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Original Article

Pathology Section

Spectrum of Haemoglobinopathies Detected on Antenatal Screening and Diagnostic Work-up in an Urban Healthcare Set-up: A Retrospective Study

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ABSTRACT

Introduction: Haemoglobinopathies are inherited disorders of Haemoglobin (Hb) and consist of Thalassemias and many other structurally variant haemoglobins. Out of these, beta-thalassemia major and clinically significant sickle cell disorders are of great public health importance in India. Lack of awareness regarding their prevalence and knowledge about diagnostic methods, has resulted in failure of community control of these otherwise totally preventable genetic disorders in India.

Aim: To study the spectrum of haemoglobinopathies in the study population and to assess the effectiveness of the antenatal screening program, in identifying the couples at-risk and providing prenatal intervention.

Materials and Methods: This retrospective cohort study was performed at a community healthcare set-up in Mumbai from August 2021 to October 2021 on medical records of 10,025 patients including women who were part of antenatal screening, patients investigated for anaemia and who underwent haemoglobin electrophoresis over 12 years (October 2007 to October 2019). Alkaline Electrophoresis test was performed using the InterlabGenios analyser on cellulose acetate and the findings were interpreted along with haemogram parameters. Finding of various conditions were presented in terms of prevalence rates and mean values of relevant blood count parameters were presented as mean±SD.

Results: An abnormal haemoglobin pattern was seen in 544 (5.42%) of the 10,025 cases with age group ranged from 1 year to 84 years. Most common haemoglobinopathies detected were beta (β) thalassemia trait 378 (3.77%) followed by sickle cell trait 93 (0.9%), Hb D trait 26 (0.26%), HbE trait 24 (0.24%) and others. Carriers of haemoglobinopathies were detected in 186 (3.74%) of 4968 women, on antenatal screening. A total of 18 couples-at-risk were identified. One child with thalassemia major and another with sickle cell disease were born in this population over 12 years.

Conclusion: The β-Thalassemia trait and HbS trait are the most common haemoglobinopathies detected. Antenatal screening programme and timely intervention is an effective strategy to control clinically significant major haemoglobinopathies.

Keywords: Electrophoresis, Genetic disorders, Haemoglobin, Sickle cell disorders, Thalassemia

INTRODUCTION

Haemoglobinopathies are a diverse group of disorders that occur due to mutation in the globin chain of Haemoglobin (Hb). These may be either due to reduced synthesis of globin chains (thalassemia syndrome) or abnormal haemoglobins with altered structure and/or function (variant Hb) [1]. These are the most common single gene disorders that follow an autosomal recessive inheritance pattern, and it is estimated that around 300,000 to 400,000 babies with a severe haemoglobin disorder are born each year [1]. Thalassemia major and Sickle cell disease are two severe haemoglobin disorders that are chronic, life restricting and require long specialised treatment. The birth of an offspring with major haemoglobinopathies can be prevented by carrier screening if there is awareness of type of haemoglobinopathies in a population [1].

While sickle cell disorders are more prevalent worldwide, the thalassaemic syndromes including α-thalassaemia, β-thalassaemia and haemoglobin-E disease are associated with high prevalence rates ranging from 2.5% to 15% in the 11 countries of the World Health Organisation (WHO) Southeast Asia (SEA) Region [2]. In a large multicentre study involving 56,780 individuals from six major cities in India, it was estimated that the prevalence of Hb disorders ranged between 3.1% and 31.8% in different regions of the country and in different ethnic groups [3]. This wide variation seen in the prevalence of Hb disorders is due to the genetic diversity of the Indian population with large numbers of endogamous ethnic, geographical, and social groupings. A study have shown that β-thalassemia is prevalent across India [3] and the haemoglobin variants like HbS are more prevalent in tribal population across India [4], HbE in eastern region of India [5] and HbD is high amongst the people of North India [6]. The Indian Government under national health mission (Ministry of Health and Family Welfare) has formulated guidelines for carrier screening with the implicit purpose of prevention of haemoglobinopathies [7].

The population covered in this study include a microcosm of people from all parts of India, unlike most other studies on haemoglobinopathies which had study subjects confined to one region of the country [4-6]. Hence this study aimed to give an insight into the spectrum of haemoglobinopathies across geographical regions as antenatal screening was implemented in the study population, and also highlights the benefits of antenatal screening and prenatal diagnosis in preventing birth of child with major haemoglobinopathy.

MATERIALS AND METHODS

This retrospective cohort study was conducted at BARC Hospital, Mumbai, Maharashtra, India, based on digital records of the patients. Study was done over a period of 3 months from August 2021 to October 2021 during which data of 12 years from October 2007 to October 2019 was analysed. The study was approved by Institutional Medical Ethics Commitee (Ethical Clearance Number_ BHMEC/NP/20/2020). Laboratory records of 10,025 patients,

which included 4968 ANC cases (49.55%) and 5057 non ANC cases (50.44%), who underwent haemoglobin electrophoresis were considered for this study.

Inclusion criteria: All patients whose haemoglobin electrophoresis was performed during the study period and with all relevant laboratory records available, were included in the study.

Exclusion criteria: Patients without complete laboratory investigations data were excluded.

Procedure

Hospital Information System (HIS) based records were used to trace the patients diagnosed with haemoglobinopathies and their laboratory findings. Those records pertaining to the patient like demographic details, clinical and laboratory findings were obtained from the electronic medical records. The demographic data included native place, mother tongue and religion, which provided a reasonable association on the ethnicity and community of the patient [8].

Complete haemogram was performed on five-part fully automated analyser (Sysmex XS1000i) in all cases. Giemsa-stained peripheral blood smears were examined for red cell morphology. In Anaemic patients, as part of diagnostic work-up ferritin, vitamin B12, folic acid were performed on Abbott Architect i1000sr based on Chemiluminiscent Immunoassay (CLIA) principle and serum iron was done in Automated Biochemistry analyser IMOLA based on spectrophotometry. Electrophoresis was performed using the InterlabGenios analyser which elutes the Hb on cellulose acetate agar at a pH of 8.6. Decreased red cell indices [Mean Cell Volume (MCV) <80 fL and Mean Cell Hb (MCH) <27 pg], a relatively high Red Blood Cell (RBC) count and normal Red cell Distribution Width (RDW) in association with HbA2 ≥3.5 percent was used to detect the β-thalassaemia carriers. Structural haemoglobin variants were detected according to the haemoglobin band on electrophoresis. Sickling test was performed on all the samples and it helped to differentiate between HbS and HbD, as they both migrate together on alkaline electrophoresis [7,9].

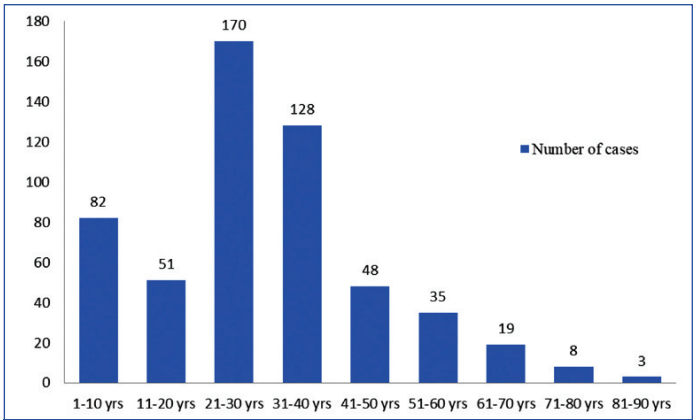
In case of antenatal screening, if a woman was detected with any haemoglobinopathy, screening of their husbands was subsequently done to identify the at risk couples. The couples were counselled regarding the possible outcomes of the pregnancy. Option for prenatal diagnosis such as Chorionic Villous Sampling (CVS) and amniocentesis were provided to the couples to prevent the birth of an affected child [7,9]. Couples at risk of having a baby with HbD-Hereditary Persistence of Foetal Haemoglobin (HPFH), HbD-β-thalassemia, homozygous HbD or HbE disease were reassured that they do not require prenatal diagnosis as affected babies will usually have a very mild clinical presentation which can be easily managed, and they can lead a normal life. The normal range of Red Blood Cell (RBC) parameters considered in this study were Hb (gm/dL) Male- 13-17/Female- 12-15; RBC count (million/mL) M- 4.5-5.5/F-3.8-4.8; Mean Corpuscular Volume (MCV) (fL)- 83-101; Mean Corpuscular Haemoglobin (MCH) (pg)- 27-32; Mean Corpuscular Haemoglobin Concentration (MCHC) (gm/dL)- 33-35 and RDW (CV%) 11.5-14.0 [10].

STATISTICAL ANALYSIS

All the data were extracted in a Microsoft Office Excel Spreadsheet. Exploratory Statistical analysis was performed to present the spectrum of finding of patient with haemoglobinopathies with Statistical Package for Social Sciences (SPSS) Software version 25.0. The number of individuals in the study group was presented in absolute numbers and percentages of the groups. Prevalence rates of various haemoglobinopathies were estimated and their haemogram parameters were presented as a mean±SD. Patient details were presented across age group wise distribution. Cases diagnosed during antenatal period were presented as a subgroup analysis.

RESULTS

Of the 10,025 patients, 544 (5.42%) patients had an abnormal haemoglobin pattern. The age of patients with abnormal haemoglobins ranged from 1 year to 84 years. The most common age group was 21 to 30 years {(170/544 (31.25%)} with the mean age being 29.92 years [Table/Fig-1]. The M:F ratio was 0.52:1 with 188 (34.55%) males and 356 (65.44%) females.



[Table/Fig-1]: Age distribution in cases of haemoglobinopathies.
yrs: Years

Of the total 544 cases with haemoglobinopathies, 186 were ANC cases, constituting 3.74% of all women who underwent antenatal screening. The remaining 358 cases included 18 spouses detected because of partner testing and 60 cases in children following the detection of the carrier state in the pregnant women on ANC screening. The physicians referred 229 cases for anaemia work-up, and 51 cases were detected following the screening in other family members of an index case with haemoglobinopathy.

Spectrum of haemoglobinopathies: In the present study population, most common haemoglobinopathies were thalassemia minor (3.77%) followed by sickle cell trait (0.9%), HbD trait (0.26%), HbE trait (0.24%) [Table/Fig-2]. The haematological parameters showed low Hb values and raised RDW indicating severe anisopoikilocytosis in thalassemia major, sickle cell disease and sickle thalassemia. In β-thalassaemia trait, RBC count was raised [Table/Fig-3]. Haemoglobin electrophoresis showed distinct bands which were quantified to analyse the abnormal HbS [Table/Fig-4].

Haemoglobin disorders	Number of cases (n=10025)	Prevalance %
Normal haemoglobin pattern	9481	94.57
Thalassemia minor	378	3.77
Thalassemia major	1	0.01
Sickle cell disease	4	0.04
Sickle cell trait	93	0.92
Sickle thalassemia	2	0.02
HbD disease	2	0.02
HbD trait	26	0.26
HbE disease	6	0.06
HbE trait	24	0.24
HPFH	5	0.05
HbD β-thalassemia	1	0.01
Delta β-thalassemia	1	0.01
β-thalassemia-Delta β-thalassemia	1	0.01

[Table/Fig-2]: Spectrum of haemoglobinopathies (n=10025).
HPFH: Hereditary persistence of foetal haemoglobin

The relative prevalence of various haemoglobinopathies in ANC population was concordant with that of the non ANC population, with most common being β-thalassemia minor followed by sickle cell trait, HbD trait and HbE trait [Table/Fig-5]. The spouses of all ANC females with haemoglobinopathy were called for screening,

Haemoglobinopathies	Hb (gm/dL)	RBC count (million/mL)	MCV (fL)	MCH (pg)	MCHC (gm/dL)	RDW (CV%)
Thalassemia minor (n=378)	10.4±1.53	5.38±0.8	61.85±8.67	19.5±2.66	32.03±2.24	17.74±2.49
Thalassemia major (n=01)	6.4	2.83	64.43	19.90	30.80	28.90
Sickle cell disease (n=04)	8.73±1.85	3.38±0.97	82.01±12.05	26.02±3.97	33.17±1.78	20.05±5
Sickle cell trait (n=93)	12.91±1.88	4.49±0.70	75.96±11.2	26.19±4.40	33.53±2.37	15.11±3.35
Sickle thalassemia (n=02)	7.6±0.74	3.36±0.15	73.12±2.32	22.97±3.27	32.11±0.69	20.12±0.11
HbD disease (n=02)	10.45±0.05	5.48±0.02	57.05±4.35	19.05±0.15	33.60±2.3	18.4±0.80
HbD trait (n=26)	11.41±2.29	4.44±0.70	76.71±7.51	25.73±3.63	33.73±±1.79	15.74±3.25
HbE disease (n=06)	10.65±1.44	5.10±0.87	60.50±7.29	21.05±1.36	33.97±1.15	18.80±1.97
HbE trait (n=24)	12.09±1.53	5.05±0.61	72.50±5.05	24.03±1.98	33.17±1.21	14.75±1.48
HPHF (n=05)	11.52±3.85	4.53±1.40	75.03±6.67	24.90±3.34	32.39±2.63	15.68±3.06
HbD β-thalassemia (n=01)	12.22	6.02	58.80	20.36	34.22	18.18
Delta β-thalassemia (n=01)	12.1	4.97	74.8	24.3	32.5	16.4
β-thalassemia-Delta β-thalassemia (n=01)	9.1	5.57	49.9	16.3	32.7	15.50

[Table/Fig-3]: Haematological parameters in various haemoglobinopathies.
Hb: Haemoglobin; RBC: Red blood cell; MCV: Mean corpuscular volume; MCH: Mean corpuscular haemoglobin; MCHC: Mean corpuscular haemoglobin concentration; RDW: Red cell distribution width; Values are expressed as mean±SD

Haemoglobinopathies (N=544)	HbA (%)	HbA2 (%)	HbF (%)	HbS (%)	HbD (%)	HbE (%)
β-thalassemia trait (n=378)	92.29±1.06	4.94±0.63	0.75±1.27	-	-	-
β-thalassemia major (n=01)	5.4	1.2	87.8	-	-	-
Sickle cell disease (n=04)	5.72±2.87	1.97±0.54	21.35±6.59	70.95±7.24	-	-
Sickle cell trait (n=93)	63.61±7.01	1.58±1.09	0.94±0.81	32.62±6.85	-	-
Sickle thalassemia (n=02)	9.8±7.29	4.25±0.15	13.3±3.4	69.75±2.45	-	-
HbD disease (n=02)	5.95±5.95	5.35±0.25	1.0±0	-	87.7±5.7	-
HbD trait (n=26)	59.50±8.28	1.33±1.29	0.55±0.59	-	36.05±5.48	
HbE disease (n=06)	3.04±2.95	*	9.77±4.22	-	-	87.33±5.57
HbE trait (n=24)	69.66±4.19	*	1.15±0.46	-	-	28.99±4.37
HPHF(n=05)	74.07±0.97	2.02±0.40	23.91±0.69	-	-	-
HbD β-thalassemia (n=01)	0	4.2	0.8	-	95	-
Delta β-thalassemia (n=01)	74.5	1.8	23.7	-	-	-
β-thalassemia-Delta β-thalassemia (n=01)	16.2	3.3	80.5	-	-	-

[Table/Fig-4]: Haemoglobin electrophoresis findings in various haemoglobin disorders.
Values are expressed as mean±SD; *As HbA2 and HbE co-migrate on alkaline electrophoresis; hence it is not possible to quantitate each separately

Total cases (N=10025)	Normal haemoglobin pattern	β-thalassemia trait	Sickle cell trait	HbD disease	HbD trait	HbE disease	HbE trait	Increased foetal haemoglobin
No. of ANC cases (n=4968) Prevalence %	4782 (96.25%)	118 (2.37%)	34 (0.68%)	1 (0.02%)	15 (0.3%)	2 (0.04%)	13 (0.26%)	3 (0.06%)
No. of non ANC cases (n=5057) Prevalence %	4699 (92.92%)	260 (5.14%)	59 (1.16%)	1 (0.02%)	11 (0.21%)	4 (0.08%)	11 (0.22%)	2 (0.04%)

[Table/Fig-5]: Prevalence of haemoglobinopathy in women on Antenatal Care (ANC) screening compared to the non-ANC cases.

18 (9.67%) showed an abnormal haemoglobin pattern, 133 (71.50%) had normal Hb pattern, while 35 (19.33%) did not turn up for screening. The high-risk couple management and outcome with two cases of major haemoglobinopathies is shown in [Table/Fig-6].

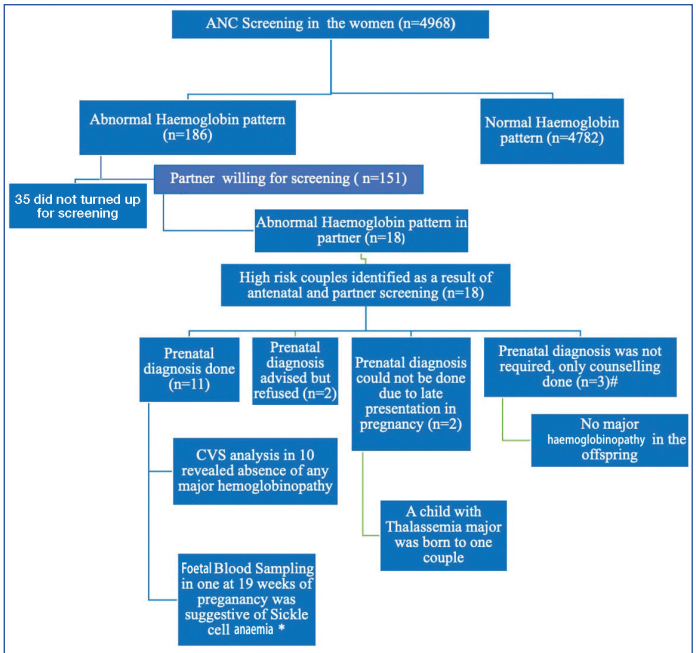
DISCUSSION

The present study was an attempt to find out the prevalence of various haemoglobinopathies in a cohort of a population which comprises of people from diverse ethnic groups and geographical regions across the country. The outcome of the ANC screening was also assessed in this study.

The overall prevalence of Hb disorders in the present study of 10,025 cases was 5.42% which is like the study of Jandial R and Gupta I with prevalence of 5.89% and much lesser than the study of Mondal SK and Mandal S who reported 12.17% [11,12]. These studies like the present study, were also hospital-based studies and included both ANC women and patients investigated for anaemia. The most common Hb disorder and their frequencies found in present study were β-thalassemia trait (3.77%), followed by HbS trait (0.92%), Hb D trait (0.26%) and HbE trait (0.24%). β-thalassemia trait was also the most common Hb disorder reported in those same previous studies [11,12]. However, compared to the present study Jandial R and Gupta I reported higher Hb D trait (0.59%) and Mondal SK and Mandal S reported higher HbE trait (3.02%). The subjects in the former were primarily North Indian and in the latter from the Eastern India [11,12].

Population-based studies covering different states have provided data on haemoglobinopathies with β-thalassaemia trait being the predominant Hb disorder in most places except in Eastern India where both β-thalassaemia trait and HbE disease were equally prevalent and sickle cell disorder has the highest frequency in the tribal population across the central belt [3,13-15].

β-thalassemia trait is usually silent at the clinical level and haematology findings are characterised by microcytosis and hypochromia with increased haemoglobin A2 value. Sachdev R et al., reported that cases with co-existent nutritional deficiencies may have borderline A2 levels, masking an underlying haemoglobinopathy. Iron deficiency anaemia may show a low level of HbA2, and cobalamin or folate deficiency may elevate HbA2 level [6]. Hence in present study and in most other studies, other red cell indices are associated with HbA2 levels for correct diagnosis [3-6,11].



[Table/Fig-6]: Outcome of the Antenatal Care (ANC) screening in preventing a major Haemoglobinopathy.
#2 couples had both partners with HbE trait and one couple had woman with β-thalassemia minor and partner with HbD trait, so there was no risk of a major haemoglobinopathy; *Prenatal genetic counselling was done for this couple, however they decided to continue the pregnancy, the offspring had Sickle cell anaemia; CVS: Chorionic villous sampling

This study showed 0.9% prevalence of sickle cell trait and 0.04% of sickle cell disease. Colah RB et al., reported that sickle gene is widespread among many tribal population groups in India with prevalence of heterozygotes varying from 1-40 percent [4]. Hockham C et al., have compiled a recent geodatabase of sickle-cell anaemia from several population surveys and reported the highest frequency of upto 10% across the central belt in India [16].

Haemoglobin E is the most common Hb variant in Southeast-Asia and the second most prevalent Hb variant worldwide after HbS. HbE disorders In India are commonly confined to north-eastern states and Bengal, with tribal communities constituting a major part of the Indian population with HbE haemoglobinopathies [13]. All subjects in present study with HbE variant hailed from West Bengal and the North-east states. On alkaline Hb electrophoresis HbA2 and HbE co-migrate; hence it is not possible to quantitate each separately. However, HbA2 is never more than 9% in β-thalassemia trait and HbE is never less than 15% in HbE trait; so this feature helps to distinguish the two. Mean HbE values in HbE trait cases in the present study was 28.99±4.37. Clinically HbE is a mild type of disorder both in homozygous and heterozygous state [5].

HbD is an uncommon haemoglobin variant prevalent in Northwest India and the heterozygous and homozygous states are usually clinically asymptomatic. The prevalence of HbD trait in this study population was 0.26% and all hailed from the north India. Srinivas U et al., reported the largest single centre study on HbD, of the total of 484 cases of structural haemoglobin variants, prevalence of HbD in Punjab was found to be 0.55% and it was 7.8% of all haemoglobin variants [17].

In present study, there were five cases with Hereditary Persistence of Foetal Haemoglobin (HPFH), with mean HbF 23.91±0.69 and normal red cell indices, comparable to study of Pandey H et al., with 28 cases with mean HbF 31% and normal red cell indices [18]. Delta-β-thalassemia is a rare form of thalassemia which presents with a raised HbF and normal or low HbA2 and near normal red cell indices making HPFH a close differential [19]. The authors found one ANC case with delta β-thalassemia, her husband was detected to have β-thalassemia on screening and the child born to them was double heterozygous for β-thalassemia and delta β-thalassemia. The mother and child showed raised HbF; HbA2 was normal in mother and 3.3% in the child.

The antenatal screening detected 186 cases of haemoglobinopathies, accounting for 3.74% of all the ANC cases in this study period. The frequency of haemoglobinopathies reported on antenatal screening in a study by Dharmarajan S et al., was 1.92%, Bhukhanvala DS et al., was 3.38% and Baxi A et al., was 3.47% [20-22].

Prenatal diagnosis using Chorionic Villous Sampling (CVS) can be carried out at 9-11 weeks, amniocentesis in 14-18 weeks, and foetal blood testing by cordocentesis in 18-20 weeks of pregnancy [9]. In this study, despite of ANC screening programme and availability of universal healthcare, there were two cases of major haemoglobinopathy. Late antenatal registration and decision to continue the pregnancy despite an adverse prenatal diagnosis were the hurdles to achieving the goal of total prevention in study population. Bhukanvala DS et al., also enlisted the same hurdles along with non cooperation of the husbands and refusal for prenatal diagnosis in their study [21]. As outlined by Colah R et al., Ministry of Health and Family Welfare (MoHFW), National health mission and Cousens NE et al., a concentrated effort to create awareness and guidelines for prevention, screening and prenatal diagnosis is essential to reduce the burden of these diseases in the society [7,23,24].

Saffi M and Howard N, concluded that those mandatory premarital and genetic counselling programmes were unsuccessful in discouraging at-risk marriages but successful in reducing the prevalence of affected births by providing prenatal detection and therapeutic abortion [25]. The strength of this study was that in view of an established antenatal screening programme, most pregnant women could be screened in early first trimester itself. Due to accessible healthcare, there was better compliance to further screening of spouses, diagnostic prenatal testing and testing on the children born thereafter.

Limitation(s)

Authors have used cellulose acetate electrophoresis, a cost-effective approach for screening as our daily sample volumes were relatively low. High-Performance Liquid Chromatography (HPLC) and capillary electrophoresis are gaining in popularity because these methods are more automated. As HPLC can identify and quantify low levels of HbA2 and HbF, it is a method of choice for diagnosis of Thalassemias. Capillary electrophoresis uses smaller volume of samples and produces better separation of the variants.

CONCLUSION(S)

The study highlighted the spectrum of haemoglobinopathies seen in a population with study subjects hailing from different geographical areas across India. Thalassemia minor and HbS trait are the most common haemoglobinopathies detected. Antenatal screening programme and timely intervention is an effective strategy to control clinically significant major haemoglobinopathies. Use of molecular studies such as Polymerase Chain Reaction (PCR) and Amplification Refractory Mutation System (ARMS) which can determine specific mutations responsible for the haemoglobin disorders may be better health management strategies for the future, to reduce the burden of major haemoglobinopathies.

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