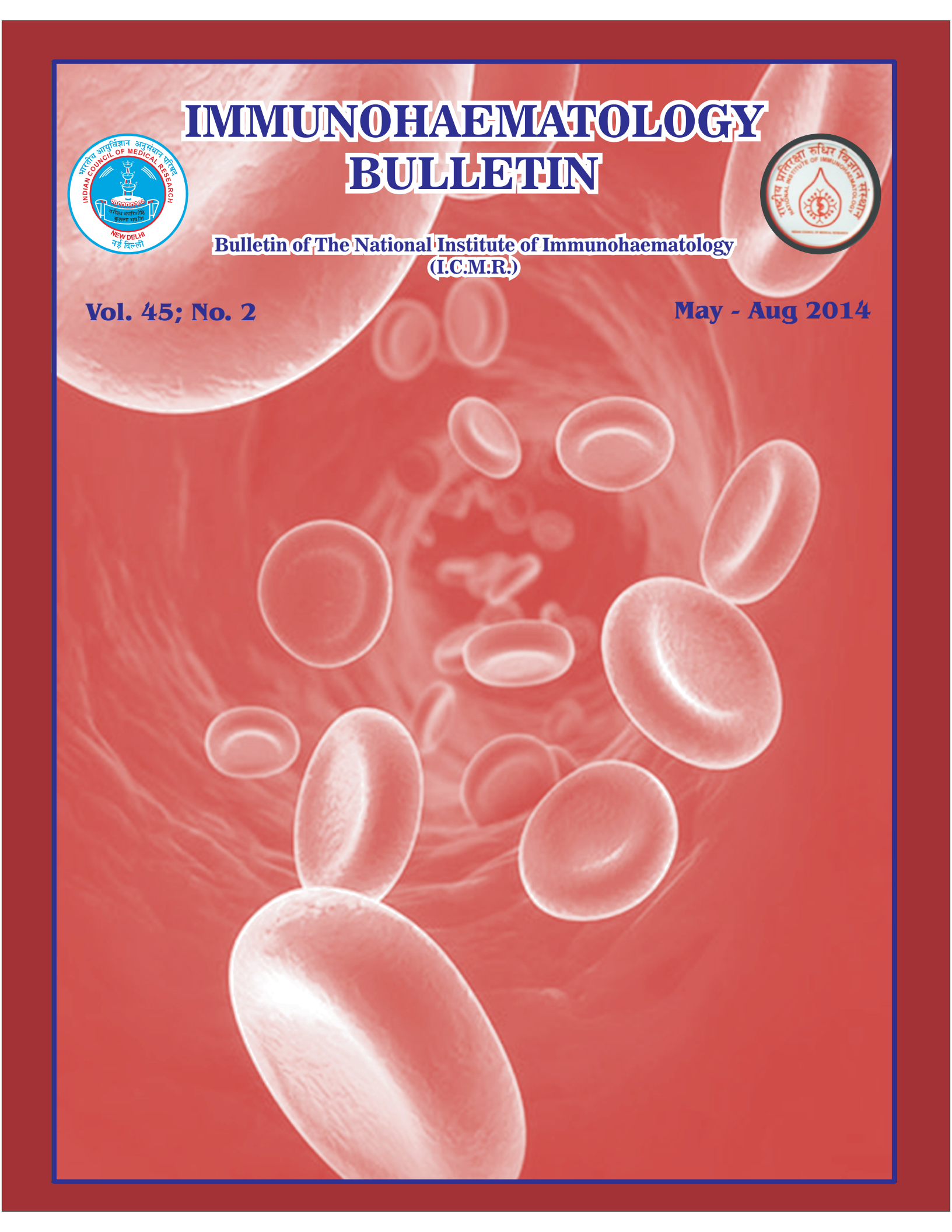
IMMUNOHAEMATOLOGY BULLETIN

Bulletin of The National Institute of Immunohaematology
(I.C.M.R.)

Vol. 45; No. 2

May - Aug 2014



Mr. T. S. Jawahar, I.A.S. Senior Deputy Director General and Chief Vigilance Officer, ICMR Visited NIIH for Cleanliness drive

Neonatal screening & Natural history of sickle cell disease

Dipti Upadhye

Summary:

Newborn screening is still a young science. Prof. Robert Guthrie conceived the concept in 1960 in USA and the first metabolic disorder tested was Phenylketonuria (PKU) (Guthrie, 1962). In 1975, Dussault described screening for congenital hypothyroidism (CH), and since then other disorders covered in some screening programmes have included congenital adrenal hyperplasia, the galactosaemias, cystic fibrosis, biotinidase deficiency, glucose-6-phosphate dehydrogenase deficiency and many others in combination with haemoglobinopathies. At present, screening of neonates for sickle cell disease (SCD) is carried out in a number of areas throughout the world which includes USA, Africa, European nations and UK. In India where the prevalence of SCD is very high, there are no such established neonatal screening programmes. This review discusses the importance of neonatal screening programs for sickle cell disease carried out across the world and two major contributions made in this field to understand the natural history of sickle cell disease.

Introduction

Sickle cell anemia is a monogenic disorder due to a single point mutation (β^6- GAG-> GTG) in the β globin gene resulting in the formation of abnormal "sickle shaped" red blood cells leading to chronic hemolysis, vaso-occlusion and other severe complications. The highest incidence is in Africa where malaria is endemic; however it is also a significant health burden in other countries like India, Saudi Arabia and the Mediterranean region. In India, the sickle gene is prevalent in the tribal populations in India as well as in some non-tribal populations with carrier frequencies ranging from 1-40% with the highest prevalence in central India.

Natural history studies for sickle cell disease from Jamaica and USA have confirmed that the greatest morbidity and mortality occur early in life especially between 6 and 12 months. The high mortality in the first year of life, and the potential to reduce this through early administration of penicillin prophylaxis and education of parents in recognising the early signs of potentially serious complications such as splenic sequestration suggests that early diagnosis of SCD could reduce morbidity and mortality. Early diagnosis and development of interventions against some serious problems gave an impetus to newborn screening.

The aim of the neonatal screening program is to provide :

1. Early diagnosis of the disease
2. Early prophylactic treatment and vaccination and
3. Parental education and counselling for appropriate home care of the babies to avoid serious complications such as splenic sequestration.

Possible methods for neonatal screening for SCD can be of 2 types:

1. **Universal -** where all the neonates delivered are screened (National screening programme).
2. **Targeted -** where sampling is done on infants where either of the parent is positive for any hemoglobinopathy or a selected high risk group is

Dipti Upadhye, Senior Research Fellow,
National Institute of Immunohaematology,
13th Floor, KEM Hospital, Parel, Mumbai 400 012, India. ● Email : dipti.upadhye@gmail.com

screened.

Apart from detection of babies with sickle cell disease it also enables detection of another group of babies – those with sickle cell trait (AS). These babies are healthy and asymptomatic and the implications of detection of this state are related principally to future reproductive risks.

The strategy adopted for neonatal screening is different in different countries depending on the prevalence and cost-effectiveness of the program. In the USA, newborn screening for sickle cell disorders was implemented in California in 1990 and is now done in all the 50 states (Michlitsch, *et al*., 2009). In the European Union, four countries have adopted a neonatal screening programme - England, France, Belgium and recently the Netherlands (Streetly, *et al*., 2008; Thuret, *et al*., 2010; Gulbis, *et al*., 2009; Bouva, 2010). The neonatal screening programmes for SCD running globally and in India are as follows:

Neonatal screening programs for SCD across the world

A. Neonatal screening in Jamaica :

Jamaica's first experience with newborn screening occurred from 1973 to 1981 with the development of the well-described Jamaican Sickle Cell Cohort Study. In this study, 100,000 consecutive live births were screened at the main Government Maternity Hospital (Victoria Jubilee Hospital, Kingston). A total of 315 babies with Hb SS disease were detected. These SS babies were followed up prospectively from birth. The Sickle Cell Cohort Study has provided invaluable information on the natural history of the disease as well as some of the major lessons in disease management, which makes it an ideal control group. The second experience started in November 1995 when newborn screening began at the main Government Maternity Hospital in Kingston (Victoria Jubilee Hospital). It was extended to include the University Hospital of the West Indies, Kingston in October 1997 and The Spanish Town Hospital, in April 1998. Together, these hospitals were responsible for ~ 43% of births in Jamaica (Serjeant and Serjeant, 2001).

B. Neonatal screening in the United States:

1. California, Illinois and New York:

All newborns in California, Illinois, and New York were screened for hemoglobinopathies. Health departments implemented screening programs in New York in 1975, Illinois in 1989 and California in 1990. During 1990-1994, a total of 2487 children with presumed or confirmed SCD were identified by the three newborn screening programs conducted in California, Illinois and New York. 29 deaths were reported among children with SCD.The mortality rate in these states declined significantly as infants with SCD were identified soon after birth. This trend occurred at the same time with the widespread use of penicillin prophylaxis and vaccinations (Morbidity, mortality weekly report, 1998).

The study was further extended from 1998-2006 to California where 5, 30,000 newborn samples were screened annually. 0.05% of the newborns had abnormal findings and referred to a specialised laboratory. Sickle cell disease was most frequently observed followed by α-thalassemia and β-thalassemia. The majority (93%) of sickle cell anemia cases (Hb SS) were reported in Black newborns, a large proportion of Hb S/β-thalassemia cases (12.5%) were observed in the Hispanic population. Most cases of α-thalassemia were reported in the South East Asian

(SEA) population (93%), whereas β-thalassemia was distributed over multiple ethnic groups (Michlitsch, *et al*., 2009). In a 4-year review, nearly 90% of infants diagnosed with SCD began prophylactic penicillin therapy by age 24 weeks (Shafer, *et al*., 1996).

2. Connecticut :

In the 11-1/2 years after universal NBS was initiated in Connecticut in 1990, there were no reported deaths among infants diagnosed at birth with sickle cell anaemia or sickle-beta thalassemia as compared to 13 deaths attributed to sickle cell diseases before any State NBS program was operated and five affected children who missed testing and died with the limited State NBS program (Frempong and Pearson, 2007). These results document a marked reduction in mortality since the introduction of NBS for haemoglobinopathies.

C. Neonatal screening in South America:

1. Venezuela:

The prevalence of hemoglobinopathies was evaluated in newborns from different areas of Venezuela, in cooperation with the neonatal screening system of the Study Unit of Inborn Errors of Metabolism. The heel blood samples of 101, 301 newborns were screened with HbS being the most frequent phenotype (67.92%) (Gimenez, *et al*., 2009).

2. Brazil :

Brazil shows a high prevalence of HbS and HbC, indicating the need for early diagnosis and proper medical care and counselling. In this study around 117, 320 newborns samples from different areas of Rio Grande do Sul State, Brazil were obtained by heel stick along with the parents sample. The most frequent hemoglobinopathy being sickle cell carriers followed by HbC carriers and HbD carriers. 26 other variant hemoglobins were also identified (Sommer, *et al*., 2006).

D. Neonatal screening in the European countries:

1. Belgium:

A total of 27,010 newborn samples were collected in Belgian maternity units between 2003 and 2005, and were examined for the presence of haemoglobin (Hb) C or S. 132 (0.49%) were positive. 106 of these babies were heterozygotes for the Hb S mutation and 3 were heterozygotes for the Hb C mutation, 3 newborns were SS homozygotes (0.011%) while 17 samples gave false positive results (Boemer, *et al*., 2006).

In Brussels, a non-selective universal neonatal screening for haemoglobinopathies was in operation between 1994 and 2002 respectively where 118,366 newborns were screened and 64 were diagnosed with a sickle cell syndrome, 6 were β-thalassaemia major, 4 had haemoglobin C disease and 3 had haemoglobin H disease (Gulbis, *et al*., 2006).

A universal neonatal haemoglobinopathy screening programme in Belgium and Liege found SCD to be the most common haemoglobinopathy alongwith other hemoglobin abnormalities. These affected children received medical care from birth. Death of 2 SCD newborns was reported of whom 1 died of septicaemia where the prophylactic treatment was interrupted and 1 died of respiratory distress (Gulbis, *et al*., 2009).

2. France:

The French national programme for the neonatal screening of SCD was set up in 1995 in metropolitan France by the French Association for the Screening and Prevention of Infant Handicaps (AFDPHE). The programme was extended progressively and had covered the whole country by 2000. The neonatal screening of sickle cell disease was applied to newborns 'at risk', defined as those born to parents

originating from sub- Saharan Africa, the Mediterranean area, the Arabic peninsula, the French overseas islands and the Indian subcontinent. To evaluate the efficiency of selection, a non-selected population was also screened. Altogether, over this 6-year period, a total of 151 017 newborns were screened of which 28 affected children were detected (1/5393). Of these 18 were in a selected population and 10 were in a non-selected population out of which 9 were from the at-risk couples but failed to be asked the screening question. The study indicated that selective screening leads to a carrier frequency that is twice as high in the selected population as compared with the nonselected population (1.23% versus 0.62%) (Thuret, *et al*., 2010).

3. Spain :

The first neonatal screening of haemoglobinopathies in Spain was done in Barcelona by Baiget, *et al.,* in 1981. In 1998, Cabot, *et al.,* performed a targeted screening study in Mataro´ (Catalonia) limited to newborns of sub-Saharan African mothers, initiated because of the high and increasing delivery rate of black origin immigrants in this geographical area. In 2003, Dulın, *et al*., performed a universal pilot screening of haemoglobinopathies in the Community of Madrid. A total number of 29253 specimens, obtained by heel prick and preserved as a dried blood spot on a filter paper (a "Guthrie spot") were collected in which 98 haemoglobinopathies were identified with an overall incidence of 0.33%.

In 2007, Cela, *et al*., reported the results of the first 32 months of running this programme, after studying of a total of 190 238 blood samples from newborns (Guthrie spots) by HPLC, 1060 haemoglobin variants were identified, corresponding to a prevalence of 0.56%. In all of these cases, prevention measures consisting of antibiotic administration, vaccination and comprehensive care were initiated. Among 3,365 newborns studied, hemoglobinophaty was detected in 26 children with the HPLC Variant, with a global incidence of 7.7 per 1,000 newborns (Joyanes, *et al*., 2006).

4. Italy :

In 2007-2009, 59% of women who delivered at the University Hospital of Ferrara had undergone HPLC. Of the 41% who were not tested, many were from areas where sickle cell disease is common. Between 2010 and 2012, 1992 neonatal tests were performed and 24 carriers of haemoglobinopathies were identified. 42.6% of the mothers of these neonates had not undergone screening during pregnancy. Currently, prevention of haemoglobinopathies in Italy is provided during the pre-conception period but only to patients with abnormal blood counts. Therefore in such cases, neonatal screening is useful to ensure early diagnosis and appropriate treatment for infants with sickle cell disease or other haemoglobinopathies (Ballardini, *et al*., 2013).

E. Neonatal screening in United Kingdom :

1. England :

A national newborn screening programme was started in England from 2003-2005. 108,255 infants were screened in London and the Midlands in the first seven months (up to March 2004). There were 125 clinically significant positive results in addition to 2950 carriers. There were also nine infants with only Hb F (suggestive but not conclusive of beta thalassaemia major).

The second year of implementation of the screening programme in 2004–2005, covered about 400,000 births (two-thirds of the population) of which 250

were positive for sickle cell disorders and about 6500 carriers were identified. Over three quarters of the affected cases were in London (Streetly, *et al*., 2008).

In 2000 the UK government implemented a National Health Service (NHS) Sickle Cell and Thalassaemia Screening Programme for appropriate newborn and antenatal screening throughout England.

In a screening programme from 2005-2007, 13 laboratories all over England were involved, which covered almost 1.2 million infants where over 17,000 carriers were identified (Streetly, *et al*., 2009).

2. Birmingham :

During 1978-81 there were about 43,500 births in Birmingham who were screened for sickle haemoglobinopathy of whom 8 babies had important haemoglobinopathies (HbSS, HbSC, HbE-β thalassaemia) whereas 534 were heterozygous for HbS/D and 205 for HbC or HbE (Griffiths, *et al*., 1982).

F. Neonatal screening in the Middle East:

1. Oman :

A neonatal screening in Oman analysed 7837 cord blood samples with 5.46% incidence of sickle haemoglobin and 9.86% incidence of other haemoglobinopathies (Alkindi, *et al*., 2011).

G. Neonatal screening in Canada:

1. Quebec :

A pilot study for 1 (1987-88) year was conducted in Canada in which 2279 cord blood samples were obtained from 9 different ethnic groups, estimated the prevalence of HbSS to 40 per 1,00,000 newborns (Yorke, *et al*., 1992). A hospital based neonatal screening program targeting infants at-risk over a 15 year period (1988-2003) was carried out in Quebec. A total of 9619 infants were screened of which 37 were classified as HbSS/ HbSβ and 35 had HbSC disease. 1012 were identified as sickle cell trait and 386 as HbC trait. This screening program was clinically effective as it identified 92.3% at risk patients.

Worldwide, Neonatal Screening Programmes for Sickle Cell Disease (SCD) have been set up in high prevalent regions. It's been around 40 years since neonatal screening programs have been started in Jamaica and in USA. Subsequently, other countries also initiated such programmes and huge number of newborns are being screened and the affected babies are getting the benefit of early comprehensive care and prophylactic treatment. An initial pilot effort was made in Raipur district in Chattisgarh in India, on screening a small number of babies (1,158), however newborn screening programmes have not been initiated in most regions in Central India where the burden of sickle cell disease is high. Therefore, there is an urgent need to implement such programmes for sickle cell disease in Central India as well as in other regions of the country. There can be little doubt that newborn screening confers benefit, is cost-effective and is urgently needed in high prevalence areas, such as sub-Saharan Africa and central India, where the disease represents a formidable public health problem.

Neonatal Screening for non-sickle haemoglobinopathies

In addition to sickle cell disorders, newborn screening programs have historically focused on identifying infants with classic β-thalassemia major. However, clinically significant forms of α-thalassemic disorders, Hb H and Hb H Constant Spring, as well as Hb E disorders are now becoming increasingly

recognized in western countries. Some of these conditions may be severe and require chronic blood transfusions. In Thailand, non-deletional Hb H disease with $\alpha^{Constant\ Spring}$ is even more common, reportedly found in 40% to 50% of patients with Hb H disease (Fucharoen, *et al*., 1988). This indicates the importance of neonatal screening for alpha thalassaemia in countries where the prevalence is high.

Concurrent screening for non-sickling disorders, such as β-thalassemia and HbH disease and HbE disorders, is not mandated and is often limited to making a presumptive diagnosis that requires confirmation outside of the newborn screening program.

A. Neonatal Screening for β and α thalassaemias :

Along with the newborn screening for haemoglobinopathies, a preliminary study for carrier detection of β-thalassaemia was done on the basis of the HbA fraction in England (Mantikou, *et al*., 2009).

Hb H disease is found in many parts of the world, including Southeast Asian, Middle Eastern, and Mediterranean populations. It is particularly prevalent in Southeast Asia and in southern China, because of high carrier frequencies of the ($--^{SEA}$), and to a lesser extent, the ($--^{FIL}$) types of α-thalassemia deletions. In Thailand with a population of 62 million people, it is estimated that 7000 infants with Hb H disease are born annually, and that there are 420 000 patients with Hb H disease in this country.

Hb H disease, a clinically significant form of α - thalassemia, is the second most common hemoglobinopathy observed in California. The newborn screening program of California from 1998-2006 reported 8 surviving α-thalassemia major newborns along with 500 Hemoglobin H disease babies. There were five cases of α-thalassemia major identified, all of whom were picked up on initial newborn screening (NBS), with Hb Barts levels 98–100%. At least two of these patients have subsequently undergone stem cell transplantation and are currently transfusion independent (Michlitsch, *et al*., 2009). This has justified NBS in the state of California. In 1999, newborn screening for Hb H disorders was incorporated in the state wide hemoglobinopathy screening program.

Cord blood screening for α-thalassemia and Hb variants in northern Thailand identified 207 out of 566 newborns (36.6%) who had thalassemia genes or Hb variants. Seventeen different genotypes were found. Nine cases (1.6%) of Hb H disease and one Hb E-β-thalassemia was identified (Charoenkwan, *et al*., 2010).

A correct diagnosis would allow affected infants to be properly cared for, and would also raise awareness for the prevention of homozygous alpha^{0}-thalassemia or Hb Bart's hydrops fetalis syndrome.

Neonatal screening programs for hemoglobinop athies cannot differentiate cases from sickle homozygous and sickle-β-thalassaemia, HbE homozygous from HbE–β thalassaemia, β thalassaemia minor and HPFH conditions.

Success of a neonatal screening programme

The success of a neonatal haemoglobinopathy screening programme is not related to the number of neonates tested, or the incidence of major haemoglobinopathies calculated, but it has enabled appropriate healthcare planning, allocation of resources, and appropriate counselling for parental

choice and prevention of disease in future. The early detection and compliance with the prophylactic measures has significantly declined the mortality rate among the SCD patients.

Natural history of sickle cell disease

The highest mortality in SCD patients (2-30%) occurs in the first five years of life (Overturf, *et al*., 1977; Vichinsky, *et al*., 1988; Serjeant and Serjeant, 2001). Around 30 % of children with SCD among some tribal communities in India die before they reach adulthood (Rupani, *et al*., 2012, Sickle cell anemia control project: Govt. of Gujrat, 2012). The only way to assess morbidity and mortality is to raise a cohort at birth and follow up the babies regularly to understand the natural history of Sickle Cell Disease.

Two major contributions to the study of natural history of sickle cell disease were made by the Jamaican cohort study from 1973 to 1981 and the Cooperative Study of Sickle Cell Disease (CSSCD) in the United States from 1978 to 1988.

A. The Jamaican Sickle Cell Cohort Study :

Out of 1,00,000 births screened , a total of 315 babies with HbSS were detected, of whom 309 babies were followed prospectively from birth. Long term follow up identified major early causes of death such as acute splenic sequestration, pneumococcal septicaemia, aplastic crisis, and the acute chest syndrome. Acute splenic sequestration (ASS), septicaemia and acute chest syndrome were the most important complications in early life in the Jamaican cohort (Thomas, *et al*., 1982). A parental education programme was followed for the increasing rate of ASS in the SCD patients. This awareness showed a 90% reduction in the mortality arising from acute splenic sequestration crisis (Emond, *et al*., 1985). A successful trial of pneumococcal prophylaxis decreased the number of deaths from pneumococcal disease (John, *et al*., 1984). In the Jamaican Sickle Cell Cohort where early age interventions had not been implemented 14% of the children died in the first two years while the number declined to less than 1% when prophylactic treatment was made available. Since the patients with sickle cell disease were at increased risk of *Haemophilus influenza* septicaemia, an introduction to this vaccine was also recommended (Powars, *et al*., 1983).

After this study, ASS and pneumococcal sepsis were no longer a significant cause of mortality and acute chest syndrome was the most common clinical non death event resulting in a decline in the mortality rate and improving the overall survival of the SCD patients in Jamaica. But much has to be still done in the area of acute chest syndrome, stroke and aplastic crisis which have reduced but still remain as major complications in SCD patients in Jamaica.

These observations from the Jamaican cohort study show that simple readily implementable measures may improve survival in sickle cell disease, but to avoid the high mortality in the first year of life neonatal detection of sickle cell disease is essential.

B. The Cooperative Study of Sickle Cell Disease :

The second major contribution to the study of the natural history of sickle cell disease was made by the Cooperative Study of Sickle Cell Disease (CSSCD) in the United States from 1978 to 1988 where 23 clinical centres entered in the study (Gaston & Rosee, *et al*., 1982). In this cohort study of more than 4000 babies, 694 had confirmed haemoglobin diagnosis out of which 60% were diagnosed as sickle homozygous. One of the earliest contributions of the CSSCD was to identify the persistent high mortality rate due to severe

pneumococcal infections in children with SCD. Twenty children, all with Hb SS, died during the study period, out of which eleven children died from infection (9 *S. pneumoniue* and 2 *H. influenze)* the mortality rate being highest between 6 months and 3 years of age. This led to the development of the landmark Prophylactic Penicillin Study (PROPS), which highlighted the importance of neonatal screening for SCD followed by prophylactic penicillin therapy in children between the age of 4 months and 5 years (Gaston, *et al*., 1986). The purpose of the study was to assess the efficacy of penicillin prophylaxis in the prevention of severe bacterial infections in children with SCA. The study was a multi-center, randomized, double-blinded, placebo-controlled clinical trial conducted in the United States. The high mortality in the first year of life, and the potential to reduce this through early administration of penicillin prophylaxis suggests that early diagnosis of SCD could reduce morbidity and mortality which can only be achieved through neonatal screening programmes.

Other CSSCD studies found that 50% of patients died before the fifth decade, and most of those who died succumbed during an episode of acute pain, acute chest syndrome, or stroke (Platt, *et al*., 1994). Parallel studies established that approximately 11% of patients with SCD will go on to develop a clinically apparent stroke by the age of 20 years, and 24% by the age of 45 years (Ohene-Frempong, *et al*., 1998). This high risk of a life-threatening complication generated the momentum for the Stroke Prevention Trial in Sickle Cell Anemia (STOP), which demonstrated the benefit of prophylactic transfusions in preventing a first stroke in patients with an elevated flow rate by transcranial Doppler (TCD) ultrasonography (Adams, *et al*., 1998). Moreover, the observation that more than 50% of patients with SCD have at least one crisis per year and the association between multiple pain episodes and early death in young adults (Platt, *et al*., 1991; 1994) provided the impetus for the Multicenter Study of Hydroxyurea (MSH). The CSSCD also shed some important light on the incidence and risk of other complicating conditions, including alloimmunization (Rosse, *et al*., 1990), pregnancy (Koshy, *et al*., 1988), and surgery (Koshy, *et al*., 1995).

C. Natural history of sickle cell disease in England:

Universal newborn screening was carried out in East London between 1983 to 2005. Umbilical cord blood samples from all babies born in maternity hospitals in the London Borough of Hackney were analyzed for haemoglobin variants. From 1998, the program was expanded to include children from the adjacent London Borough of Tower Hamlets, and continued until replaced in 2004 by the newborn bloodspot screening programme for SCD (http://www.kcl-phs.org.uk/haemscreening). 252 children who were homozygous/compound heterozygous for abnormal haemoglobin variants (SS/Sβ/SC) were identified and were followed in a hospital and community-based program. All children received penicillin V prophylaxis from 3 months of age, 23-valent pneumococcal polysaccharide vaccine from 1993, conjugate pneumococcal vaccine from 2002 and transcranial Doppler screening from 1991. Hydroxyurea was recommended to children with recurrent painful crises (more than three admissions per year) or recurrent chest crises (more than two episodes per year) after 1999. Out of the 253 children, 7 patients (2.8%) were lost of follow up. Pain crisis followed by acute chest syndrome and acute splenic

sequestration crisis were the main clinical complications in this cohort. Infections included Pneumococcal sepsis, bacterial meningitis, septicaemia and osteomyelitis. The documented infection rates were about ten-fold lower than those in cohorts not given penicillin prophylaxis (Gaston, *et al*., 1986). There were no SCD-related deaths in under 5-year olds in SS/SC and in Sβ- thalassaemia babies. One boy with HbSS underwent matched sibling allogeneic bone marrow transplantation aged 5 years, and is crisis-free after 6 years of follow-up (Telfer, *et al*., 2007).

The lessons learned from the study of the natural history of SCD underscored the fact that this disease, which is caused by a single missense mutation in a gene whose expression is restricted to the hematopoietic system, can have wide-ranging manifestations and complications that affect every aspect of the life of affected patients. The natural history of SCD has not been studied in Indian patients. Looking at the huge population of SCD patients in India, their diverse clinical complications and high mortality rates such studies will contribute to the existing knowledge of SCD in India and allow us to understand the associated complications and the cause of mortality in these patients in their early years of life.

NIIH Experiences :

Neonatal screening for sickle cell disease was done in Central India in collaboration with the Govt. Medical College, Nagpur. A targeted newborn screening approach was used where solubility test was done for the presence of HbS in the pregnant mothers coming to the antenatal clinic at Govt. Medical College, Nagpur. Babies were screened for HbS in all the cases where either of the parents was found to be positive. In 3 years (2009-2012) 10,181 parents were screened by the solubility test and of them 1863 mothers and 271 fathers were positive. A total of 2134 newborns were screened of whom 1040 (49.0%) were found to be normal and 978 (46.0 %) were HbS traits. 104 (4.9%) newborns were diagnosed as sickle homozygous and **7** were identified as sickle thalassaemia (Jain, *et al*.,2012). Few babies with other haemoglobinop athies were also identified. 3 rare alpha chain variants - Hb Fontainebleau, HbO Indonesia and Hb Koya Dora were also identified (Upadhye, *et al*., 2012). The initial screening of the babies on HPLC was done using the sickle short programme in Nagpur and β thal short program in NIIH, Mumbai. Comparison of both the programmes showed that β thal short program was more superior for screening of newborns than sickle cell short programme (Upadhye, *et al*., 2014). Of the 104 sickle homozygous babies, majority (92%) belonged to the non-tribal communities specifically Mahars, Teli, Kunbi, Gawli and Gowari castes while 8 % belonged to the tribal communities Gond and Pinjari. Timely intervention and prophylactic treatment (HiB & Pneumococcal vaccination, Penicillin prophylaxis, and folic acid) was given to the babies as per their age and requirement. The babies were followed up every 6 months for 2-4 years for hematological and clinical evaluation. A decrease in the levels of RBC, Hb, HCT and increase in the MCV, MCH and RDW levels were found in the SS newborns from 6 months to 2 years of age and thereafter. Clinically, painful events followed by blood transfusions and acute febrile illness were the main clinical complications observed in this study. Dactylitis, vasoocclusive crisis, acute splenic sequestration crisis, stroke, splenomegaly, pneumonia, sepsis and other infections were also seen

among the sickle cell disease babies. 6 SS babies died during the study. 90% of the SS babies had Xmn I (+/+) while 10% of the babies had Xmn I (+/-). Majority of the babies belonged to the Arab-Indian haplotype while Atypical haplotype and Bantu A2 haplotype were also found in this cohort. Even though majority of the SS babies were linked to the Arab- Indian haplotype, the babies had serious complications. Alpha thalassaemia significantly affected the RBC counts in the normal, sickle traits and sickle homozygous babies.

Apart from Nagpur and nearby areas, neonatal screening is also been carried out in South Gujrat where the tribal population is screened for sickle cell disease.

References :

1. Adams RJ, McKie VC, Hsu L, Files B, Vichinsky E, Pegelow C, Abboud M, Gallagher D, Kutlar A, Nichols FT, Bonds DR, Brambilla D. Prevention of a first stroke by transfusions in children with sickle cell anemia and abnormal results on transcranial Doppler ultrasonography. N. Engl. J. Med. 1998; 339: 5–11.
2. Alkindi S, Pathare A, Al-Madhani A, Al-Zadjali S, Al-Haddabi H, Al-Abri Q, Gravell D, Mathew M, Krishnamoorthy R . Neonatal Screening: Mean haemoglobin and red cell indices in cord blood from Omani neonates. Sultan Qaboos Univ. Med. J. 2011; 11: 462-9.
3. Baiget M, del Rı ´o E, Dome´nech M, et al. Escrutinio de hemoglobinopatı ´as en sangre de cordon umbilical. Biologı ´a Clı ´n Hematol 1981; 3: 251–6.
4. Ballardini E1, Tarocco A, Marsella M, Bernardoni R, Carandina G, Melandri C, Guerra G, Patella A, Zucchelli M, Ferlini A, Bigoni S, Ravani A, Garani G, Borgna-Pignatti C. Universal neonatal screening for sickle cell disease and other haemoglobinopathies in Ferrara, Italy. Blood Transfus. 2013; 11: 245-9
5. Boemer F, Vanbellinghen JF, Bours V, Schoos R. Screening for sickle cell disease on dried blood: a new approach evaluated on 27,000 Belgian newborns. J. Med. Screen. 2006; 13: 132–6.
6. Bouva MJ, Mohrmann K, Brinkman H, Kemper-Proper EA, Elvers B, Loeber JG, Verheul F, Giordano PC. Implementing neonatal screening for haemoglobinopathies in the Netherlands. J Med Screen. 2010; 17: 58–65.
7. Cabot Dalmau A, Casado Toda M, Barberán Pérez J, Roqueta Sureda M, Martorell Aymerich Q, , Rovira Fernández JM. Neonatal screening for sickle cell disease in the Consorci Sanitari de Mataro. Rationale and first results. An Esp Pediatr. 1998; 49: 157–60.
8. Cela de Julián E, Dulín Iñiguez E, Guerrero Soler M, Arranz Leirado M, Galarón García P, Beléndez Bieler C, Bellón Cano JM, García Arias M, Cantalejo López A. Evaluation of systematic neonatal screening for sickle cell diseases in Madrid three years after its introduction. An. Pediatr. (Barc). 2007; 66: 382-6.
9. Charoenkwan P, Taweephol R, Sirichotiyakul S, Tantiprabha W, Sae-Tung R, Suanta S. Cord blood screening for α-thalassemia and hemoglobin variants by isoelectric focusing in northern Thai neonates: Correlation with genotypes and hematologic parameters. Blood Cells, Mol. Dis. 2010; 45: 53–57.
10. Dulín Iñígueza E, Cantalejo López MA, Cela de Julián ME, Galarón García P. Early detection of

sickle cell anemia and other hemoglobinopathies in neonates in the Autonomous Community of Madrid. A pilot study. An. Pediatr. (Barc). 2003; 58: 146–55.

11. Dussault JH, Coulombe P, Laberge C, Letarte J, Guyda H, Khoury K. Preliminary report on a mass screening program for neonatal hypothyroidism. J. Pediatr. 1975; 86: 670–674.
12. Emond AM, Collis R, Darvill D, Higgs DR, Maude GH, Serjeant GR. Acute splenic sequestration in homozygous sickle cell disease: Natural history and management. J. Pediatr. 1985; 107: 201–206.
13. Frempong T, Pearson HA. Newborn screening coupled with comprehensive follow-up reduced early mortality of sickle cell disease in Connecticut. Conn. Med. 2007; 71: 9-12.
14. Fucharoen S, Winichagoon P, Pootrakul P, Piankijagum A, Wasi P. Differences between two types of Hb H disease, alpha-thalassemia 1/alpha-thalassemia 2 and alpha-thalassemia1/Hb constant spring. Birth Defects Orig Artic Ser. 1988; 23: 309-315.
15. Gaston M, Rosse WF. The Cooperative Study of Sickle Cell Disease: Review of study design and objectives. Am J Pediatr Hematol.1982;4:197-201.
16. Gaston MH, Verter JI, Woods G, Pegelow C, Kelleher J, Presbury G, Zarkowsky H, Vichinsky E, Iver R, Lobe J, Diamond S, Holbrook CT, Gill FM, Ritchey K, and Falletta JM. Prophylaxis with oral penicillin in children with sickle cell anemia. N. Engl. J. Med. 1986; 314: 1593-1599.
17. Gimenez OG, Torrealba MC, Urquiola MB, Ortiz GG, Fonseca SM, Merzon R, de Donato M, Castillo O, Arends de Pérez A. Diagnosis of hemoglobinopathies in newborns in Venezuela hospitals. An. Pediatr. (Barc). 2009; 71: 314-8.
18. Griffiths KD, Raine DN, Mann JR. Neonatal screening for sickle haemoglobinopathies in Birmingham. Br. Med. J. (Clin. Res. Ed.). 1982; 284: 933-5.
19. Gulbis B, Ferster A, Cotton F, Lebouchard M, Cochaux P, Vertongen F. Neonatal haemoglobinopathy screening: review of a 10-year programme in Brussels. J. Med. Screen. 2006; 13: 76–78.
20. Gulbis B, Cotton F, Ferster A, Ketelslegers O, Dresse MF, Rongé-Collard E, Minon JM, Lé PQ, Vertongen F. Neonatal haemoglobinopathy screening in Belgium. J. Clin. Pathol. 2009; 62: 49–52.
21. Guthrie R. Screening for Inborn errors of metabolism in the newborn Infant- A multiple test program. Birth defects original article. 1962; series IV: 92-98.
22. http://www.kcl-phs.org.uk/haemscreening
23. Jain D, Sarathi V, Upadhye D, Gulhane R, Nadkarni A, Ghosh K, Colah R. Newborn screening shows a high incidence of sickle cell anemia in Central India. Hemoglobin. 2012; 36: 316-22.
24. John AB, Ramlal A, Jackson H, Maude GH, Waight Sharma A, Serjeant GR. Prevention of pneumococcal infection in children with homozygous sickle cell disease. BMJ. 1984; 2A8: 1567-70.
25. Joyanes B, Moro M, Ropero P, Briceño O, Dulín E, Villegas A. Screening of hemoglobinopathies in a cohort of newborns in Madrid.Med. Clin. (Barc). 2006; 126: 290-2.
26. Koshy M, Burd L, Wallace D, Moawad A, Baron J. Prophylactic red-cell transfusions in pregnant patients with sickle cell disease. A randomized cooperative study. N. Engl. J. Med. 1988; 319: 1447–1452.

27. Koshy M, Weiner SJ, Miller ST, Sleeper LA, Vichinsky E, Brown AK, Khakoo Y, Kinney TR. Surgery and anesthesia in sickle cell disease. Cooperative Study of Sickle Cell Diseases. Blood. 1995; 86: 3676–3684.
28. Mantikou E, Arkesteijn SG, Beckhoven van JM, Kerkhoffs JL, Harteveld CL, Giordano PC. A brief review on newborn screening methods for hemoglobinopathies and preliminary results selecting beta thalassemia carriers at birth by quantitative estimation of the HbA fraction. Clin. Biochem. 2009; 42: 1780-5.
29. Michlitsch J, Azimi M, Hoppe C, Walters MC, Lubin B, Lorey F, Vichinsky E. Newborn screening for hemoglobinopathies in California. Pediatr. Blood Cancer. 2009; 52: 486-490.
30. Mortality among children with sickle cell disease identified by newborn screening during 1990-1994--California, Illinois, and New York. MMWR Morb Mortal Wkly Rep. Centres for Disease Control and Prevention (CDC). 1998; 47: 169-72.
31. Ohene-Frempong K, Weiner SJ, Sleeper LA, Miller ST, Embury S, Moohr JW, Wethers DL, Pegelow CH, Gill FM. Cerebrovascular accidents in sickle cell disease: rates and risk factors. Blood. 1998; 91: 288-94.
32. Overturf GD, Powars D, Barraf LJ. Bacterial meningitis and septicemia in sickle cell disease. Am J. Dis. Child. 1977; 131: 784–7.
33. Platt OS, Thorington BD, Brambilla DJ, , Rosse WF, Vichinsky E, Kinney TR. Pain in sickle cell disease. Rates and risk factors. N. Engl. J. Med. 1991; 325: 11–16.
34. Platt OS, Brambilla DJ, Rosse WF, Milner PF, Castro O, Steinberg MH, Klug PP. Mortality in sickle cell disease. Life expectancy and risk factors for early death. N. Engl. J. Med. 1994; 330: 1639–44.
35. Powars D, Overturf G, Turner E. Is there an increased risk of Haemophilus influenzae septicemia in children with sickle cell anemia? Pediatrics. 1983; 7r: 927-31.
36. Rosse WF, Gallagher D, Kinney TR, Castro O, Dosik H, Moohr J, Wang W, Levy PS. Transfusion and alloimmunization in sickle cell disease. The Cooperative Study of Sickle Cell Disease. Blood. 1990; 76: 1431–1437.
37. Rupani MP, Vasava BC, Mallick KH, Gharat VV, Bansal R. Reaching community through school going children for sickle cell disease in Zankhvav village of Surat district, western India. Online J Health Allied Sci. 2012; 11: 4.
38. Serjeant GR. and Serjeant BE. Screening and Sickle cell clinics Eds: Serjeant GR and Serjeant BE. Oxford Medical Publications. 2001: 537-544
39. Shafer FE, Lorey F, Cunningham GC, Klumpp C, Vichinsky E, Lubin B. Newborn screening for sickle cell disease: 4 years of experience from California's newborn screening program. J. Pediatr. Hematol. Oncol. 1996; 18: 36–41.
40. Sommer CK, Goldbeck AS, Wagner SC, Castro SM. Neonatal screening for hemoglobinopathies: a one-year experience in the public health system in Rio Grande do Sul State, Brazil. Cad Saude Publica. 2006; 22: 1709-14.
41. Streetly A, Clarke M, Downing M, Farrar L, Foo Y, Hall K, Kemp H, Newbold J, Walsh P, Yates J, Henthorn J. Implementation of the newborn screening programme for sickle cell disease in England: results for 2003–2005. J. Med. Screen. 2008; 15: 9–13.

42. Streetly A, Latinovic R, Hall K, Henthorn J. Implementation of universal newborn bloodspot screening for sickle cell disease and other clinically significant haemoglobinopathies in England: screening results for 2005-7. J Clin Pathol. 2009; 62:26-30.
43. Telfer P, Coen P, Chakravorty S, Wilkey O, Evans J, Newell H, Smalling B, Amos R, Stephens A, Rogers D, Kirkham F. Clinical outcomes in children with sickle cell disease living in England: a neonatal cohort in East London . Haematologica. 2007; 92: 905-912.
44. Thomas AN, Pattison C, Serjeant GR. Causes of death in sickle-cell disease in Jamaica. Br. Med. J. 1982; 285: 633–635.
45. Thuret I, Sarles J, Merono F, Suzineau E, Collomb J, Lena-Russo D, Levy N, Bardakdjian J, Badens C. Neonatal screening for sickle cell disease in France: evaluation of the selective process. J. Clin. Pathol. 2010; 63: 548-551.
46. Upadhye D, Jain D, Nair S, Nadkarni A, Ghosh K, Colah R. First case of Hb Fontainebleau with sickle hemoglobin and other non deletional α gene variants identified in neonates during newborn screening for sickle cell disorders. J. Clin. Pathol. 2012; 65: 654-9.
47. Upadhye DS, Jain DL, Trivedi YL, Nadkarni AH, Ghosh K, Colah RB. Newborn screening for haemoglobinopathies by high performance liquid chromatography (HPLC): diagnostic utility of different approaches in resource-poor settings. Clin Chem Lab Med. 2014. [Epub ahead of print].
48. Vichinsky E, Hurst D, Earles A, Kleman K, Lubin B. Newborn screening for sickle cell disease: Effect on mortality. Pediatrics. 1988; 81: 749–54.
49. Yorke D, Mitchell J, Clow C, Nuguid E, Cadogan R, Sinclair D, Luscombe S, Rozen R, Meredith P, Esseltine D. Newborn screening for sickle cell and other hemolobinopathies. A Canadian pilot study. Clin. Invest. Med. 1992; 15: 376-83.

NIIH Happenings

Dr. K. Ghosh, Director

Attended the Homeopathic meeting on Dengue medicine at ICMR, New Delhi on 7th May, 2014.

Attended the WFH 2014 World Congress on Haemophlia held at Melbourne, Australia from 11th – 15th May 2014 and present a paper "Molecular Pathology of Haemophilia in patients from Western India".

Attended the meeting on Use of Hemglobin colour scales for large scale evaluation of anaemia in the population at AIIMS, New Delhi on 21st May 2014.

Attended the PRC meeting of maternal and child health at ICMR, New Delhi on 22nd May 2014

Delivered lectures on " How to see at Blood Smear" and " Coagulation Biology and its deeper indication " at NRS Medical College, Kolkata and Govt. Medical College, Kolkata on 16th and 17th June 2014.

Visited Valsad and Navsari, Gujrat in relation to the Tribal Health Unit and New born screening programme from 15th to 17th July 2014.

Visited Chandrapur for Completion of Stamp Duty and Registration formalities of Chandrapur MHADA Land from 22nd to 23rd July, 2014.

Invited as an expert to the Meeting of the Review Committee for enhancing research activities in North East Region at RMRC, Dibrugarh from 30th to 31st July 2014.

Invited to attend the Annual Meeting of Haemophilia at Kolkata from 1st to 3rd August 2014.

Attended the Tribal Health Forum Meeting at NITR, Chennai from 9th to 10th August 2014.

Attended the ICMR Translational Immunology Meeting at New Delhi on 11th August 2014.

Delivered a Lecture on "Molecular Biology and Coagulation Disorder" at PG Hospital, Kolkata from 22nd to 23rd August, 2014.

Roshan B Colah, Scientist F

Invited to participate in the Workshop on "Implementing G6PD testing to ensure safe radical cure of P.vivax malaria" organized by NIMR, Delhi, PATH and MMV at New Delhi on 1st May 2014.

Visited Valsad, Navsari and Pardi for the Newborn screening project for Sickle cell disorders under the Tribal Health Research Forum of ICMR from 15th to 17th July, 2014.

Invited to participate in the Round Table Meet on "Non-Transfusion Dependent Thalassemia" organized by Novartis Health Care at Mumbai on 2nd Aug, 2014.

Attended the annual Tribal Health Research Forum Meeting of ICMR at NIRT, Chennai on 9th and 10th Aug, 2014 and presented the work done at our centre.

Invited to give a talk on Thalassemia and evaluate the Thalassemia screening programme at Indian Medical Science Research Foundation, Rajkot on 20th and 21st Aug, 2014.

Invited to give a talk on "Overview of hemoglobinopathies in India" at the CME on "Updates in Laboratory Medicine" organized by the Gujarat Association of Pathologists and Microbiologists at Ahmedabad on 31st Aug, 2014.

Dr Manisha Madkaikar, Scientist E

Invited to attend brain Storming Meeting for Translational Immunology on 11th August 2014 at NIOP (ICMR), New Delhi.

Invited to deliver a talk on ' Immunogenetics' at UG Workshop on Human Genetics held jointly by the Moving Academy of Medicine and ICMR-Genetic Research Center (GRC) at GRC Mumbai from 25th to 27th August, 2014.

Dr. V. Babu Rao, Scientist D

Iinvited as a member of the selection committee for the selection of Scientist at the Sickle cell Institute, Chattisgarh, Raipur on 26th May 2014.

Iinvited as a member of selection committee for the selection of Technical Officer at ACTREC on 19th June 2014.

Iinvited as a member of selection committee to select Research Associate, at NIRRH, Mumbai on 7th July 2014.

Appointed as a member of selection committee for the selection of Ph.D students for the academic year 2014-15, at NIRRH, on 17th July 2014.

Attended Accreditation of NIRRH ethics committee for clinical studies at NIRRH, Mumbai, from 11th to 14th August 2014.

Invited to deliver a talk on "Cytogenetics: Microscope to Molecules" in Human Genetics workshop, jointly organized by the Moving academy of Medicine and ICMR at Genetic Research Centre on 26th August 2014.

Dr Malay Mukherjee, scientist D

Visited Valsad, Navsari and Dang districts of South Gujarat in relation to Newborn screening programme for sickle cell disorders and Tribal Health Unit from 15th to 17th July 2014.

Attended Tribal Health Research Forum Meeting of ICMR held at NIRT, Chennai from 9th to 10th August 2014 and presented the work done at NIIH, Mumbai.

Ms Seema Korgaonkar, TO

Attended 2nd ACE meetings held at Mumbai on 26th and 27th July 2014 and awarded 3rd prize for the poster presentation entitled "Cytogenetic and molecular study in Myelodysplastic syndrome".

Ms Swati Garg, SRF

Received 'Student travel Award' for Cyto 2014 held at Ft. Lauderdale, Florida, USA from 17th to 22nd May 2014 and presented a poster entitles "Characterization of leukemic stem cells and associated cell signaling pathways can predict the outcome in acute leukemia".

Vijay Padwal, ALIO

Attended zonal one day workshop on J-Gate Plus along with JCCC@ICMR for the Western Region of ICMR institutes at NIOH, Ahmedabad on 5th June, 2014.

Anita Mukherjee, PS to Director

Visited Chandrapur with the Director for Completion of Stamp Duty and Registration formalities of Chandrapur MHADA Land from 22nd – 23rd July, 2014.

Mr. Lakshminarayana, Senior Administrative Officer and his team from NIV Pune delivered Administrative Lecture to the NIIH Staff

Participation of NIIH Scientists, Staff and Students in NML-ERMED E-journal Consortium library training program

EDITORIAL BOARD

Immunohaematology Buttetin is Brought out by :

NATIONAL INSTITUTE OF IMMUNOHAEMATOLOGY

13th Floor, NMS Building, KEM Hospital, Parel, Mumbai 400 012. (INDIA) Web : www.niih.org.in