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Original Article

Pathology Section

Burden of Congenital Haemolytic Anaemias among the Rural Paediatric Population of Central India

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ABSTRACT

Introduction: Childhood anaemia may be caused due to many factors, including malnutrition, chronic infections, deficiency of iron and vitamins, parasitic infections. On haematological work up the most common finding is microcytic hypochromic anaemia. Inherited defects in Red Blood Cell (RBC) and haemoglobin structure and metabolism result in congenital haemolytic anaemia. About 4.2% of all paediatric microcytic anaemias are diagnosed with haemoglobinopathies. Thalessaemia and other haemoglobinopathies have a large representation in the Indian subcontinent. A study of the extent of these hereditary anaemias will help in formulating a targeted approach of spreading awareness and educating couples in target population, who are planning conception.

Aim: To comprehend the incidence of congenital haemolytic anaemias in the paediatric population of central India, and to evaluate their clinico-haematological status.

Materials and Methods: This cross-sectional observational study was conducted in the Paediatric Anaemia Clinic at Chirayu Medical College and Hospital (teaching, tertiary care hospital), Bhopal, Madhya Pradesh, India, from January to December 2021. All relevant details regarding clinical history, consanguinity, family history of the patients were recorded. Investigations done included-complete blood count, reticulocyte count, sickling test, Naked Eye Single Tube Red Cell Osmotic Fragility Test (NESTROFT) and High Performance Liquid Chromatography (HPLC). All the relevant data collected was entered into Microsoft excel and frequency, distribution, standard deviation were analysed and calculated.

Results: Amongst the total 1080 cases of microcytic hypochromic anaemia in the Paediatric Anaemia Clinic, there were 55 cases of congenital haemolytic anaemias. Of these 55 cases of congenital haemolytic anaemias, there were 41 (74.54%) cases of thalassaemia (four major and 37 minor thalassaemia), 11 (20%) cases of sickle cell anaemia, 1 (1.81%) case of Hereditary Persistence of Foetal Haemoglobin (HPFH) and 2 (3.63%) cases of Haemoglobin J variant (HbJ). The haemoglobin values were the lowest in the β-thalassaemia major patients, followed by sickle cell disease. Foetal Hb (HbF) was highest in β-thalassaemia major at and the HPFH case had foetal haemoglobin of 35%.

Conclusion: In the current study, 5.09% of the paediatric population presenting to the clinic were diagnosed with congenital haemolytic anaemias, with major representation of β-thalassaemia, followed by sickle cell trait. This lifelong burden of haemolytic anaemias on the children and their families may be reduced by active counseling and awareness programs.

Keywords: Awareness programmes, Haemoglobinopathies, Hereditary persistence of foetal haemoglobin, Thalassaemia

INTRODUCTION

Childhood anaemia may be caused due to many factors, including malnutrition, chronic infections, deficiency of iron and vitamins, parasitic infections. All of these come with the complaints of pallor, weakness and failure to thrive, amongst others, and on further investigations are diagnosed as microcytic hypochromic anaemia. Inherited defects in Red Blood Cell (RBC) and haemoglobin structure result in congenital haemolytic anaemias, which are further categorised broadly as membrane defects, enzyme defects and those with abnormal haemoglobins. Amongst all children presenting with anaemia in the Pediatric Outpatient Department, about 4.2% are diagnosed with haemoglobinopathies, with about 30 million being carriers [1]. Thalessaemia and other haemoglobinopathies have a large representation in the Indian subcontinent [2]. The three most common abnormal haemoglobins; HbS, HbD and HbE have a collective gene frequency of 5.35% in India [3].

The objective of the present study was to estimate the proportion of the paediatric population in rural central India who were suffering from congenital haemolytic anaemias and to evaluate their clinico-haematological status. The results will help in targeted screening and counselling of couples with a family history of haemoglobinopathies and are thus at an increased risk of having offspring with the same, and also to diagnose and help affected children lead a more productive life.

MATERIALS AND METHODS

This cross-sectional observational study was conducted in the Paediatric Anaemia Clinic at Chirayu Medical College and Hospital (teaching, tertiary care hospital), Bhopal, Madhya Pradesh, India, from January to December 2021. The study was conducted after obtaining the approval from the Ethics Committee (EC/2021/34.11). Socio-demographic details were collected after taking parents consent and the purpose of the study was explained to them.

Total 1080 children with anaemia presented to the Paediatric Anaemia Clinic. There were 55 cases of congenital haemolytic anaemias after the confirmatory test.

Inclusion criteria: All patients between the age group of 6 months to 15 years, with haemoglobin <10 gm/dL, with microcytic hypochromic anaemia and with the presence of an abnormal haemoglobin band on High Performance Liquid Chromatography (HPLC) were included in the study.

Exclusion criteria: Anaemic paediatric patients who showed no evidence of haemoglobinopathy or thalessaemia on HPLC, and those who did not get HPLC testing were excluded from the study. Also patients who were known cases of cardiovascular abnormalities and undergoing chemotherapy were excluded from the study.

Data Collection

Routine investigations were performed on all patients who came to the Paediatric Clinic for Anaemia. These included haemoglobin estimation, blood indices, (done on automated blood cell counter MINDRAY BC 6000) and peripheral blood smear examination. All patients with microcytic hypochromic anaemia were further worked up for haemolytic anaemias by using different screening and confirmatory investigations. After preliminary tests performed on patients, they were confirmed as congenital haemolytic anaemia by using HPLC.

Study tool: All relevant details regarding clinical history, consanguinity, family history of the patients were recorded. Investigations done included;

a) Complete blood count performed on automated blood cell counter MINDRAY BC 6000

b) Reticulocyte count

c) Sickling test

d) Naked Eye Single Tube Red Cell Osmotic Fragility Test (NESTROFT)

e) HPLC

STATISTICAL ANALYSIS

Data was entered in Microsoft excel and relevant percentage, frequency, mean and standard deviation measured with the help of Microsoft excel (2013).

RESULTS

Amongst the total 1080 cases of microcytic hypochromic anaemia in Paediatric Clinic, there were 55 (5.09%) cases of congenital haemolytic anaemias. Amongst these, there were 41 (74.54%) cases of thalassemia, 4 (7.27%) major thalassaemia and 37 (67.27%) minor thalassaemia; 10 (18.18%) cases of sickle cell trait , 1 (1.81%) sickle cell disease; 1 (1.81%) case of Hereditary Persistence of Foetal Haemoglobin (HPFH) and 2 (3.63%) cases of Haemoglobin J variant (HbJ). There was an overall male predominance in all the anaemias [Table/Fig-1]. Two patients of β-thalassaemia major had a history of second degree consanguinity, other patients did not give significant family history. β-Thalassaemia trait was the most common at 67.27%, followed by sickle cell trait at 18.18%. Among the homozygous forms of both haemoglobinopathies, β-thalassaemia major has a larger share of 7.27% as compared to the 1.81% of sickle cell disease in the population studied. The age of patients ranged from youngest of six months to oldest being 10 years; mean age was 5.72 years.

Type of anaemia	Male	Female	Total number	Percentage
β-thalassaemia major	3	1	4	7.27%
β-thalassaemia trait	24	13	37	67.27%
Sickle cell disease	1	00	1	1.81%
Sickle cell trait	7	3	10	18.18%
HPFH	1	00	1	1.81%
HbJ	1	1	2	3.63%

[Table/Fig-1]: Types of congenital haemolytic anaemia, their prevalence and sex-wise distribution.

All the patients of sickle cell disease and β-thalassaemia major presented with complaints of failure to thrive. Three of the sickle cell trait also presented with the same. None of the other congenital anaemias had this complaint. Except for HbJ and HPFH all other patients had varying degrees of pallor, with the majority in β-thalassaemia major and sickle cell disease. A 40% of the sickle cell trait patients had pallor and among β-thalassaemia trait patients, 29.72% were clinically assessed to have pallor [Table/Fig-2].

Joint pain with difficulty in walking was seen in all patients with sickle cell disease (100%), 7 (70%) cases in sickle cell trait and only 1 (25%) case in β-thalassaemia major. Spleenomegaly was present in all patients of both the major congenital haemolytic anaemias. Bossing of skull with haemolytic facies was classically seen in all patients of β-thalassaemia major [Table/Fig-2].

The Haemoglobin (Hb) values were the lowest in the β-thalassaemia major patients ranging at a mean of 4.3±0.2 gm/dL, followed by sickle cell disease with a mean Hb value of 5.6±0.2 gm/dL. The less severe forms of both diseases had mean values of 7.2±1.4 gm/dL and 6.2±0.3 gm/dL for β-thalassaemia trait and sickle cell trait, respectively [Table/Fig-3]. The two other haemoglobinopathies of HbJ and hereditary persistence of foetal haemoglobin did not have significant drop in the haemoglobin values. The total RBC counts also reflected the low haemoglobin of β-thalassaemia major, whilst the other haemoglobinopathies did not cause the RBC count to drop as significantly.

The mean corpuscular volume was the lowest in β-thalassaemia major at 61.2±8.1 fL [Table/Fig-3]. The mean MCV in other congenital haemolytic anaemias were noted as 77.4±0.5 fL in β-thalassaemia trait, 78 fL in HPFH, 80±1.2 fL in HbJ, 82.2±0.6 fL in sickle cell disease and 98.8±12.2 fL in sickle cell trait [Table/Fig-3]. Anisopoikilocytosis as defined by Red cell Distribution Width (RDW) with a normal range of 11.5-14.5% and was the most significant in the major form of β-thalassaemia with a mean of 28.4±1.7%, followed by sickle cell trait 26.1±4.2% and sickle cell disease had a mean 21.4±2.6%. RDW of β-thalassaemia trait was near normal at a mean of 15.1±0.6%, as was expected.

On HPLC the Foetal Hb (HbF) was highest in β-thalassaemia major at a mean of 83.8±5.8%, thus effectively becoming the predominant haemoglobin fraction [Table/Fig-3]. The HPFH case had foetal Hb of 35%, with an HbA of 55.3%. Sickle cell disease had a foetal Hb of 19.6±0.2%, sickle cell trait had a mean HbF of 6.1±5%. The HbA2 was significantly raised in β-thalassaemia trait upto a mean of 26.0±1.4%. In all other haemoglobinopathies the HbA2 ranged from 1.3 to 5.6%. HbJ variants had 35.7±0.3% HbJ, 61.2±0.3% of HbA and 1.8±0.5% of HbF, and was clinically silent.

DISCUSSION

Anaemias are an omnipresent entity in the Indian population. Congenital haemolytic anaemias surface within the first few months of life, and are often amplified by the presence of nutritional anaemias. The prevalence of anaemia in children below five years is thought to be 20% in industrialised countries and 39% in non industrialised countries [4]. Congenital haemolytic anaemias are represented by haemoglobinopathies, comprising α and β-thalessaemias and other haemoglobin variants like HbS, HbD, HbE. These Hb variants arise

Clinical findings	β-thalassaemia major (n=4) n (%)	β-thalassaemia trait (n=37) n (%)	Sickle cell disease (n=1) n (%)	Sickle cell trait (n=10) n (%)	HPFH (n=1) n (%)	Haemoglobin J variant (n=2) n (%)
Failure to thrive	4 (100%)	0	1 (100%)	3 (30%)	0	0
Pallor	4 (100%)	11 (29.72%)	1 (100%)	4 (40%)	0	0
Joint pain	1 (25%)	0	1 (100%)	7 (70%)	0	0
Splenomegaly	4 (100%)	0	1 (100%)	0	0	0
Haemolytic facies	4 (100%)	0	0	0	0	0

[Table/Fig-2]: Clinical spectrum of congenital haemolytic anaemias.

Type of anaemia	Haemoglobin (gm/dL) (Mean±SD)	RBC (10^6 /µL) (Mean±SD)	MCV (fL) (Mean±SD)	RDW (%) (Mean±SD)	HbA (%) (Mean±SD)	HbA2 (%) (Mean±SD)	HbF (%) (Mean±SD)	HbS (%) (Mean±SD)	HbJ (%) (Mean±SD)
β-thalassaemia major	4.3±0.2	3.2±1.2	61.2±8.1	28.4±1.7	7.4±1.6	4.9±3.4	83.8±5.8	-	-
β-thalassaemia trait	7.2±1.4	4.1±1.5	77.4±0.5	15.1±0.6	53.8±0.4	26.0±1.4	7.9±0.5	-	-
Sickle cell disease	5.6±0.2	4.4±0.2	82.2±0.6	21.4±2.6	2.6±1.5	3.6±0.4	19.6±0.2	71.2±1.3	-
Sickle cell trait	6.2±0.3	4.2±0.4	98.8±12.2	26.1±4.2	53.3±8.2	5.6±0.8	6.1±5.0	28.3±7.8	-
HPFH	11.4±0	4.8±0.0	78±0.0	15.6±0.0	55.3±0.0	2.2±0.0	35.0±0.0	-	-
HbJ	10.2±0.05	4.5±0.03	80±1.2	14.0±0.5	61.2±0.3	1.3±0.04	1.8±0.5	-	35.7±0.3

[Table/Fig-3]: Hematological parameters of the congenital haemolytic anaemias.

due to mutations for the genes encoding for the α and β chains and result in change of amino acids [5]. Consanguinity plays a major in the inheritance, severity and prevalence of congenital haemolytic anaemias, especially in thalessaemias. This is evidenced by the predominance of HbE-thalessaemia in the north eastern states of India [6]. Certain communities such as Sindhi, Gujarati, Punjabi, and Bengali are more commonly affected with β-thalassaemia with incidence varying from 1-17% [7].

The study by Sanghavi J and Asati A, partially agrees with the present study which shows a predominance of β-thalassaemia trait at 67.27%, followed by sickle cell trait at 18.81%, both studies were done on a similar population. Sanghavi J and Asati A, had an equal incidence of β-thalassaemia trait and sickle cell anaemia [8].

The study by Saba F et al., on the paediatric population contrasted from the present study by having a larger proportion of β-thalassaemia major patients, this was probably due to the fact that they had only included admitted patients in their study, while the study population in this study comprised of patients from the outpatient anaemia clinic [9]. The present study population did not yield any cases of HbE or HbE-β-thalassaemia, HbE trait or HbE disease. Other studies like Mondal UK et al., and Konar K et al., which were conducted amongst the population of the eastern belt of the Indian subcontinent, including Assam, West Bengal and Bihar showed a significant representation of HbE disease and trait [10,11].

After 13 weeks of foetal age HbF is the major haemoglobin and has strong oxygen affinity. By the end of the first year of life these levels drop from 80% to less than 2%, this is due to decreased synthesis of γ chains. Levels of HbF >2% are an indication of an abnormality, marked by the presence of an abnormal haemoglobin [12]. In the present study, the mean HbF levels ranged from 83.8± standard deviation of 5.8, with the maximum in β-thalassaemia major and least of 1.8±0.5 in HbJ. This was similar to the findings of Mondal et al., who had a mean HbF of 82.9% for β-thalassaemia, the HbA levels were also similar [10]. The HbA2 levels of sickle cell disease and sickle cell trait were reflective of the study by Mondal UK et al., [10].

The mean haemoglobin of β-thalassaemia in the present study was 4.3±0.2 mg/dL, which was less as compared to the studies by Sanghavi J and Asati A, Mondal UK et al., and Konar K et al., [8,10,11]. The mean red cell distribution width, an indicator of the varied morphology of the RBCs was similar to the findings of Mondal UK et al., with respect to thalessaemia and sickle cell disease and trait [10].

In the present study, all the patients of β-thalassaemia major and sickle disease with pallor, failure to thrive and splenomegaly; similar findings were seen in the study by Mondal UK et al., in the β-thalassaemia major group, however amongst their sickle cell disease patients only 60% showed failure to thrive [10]. In the study by Konar K et al., among the β-thalassaemia major patients only 58.8% had splenomegaly, which was significantly less as compared to the present study [11]. Joint pain was equally present in patients of sickle cell trait in both studies. None of the sickle cell disease patients of the current study showed features of haemolytic facies in the present study, however Mondal UK et al., had 30.4% of the same [10].

The entity of HbJ is a fairly new entrant in the haemoglobinopathies and is usually incidentally diagnosed on HPLC. It is a fast moving α globin derived haemoglobin, with a large family comprising HbJ Meerut, HbJ Birmingham, HbJ Bangkok [13]. This variant is also detected in cases of glycosylated Hb analysis, where patient has a low HbA1c value and high blood glucose levels, due to the presence of the abnormal haemoglobin [14].

Limitation(s)

This study was limited by a small sample size as all patients with microcytic hypochromic anaemia did not agree to further screening and confirmatory testing and thus could not be conclusively included or excluded from the study.

CONCLUSION(S)

Congenital haemolytic anaemias are largely represented by thalassaemias and sickle cell anaemias in the paediatric population of central India. Red cell enzyme defects and membrane disorders are not seen as commonly as the abnormal haemoglobin associated haemolytic anaemias. This predominance of abnormal haemoglobins, especially β-thalassaemia trait is attributable to the tribal population and the large presence of migrant Sindhi population in the nearby areas. This lifelong burden of haemolytic anaemias on the children and their families may be reduced by active counseling and awareness programs. These programs are now proposed and will be implemented towards the specific groups, at the premarital stage, with the inclusion of genetic counseling, to allow people to make informed decisions.

REFERENCES

[1] Chandran P, Laxmi MS, Yadagiri B, Noorjahan M, Rao MN. Biochemical characterization of spectrum of haemoglobinopathies and thalassaemia syndromes- Experience with 689 cases in a tertiary care hospital in South India. IJUPBS. 2013;4(4):1234-42.

[2] Christianson A, Howson C, Modell B. March of Dimes global report on birth defects. March of Dimes Birth Defects Foundation. 2006.

[3] Balgir RS. Genetic epidemiology of the three predominant abnormal hemoglobins in India. J Assoc Physicians India. 1996;44(1):25-28.

[4] Keikhaei B, Zandian K, Ghasemi A, Tabibi R. Iron-deficiency anemia among children in southwest Iran. Food and Nutrition Bulletin. The United Nations University. 2007;28(4):406-11.

[5] Tan JA, Tan KL, Omar KZ, Chan LL, Wee YC, George E. Interaction of Hb South Florida (codon1;GTG→ATG)and HbE, with β thalessemia (IVS1-1;G→A): Expression of different clinical phenotypes. Eur J Pediatr. 2009;168:1049-54.

[6] Ghai OP. Gupta P, Paul VK. Essential pediatrics. 6th edition. New Delhi: Interprint; 2004. Hematological disorders.100-101.

[7] Gupta A, Hattori Y, Gupta UR, Sarwai S, Nigam N, Singhal P, et al. Molecular genetic testing of beta-thalassemia patients of Indian origin and a novel 8-bp deletion mutation at codons 36/37/38/39. Genet Test. 2003;7(2):163-68.

[8] Sanghavi J, Asati A. Profile of anemia with special reference to hemoglobinopathies in a tertiary care centre in Madhya Pradesh. Pediatr Rev: Int J Pediatr Res. 2015;2(4):143-51.

[9] Saba F, Poornima S, Balaji PAR, Varne SRR, Jayashree K. Anemia among hospitalized children at a multispecialty hospital, Bangalore (Karnataka), India. J Family Med Prim Care. 2014;3(1):48-53.

[10] Mondal UK, Dowerah P, Mukherjee R. Hemoglobinopathy in pediatric population: A cross sectional study at tertiary care center in Assam. Indian J Med Sci 2021;73:327-30.

[11] Konar K, Karmakar A, Mondal BC. Clinico-hematological pattern of thalassemias and hemoglobinopathies in children presenting with microcytic anemia: An outdoor-based study at Burdwan, West Bengal. Int J Cur Res Rev. 2018;10(10):2734.

[12] Singh T. Textbook of Haematology, 2nd Ed. In: Arya Publishers; 2010;81-91.

[13] Srinivas U, Mahapatra M, Pati HP. Hb J Meerut, a fast-moving hemoglobin: A study of seven cases from India and a review of literature. Am J Hematol Oncol. 2007;82(7):666-67.

[14] Neelima V, Jyoti C. Detection of a rare hemoglobin variant- HbJ during glycosylated hemoglobin analysis- A rare single case report. Anatomy Physiol Biochem Int J. 2018;4(4):555650.

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