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New Born Screening for Hemoglobinopathies & Thalassemia in Chennai, Tamilnadu, South India

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ABSTRACT

Hemoglobin disorders are considered to be a serious health problem by World Health Organization. This hereditary problem has raised global concern including the region of Mediterranean, Southeast Asia, Indian subcontinent and southern part of China. Although there has been a significant decline in infant and childhood mortality rates in most countries, due to effective and successful use of Immunization and improvement in health services, birth defects are responsible for a greater proportion of infant and childhood mortality (WHO, 2013). It is proposed to study the prevalence of hemoglobinopathies and thalassemia among newborn babies in chennai population. A total number of 474 dried blood spot samples were collected during the period of April 2016 to July 2016 which was referred for other inherited conditions (Expanded Newborn Screening) by the physician. These dried blood spot samples were used for assessing various hemoglobin variants among the newborns using automated Capillary electrophoresis system (Capillarys 2; Sebia, Evry, France). In this present study among 474 cases, 233 male and 241 female newborns were noted. Out of these, 20 babies showed hemoglobin variants. Among 20 cases, 3 cases of Hb Bart's (Alpha thalassemia 2), 1 case of HbSS (Sickle homozygous), 3 cases of sickle cell trait, 8 cases of HbE, 1 case of HbSC (compound heterozygous), 1 case of HbS/beta thalassemia and 3 cases of beta thalassemia were noted. Newborn screening for hemoglobinopathies enables to detect the affected newborns and helps in reduction of mortality among newborns.

Key words: Hemoglobinopathies, Dried blood spot (DBS), Capillary electrophoresis, Thalassemia, Sickle cell disease-Bart's.

INTRODUCTION

In India, hemoglobinopathies and thalassemia are the major non-communicable genetic disease (Balgir, 2000). Hemoglobin disorders are endemic in 60% of 229 countries which affects 75% of births but it significantly increased to 71% of countries affecting 89% of births, 5.2 % of world population (7% of pregnant women) carry a significant variant. The prevalence of hemoglobin disorder ranges from 0.3-25 per 1000 live births (Modell and Darliason, 2008). In India, genetic disorders and birth defects are relatively common due to consanguineous marriage among the communities which comprise about 60-70%. Among that, single gene disorders like alpha and beta thalassemia, sickle cell disorders, glucose 6 phosphate dehydrogenase deficiency, albinism, cystic fibrosis, phenylketonuria, hemophilia A and B occurs with frequency of 1 in 81 births. The estimated frequency of birth defects and genetic disorder for beta thalassemia and sickle cell disease in India is about (1:2700) i.e. 16,700 births/year. About 17, 22,404 infants are born each year with defects, of these 1.2% of hemoglobinopathies (Christianson, 2006 and WHO 2013). WHO recommends that the country should introduce genetic services with an infant mortality rate (IMR) less than 50 (Kamath, 2015). According to National Health Mission report, India have IMR of 42 and Tamil Nadu have IMR of 21(sample registration scheme 2016), which should introduce new born

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screening and genetic services. The Indian Academy of Pediatrics strongly emphasis the importance and inclusion of new born screening in our public health policy. In Indian scenario, new born screening is categorized into three types which includes Category A (all newborns), Category B (High risk screening) and Category C (Expanded screening) (Kamath, 2015). For new born screening, blood collection after 72 hours and within 7 days of life on Whatman 903 filter paper is the standard method, however in India due to early discharge from hospitals and higher number of home birth has limited the newborn screening (Kapoor and Kabra, 2010 and Mukhopadhyay and Balachandran, 2014). About 1, 25,000 infants screened for the most common metabolic disorders in southern India (Rao, 1988) and similar studies were carried out by Devi *et al.* (2004). In the present study, newborns have been screened based on (Category B- High risk screening) which emphasized on screening of sickle cell anemia and other haemoglobinopathies.

Genetic Background

Hemoglobin comprises four globin chains: two alpha chains and two beta chains (α2β2). Expression of alpha globin and beta globin chains is regulated by cluster of genes on chromosomes 16 and 11 (Higgs, 2013). Structural variants that change amino acid sequence and produce an abnormal hemoglobin (qualitative) like Hb D, E, H, J , K, L, M, Q, S, Lepore, Norfolk, Koya Dora, Chandigarh and hereditary persistence of fetal hemoglobin (HPFH) and thalassemia-lower or abolish globin chain production (quantitative) - α thalassemia, β thalassemia. (Balgir, 2000).

Fetal hemoglobin (HbF) has two alpha and two gamma chains (α2 γ2), Adult hemoglobin (HbA) has two alpha and beta chains (α2β2) and HbA2 has two alpha chains and two delta chains (α2δ2) (Higgs, 2013). The globin moiety of hemoglobin molecules is made of seven different polypeptide chains and they are designated by the Greek letters alpha (α), beta (β), gamma (γ), delta (δ), epsilon (ε) and zeta (ζ).The alpha and zeta contain 141 amino acid, whereas beta, gamma, delta and epsilon contain 146 amino acid. Epsilon, zeta and alpha are synthesized in early embryonic life. Alpha and gamma are synthesized in fetal life and alpha and delta in post natal life (Balgir, 2000).

In the earliest embryonic (fetal) life, zeta and epsilon (ζ2 ε2) chains combine to form Hb Gower 1, alpha and epsilon chains (α2 ε2) combine to form Hb Gower II and zeta and gamma chains form Hb Portland (ζ2 γ2). By the end of the first trimester zeta replaced by alpha chains and epsilon is replaced by gamma chains (Balgir, 2000). During 34-36 weeks of gestation, fetal hemoglobin (HbF) constitutes about 90-95% and adult hemoglobin accounts for 4 to 13% of all hemoglobin. HbF production falls and HbA production significantly increases after 34 weeks of gestation. At term HbF falls to 53-95% and HbA level increased by 20-30%. (Bain, 2006 and Ryan *et al.*, 2010). During birth, HbA2 (α2δ2) starts producing in small amounts and reaches adult level at the end of six months, hence mutations involved in beta chains cannot be diagnosed in early fetal life. However reduction in the production of HbA indicates the beta thalassemia (Ryan *et al.*, 2010). Moreover alpha chain is common to all these three hemoglobin's, mutation in this chain leads major problem in neonates.

MATERIALS AND METHODS

A total of 474 dried blood spot samples were collected from the newborn babies with the age group of 3 to 7 days during the period from April 2016 to July 2016 which were referred for other inherited conditions which includes metabolic screening of amino acid and fatty acid metabolism, Congenital Adrenal hyperplasia (CAH), Congenital Hypothyroidism (CH), Glucose-6-phosphate dehydrogenase deficiency (G6PD), Galactosemia, Phenylketonuria (PKU), Biotinindase deficiency and Cystic Fibrosis (CF) by the physician. 56 older newborns with age group of 8 to 60 days also included in the present study. For newborn screening, blood samples were collected by direct heel prick procedure compliance with Clinical Laboratory Standards Institute [(CLSI): LA4-A5] guidelines, on whatman 903 filter paper. These dried blood spot samples were used for assessing various hemoglobin variants among the neonates using automated Capillary electrophoresis system (Capillarys 2; Sebia, Evry, France) which has high resolution, on-line detection and direct quantification for normal hemoglobin (F and A), detection of major hemoglobin variants (S, C, E, D and Bart's) and easier to interpret (Renom *et al.*, 2009; Wajeman and Moradkhani, 2011 and Frommel, 2014) by CAPILLARYS NEONAT Hb kit. Newborn screening using capillary electrophoresis (CE) by the

capillarys2 system detects complete hemoglobin profile. Further Capillary electrophoresis enables to detect both hemoglobinopathies and thalassemia. CE highly useful in mass screening, which replaces the conventional techniques and also compared with well-established techniques like iso electric focusing (IEF) and high performance liquid chromatography (HPLC) (Gulbis *et al.*, 2003; Louahabi *et al.*, 2006 and Renom *et al.*, 2009; Alauddin *et al.*, 2012). Diagnosis of thalassemia in Tandem mass spectrometry (TMS) has a major drawback which fails to detect Bart's a marker for alpha thalassemia (Boemer *et al*, 2008), however the CE has a greater sensitivity to detect Bart's even with the 1% of concentration. Moreover tandem mass spectrometry serves as an excellent methodology for screening Hb variants other than alpha thalassemia. Sebia HbAF control (PN 4777) were run along with the samples every run (Fig. 1). Consents were obtained from the patient including the history of blood transfusion, gender, weeks of gestation, consanguinity and ethnicity. The present study was approved by Institutional Human Ethical Committee. (HDC/ IHEC/03).

RESULTS AND DISCUSSION

During the study period, 474 newborns were analyzed for Hb variants using capillary electrophoresis. In the present investigation, 418 newborns with age of 3 to 7 days and 56 older newborns with the age of 8 to 60 days were observed. Among 474 newborns, 233 newborns were male and 241 were female. The study revealed that, 19 babies (4.0%) were premature delivery with gestational period ranges between 32 to 36 weeks and 455 babies (95.9%) had normal gestational age (more than 38 weeks). Out of 474 newborns, 22 newborns with sickness were recorded (Table-1).

Table 1 : Distribution of age, gender, status of newborns for hemoglobinopathies and thalassemia in Chennai and in some places of Tamil Nadu (n=474).

Place	No. of babies screened	Male	Female	Infants	Older infants	Premature	SICK
				3-7 Days	8-60 Days	32-36 Weeks	
CHENNAI	436	217	219	385	51	17	17
COIMBATORE	9	2	7	9	-	-	-
ERODE	1	1	0	1	-	-	-
KANCHIPURAM	7	4	3	7	-	2	-
MADURAI	4	2	2	3	1	-	1
NAGAPATTINAM	1	1	0	1	-	-	1
SALEM	3	1	2	1	2	-	-
THANJAVUR	2	1	1	2	-	-	2
THIRUVAROOR	1	1	0	1	-	-	-
THIRUVALUR	6	2	4	6	-	-	-
VELLORE	3	1	2	1	2	-	1
VILLUPURAM	1	0	1	1	-	-	-
Total	474	233	241	418	56	19	22

The newborn babies were screened for the identification of hemoglobinopathies, including sickle cell disease and thalassemia using The CAPILLARYS NEONAT Hb kit. In the software, the normal hemoglobin fractions and variants were detected at different migration zones of N1 to N13 respectively (Table 2).

Table 2 : Detection of normal and Hb variations on migration zone

N1	N2	N3	N4	N5	N6	N7	N8	N9	N10	N11	N12	N13
HbC	HbA2, HbO Arab	HbE	HbS	HbD-Punjab		HbF		De-graded HbF acety-lated HbF	HbA	Hb Hope		Hb Barts

In the present study among 474 cases, 454 normal pattern and 20 Hb variants were observed. Among 454 normal patterns, 411 normal newborns without HbA2, 25 newborns with HbA2 and 18 premature babies without HbA2 were found. All the abnormal Hb variants were repeated on capillarys2 before taken into account. Among 20 cases, 3 cases of Hb Bart's (Alpha thalassemia 2), 1 case of HbSS (sickle homozygous), 3 cases of sickle cell trait, 8 cases of HbE, 1 case of HbSC (compound heterozygous), 1 case of HbS/beta thalassemia and 3 cases of beta thalassemia were noted (Table-3). The reference range for the normal newborns reported by Ivaldi *et al.*, (2007) was adapted which was mentioned in Mosca *et al.*, (2009). The present study also showed the similar observations with the above reference range. Based on the gestational age, HbA concentrations were reported by Galacteros *et al.*, (1991) for both male and female newborns. In the present study, among 19 premature babies, 18 babies were normal with gestational age of 32-36 weeks in which HbA concentration were comparable with the reference range reported by Galacteros *et al.*, (1991).

Table 3 : Table-3 Hemoglobin fractions on Capillary Electrophoresis among Newborns. n=474 [419 neonates (3-7 days) and 56 Older newborns (8-60 days)]

Presumptive CE Diagnosis	No of screened	HbA %	Acety-lated HbF	HbF %	HbA2 %	HbS %	HbE %	Hb Bart's	HbC
		Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
Normal	411 (86.70%)	24.23 ±9.09		75.61 ±9.54					
Normal with HbA2	25 (5.27%)	33.63 ±14.65		65.08 ±14.77	0.92 ±0.63				
Premature	18 (3.79%)	13.37 ±3.37		86.27 ±3.21					
HbS trait	3 (0.63%)	17.13 ±0.98		77.63 ±2.30	0.9	4.93 ±3.38			
HbS Homo	1 (0.21%)		1.9	87.8		10.3			
HbE Trait	8 (1.68%)	16.46 ±6.6		80.02 ±7.04			3.51 ±2.63		
Hb Bart's	3 (0.63%)	17.53 ± 1.86		80.5 ±1.31				1.97 ±1.79	
HbSC	1 (0.21%)		1.3	92.5		3.9			2.3
Suspected Hb S/β+	1 (0.21%)	8.6		82.7		8.7			
Suspected Beta Thalassemia	3 (0.63%)	8.7± 0.28		91.3± 0.28					

**Reference range adapted from Ivaldi. et.al. 2007 for normal infants: At birth- (HbA: 15-40%, HbF: 58-84%, HbA2: 0-1%), 1-3 month- (HbA: 38-70%, HbF: 29-61%, HbA2: 0.5-1.5%).
***Reference range adapted from Galacteros et.al. 2007 based on gestational age: (<33 – 36 weeks - 11.2 – 14.1 % of HbA).

Normal and Abnormal patterns

According to Proycheva (2009), the term neonates [normal gestation: more than 38 weeks (Adorno, 2005)] have 50% to 80% of hemoglobin F and 15% to 50% of hemoglobin A. In the present study, out of 474 cases, 411 were observed normal with normal HbAF values with HbF (75.61%) and HbA (24.23%). 25 newborns were observed normal with normal HbAF and A2 values of 33.63%, 65.08% and 0.92% respectively. Fig 2 shows the normal newborn with normal Hb A, HbF (at zone N10 and N7), normal gestation of 39 weeks and without any Hb variants. Among 474 newborns, 19 premature babies (4.0%) with gestational weeks of 32-36 weeks were presented with low HbA values of (13.37%) for 18 newborns (Fig 3) and one premature newborn presented HbE variant with low HbA of 8%. About 22 of sick babies (4.64%) with HbA of 27.62% and HbF of 71.87% were observed.

Newborns with HbS variant

Sickle hemoglobin was first detected in Nilgiris among the tribal population (Lehman and Cutbush, 1952). Carrier frequencies for various genetic disorders like sickle cell anemia and thalassemia are high among Indians (Weatherall and Clegg, 2001; Adorna *et al.*, 2005). In beta globin gene, valine is substituted for glutamic acid at 6th position which causes HbS variant, on exposure to low oxygen, HbS precipitates and appears as sickled (Edoh *et al*., 2006) and have a reduced lifespan from 120 days to 10-20 days (Kanter, 2013) which leads to chronic hemolytic anemia, microcirculatory vaso-occlusive crisis and functional asplenia with increased susceptibility to infections by encapsulated bacteria (Streptococcus pneumonia) (Rees *et al.*, 2010 ; Benkerrou *et al.*, 2013 and Frommel *et al.*, 2014). In capillary electrophoresis HbS peak observed in N4 zone. Among 474 cases, four (0.84%) cases of HbS variants were observed. Among four cases of HbS variant, three HbS heterozygous (F/AS) were observed with 17.13% (HbA in N10), 77.63% (HbF in N7) and 4.93% (HbS in N4) respectively (Fig 4) and one case (9 days) of HbS homozygous (F/S) pattern and absence of HbA in N10, with 1.9% of acetylated HbF, 87.8% of HbF and 10.3% of HbS were observed. In the present study, all the four newborns, born with normal gestational age with the average weight of 3.2 kg were found. Table: 4 shows prevalence rate of the present study and different studies carried out in various parts of India on sickle cell disease (Panigrahi *et al.*, 2012; Jain *et al.*, 2012; Italia *et al.*, 2014; Dixit *et al.*, 2015 and Upadhye *et al.*, 2016). In the present study, the prevalence rate is moderate when compared to the other studies referred in different parts of India.

Table 4: Prevalence rate of Sickle Cell Disorder among newborns-Various studies carried out in India.

Author	Present study 2016	Panigrahi *et al.*, 2012	Jain *et al.*, 2012	Upadhye *et al.*, 2016	Italia *et al.*, 2014	Dixit *et al.*,2015
Place	Southern India	Central India	Central India		Western India	Eastern India
	Chennai	Chhattisgarh	Nagpur		South Gujarat	Kalahandi
No screened	n=474	At birth n=1158	n=8243(mothers) n=1178 newborns with sickle positive	n=10,181(mothers) n=2134 newborns with sickle positive	n=5467	n=761
Method	Sebia Capillary Electrophoresis	BioRad HPLC	BioRad HPLC	BioRad HPLC	BioRad HPLC	BioRad HPLC
Sickle Cell Disease	0.21%	0.40%	1.06%	4.90%	0.60%	1.70%
Sickle cell Trait	0.63%	5.26%	6.50%	45.80%	12.50%	14.71%
Sickle beta thalassemia	0.21%	0.08%	-	0.32%	0.23%	-

Newborns with compound heterozygous HbS/C

In beta globin gene, glutamic acid is substituted by lysine at 6th position which results HbC variant. In capillary electrophoresis HbC observed in N1 zone. In the present study, one baby with normal gestational age was observed with compound heterozygous condition (HbF/SC) with HbF of 92.5%, HbS of 3.9% and HbC of 2.3% were observed (Fig 5). HbC disease is of

African origin and also in Hispanic and Sicilian ethnicity (Carter. 2008). Chennai is composed of mixed population, which results from national and international migration of people from different parts of the world which results in introduction of single gene defect into new populations and the gene become embedded in the host population as the result sickle cell disease and other hemoglobinopathies are common. Compared to sickle cell anemia, HbC disease has less pathology which results anemia with normal life expectancy and remains asymptomatic (Bernal, 2010). However, when it is presented with compound heterozygous condition with sickle cell anemia which may leads to various complications in neonatal period. Molecular DNA study is recommended for the confirmation of the condition.

Newborns with HbE variant

In beta globin gene, glutamic acid is substituted for lysine at 26th position which causes HbE variant. Among 474 cases, eight cases (1.68%) of HbE Heterozygous (F/AE) were observed in which HbE observed in N3 zone on capillary electrophoresis. Among eight newborns, seven have normal gestational age and average weight of 2.6 kg at birth and one premature newborn with 33 weeks and weight of 2.02 kg were observed. HbE heterozygous pattern associated with HbE in N3, sharp peak in N10 (HbA) and N7 (HbF) with concentration of 3.51%, 80.03% and 16.46% respectively (Fig 6). Patients with HbE disease is presented with only anemia, which is less severe, however it may leads to severe complications when it presented with beta thalassemia in compound heterozygous condition (Ziwanitkit, 2013).

Newborns with Hb Bart's (γ4) (Alpha thalassemia)

Alpha thalassemia results from decreased synthesis of alpha chains. The absence or reduced synthesis of alpha chains results abnormal production of Hb Bart's in fetus (Tang et al., 2012 and Yaish et al., 2015). Alpha Thalassemia Major (Fetal Hydrops Syndrome) is the condition results from complete deletion or inactivation of four alpha genes in fetus leads to severe hemolytic anemia and severe toxemia occurs during the pregnancy in women carrying fetus with fetal hydrops syndrome. In capillary electrophoresis, low level of Hb Bart's (0.1%) could be detected (Tang et al., 2012). In CE, for the diagnosis of alpha thalassemia, detection of Hb Barts with cutoff point of 0.2% is used in newborns (Munkongdee et al., 2011). Among 474 cases, three newborns presented with Hb Bart's in N13 zone on capillary electrophoresis. All the three newborns born with normal gestational age and average weight of 2.43 kg. In the present study, two cases observed with Hb Bart's of 0.6 and 0.8 % respectively and 4.5% of Hb Bart's observed in one case (Fig 7). However to prevent the risk of false negative or positive diagnosis, DNA analysis must be carried out to rule out deletional and non-deletional alpha thalassemia.

Newborns with beta thalassemia

During embryonic and early fetal life, the expression of beta globin gene is absent and it's become measurable only around 12 weeks of gestation and increases about 20% at birth (Mantikou et al., 2010). In beta thalassemia major, HbA is absent due to inactive beta genes, highly decreased in beta thalassemia intermedia and partially decreased in beta thalassemia carriers (Mantikou et al., 2009). In the present study, among normal newborns 23.84% HbA were observed which is similar to the report of Mantikou et al. (2010) (HbA 21.8%). Further, using the cut off < 15% HbA, 4 carriers of point mutations defects were reported by Mantikou et al., 2009. Mantikou et al. (2009) reported the reduced HbA in newborns was considered for beta thalassemia carrier and Mosca et al. (2009) showed that an increased HbF observed in beta thalassemia condition. In the present study among 474 cases, three newborns with elevated HbF of 91.3%, reduced HbA of 8.7% with the average weight of 2.1kg with normal gestational age were observed, indicating of high risk for beta thalassemia (Fig 8). But in the present study, both the reduced HbA and elevated HbF has been noted in the newborns indicates the possibility of beta thalassemia. However parental screening and molecular analysis is recommended for confirmation.

Newborns compound heterozygous HbS/β+ thalassemia

In the present study, one case of F/AS with β+-thalassemia (S/ β+-thalassemia compound heterozygous) HbA of 8.6%, HbF of 82.7% and HbS of 8.7% were observed. However parental screening and molecular analysis helps to confirm and identify the double heterozygous condition as well as by screening the newborn after 6 months of age helps to identify the same.

Religious composition of newborn babies were categorized for 474 newborns: 385 babies were Hindu (81.22%), 36 babies as Christian (7.59%), 48 babies as Muslim (10.12%) and 5 babies as Jain (1.05%). Table 5 and Graph 1 shows the religious wise distribution of hemoglobinopathies and thalassemia among newborns. The religion wise data on hemoglobinopathies and thalassemia revealed that Among the religious distribution high prevalence was found in Jain (40%) followed by Muslim (8.16%), Christian (5.56%) and Hindu (3.13%). (Table 5).

However the prevalence rate shows higher in Jain religion, whereas only five samples has been screened, among five newborns, two newborns presented with abnormal pattern. In the present study only limited samples were available for the religion other than Hindu. However the prevalence rate may either increase or decrease, if more number of samples are screened among those religion.

Table 5: Religion wise distribution of Hemoglobinopathies and thalassemia(n=474).

RELIGION	Total	Normal	Abnormal
HINDU	384 (81.01%)	372 (78.48%)	12 (2.53%)
MUSLIM	49 (10.33%)	45 (9.49%)	4 (0.84%)
CHRISTIAN	36 (7.59%)	34 (7.17%)	2 (0.42%)
JAIN	5 (1.05%)	3 (0.63%)	2 (0.42%)

Based on recent estimates, the consanguinity rates in India varies from 1-4% in northern India and 40-50% in southern India (Bittles *et al.,* 2009 and Sharma, 2013). In the present study, about 54.43% of non-consanguineous couples and 45.57% of consanguineous couples were observed. Among 20 (4.21%) newborns with Hb variants, 11 (2.32%) of Hb variants found among consanguineous couples and 9 (1.89%) of Hb variants found among non-consanguineous couples. The congenital birth defect is slightly higher among consanguineous marriage.

The majority of newborn screening fails to establish the comprehensive care centers, providing Pneumococcal vaccination, hematological work-up, folic acid supplementation and reinvestigation (Italia et al., 2014). Most of the SCD newborns are regularly monitored and follow-up were done for the maximum cases in Chhattisgarh, Central India and South Gujarat which were mentioned in the respective studies. As the most of study were carried out in hospitals, vaccination, follow-up and reinvestigation were done successfully with exceptions of few cases. However the present study was carried out in laboratory based centres patient follow-up, vaccination, supplementation of nutrients are difficult to carry out, however the newborns presented with significant Hb variants are informed to their referring physician and to those parents for parental screening.

CONCLUSION

Lower percentage of hemoglobinopathies and thalassemia has been found among the newborns in chennai population. Capillary electrophoresis method is found to be accurate to diagnose the hemoglobinopathies and thalassemia over HPLC method for better accuracy. Genetic counseling is advisable for families those affected by sickle and other thalassemia conditions to create awareness among the parents and for the wellness of their offspring in future. Most of the screening studies carried out in various parts of the India have been funded by various authorities which enable to monitor and follow-up the affected babies. However the present study attempted to screen the newborns for hemoglobinopathies and the findings revealed the prevalence rate of Hb variants among the newborns in chennai.

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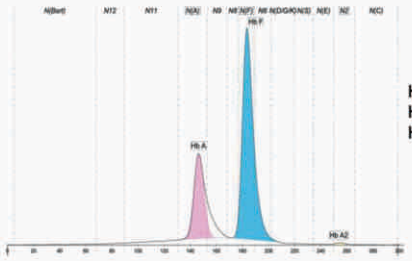
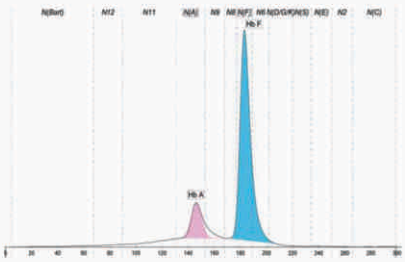
Neonatal Haemoglobin Electrophoresis

Figure - 1 HbAF CONTROL-1



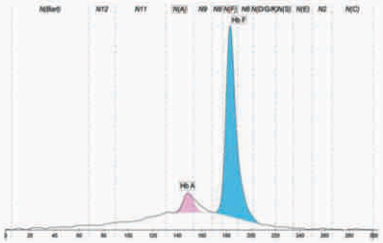
HbA 28.5%
HbF 71.1%
HbA_2 0.4%

Figure - 2 NORMAL



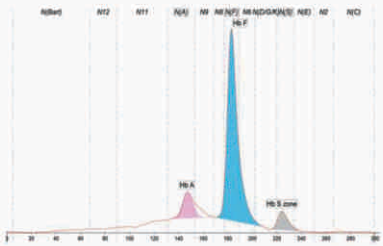
HbA 19.9%
HbF 80.1%

Figure - 3 PREMATURE



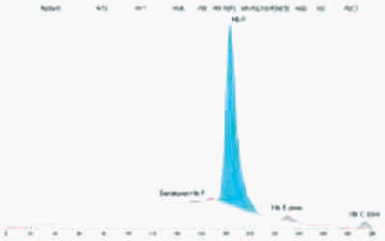
HbA 8.9%
HbF 91.1%

Figure - 4 HbS Heterozygous



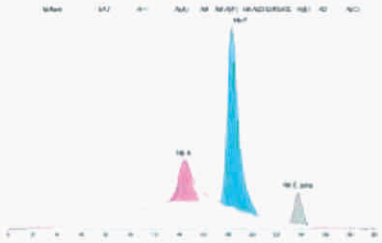
HbA 10.6%
HbF 81.7%
HbS 7.7%

Figure - 5 HbSC Compound Heterozygous



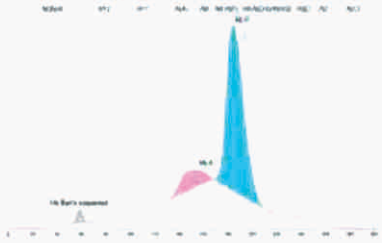
HbF 92.5%
HbS 3.9%
HbC 2.3%

Figure - 6 HbE Heterozygous



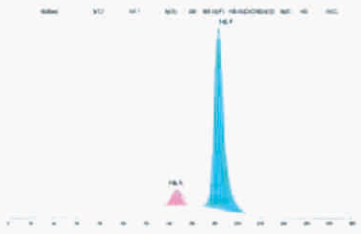
HbA 18.4%
HbF 71.5%
HbE 10%

Figure - 7 Hb Barts



HbA 15.5%
HbF 80%
Hb Barts 4.5%

Figure - 8 BETA THALASSEMIA



↓HbA 8.3%
↑HbF 91.7%

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