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Diagnosis And Haematological Parameters of VariousHaemoglobinopathies in Paediatric Age Group by Using Cation Exchange High Performance Liquid Chromatography-A Hospital Based Crosssectional Study

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***Abstract*:** *Haemoglobinopathies particularly β thalassaemia and sickle cell anemia are the most commonly encountered single gene disorders in the world; as so in India. About 4.5% of the world's population carries genes responsible for haemoglobinopathies, and are especially common in populations of tropical Africa, Asia and mediterannaean region and have spread via migration throughout the world.The objective of current study is to find occurrence of haemoglobinopathies in paediatric patient and various haematological parameters in different haemoglobinopathies. In this study various abnormal haemoglobin fractions on HPLC were observed in 158 cases out of the total 300 screened paediatric anaemic cases. Of the 300 paediatric cases, samples analyzed on CE-HPLC for haemoglobinopathies, maximum 67 (22.33%) cases were diagnosed as S-β double heterozygous, 39(13%) as sickle cell trait, 27 (9 %) as β-thalassaemia major, 10 (3.33 %) as sickle cell disease, 05 (1.66 %) were diagnosed as β-thalassaemia trait, 03 (1%) were unknown haemoglobins , 02 (0.66 %) as Hb E-β-thalassaemia double heterozygous, 02 (0.66 %) as SCT + Hb D trait and 01 (0.3%) as Hb E trait. The prevalence of haemoglobinopathies among children is more commonly seen in countries with limited resources, where priority tends to be given to tackling infant and child mortality from infections and malnutrition.*

I. Introduction

Haemoglobinopathies, which include thalassaemia and sickle-cell disease, are major public health problems especially in the Mediterranean area, Middle East, Indian subcontinent, Far East and tropical Africa. It has been estimated that about 250 million people are heterozygous for these disorders and at least 300 000 lethally affected homozygotes are born annually throughout the world.[1]The cumulative gene frequency ofhaemoglobinopathies in India is 4.2%.Sickle cell anaemia is especially prevalent in central and southern parts of the country, with an estimated prevalence of 0-18% in North eastern India, 0-33.5% in Western India, 22.5-44.4% in Central India and 1-40% in Southern India.[2] Sickle cell gene is widely spread in all districts of Eastern Maharashtra i.e. Vidarbha, North Maharashtra i.e. Satpuda ranges, Nandurbar and some parts of Marathwada region[3,4]. Estimated 10 %(30-40 million) of the worldsthalassaemics belong to the Indian subcontinent with carrier rates ranging from 1-17% with an average of 3.2%.[5]

Majority of patients homozygous for β-thalassaemia or SCD; or double heterozygotes of β-thalassaemia with HbS or HbE have severe disease starting from infancy and require regular blood transfusion for survival and succumb eventually to myriad range of causes by adolescence or adulthood.[3,5,6]

The cost of optimal management for a child with thalassaemia including regular blood transfusions, chelation therapy, investigations, hospitalization and specific vaccines is approximately Rs 125 000 per annum, not an affordable amount by most Indian families. The result is irregular transfusions without chelation therapy, leading to complications of iron overload and early death. Patients with sickle-cell disease do not require transfusions but are disabled due to severe pain as a result of vaso-occlusive episodes and susceptibility to infections. Splenic sequestration, haemolytic and aplastic crises may be Life threatening.They require antibiotic prophylaxis, specific immunizations and frequent hospitalization[7]. Haemoglobinopathies form a significant proportion of hereditary disorders in paediatric population leading to a range of myriad complication, leading to mortality in large number of afflicted patients. Most common forms of these includes thalassaemia and sickle cell anaemias. Published literature includes various reports on screening patient using HPLC in adults as well as paediatric population. However, there is paucity of literature on studies on exclusive paediatric population.

II. Material And Methods

This study cleared by institutional ethic commette of Government medical college NANDED Maharashtra,india.This is a hospital based cross sectional study in which a total of 300 anaemic paediatric patients (6months to 14 years of age) along with there parents blood samples were analysed for Hb variants who were referred from pediatric department of GMC nandedmaharashtra,from various Primary health centre and rural health centre around nandedregion of maharashtra.The study was conducted for 18 month from January 2013 to June 2014.patients were having clinical manifestation like anaemia ,hepatospleenomegaly,weakness,repeatedinfection,aches and pain,feveretc.Detail clinical history including ethnic origin,agesex,blood transfusion etc along with family history was taken.

After consent from parents, 2 ml of venous blood collected in EDTA (Ethylene diamine tetra-acetic acid) coated vaccutainers from each patient and there parents wherever required.Patients transfused in last 3 months anfage lessthen 6 month were not included in study.TheHematological profile of cases was done, which included PS, CBC including RBC Indices, reticulocyte count etc. The complete blood count was done by using orphee-mythic cell counter. With the help of Bio rad thalassemia shortprogrammHPLC exact percentage of HbS, HbF, HbA_2 and HbA_0 was estimated to classify the cases.

Then study of family members accompanying the cases was done to confirm the diagnosis and to determine ethnic background.The statistical analysis of all data was carried out using Microsoft Office Excel and SPS 16.

III. Results

Of 300 (159 male+141 female) patients screened for haemoglobinopathies. Out of 300 patients 158 (81 male+77 female) variant haemoglobinopathies cases were diagnosed.Of 158 variant haemoglobinopathy cases detected, 57 (36.07 %) (32males + 25 females) belonged to the 0-5 year age group, followed by 53 (33.54 %) (24 males + 29 females) to the 6-10 year age group and 48 (30.37 %) (25 males + 23 females) to the 11-14 year age group.

Figno. 1Of the 300 patients screened for haemoglobinopathies, Pallor (Anaemia) was the most common clinical feature seen in 261(87%) patients, followed by Weakness in 202 (67.33%) cases, other presenting complaints include fever, hepatomegaly, spleenomegaly, Aches and pains, Paleness of body (jaundice), growth stunting, repeated blood transfusions, Failure repeated blood transfusions, Failure to thrive in infants, convulsion and a positive family history.

Of the 158 paediatric cases with variant haemoglobins, 59 (37.34 %) parents have history of consanguineous marriage.

Table no 1 shows :- Of the 300 paediatric cases, samples analyzed on CE-HPLC for haemoglobinopathies, 67 (22.33%) were diagnosed as S-β double heterozygous, 39(13%) as sickle cell trait, 27 (9 %) as β-thalassaemia major, 10 (3.33 %) as sickle cell disease, 05 (1.66 %) were diagnosed as β-thalassaemia trait, 03 (1%) were unknown haemoglobins , 02 (0.66 %) as Hb E-β-thalassaemia double heterozygous, 02 (0.66 %) as SCT + Hb D trait and 01 (0.3%) as Hb E trait.Alphathalasssaemia and unknown haemoglobinopathy cases were suspected cases and referred to higher center for genetic analysis.

As per table no 2 Of the 300 patients screened for haemoglobinopathies, 100 (33.33 %) belonged to the Boudha community, followed by 56 (18.66 %) and 32 (10.66%) to the Banjara and Muslim community respectively.Others were, Gond, Matang, Andh, Maratha, Mahar, Gowari, Sikh, Hatkar, Kumbhar, Manelwarli, Wadar, Koli and Navhi.Of the 158 variant haemoglobinopathy cases found, 69 (43.67 %) belonged to the bouddha community, followed by 38 (24.05 %) to the Banjara community, followed by 10(6.32%) and 09(5.69%) to Andh and Gond community respectively.

Table 3 and 4 shows the various haematological parameters in variant haemoglobinopathies.

IV. Discussion

In our study Out of 300 patients 158 (52.67%) variant haemoglobinopathies cases diagnosed on CE-HPLC, In 2014 Dr. Mauchumisakia Pathak et al[8]studied 800 anaemicpaediatric patients and found 522(65.25%) variant haemoglobinopathypatients.During the present study, majority cases of haemoglobinopathies were found in the age group of 0 – 5 years, Similar results were seen in the study by S.S.Ambekar et al (2001).[9]

And out of 158 cases of variant haemoglobinopathies detected, 81(51.27%) were males and 77(48.73%) females and found comparable with Dr.MauchumiSaikia Pathak et al [8]in 2014 that is 522 cases , 268(51.34%) were male and 254(48.66%) were female.

Endogamy and consanguinity are a common practice in the Indian subcontinent and it poses a major risk factor for the homozygous inheritance of haemoglobinopathies. Of the 158 cases with variant haemoglobins, 59 (37.34 %) parents have history of consanguineous marriage.The study done by Shivashankara A.R et al in 2008[10], J Sana et al[11] in 2008 and Colah et al[12] in 2010 reported 20%,24.3% and 21% cansanguinity rate in haemoglobinopathy cases.

It was found significantly very high in haemoglobinopathy detected cases. Majority of the cases in our study belonged to tribal and low socioeconomic communities, where in the tradition of consanguineous marriages is common, thus the reason of higher percentage of consanguinity in our study.

T.sahu et al (2003)[13], Shah. Sejal et al (2012)[14], Dr.MauchumiSaikia Pathak et al (2014) [8]found pallor (anaemia) as most common presenting complaint in their studies which is comparable to the present study in which the most common presenting complaint was pallor in the afflicted (89.24%) cases.Hepatomegaly (65.16%) and splenomegaly (62.65%) was found next most common clinical presentation in afflicted cases. 45(67.16%) cases out of 67 cases of S β-thalassaemia had hepato-splenomegaly.Tyagi et al (2003) [15]also found that 75% splenomegaly in S β-thalassaemiacases.The prevalence of Aches and pain in the study population was in 55.69% of afflicted cases.The prevalence of paleness of body (jaundice) in the study population was 35% but 47.46% in the afflicted group.Also, Family history (22.15%), growth stunting (40.50%) was significantly higher in the afflicted patients as compared to the general study population.There were only 01 afflicted patient came with presentation of convulsion, later diagnosed as sickle cell disease with transient ischaemic attack.

As per table no 1The most common haemoglobinopathy detected was Sβ-thalassaemia double heterozygous in 67(22.33%) of the cases, which is significantly raised. 39(13%) as sickle cell trait, it is comparable to Shah Sejal J et al (2012) [14] 27(9%) as β-thalassaemia major it is comparable with S.S.Ambekar et al (2001)[9]10(3%) as sickle cell disease it is comparable toDr.MauchumiSaikia Pathak et al (2014)[8]found 2% cases of Sickle cel disease in his study.β-thalassaemia trait 05(1.66%) cases S. S. Ambekar et al (2001) [9] in his study also got very low percentage of thalassaemia trait (0.5%).The reason may be because thalasaemia trait patients are asymptomatic.Hb E-β-thalassaemia double heterozygous, 02 (0.66%) cases, this variant of haemoglobinopathies is more prevelant in eastern state of india, but rarely found in other parts of india.02(0.66%) cases of compound heterozygous for Sickle cell trait with HbD trait (HbSD), Mukherjee M.B. et al (2005)[16] reported this rare case in 10 year old female. 1 case (0.3%) was suspected as alpha thalassaemia* and 3 cases (1 %) were unknown haemoglobins; these unknown haemoglobins after relevant family studies were referred to higher center at Mumbai for further evaluation and diagnosis.

We came across that there is rise in double heterozygous cases ,it is a alarming sign that tells there is rise in heterozygous cases in society of this area.These cases could have avoided only by doing premarriatal screening test of pre conceptional diagnostic tests.

In present study, out of the total 300 paediatric cases screened, 100(33.33%) children belonged to Boudha community while those in Banjara were 56 (18.66%). 32 (10.66%) children belonged Muslim community, 17(5.66%) to the Gond and matang each community.

Of the total 158 affected paediatric cases found positive on HPLC, 69 (43.67%) children belonged to Boudha community while those in Banjara were 38 (24.05%). 10 (6.32%) children belonged Andh community,9 (5.69%) to the Gond and 07 (4.43%) muslimcommunity.InAmbekar SS et al (2001) [9] maximum cases were of Navbudha caste(33.5%) while The present study reveals that the occurrence of haemoglobinopathies in general is higher among the Boudha (43.67%) and banjara (24.05%) populations in our region, followed by Andh, Gond, and Muslim. The reasons are varied but the main reason are high incidence of endogamy and consanguineous marriage, and the problem compounded with illiteracy and low socio-economic development. In the present study 2 out of 2 cases (100%) of HbEβ Thal patients were Muslims and found comparable with study done byShahSejal et al (2012)[14].Also 02 cases of compound heterozygous for sickle cell trait and HbD Punjab trait found in our study, both the children blonged to sikh community.

The haematological parameters as per table no 3 Of the 142 cases with normal HPLC studies,Meanhaemoglobin was 8.96 gm% with a S.D of 2.1. ,Mean PCV was 27.53% with a S.D of 5.96.,Mean MCV was 75.6fl with a S.D of 10.97.,Mean MCH was 22.06pg with S.D of 3.85.Of the 158 cases with variant haemoglobins, the Mean haemoglobin was 7.98gm% with a S.D of 2.30. ,Mean PCV was 24.68% with a S.D of 6.57.,Mean MCV was 74.42fl with a S.D of 9.80.,Mean MCH was 23.3pg with S.D of 3.58.,Mean RDW was 17.26 with a S.D of 4.04.

After applying unpaired t test to blood indices of normal and variant cases the P value was found highly significany for Hb gm%(haemoglobin) PCV% and RDW. Also it was significant for MCH.But P value was not significant for MCV in the present studyThalassaemia major patients had low Haemoglobin levels (5.15±1.27), In Thalassaemia trait patients the mean haemoglobin levels were (8.32±1.95) in S-beta thalassaemia cases mean Hb levels were 8.17±2.16 compared to the other groups, in 2010 Rao Seema et al [17]found Hb level (5.4 ± 1.7)in thalassaemia major,Hb level (10.3 ± 2.1) in thalassaemia trait and Hb level (7.6 ± 1.2).The study done by raoseema et al was not exclusively on paediatric population leading to variation of result in thalassaemia trait patients as thalassaemia trait is asymptomatic.

On CE-HPLC,InThal Major Group the average HbF levels were the high as compared to the other groups and it was (88.30±11.92.).In 2011 C.Vaniet al[18] reported Hbf 88%. Elevated HbF levels were encountered in double heterozygous states of Sβ thal and Eβ thalpatients.The cut off value of HbA2 >3.9%, was used to diagnose Thalassemia trait, after exclusion of the other causes of increase HbA2 .In the present study in Thal trait patients the average HbA2 level was found to be (4.34±0.87)in 1993 G.B.Tan et al[19] reported 4.6%

HbA2 in thalassaemia trait **.**The mean HBS levels in Sickle cell disease patients was found 73.77±10.31, in Sickle cell trait 33.81±6.32 and in SB Thalassaemia patients was 42.74±16.94.

Figure And Tables

Sr.no.	Haemoglobin Pattern	No. of Cases	Percentage %
1.	Normal	142	47.33 %
2.	Sickle cell trait	39	13 %
3.	Sickle cell disease	10	3.33 %
4.	β thalassaemia major	27	9 %
5.	β thalassaemiaintermedia	01	0.33 %
6.	β thalassaemia trait	05	1.66 %
7.	S β-thalassaemia	67	22.33 %
8.	E β-thalassaemia	02	0.66 %
9.	Heterozygous HbE	01	0.33 %
10.	Homozygous HbE	00	00 %
11.	SCT + HbD trait	02	0.66 %
12.	Alpha thalassaemia	01	0.33 %
13.	Unknown	03	1%
	Total	**300**	**100%**

Table no.1 - Haemoglobin patterns found during screening all cases.

Sr.no	Caste	No. of patients screened	Cases having variant haemoglobin
1.	Boudha	100(33.33%)	69(43.67 %)
2.	Banjara	56(18.66%)	38(24.05 %)
3.	Muslim	32(10.66%)	07(4.43 %)
4.	Gond	17(5.66%)	09(5.69 %)
5.	Matang	17(5.66%)	04(2.53 %)
6.	Andh	14(4.66%)	10(6.32 %)
7.	Maratha	12(4%)	02(1.26 %)
8.	Mang	08(2.66%)	03(1.89 %)
9.	Mahar	07(2.33%)	06(3.79 %)
10.	Dhangar	07(2.33%)	01(0.63 %)
11.	Gowari	06(2%)	02(1.26 %)
12.	Sikh	06(2%)	02(1.26 %)
13.	Hatakar	05(1.66%)	0
14.	Kumbhar	04(1.33%)	02(1.26 %)
15.	Manelwarli	03(1%)	0
16.	Wadhar	02(0.66%)	02(1.26 %)
17.	Koli	02(0.66%)	0
18.	Navhi	02(0.66%)	01(0.63 %)

Table no. 2 - Distribution of variant haemoglobinopathy cases according to the Caste.

Para-Meters	**Stati-stics**	**Hb (gm%)**	**PCV (%)**	**MCV (fl)**	**MCH (pg)**	**RDW**
Normal Hb		8.96	27.53	75.6	22.06	18.57
Variant Hb	**Mean**	7.98	24.68	74.42	23.3	17.26
Normal Hb		2.1	5.96	10.97	3.85	1.52
Variant Hb	**SD**	2.30	6.57	9.80	3.58	4.04
	P value	**<0.001**	**<0.001**	**>0.05**	**<0.05**	**<0.001**

Table no. 3 -Comparison of haematological parameters among the normal and variant haemoglobin cases.

Variable (N)	**TM (27)**	**TT (05)**	**SCD (10)**	**SCT (39)**	**SB thal (67)**
Hb(g/dl)	5.15±1.27	8.32±1.95	7.93±1.24	9.89±1.29	8.17±2.16
RBC(10/ul)	3.42±1.13	4.14±0.41	3.93±1.00	4.23±0.57	3.9±0.90
PCV(%)	16±3.87	26.31±6.06	23.59±3.29	29.23±3.41	25.74±6.34
MCV(fl)	64.16±6.84	81.84±6.13	77.42±4.55	78.85±7.99	75.74±9.6
MCH(pg)	21.74±1.52	21.54±1.53	24.70±2.68	24.38±3.78	23.65±4.00
MCHC(g/dl)	26.18±4.76	21.96±4.07	29.73±1.17	30.65±2.64	28.65±4.12
HBA0(%)	12.97±10.65	84.62±1.15	4.29±2.76	51.78±6.73	34.90±20.67
HBA2(%)	2.32±2.13	4.34±0.87	3.31±0.38	3.46±0.33	6.11±2.27
HBF(%)	88.30±11.92	0.78±0.44	20.35±10.16	1.60±1.34	7.54±10.58
HBS(%)	-	-	73.77±10.31	33.81±6.32	42.74±16.94

Note:-

1. Data are mean±SD, **N –** Number of Patients.
2. **HBA0**-Adult haemoglobin, **HBA2**-Adult haemoglobin A_2, **HBF**-Fetal haemoglobin, **HBS**-Sickle cell haemoglobin.

3. **TM-**Thalassaemia major, **TT-**Thalassaemia trait, **SCT-**Sickle cell tr**SCD-**Sickle cell disease, **SB Thal-** Sβ-Thalassaemia

Table no 4 - Haematological features of different types of haemoglobinopathies In the present study

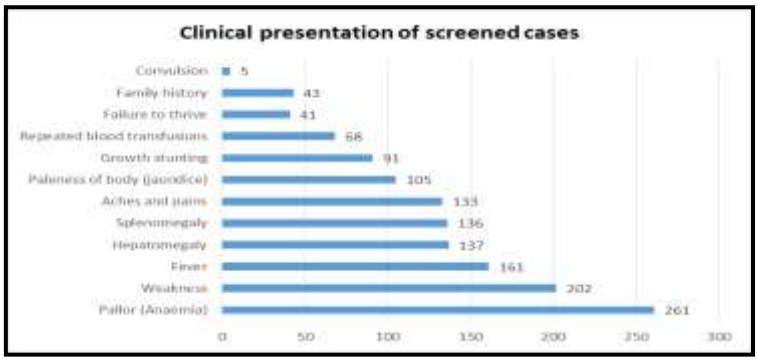
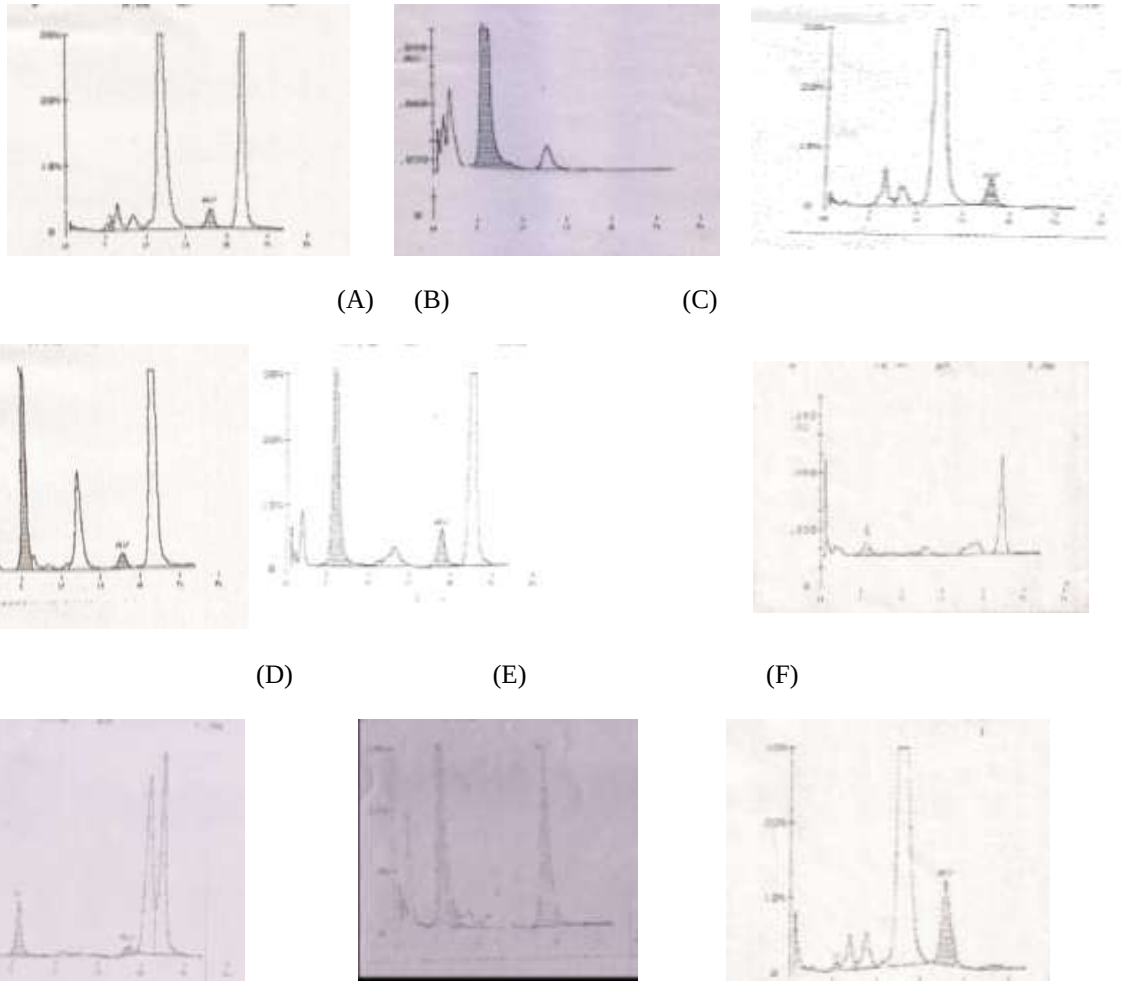


Fig no. 1: Clinical presentation of screened cases.



(A) (B) (C)

(D) (E) (F)

(G) (H)(I)

FIG 2:-Various chromatogram obtained by using BIORAD HPLC machine with thalassaemia short program, on x axis retention time in seconds and on Y axis absorption.Gragh (A)Sickle cell trait (B) β thalassaemia major (C)β-thalassaemia trait(D)Sickle cell disease (E) S-β thalassaemia (F)Hb D trait (G)Sickle cell trait + HbD trait(H) E-β thalassaemia (I)HbE trait

V. Conclusion

Genetic haemoglobin disorders with severe anaemia cause considerable pain and suffering to the patients and their families and are major drain on health resources in India. Frequencies found in the present study confirm that haemoglobinopathies and thalassaemia are public health problem in this region of India, emphasizing the need for neonatal screening and genetic counselling programs.The HPLC based Haemoglobin Testing system forms a rapid and easy tool early detection and management of various haemoglobin disorders. Nationwide Government sponsored programme can effectively reduce the occurrence of new cases of serious haemoglobin variants as well as thalassaemia major cases and thus making it possible to direct the available resources towards the optimization of treatment of the patients who are already present. Detection of these patients with abnormal haemoglobins will help in prevention of more serious Hb variant cases.

The comprehensive data thus obtained can help us formulate, develop and shape infrastructure and policies for afflicted children care and provide impetus for research in the development of advanced techniques, newer drugs and diagnostic modalities.

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