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Original Research Article

SPECTRUM OF HEMOGLOBINOPATHIES USING HPLC IN PEDIATRIC PATIENTS IN SOUTHEASTERN MAHARASHTRA – A SINGLE CENTER STUDY

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Abstract

Background: Hemoglobinopathies (thalassemias and structural hemoglobin variants) are major causes of microcytic hypochromic anemia in children and contribute substantially to pediatric morbidity in many regions. Early detection and characterization of the hemoglobinopathies is essential to provide necessary counseling and avoid unnecessary iron overload. HPLC has emerged as a rapid, accurate and reproducible tool for detection and quantification of hemoglobin variants. **Aims and objectives:** To determine the frequency and spectrum of hemoglobinopathies among pediatric patients presenting with microcytic hypochromic anemia and their correlation with significant hematological parameters. **Materials and Methods:** This retrospective descriptive study reviewed pediatric (age 0–18 years) cases presenting with microcytic hypochromic anemia who underwent HPLCat the referral laboratory were studied for hemoglobinopathies. Samples were received from various private hospitals and laboratoriesFromnov2022 to october 2025.Demographic and hematological data (CBC, red cell indices, peripheral smear) and HPL data were collected and analyzed. **Result:** Of 169 children analyzed, 48 (38.7%) had an underlying hemoglobinopathy identified on HPLC. The most frequent findings were β-thalassemia trait (n = 40, 23.6%). Also found were Compound heterozygous HbS with Beta Thalassemia (n = 3, 1.7%), 1 case (0.6%) each of sickle cell disease and sickle cell trait and 3 cases with raised HbF (1.7%). Mean HbA2 for Thalssemia trait was 4.6%. Mean MCV in Thalassemia trait is 55.2±1.84 fl. Majority of the Thalassemia cases had Hb level in the range of 9-12 g/dl.Maximum cases had RBC count >5x106/µl.
Conclusion: Although nutritional defiecincy is a leading cause for pediatric microcytic hypochormic anemia, as ubstantial proportion of cases harbor hemoglobinopathies. HPLC is an accurate, simple and superior technique in detection of various haemoglobinvariants. Routine screening protocols in high-prevalence areas along with premarital and antenatal screening can help in early diagnosis, direct management and prevent child birth with severe hemoglobin disorders.

INTRODUCTION

Haemoglobinopathies are the group of genetic disorders of haemoglobin in which there is a quantitative or qualitative abnormal production or defect in the structure of haemoglobin.[1,2]
The two main groups are structural Hb variants including HbS, HbE, HbC and thalassemia syndromes.[3]
WHO estimates that 7% of the world population are carriers of hemoglobinopathies.
Haemoglobin disorders present a significant health problem in 71% of 229 countries, and these 71% of countries include 89% of all births worldwide. Over 330 000 affected infants are born annually (83% sickle cell disorders, 17% thalassaemias).[4] Among them around 56,000 conceptions are born with thalassemia disorder and 30,000 of them would be beta thalassemia, the majority of them being born in developing countries [5]
The β thalassemias and sickle cell disorders pose a significant health burden in india. The average prevalence of β thalassemia carriers in india is 3–4% which translates to 35 to 45 million carriers which is largest in the world.[6] The prevalence of various hemoglobinopathies

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exhibits significant regional and ethnic variability. Notably, β-thalassemia prevalence is 6.5% in Punjab, 8.4% in Tamil Nadu, 4.3% in southern India, and 3.5% in Bengal. In Gujarat, the incidence ranges from 10% to 15%, illustrating the geographical heterogeneity of prevalence. A high prevalence of sickle cell anaemia is observed, particularly in Madhya Pradesh, Chhattisgarh, and Odisha, with carrier frequencies reaching 20–30% in tribal populations.[7] There is an uneven distribution in frequencies of β thalassemia carriers in different districts in Maharashtra (1–6%) and Gujarat (0–9.5%) within small geographic regions. The prevalence of sickle cell trait and disease is very high amongst the tribal population groups from Nandurbar and Gadchiroli district of Maharashtra.[8]

Therefore populations at increased risk should be screened for hemoglobinopathies to reduce number of affected births and in case of sickle cell disease, reduce childhood mortalities [9]. This can further help in management (transfusion planning, iron-chelation when needed), genetic counseling and prevention programs. HPLC isa simple and rapid automated system with reproducible and precise results.[10] This study describes the frequency and hematological characteristics of hemoglobinopathies in children presenting with microcytic hypochromic anemia in southeast maharashtra.

MATERIALS AND METHODS

A retrospective descriptive study was conducted over a period of 3 years from November ,2022 to October,2025 at a referral laboratory in south east maharsahtra. Samples were received from various private pediatric hospitals and other laboratories.

Cases presenting with microcytic hypochromic anemia with clinical suspicion of hemoglobinoathy were advised HPLC by clinicians and also some cases as reflex testing by pathology laboratory after review of peripheral blood smear and RBC indices were included in study. Clinical history, demographic details were retrived from hospital records.

After informed consent from parents/guardian, blood samples were collected in EDTA vacutainers and were analyzed in automated hematological analyzer Sysmex XN 350 for complete blood count.

Basic hematological workup including complete blood count, hemoglobin. RBC indices and peripheral smear examination was done for all cases.

- ❖ Hematological parameters considered were as follows:
- ➢ Hemoglobin (g/dl)
- ➢ MCV(fl)
  - ➢ RBC count (10^6 millions/µl)
  - ➢ RDW

Inclusion Criteria

- ➢ Samples of all patients of both sexes from 6 months to 18 years in whom hemoglobinopathies were suspected by treating clinician, family history and advised HPLC by consultant pathologist at the referrallaboratory after review of peripheral blood smear and RBC indices.
- ➢ Siblings of index cases till 18 years of age.

Exclusion Criteria

- ➢ Previously diagnosed cases of hemoglobinopathies
- ➢ Within 90 days of blood transfusion
- ➢ Age < 6 months and > 18 years

The HPLC samples were collected in an EDTA tube and were evaluated utilising Fully automated Matrix - LIFOTRONIC H8 Heamoglobin analyzer which functions on the principle of High-Pressure Liquid Chromatography (HPLC), to separate HbF, HbA2 and other variants including HbE, HbS, HbD directly with measuring the absorbance points continually to form chromatogram. Using normal distribution curve fitting auto-iterative algorithm and the column comprises of a small cation exchange cartridge with a requirement of only 12µl of blood sample, and each sample taking only 7.5 minutes for analysis. The samples are injected into the analysis stream and separated by the cation exchange cartridge using a phosphate ion gradient generated by mixing 2 buffers of different ionic strengths to elute the different haemoglobins. A dual wavelength filter photometer monitors the eluent from the cartridge as it passes through the photometer cell. Changes in optical density at 415nm are measured. A secondary filter at 690nm corrects the effects caused by mixing buffers of different ionic strengths. The data is processed and the report giving the chromatogram where the different peaks are identified in defined windows with relevant information like retention time, relative percentage and area.[11]

A haemoglobin A2/ haemoglobin F calibrator and two levels of controls) were included in each run of samples

The data collected were systematically compiled and analysed. Qualitative variables were expressed as percentages, while quantitative variables were represented as percentages or as means accompanied by standard deviations.

RESULTS

The present study is a retrospective study for a period of 3years conducted in a referral laboratory. A total of 169 cases were studied, out of which 48(70%) cases were detected of abnormal haemoglobin and the results are put forward in tabular form.

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Table 1: Distribution of all case (169) study

Hb Pattern	No of cases	Percentage
Normal Hb pattern	121	71.5 %
Beta Thalassemia Trait	40	23.6 %
Compound heterozygous HbS with Beta Thalassemia	03	1.7 %
Sickle cell trait	1	0.6 %
Sickle cell disease	1	0.6 %
Raised HBF	3	1.7 %
Total	169	

Table 2: Age distribution in hemoglobinopathies

Age group	Thalassemia minor	Sickle cell Trait	Sickle cell Disease	Compound heterozygous HbS with Beta thalassemia minor	High HbF	Total
6 months- 1 yr	2				2	4 (8.3%)
1 – 6 yrs	16				1	17(35%)
6-12 yrs	15			1		16(33.33)
12-18 yrs	8	1	1	2		12(25%)

Table 3: Gender distribution of Cases

Gender	% of total
30	62%
18	38%

Table 4: Haemoglobin profile in each case obtained on HPLC (mean±SD)

Diagnosis	HbA0 (%±SD)	HbA2(%±SD)	HbF(%±SD)	Variant Hb (HbS) (%±SD)
Beta Thalassemia minor	90.95	4.6	1.8	
Sickle cell trait	63.9	5.0	0	31.1
Sickle cell disease	0.1	2.6	8.9	88.4
Sickle cell Beta Thalassemia	56.0	5.1	17.4	32.9
High HbF	24.7	0.3	74.3	

Table 5: Comparative analysis of MCV in various hemoglobinopathies

MCV (fl)	Thalassemia Trait	Compound heterozygous HbS with Beta Thalassemia	Sickle cell trait	Sickle cell disease	High HbF	Total
50-65	38 (95%)	3 (100%)			2 (66.6%)	**43 (89%)**
65-80	2 (5%)			1 (100%)	1 (33.3%)	4(8.3%)
80-90			1 (100%)			1 (2%)

Table 6: HPLC Interpretation with CBC Hb(g/dl)

Hb(g/dl)	HPLC findings					Total
	Thalassemia Trait	Compound heterozygous HbS with Beta Thalassemia	Sickle cell trait	Sickle cell disease	High HbF	
< 5 g/dl				1		1(2%)
5 - 7g/dl		2	1		2	5(10%)
7-9 g/dl	06	1			1	8 (16.6%)
9-12 g/dl	34					**34(70%)**
Total	40	3	1	1	3	48

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Table 7: HPLC interpretation with CBC RDW (CV)

RDW (CV)	HPLC findings					Total
	Thalassemia Trait	Compound heterozygous HbS with Beta Thalassemia	Sickle cell trait	Sickle cell disease	High HbF	
10-15	6	1				7 (14.5%)
15-20	32	2	1		1	**36 (75%)**
20-30	2			1	2	5 (10.4%)
Total	40	3	1	1	3	48

Table 8: HPLC interpretation with CBC TRBC (106/ul).

RBC count (106/µl)	HPLC findings					Total
	Thalassemia Trait	Compound heterozygous HbS with Beta Thalassemia	Sickle cell trait	Sickle cell disease	High HbF	
< 2						0
2- 3				1		1(2%)
3-4		1	1		2	4(8.3%)
4-5	05	2			1	8(16.6%)
>5	35					**41**(85%)
Total	40	3	1	1	3	48

Table 9: Haematological parameters (Mean) in different group of hemoglobinopathies

Hemoglobinopathies (n)	Hb(mean±SD)	RBC count (mean±SD)	MCV(mean±SD)	RDW(mean±SD)
β Thalassemia Trait (40)	9.6+0.72	5.14+0.82	55.2+1.84	16=2.19
Compound heterozygous HbS with Beta Thalassemia (3)	7.8+1.8	4.8+0.73	69.5+4.5	19.1+3.4
Sickle cell trait (1)	7.7	4.4	65	18
Sickle cell disease (1)	6.2	4.1	72	
High HbF (3)	6.4+0.3	5.1+0.4	61+3.2	17+2.1

Statistical data analysis was done on Microsoft Excel 2012. Continuous variables were expressed as mean SD. Categorical variables were expressed in frequencies and percentages. Data obtained was tabulated using version 22 of the statistical package for social sciences

DISCUSSION

Anemia is defined as a reduction of the total circulating red cell mass below normal limits. In India microcytic hypochromic anaemias are caused predominantly by Iron deficiency and disorders of haemoglobin synthesis due to abnormal variants of Hb. Many of the abnormal variants of Hb are of little clinical significance in heterozygous state, but when in the homozygous state or combined with other variants, they may give rise to severe disease. In any given population, it is the children that are both most vulnerable as well as most suitable for timely intervention and efficacious treatment. Itis also this pediatric age group that genuinely reflects the challenges we are facing as a society from hemoglobinopathies. India is an ethnically diverse country with marked regional variation. This diversity is reflected in the presence of different hemoglobin variants in different ethnic groups. Moreover due to migration, there is constant mixing of people from different regions. Our study on hemoglobinopathies in 6 months-18yrs of age group was carried out in southeast Maharashtra.

In this study, total 169 cases were studied, normal Hb pattern was found among more than half of children.i.e.121 (70%) similar to study by Setia and Bagga et al. & hemoglobinopathies was seen in 48 (30%) of cases similar to study by Mondol.[12]

Among the children with hemoglobinopathies, the most common hemoglobinopathy found was Thalassemia trait amounting to 24.2 % of total HPLCs performed. Second most common hemoglobin pattern was sickle thalassemia trait and least in number were sickle cell disease and sickle cell trait. A study by ankita et al. shows Thalassemia cases as predominant hemoglobinopathy.[13] In a study by Behera and et al. sickle cell trait and disease were the predominant hemoglobin patterns.[14]

In a study by shivangisolanki, sudhajain et al. Sickle cell trait was the most frequent Hemoglobin disorder.[15]

Along with above three studies on pediatric population, results of few other studies are mentioned below in tabular form.

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Table 10: Comparative studies of abnormal haemoglobin variants with the present study.

Studies	Total no. of samples analysed	Total no. of normal pattern observed	Total no. of abnormal pattern observed	Most common haemoglobinopathy observed	No. of most common Haemoglobinopathy observed
Campbell et al. [17]	25750	24587	1163	Sickle cell trait	568
Sachdev et al. [18]	2600	2273	327	Beta thalassemia trait	232
Rao et al. [19]	800	553	247	Beta thalassemia trait	145
Chandrashekar et al [20]	543	00	543	Beta thalassemia trait	206
Bhalodia et al. [21]	500	457	43	Beta thalassemia trait	26
Pant et al. [22]	4800	4510	290	Beta thalassemia trait	216
Mondal et al. [23]	119336	104804	14532	Beta thalassemia trait	5488
Banerjee et al. [24]	1048	444	604	Beta thalassemia trait	156
Present Study	169	121	48	Beta thalassemia trait	40

Our study corresponds to most of the studies in above table showing Beta Thalassemia trait as the predominant hemoglobinopathy. The resident population in India is multi-cultural and multi-regional. This mixed ethnicity is reason for variable distribution of hemoglobinopatheis across different regions of India.[16]

Most of our patients belong to areas of south-East Maharashtra.

Hemoglobinopathies were reported in children who were between 1-6 years (35%), followed by 6-12 years of age (33.3%) and >12 years of age (25%) Males were predominantly affected (62%) similar to study by Setia and Bagga et al.[12] And study by Ankita et al.[14]

Mean HbA2 for Thalssemia trait was 4.6%, Mean HbS in sickle cell trait and sickle cell disease were 31.1% and 54.4 respectively. Three cases of High HbF (70-80%) were found which were suspected to be Thalassemia major clinically but their genetic study results couldn't be retrieved. In a study by Syeda degermenci et al. Mean HbA2 in Thalassemia trait in iron sufficient and iron deficient children was 4.9% and 4.1%respectively which corresponds to our study as iron status was not determined in our study.[28] In a study by Ankita garg et al. mean HbA2 was 5.17%.[13] Different authors have established different cutoff values for HbA2 for diagnosis of Beta Thalassemia Trait, which ranges from 3.5% to 4%. It has been recommended that each laboratory should establish individual normal ranges [19] In our study the cut off value considered for Beta Thalassemia Trait was >3.5%.

Comparative analysis of MCV showed that maximum cases (89%) were having MCV in the bracket of 50-65 fl. This could be due to predominant case load of Thalassemia Trait in our study as MCV values are low in Thalassemia traits.[25]

Mean MCV in Thalassemia trait (Table no 9) is 55.2+1.84 fl. A study by Bhalodia et al. shows 59.5+6.1 fl [21] and a study by sudke et al. which is a pan india study shows mean MCV 57.9628± 4.79 [30]. Both these studies show similar results as our study.

Evaluation of Hemoglobin level amongst different Hemoglobinopathies reflected that majority of the Thalassemia cases had Hb level in the range of 9-12 g/dl. While sickle cell trait and sickle cell disease and sickle –thalassemia cases had Hb in slightly lower range i.e.5-7 g/dl and 7-9 g/dl. Levels of MCV and Hb in our study correspond to a study by Richa jain et al.[27]

Assessment of RBC count showed maximum cases (85%) with RBC count >5x10^6/µl.RDW was in the range of 15-20 cv for maximum cases (75%). In a study by **Marwa Hasanet al.** In the β-thalassemia minor group, the mean (RBC count and RDW) were 5.96±0.71×10^6/µL and 15.33±1.29, RDW being highly specific for diagnosis of Thalassemia Minor, Which corresponds with our study.[29]

Results of Comparative evaluation of Mean+ SD of all hematological parameters (Hb,MCv,RBC and RDW) in different hemoglobinopathies are largely comparable with a study by Bhalodia et al.[21] which involves western Indian population and and a study by Sudke et al., which is a nationwide study of a network of reference laboratories.[30]

CONCLUSION

A high frequency of hemoglobinopathies was found in this region. It should be considered as a significant cause of anemia in children and need to be diagnosed early in order to prevent indiscriminate administration of iron supplementation in all cases of microcytic hypochromic anemia and also to reduce morbidity and mortality in homozygous conditions.

premarital and antenatal screening are helpful in detecting abnormal hemoglobin disorders and identifying carrier states as well as double heterozygous states which may lead to severe haematological abnormalities.

HPLC is highly sensitive and specific and helps in identifying hemoglobins and eluting hemoglobin fractions like HbA, HbA2, HbF, HbS, HbD, HbE and HbC

REFERENCES

1. WHO. Management of haemoglobin disorders. Report of joint WHO-TIF meeting on the management of haemoglobin disorders. Nicosia, Cyprus, 16-18 November 2007. World Health Organization 2008; 1-2. Available from: http:// www. who. int/ genomics/ WHO TIF genetics
2. Vaz FE, Thakur CB, Banerjee MK, Gangal SG. Distribution of beta-thalassemia mutations in the Indian population referred to a diagnostic center. Hemoglob. 2000;24 (3):181-194. doi: 10.3109/03630260008997526

ISSN (O): 2687-5365; ISSN (P): 2753-6556

3. Kohne E. Hemoglobinopathies. DtschArztebl Int. 2011 Aug;108(31-32):532-540.
4. Modell B, Darlison M. Global epidemiology of haemoglobin disorders and derived service indicators. Bull World Health Organ. 2008;86(6):480–7. ([PMC][1])
5. Colah R, Italia K, Gorakshar A. Burden of thalassemia in India: The road map for control. PediatrHematol Oncol J. 2017 Dec 1;2(4):79-84.
6. Campbell M, Henthron JS, Davies SC. Evaluation of cation-exchange HPLC compared with isoelectric focusing for neonatal hemoglobinopathy screening. Clin Chem. 1999;45:969-75.
7. Ambekar SS, Phadke MA, Mokashi GD, Bankar MP, Khedkar VA, Venkat V, *et al.* Pattern of hemoglobinopathies in western Maharashtra. Indian Pediatr. 2001; 38(5):530-534.
8. Varsha S Zade, Sandeep Chede, VG Thakare, NW Warghat. The prevalence of sickle cell disease phenotypes and sickle cell gene frequency in some tribals of Melghat forest region of Amravati, Maharashtra (India). Bioscience Biotech Res Comm. 2011; 4(1):70-3
9. Tyagi S. Hematography in high performance liquid chromatography in hematology. In: Saxena R, Pati HP, Mahapatra M, editors. De Gruchy's Clinical Haematology in Medical Practice. 6th Ed. New Delhi, India: Wiley India, 2013, p. 481-90.
10. Warghade S, Britto J, Haryan R, Dalvi T, Bendre R, Chheda P, *et al.* Prevalence of hemoglobin variants and hemoglobinopathies using cation-exchange high-performance liquid chromatography in central reference laboratory of India: A report of 65779 cases. J Lab Physicians 2018; 10:73-9. 11.https://www.matrixlabs.in/hemotology-h8.php
11. Setia S, Bagga P. Spectrum of hemoglobinopathies in children by HPLC in a tertiary care hospital. Journal of Cardiovascular Disease Research. 2023;14(4).
12. Ankita Garg,Pooja Agarwal 2 , Prashant Bhardwaj , Ranjan Agrawal , Rajesh Bansal, A Study of Different types of Hemoglobinopathies in Pediatric Population with Anemia by High Performance Liquid Chromatography in a Tertiary Care Centre.International Journal of Toxicological and Pharmacological Research 2023; 13(1); 171-178
13. Behera SK, Mohanty SR, Nayak J, *et al.* Spectrum of hemoglobinopathies in pediatric population in Southern Odisha: An institutional study. J Med Sci Clin Res. 2019;7(7):935-942.
14. Shivangi Solanki, Sudha Jain, Bhumika Patel and Hardik Jain. **Study of hemoglobinopathies using HPLC in paediatric patients at a tertiary care hospital**. Int. J. Clin. Diagn. Pathol. 2024;7(4):105-108. DOI: 10.33545/pathol.2024.v7.i4b.2039
15. Sandhya iyer *et al.* Hemoglobinopathy in India.Clinicachimcaatta.Vol 444.229-233
16. Campbell M, Henthorn JS, Davies SC. Evaluation of cation-exchange HPLC compared with isoelectric focusing for neonatal hemoglobinopathy screening. Clin Chem. 1999; 45(7):969-975.
17. Sachdev R, Dam AR, Tyagi G. Detection of Hb variants and hemoglobinopathies in Indian population using HPLC: report of 2600 cases. Indian J Pathol Microbiol. 2010;53(1): 57-62. doi: 10.4103/0377-4929.59185
18. Rao S, Kar R, Gupta SK, Chopra A, Saxena R. Spectrum of haemoglobinopathies diagnosed by cation exchange-HPLC & modulating effects of nutritional deficiency anaemias from north India. Indian J Med Res.
19. Chandrashekar V, Soni M. Hemoglobin disorders in South India. ISRN Hematol. 2011;2011:748939. doi: 10.5402
20. Bhalodia JN, Oza HV, Modi PJ, Shah AM, Patel KA, Patel HB. Study of hemoglobinopathies in patients of anemia using high performance liquid chromatography (HPLC) in Western India. Natl J Community Med. 2015; 6(1): 35-40./2011/ 748939. Epub 2011 Jun 28.0; 132:513-519.
21. Pant L, Kalita D, Singh S, Kudesia M, Mendiratta S, Mittal M, Mathur A. Detection of abnormal hemoglobin variants by HPLC method: common problems with suggested solutions. Int Scholar Res Not. 2014;2014. doi:http://dx.doi.org/ 10.1155/2014/257805
22. Mondal SK, Mandal S. Prevalence of thalassemia and hemoglobinopathy in eastern India: a 10-year highperformance liquid chromatography study of 119,336 cases. Asian J Transfus Sci. 2016;10(1):105-110. doi: 10.4103/0973-6247.175424.
23. Banerjee S, Singh RK, Shrivastava RK, Mahto SK. Study of haemoglobinopathies in patients of anaemia using High Performance Liquid Chromatography (HPLC) in rims (a premier institute of Jharkhand). J Evol Med Dent Sci-JEMDS. 2016; 5(46): 3029-3033. doi: 10.14260/jemds /2016 / 681.
24. Kawthalkar SM. Essentials of clinical pathology. Jaypee Brothers, Medical Publishers Pvt. Limi
25. Khera R, Singh T, Khuana N, Gupta N, Dubey AP. HPLC in characterization of hemoglobin profile in thalassemia syndromes and hemoglobinopathies: a clinicohematological correlation. Indian J Hematol Blood Transfus. 2015 Mar;31(1):110-5. doi: 10.1007/s12288-014-0409-x. Epub 2014 Jun 5. PMID: 25548455; PMCID: PMC4275515.ted; 2018.
26. Dr. Richa Jain, Assistant Professor, 2Dr. Shubhi SaxenaStudy of abnormal haemoglobin variants in patients of anaemia using high performance liquid chromatography (HPLC) in Gujarat, India. **Tropical Journal of Pathology & Microbiology** November, 2019/ Vol 5/ Issue 11
27. Değermenci Ş, Aslan D. HbA2 levels in children with β-thalassemia trait associated with iron deficiency: A perspective for pediatricians. Am J Clin Pathol. 2024 Jul 23;162(6):544–8. doi: 10.1093/ajcp/aqae085. Epub ahead of print. PMID: 39045640; PMCID: PMC11637608.
28. Marwa Hasan Abbas.1, Zuhair M Almusawi.2, & Hosna Hasan Abbas.3. (2024). Utility of Red Blood Cell Indices in the Diagnosis of β-Thalassemia Minor. *International Journal of Medical Science in Clinical Research and Review*, *7*(01), Page: 1–7. Retrieved from https://ijmscrr.in/index.php/ijmscrr/article/view/663
29. **Dr Smita Hiras Sudke1, Dr Moindrila Halder2, Raj Jatale3** Thalassemia and Hemoglobinopathies In Pediatric Populations: Prevalence, Diagnostic Insights, And Regional Patterns. Clinical Medicine and Health Research Journal (CMHRJ) Volume 05, Issue 02, March - April, 2025 Page No. 1241-1247.

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