sickle cell Trait	HbS/Beta Beta Thalassemia	Total
0-5	02	00	04	04	06	00	16
6-10	00	00	02	00	04	02	08
11-15	02	02	02	00	02	00	08
16-20	00	00	04	00	04	04	12
21-25	00	00	14	00	16	00	30
26-30	00	00	12	00	04	00	16
>30	00	00	10	00	00	00	10
Total	04	02	48	04	36	06	100

As shown in above table, Beta Thalassemia major and sickle cell anemia were most common in 0-5 years age group while Beta Thalassemia trait and sickle cell trait were most common in age group of 21-25 years.

Table-3- Gender distribution

Gender	Beta Thalassemia	Sickle cell disorder	HbS/Beta Beta Thalassemia	Total
Male	29	23	04	56
Female	25	17	02	44
Total	54	40	06	100

As shown in above table, all the hemoglobinopathies were showing slight male preponderance.

Table-4- Racial distribution

Race/ Community	Beta Thalassemia	Sickle cell disorder	HbS/Beta Thalassemia	Total
Lohana	04	14	02	20
Ahir	08	02	00	10
Bharwad	06	00	00	06
Patel	08	02	00	10
Tribal	06	04	00	10
Bhanushali	12	04	00	16
Jain	00	04	02	06
Sathwara	00	02	00	02
Muslim	08	08	02	18
Sindhi	02	00	00	02
Total	54	40	06	100

As shown in above table, Beta Thalassemia was most common in Bhanushali community and Sickle cell disorder was most common in Lohana community.

Table-5- Peripheral smear findings in various Hemoglobinopathies

Peripheral smear findings	Beta Thalassemia	Sickle cell disorder	HbS/Beta Thalassemia
Microcytic Hypochromic Cells with target cells	54	00	06
Hemolytic picture with Sickle cells	00	40	06

As shown in above table, all the Beta Thalassemia patients were showing microcytic hypochromic RBCs with target cells & all the sickle cell disorder patients were showing sickle cells in peripheral smear.

Table-6- Mentzer's Index

	Patients with MI <13	Patients with MI >13
Patients with HbA2 >4%	48	00
Patients with HbA2 <3.5%	00	52

As shown in above table, majority of Beta Thalassemia minor patients who had HbA2 level more than 4% were showing Mentzer's Index <13 while other patients who had normal HbA2 levels (<3.5%) were showing Mentzer's Index >13.

Table-7- Sickling test

	Patients with positive Sickling test	Patients with negative Sickling test
Patients with HbS	46	00
Patients without HbS	00	54

As shown in above table all the known sickle cell disorder patients were showing positive Sickling test while other patients were showing negative sickling test.

Discussion

Various Hemoglobinopathies are quite common in India especially Beta Thalassemia and Sickle cell anemia. The incidence of Beta Thalassemia is very high in Saurashtra region of Gujarat state while Sickle cell disorders in south Gujarat region. Present study was carried out to analyze Hemoglobinopathies like Beta Thalassemia and Sickle cell disorders in high risk communities along with it was conducted to establish simple, rapid and cost effective screening methods like Mentzer's Index using Blood indices, Sickling test and peripheral blood smear examination.

Observations of present study have been compared with those of various studies in India who have evaluated the problem of anaemia in infancy and childhood and some studies have evaluated and surveyed high risk population. On account of diversity in ethnic composition of different parts of country, there are wide differences in results obtained by different studies from different states.

Table -8- Comparison of peak age groups

	Beta Thalassemia Major	Beta Thalassemia Trait	Sickle cell anemia	Sickle cell Trait
Ambekar et al (2001)[6]	0-5 years	16-20 years	3-7 years	12-16 years
Jagruti et al (2005) [7]	0-5 years	16-20 years	3-7 years	12-16 years
Present study	0-5 years	21-25 years	0-5 years	21-25 years

Present study showed all cases of Beta Thalassemia major and sickle cell anaemia were mainly in pediatric age group and this was comparable with other studies. In present study Beta Thalassemia minor and sickle cell trait cases were detected mainly in adult age group and this was also partially comparable to other studies [6],[7].

Table -9- Comparison of gender groups

	Beta Thalassemia Major	Beta Thalassemia Trait	Sickle cell disorder
Jagruti et al (2005)[7]	Male	Male	Male
Present study	Male	Male	Male

In present study, there was male preponderance in all the hemoglobinopathies and this was comparable with other studies [7].

Table -10- Comparison of various Hemoglobinopathies

	Giri et al (1984)[8]	Babikar et al (1999)[9]	Ambekar et al (2001)[6]	Jagruti et al (2005)[7]	Phillip et al (2013)[10]	Present study
Beta Thalassemia Major + Intermedia	30(58.8%)	01(1.7%)	76(38.57%)	34(22.22%)	44(6.3%)	06(6%)
Beta Thalassemia Trait	0	31(55.35%)	91(46.19%)	70(45.75%)	455(66.1%)	48(48%)
Sickle cell anemia	15 (29.4%)	0	30(15.24%)	22(14.37%)	05(0.7%)	04 (4%)
Sickle cell Trait	01(0.2%)	0	30(15.24%)	12(7.88%)	54(7.8%)	36 (36%)
HbS/Beta Thalassemia	04(7.8%)	0	0	03(1.9%)	21(3%)	06(6%)

As shown in table the commonest Hemoglobinopathy in present study was Beta Thalassemia trait that was comparable with Ambekar et al (2001) [6] and Jagruti et al (2005)[7]. Cases of Beta Thalassemia major and Intermedia were comparable with Phillip et al (2013) [10]. Cases of sickle/Beta Thalassemia combination were comparable with Giri et al (1984) [8].

Regarding hematological findings in Beta Thalassemia minor, majority patients had mentzer's index below 13. Peripheral smear examination shows microcytic hypochromic RBCs with predominant target cells.in case of Beta Thalassemia major, there is hemolytic picture along with nucleated red blood cells predominate the findings. These hematological parameters are correlate well with other studies like Ambekar et al (2001) [6] and Jagruti et al (2005) [7].

Hematological findings in sickling disorders includes hemolytic peripheral smear picture with predominant

sickle cells along with fragmented cells, tear drop cells, eliptocytes. which is get confirmed by positive sickling test. These hematological findings are correlate well with other studies like Jagruti et al (2005) [7] Giri et al (1984) [8]

Hematological parameters and associated other findings like mentzer's index and sickling test are quite cost effective and quick test to access various major hemoglobinopathies in resource limited countries which should be later on get confirmed by HPLC

Conclusion

As a consequence, to the aims of the present study, Beta Thalassemia trait was the most common Hemoglobinopathy. Beta Thalassemia major and Sickle cell anaemia are detectable in pediatric age group while Beta Thalassemia trait and Sickle cell trait are generally detected in adult age group with male preponderance. Bhanushali and Muslim communities are most vulnerable for Beta Thalassemia while Lohana and Muslim communities are vulnerable for Sickle cell disease. Beta Thalassemia is associated with many other Hemoglobinopathies. In this study, common association with Sickle cell disease was found. Mentzer's Index (MI) and Sickling test are simple, easy, rapid and cost effective methods of mass screening programme of Beta Thalassemia trait and Sickle cell disorder detection, respectively in high risk community. Screening of Hemoglobinopathies is very essential particularly for high risk community, where consanguineous marriages are common and for screening of students of colleges and this screening saves the society and parent by preventing birth of child with various Hemoglobinopathies. For marriages, instead of matching HOROSCOPE of Brides and Groom, certificate of Hb Electrophoresis test should be matched. It will be a great help to mankind by preventing the Devil's disease – "BETA THALASSEMIA MAJOR" and giving birth to healthy baby and healthy society.

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