The Child and newborn

West Bengal Academy of Pediatrics

The Journal of West Bengal Academy of Pediatrics

RNI Registration No. : RNI/68911/97
ISSN 0975-0894

Volume 27 No.2, April – June 2023

Tufpro

Bacillus clausii spores 2 billion/5 ml suspension

The Tough Probiotic

Ready to Drink Formulation

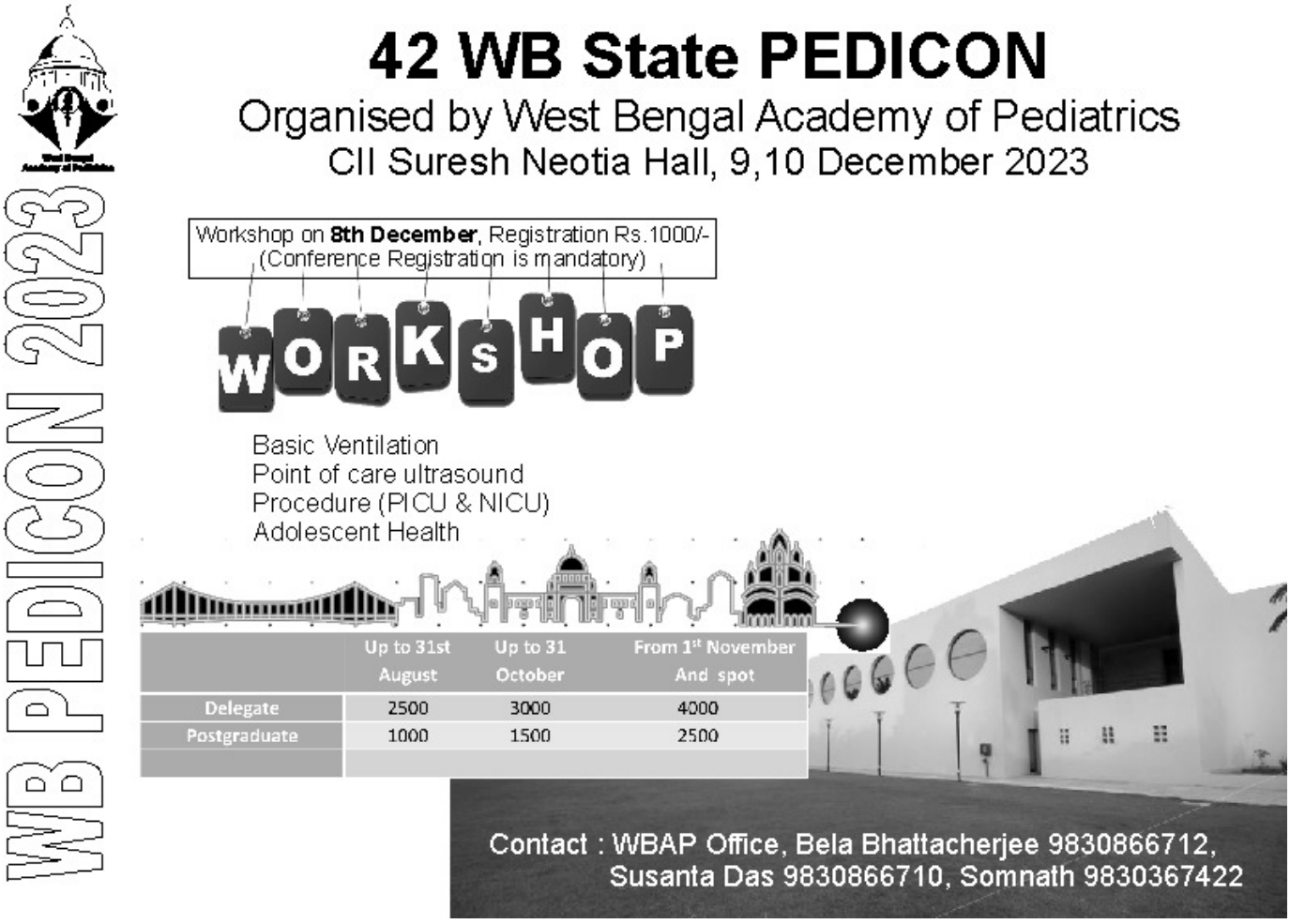
In Memories

Dr Amitava Sen
(1949-2023)

Dr Amitava Sen is a name in neonatology in India. He has dedicated his life in development of this subject not only in the Eastern states but his work has received international acceptance. Some of his achievements in spite of poor health for more than a decade, which all happened for the first time in the state are mentioned below :

1. Only one from East Zone to receive inaugural Fellowship of National Neonatology Forum (FNNF) during NNF Silver Jubilee, 2005 at Indore Convention
2. First and only one from East Zone so far, nominated to deliver the prestigious NNF Oration at Mumbai Convention, 2000. The title of Oration was “In Search of Newborn Care”
3. On behalf of Society for Applied Studies (A non-profit Research Organization) under guidance of Dr. Dilip Mahalanabis planned & with co-workers conducted a Research to accelerate reduction of high neonatal mortality rate (NMR) by establishment of a near Level-II neonatal special care unit (SNCU) in the district hospital of a backward district (Purulia) in 2003, a network of neonatal stabilization units at BPHCs and Rural Hospitals (SNSUs), trained local girls and utilized them successfully as Newborn Aides. This successful model is now famous as the Purulia Model.
4. On behalf of Society for Applied Studies replicated functioning near Level-II SNCUs in the district hospitals of Birbhum and Koochbehar and sub-division hospital at Islampur and planned for Nadia, Siliguri and Malda till the former Principal Secretary, Health, Govt. of West Bengal stopped this successful project. The team also developed the first SNCU of Andaman & Nicobor Islands at Port Blair. Dr. Sen also planned the Neonatal Intensive Care Unit for Institute of Child Health.
5. Received National Neonatology Forum Fellowship in Leadership Training Program in Neonatology in USA under Professor D Vidyasagar at University of Illinois at Chicago, 1991
6. Received a “Certificate in Recognition of Multi-faceted Contribution to the Activities of Forum during 1991-92” from the President, NNF, 1993 at Baroda Convention
7. Nominated by NNF to Operationalize Newborn Care in South 24 Paraganas district (to train the doctors and nurses in state government facilities up to BPHC to use common newborn care equipments supplied) under Govt. of India-NNF project, 1994
8. Nominated by NNF to survey and submit feed-back report of the state of the equipments supplied under Govt. of India-NNF project, 1994 from South 24 Paraganas and North Dinajpur districts in 1998. The detailed Illustrated report was submitted.
9. Conceived & edited The Newborn, the mouthpiece of National Neonatology Forum, West Bengal from 1995 to 2000. It was the first of its kind among state branches of NNF. (Now discontinued)
10. Conceived and developed the first Near-Level II Neonatal Special Care Unit at Nilratan Sarkar Medical College & Hospital, Kolkata in 1989. The unit was accredited by NNF as Level II Unit and the Regional Training Centre in 1992 but it did not finally happen as Dr. Sen was removed from that unit by then.
11. Established the first Department of Neonatology in Assembly of God Hospital & Research Centre, Kolkata, 2001. It was the First Unit in the state to get NNF Accreditation as Level-II Special care Neonatal Unit, 2001.
12. Conceived the idea of Newborn Care, Training & Operationalization Program (NBCTOP) for the state and introduced the program with NNF, West Bengal, 1998 with UNICEF support.

Announcement



Request

Members are generously requested to provide News, Views, Reviews, Case Reports, Articles to our esteemed journal.

Contact :
Dr Kaustav Nayek
Editor-in-Chief, The Child and Newborn

"Oriental Apartments" Flat H1, 15C, Canal Street, Kolkata 700 014,
Email : kaustav25@yahoo.co.in

The Child and Newborn

West Bengal Academy of Pediatrics, Oriental Apartments, Flat H1
15C, Canal Street, Kolkata 700 014
Phone : 033 2265 4072, Email : wbap2013@gmail.com, Website : www.wbap.in
E-version of this journal available at website.

ISSN 0975-0894 RNI Registration No.:RNI/68911/97

West Bengal Academy of Pediatrics

Vol.27, No.2 April - June 2023

CONTENTS

Special Correspondance
Dr Kaustav Nayek
Editor-in-Chief, The Child and Newborn
"Oriental Apartments" Flat H1
15C, Canal Street, Kolkata 700 014
Email : kaustav25@yahoo.co.in

President's Address

Dear Friends,

Wishing you and your family a very happy and healthy 2023! Shuvo Nababorsho!

We have published first issue of this year and intend to publish regularly with the participation of you all. Medical knowledge is progressing far faster than the ability to publish and disseminate. The Child and New-born journal cannot be nor is it intended to be authoritative; rather it is intended to be a reservoir of what collaborative thinking can accomplish.

West Bengal still has a long way to go to ensure every child is adequately nourished and receives the positive environment to grow to the full potential. NFHS-5 data, released about increase in the double burden of malnutrition in the state with increase in micronutrient deficiencies. The data shows an increase in severe wasting, stunting and underweight in children less than five years of age (7.1%,33.8%, 32.2% respectively) and increase in overweight (4.3%). NFHS-5 also includes some new topics, such as preschool education, disability, access to a toilet facility, death registration, bathing practices during menstruation, and methods and reasons for abortion. The scope of clinical, anthropometric, and biochemical testing (CAB) has also been expanded to include measurement of waist and hip circumferences, and the age range for the measurement of blood pressure and blood glucose has been expanded.

However, HIV testing has been dropped. However, estimates of indicators of sexual behaviour; husband's background and woman's work; HIV/AIDS knowledge, attitudes and behaviour; and domestic violence are available only at the state/union territory (UT) and national level (National Family Health Survey – 5, 2019-20).

Another worrisome fact in our state is teenage pregnancy though decreased in NFHS 5 data as compared to NFHS 4. Adolescent fertility rate for women age 15-19 years is still high (81 in NFHS 5).

So dear academician we have to focus on specific issues of health indicators and have to work for that.

Miles to go before we…..

In order to increase academically rich contents and publication patterns, we are very much interested in encouraging authors to submit clinically relevant articles, case reports and series, review articles and to The Child and Newborn.

My most sincere thanks to the Editorial team of Child & Newborn for putting so much efforts in bringing out this long waited periodical journal which has become immensely popular amongst all our members. We are trying to our best to be indexed.

Your feedback and valuable suggestions are always welcome.

Thank you IAP for all. Jai Hind

Prof. Kalpana Datta
President, WBAP 2023

Editorial

It gives me great pleasure and immence satisfaction that I'm writing this preface for the second issue of Child and Newborn . As promised it is going to be published in time. This prospect which was once considered a gigantic task is now a reallity. Iam sure the future of this journal is bright.I am overwhelmed by the responce of fellow pediatricians towards this journal. The first edition has reached most of our members.Those remaining will receive it very soon.I do appologize to you those who have not yet received the issue. We are trying to work out a mechanism by which it reaches are valued members at the earliest. I request you to bear with us.

The journey has just started . With your contribution it is destined to travel a long distance. As promised we are working towards indexing of Child and Newborn. Very soon it will be peer reviewed.Once that is done the academic quality will be even better.It is a very good platform for young post graduates to publish their work. I take this opportunity to request faculties of different medical colleges to encourage residents to use our journal as a first step in their publication career.

We are very proud of your learned editorial board. The initial urgency for the publication has gone by. Next we will be working towards qualitative improvement. There are lots of wonderful plans to take this journal to the next step. I'm confident we will make Child and Newborn very popular amongst pediatricians with the full fledged involvement of the editorial board. I am deeply indebted to the authors who have provided articles in such short time. You all made it possible to bring out the second issue in time. I thank you again. I invite constructive cititisim. With all our inputs and active participation, I'm sure Child and Newborn will soon be the face of West Bengal Academy of Pediatrics.

Prof. Kaustav Nayek
Editor-in-Chief

Role of Clinical Evaluation In Initial Management of Congenital Heart Disease

Dr Anil Kumar Singhi
Senior Consultant and Head
Department of Pediatric and Congenital Heart Disease, Medica Superspeciality Hospital

Introduction

Congenital heart disease (CHD) is seen in 8 to 12 newborns

per 1000 live births.(1) Recent advancement in the pediatric care has led to reduction in the Incidences of acute diarrheal disease, and respiratory tract infection. In the list of diseases causing under five and infant mortality congenital heart disease climbed the chart . The common belief is that we need skilled human resources and advanced Imaging technology for the diagnosis of CHD. Actually a thorough clinical evaluation backed by chest x-ray, oxygen saturation in all limbs and electrocardiogram (ECG) is sufficient for the initial functional diagnosis. It helps to categorize the patient based on the different physiologic subgroups as discussed below and to reach a reasonable treatment plan.

The Preamble for clinical diagnosis : The NADAs criteria

Dr Alexander Nada described a simple criteria hundred years ago which still is relevant for approaching congenital heart disease.

2.1 :NADA's Criteria (1920) for Congenital heart disease (2) :

Major Criteria	Minor Criteria
• Systolic murmur grade 3 (III) or more	§ Systolic murmur less than grade 3 (III)
• Diastolic murmur	§ Abnormal Second Heart sound (S2)
• Cyanosis	§ Abnormal ECG
• Congestive heart failure	§ Abnormal chest X ray
	§ Abnormal Blood Pressure

Presence of one major or two minor criteria indicate a very high probability of a congenital heart disease.

Correspondance : Anil Kr Singhi, *Senior Consultant and Head Department of Pediatric and Congenital Heart Disease, Medica Superspeciality Hospital. Email :draksinghi@yahoo.com*

2.2:The role of oxygen saturation: Need of the hour :

The estimation of oxygen saturation in all limbs by age appropriate oxygen probe is very important . Any value <95% is abnormal after 48 hours of birth. Clinical detection of cyanosis requires SpO2 below 85%. The 85- 94% range detection needs a good oxygen saturation probe. They are an integral part of congenital heart disease assessment and are available easily in the majority of the set ups.

2.3: The role of Chest X Ray and electro-cardiogram (ECG) : relevance in current era

The chest x-ray and the ECG are considered an extension of clinical evaluation. The chest X-ray tells us two important points regarding congenital heart disease. One is cardiac size and pulmonary blood flow status. Both again, helps to understand the underlying physiology of the CHD. The pulmonary blood flow can be increased ,normal or reduced. The chest X-ray is very sensitive for conditions, having reduced pulmonary blood flow. It will show a blackout type of lung field. Some of the patients may get a specific shape pattern, which will be characteristic of certain diseases. For example, snowman appearance or figure-of-eight appearance seen in bigger patients with supra cardiac total anomalous pulmonary venous drainage (TAPVC) , a boot shape at heart is described with Tetralogy of Fallot , egg on side appearance is discussed in transposition of the greater vessels though it is not specific to it.

The ECG is an underutilized tool in congenital heart disease. It is an extremely helpful adjunct to understand cardiac physiology and could add value to the clinical evaluation and management. The dilatation of the chamber as interpreted by the ECG waves corroborating with the clinical and x-ray data strengthen the bedside physiologic diagnosis. For

example, significant left ventricular forces or biventricular forces in an acyanotic child may reflect shunt lesion. Ventricular hypertrophy which strain pattern can indicate and corresponding ventricular outflow obstruction pathology. In a cyanotic baby with predominantly left ventricular forces with left axis deviation suggests an atretic or hypoplastic tricuspid valve or right heart hypoplasia. The rhythm analysis also is useful because some congenital heart disease can have rhythm disorder like heart block, pre-excitation or abnormal atrial rhythm. Prof NADA rightfully incorporated ECG and Chest X ray in the clinical analysis tool.

Table 1 : The Congenital Heart disease spectrum: (1)

Heart Disease	% of CHD
VSD (Ventricular Septal defect)	37.2%
ASD (Atrial Septal Defect)	9.8%
PDA (Patent Ductus Arteriosus)	8.3%
AVSD (Atrioventricular Septal defect)	3.6%
PS (Pulmonary Stenosis)	7.6%
AS (Aortic Stenosis)	4.1%
CoA (Coarctation of Aorta)	4.2%
TOF (Tetralogy Of Fallot)	4.4%
TGA (Transposition of Great artery)	3.3%
(HLHS) Hypoplastic Left Heart Syndrome	2.7%
TAPVC , Tricuspid atresia , Truncus etc	0.8-1.5% each
All Cyanotic Heart Disease	13.2%

Once a diagnosis of possible congenital heart disease is made at the bedside, then effort should be made to classify the heart disease based on Its physiology. The traditional system of classifying heart disease by presence or absence of cyanosis still has some relevance. The second question should be answered about pulmonary blood flow. Whether it is elevated , normal or reduced?

The features of cyanotic patients are cyanosis, clubbing and polycythaemia. In cyanotic heart disease there will be right to left shunting at some level . Uniform cyanosis means it is intracardiac shunting or at the level of ascending aorta. In differentials cyanosis one half of the body will be more cyanotic in comparison to another half. It means the right to left shunting is happening at the level of ductus arteriosus with ductal flow in one half (usually lower one) .Absence of cyanosis does not rule out cyanotic heart disease. At times cyanosis is evident only during activity or cryingIn a single Ventricle group with very high pulmonary blood flow can have very high saturation. Pink tetralogy of Fallot and tetralogy with absent pulmonary valve patients can be pink.

2.4 Differentiation of cyanosis caused by respiratory vs Cardiac cause :

Cardiac	Respiratory
Does not significantly improve with oxygen (Hyperoxia test can be done)	Improves with oxygen
Cyanosis increases with crying (More intracardiac right to left shunt)	Cyanosis decreases with crying (Better Oxygenation)
Tachypnea	Respiratory distress (Retraction/grunting)

3. Hyperoxia test in bedside:

100% oxygen given to the baby for 10 minutes. Improvement of arterial PO2 seen in respiratory causes. In healthy lungs PO2 > 150 mm hg. If the PO2 remains < 100 mm hg –cyanosis is more likely due to cardiac causes.

3.1 Features of Increased Pulmonary Blood flow:

History ofpoor feeding and interrupted feeding, excessive forehead sweating, breathing difficulty, excessive cardiac activity noted by parents, respiratory infections that are frequent, prolonged and difficult to treat and failure to thrive. Clinical features for increased pulmonary blood flow are cardiomegaly ,visible precordial activity,ejection murmur in the pulmonary area,diastolic flow murmur in the apical area , Intercostal and subcostal retractions.

The absence of increased pulmonary blood flow features indicate the pulmonary blood flow may be normal or reduced.

3.2 Left to right shunt lesion physiology:

There will be absence of cyanosis, precordial bulge and tendency for development of congestive cardiac failure. Chest x-ray will show cardiomegaly proportional to the level of shunt and evidence of increased pulmonary blood flow. The left to right shunt lesions can be pre tricuspid (eg : ASD) or post tricuspid (eg: VSD, PDA)

3.3 Obstructive lesion physiology:

There will not be any history of frequent chest,

infection, or cyanosis. The precordial shape will be normal. There will be forcible or heave cardiac impulses without cardiomegaly. There will delay the corresponding component of the second. Heart sound. A loud ejection systolic murmur with presence of thrill will be heard depending on the degree of obstruction. The electrocardiogram will show ventricular hypertrophy depending on the chamber involved. The examples of obstructive lesions are pulmonary stenosis , aortic stenosis or coarctation of aorta.

4. Cyanotic congenital heart disease:

The cyanotic patients have some important subgroups based on the hemodynamic feature. These cyanotic heart diseases can be grouped based on the hemodynamic of the lesion in different physiologies, which helps immensely in deciding the plan of management(3).

The common cyanotic physiologies:

1. Tetralogy physiology
2. Transposition physiology
3. Admixture physiology
4. Eisenmenger Physiology

4.1. Tetralogy physiology :

The feature of Fallot's physiology is unrestricted ventricular septal defect with pulmonary stenosis. The heart size is usually not enlarged. There will be a single second, heart sound. There will be ejection systolic murmur depending on the amount of pulmonary blood flow. The X-ray will show features of reduced Pulmonary blood flow without any cardiomegaly.

Examples of Tetralogy physiology :

- Tetralogy of Fallot – (most common)
- Double outlet right ventricle VSD with Pulmonary stenosis
- Transposition of great arteries TGA .(VSD and PS)
- Tricuspid atresia (small ventricular septal defect limiting pulmonary blood flow).
- Single ventricle (physiologically a very large ventricular septal defect) and PS

4.2.Transposition physiology :

The transportation physiology is unique . Usually it presents in small symptomatic neonates or infants. The cyanosis can vary from mild to severe. There will be failure to thrive features of congestive cardiac failure and cardiomegaly (2 week). The second heart sound would be single. There may be gallop and x-ray showing cardiomegaly with features of increased pulmonary blood flow. Thymic hypoplasia is common.

4.3. Admixture physiology:

In admixture physiology there will be unrestricted communication between two cardiac chambers. Pulmonary arterial pressure will be elevated in the admixture happening in the ventricular or arterial label with no pulmonary stenosis. In the atrial level admixture PAH will be present when there is associated pulmonary venous hypertension.The Oxygen saturation is directly proportional to pulmonary blood flow , a 90% saturation indicating 3:1 shunt. Features of increased pulmonary blood flow as mentioned earlier will be there. The pulmonic component of the second heart sound will be accentuated and there will be flow murmur.

Admixture Physiology: Common examples

- Tricuspid atresia with increased pulmonary blood flow
- Single ventricle without pulmonic stenosis
- Persistent truncus arteriosus
- Total anomalous pulmonary venous connection (TAPVC)

4.4. The Eisenmenger physiology:

The patient will give a history of heart disease in infancy and history of frequent chest infection in the infancy and early childhood. The cyanosis usually will appear late. Usually there will be no significant cardiomegaly except in cases of ASD where cardiomegaly may occur due to atrial enlargement .There may be vascular ejection click due to pulmonary hypertension, accentuated and palpable pulmonic component of the second heart sound. Usually there will not be significant systolic murmur of the antegrade flow or shunt however, tricuspid regurgitation murmur can be heard. A diastolic murmur of pulmonic regurgitation can be heard at an advanced level.

4.5 The Missed Congenital heart disease:

The CHD can be missed when the baby is

asymptomatic, having subtle clinical features difficult to detect. ASD is common to be missed as it remains asymptomatic till late age. The fixed split is sometimes difficult to appreciate in a busy clinic. The second heart sound (S2) fixed Split present in up to 85% of cases. In 15% it can be heard as normal split S2. Short Systolic murmur present in ASD may be missed or considered functional. Detailed examination in a quiet room tailored to clinical detail can help to avoid missing CHD.

5.Conclusion:

The non-availability of expert cardiac care and imaging in the bedside is not a bar for reaching a reasonably accurate functional cardiac diagnosis and optimum treatment plan for kids suffering with congenital heart disease. A very sick symptomatic newborn or baby may require immediatestabilization, maintenance of airway, breathing ,circulation (ABC). In a very sick cyanotic baby, a possibility of duct dependent lesions can be thought and prostaglandin infusion can be started. After consultation with a pediatric cardiologist the child can be planned to be transported with medical escort. Detailed cardiac evaluation with echocardiogram will get a reasonable functional as well as anatomic diagnosis. A management plan can be reached after discussion with the cardiac team. Eventually it is a teamwork where the pediatric cardiac unit and the pediatric team coordinate and take steps for comprehensive cardiac care.

References

1. Hoffman JIE. Incidence of congenital heart disease: I. Postnatal incidence. PediatrCardiol. 1995;16:103-13.
2. Nadas AS. Approach to diagnosis of congenital heart disease without recourse to special tests. Circulation. 1959;20:602-605. doi:10.1161/01.cir.20.4.602
3. Tandon R. Bedside Approach In The Diagnosis of Congenital Heart Diseases. First edition ,1998, B.I. Churchill Livingstone Pvt.Ltd.New Delhi, India

AGT Gene Expression Profiles In Aplastic Anaemia

***Dr Sarmistha Adhikari, **Dr Kaustav Nayek, ***Dr Paramita Mandal**

**Biomedical Genetics Laboratory, Department of Zoology, The University of Burdwan, West Bengal*

***Professor, Department of Paediatric Medicine, Burdwan Medical College & Hospital, West Bengal*

**** PHD, *Biomedical Genetics Laboratory, Department of Zoology, The University of Burdwan, West Bengal*

Objectives : *Severe aplastic anemia is characterized by a hypocellular bone marrow and peripheral cytopenia. Mesenchymal stem cells (MSCs) play a crucial role in haematopoietic stem cells (HSCs) development and the microenvironment suitable for haematopoiesis. Investigation of the therapeutic targets by paediatric patient-specific gene expression analysis of the MSCs can be important for diagnosis.*

Methods : *The study was based on freely available miRNA and host gene expression in NCBI GEO dataset. Microarray based gene expression profiles (GSE33812) of MSCs for five paediatric aplastic anaemia patients and healthy controls were generated using Agilent-014850 platform and the data was downloaded from the database.*

Results : *MSCs gene expression profiling distinguished between healthy controls, children with aplastic anemia. Angioteninogen (AGT) gene involved in ERK1/ERK2 cascade, cyotokine secretion, metabolic processes were strongly downregulated among all the patients with aplastic anemia. Emerging role of various transcription factors binding to this gene was identified as a new avenue of therapeutic application.*

Conclusions : *As a potential diagnostic tool, patient-specific gene expression profiling of MSCs made it possible to make the difficult diagnosis of most patients with aplastic anemia.*

Keywords: *aplastic anemia, mesenchymal stem cells, angiotensinogen,transcription factor.*

Introduction:

Aplastic anemia (AA) is a rare, immune-mediated hematopoietic disorder associated with significant morbidity and mortality [1]. AA can be diagnosed in patients presenting with pancytopenia and a hypocellular bone marrow. Typical symptoms include fatigue and easy bruising or bleeding; infections may be present, but generally there is no long-standing illness [2]. In patients with suspected AA, rapid and accurate diagnosis and concomitant supportive care are critical. Historically, immunosuppressive therapy (IST) and bone marrow transplantation (BMT) in eligible patients have been the mainstay of AA treatment [1]. In pediatric patients, new transplant strategies and improvements in supportive care have led to greatly improved outcomes and increasing use of BMT in both upfront and refractory settings [1,3].

The incidence of AA varies with geography, and it was found to be higher in Asia and lower in Europe, North America, and Brazil according to the International Agranulocytosis and Aplastic Anemia Study [IAAAS] [4-7]. It was also identified that the incidence of that disease was 2-to 3-fold higher in Asia than in the West [8]. The great variation of the incidence of the disease is due to differential environmental exposure such as use of certain drugs and chemicals or by infectious agents such as viruses and bacteria. Besides the environmental agents the genetic background of different ethnic population may confer the risk of that disease [9-11]. It is really complicated to characterize the paediatric patients with aplastic anaemia than an adult because numerous inherited bone marrow failures can also present with aplastic anaemia without any obvious somatic features. Therefore, a precise diagnostic technique is essential for the children for the application of therapeutics [12]. Different demographic factors were already reported to be associated with aplastic anaemia among pediatric individuals and disease severity [13]. As the children are more sensitive to newer therapeutic agents in respect to their tolerability and

Correspondance : Dr Paramita Mandal, PHD, *Biomedical Genetics Laboratory, Department of Zoology, The University of Burdwan. Email paramita.mandal2@gmail.com

suitability in contrast with chemotherapy or stem cell transplant, it is necessary to establish and then incorporate into optimal treatment strategies.

Transcriptome analysis can clearly differentiate healthy controls from samples of AA patients. A study on transcriptome analysis among paediatric aplastic anaemia patients identified differentially expressed genes are involved in cell metabolism and cell communication or adhesion [14]. Fischer et al., 2012 able to identify that the transcription of major integrins were dramatically down-regulated in the few remaining CD34+ bone marrow cells from children with severe aplastic anaemia [15]. MSCs usually resides within the stroma and they are derived from bone marrow. They play significant role in hematopoiesis and immunomodulation. Various studies have reported the differential gene expression in MSCs among patients with aplastic anaemia compared to healthy controls. A study by Li et al., 2012 identified over 300 differentially expressed genes among aplastic anaemia compared with healthy controls [16]. These differentially expressed gene are involved in apoptosis, adipogenesis, and the immune response. Another study also reported increased MSC apoptosis in AA patients [17]. Consequent studies also revealed that MSCs among AA patients have lower proliferation potential [18, 19].

Various studies have already reported gene expression profiling of bone marrow MSCs from aplastic anaemia patients and they identified several genes those are involved in various biological processes such as cell cycle, cell division, proliferation, chemotaxis, adipogenesis- cytokine signalling and haematopoietic cell lineage differentiation which suggests that impaired cellular function is a hallmark of this disease [20-22]. It is well documented in several studies that transcription factor deregulation was also involved in aplastic anaemia. It was reported that GATA2 transcription factor downregulation in bone marrow MSCs can accelerate adipocyte differentiation which is one of the chief characteristics of aplastic anaemia [23].

Therefore, we undertook the current study to determine the deregulated genes associated with pediatric patients with aplastic anaemia and their transcription factor binding motifs which can regulate this disease progression.

Materials and Method

Source data

The expression analysis of the microarray datasets from the Gene Expression Omnibus [GEO] database of the National Center for Biotechnology Information [NCBI] of the U.S. National Library of Medicine was used for the current study. There was single gene expression profiling dataset for aplastic anemia patients. This dataset included 5 severe pediatric aplastic anemia patients.

Definition of clinical dataset

For gene expression profiling, we have used GEO dataset with accession id GSE33812 [24] which included bone marrow mesenchymal stem cells of 5 pediatric aplastic anemia patients and healthy donors. Agilent-014850 Whole Human Genome Microarray 4x44K G4112F GeneChips were used for gene expression profiling.

Microarray based gene expression analysis

Gene expression profiles for all the categories of aplastic anaemia patients and healthy controls were generated using Agilent-014850 Whole Human Genome Microarray 4x44K G4112F probes. The probe quality of the array was assessed before and after normalization and the background correction was done using bioconductor based limma package. To improve data quality, a filtering of the probes was applied. The probes containing repetitive sequences, binding to multiple sites of human transcriptome, were removed for further analysis. Downstream analysis was done to identify the differentially expressed genes, based on t-test among both the categories of pediatric aplastic anemia patients compared to healthy controls. The p-values were determined and multiple testing corrections [Benjamini Hochberg method] done to remove the false discovery rate. The differentially expressed genes were selected based on p value< 0.05 and fold change of gene expression, compared to controls [for up-regulation, fold change =2 and for down-regulation, fold change =-2].

Identification of biological processes of significantly enriched genes

To get a global view of relevant biological pathways associated with disease progression, we used GeneCodis 3 [25, 26, 27] and we considered significant p value (<0.05) for identification of relevant biological processes.

Cellular localization of deregulated genes

To know about the cellular localization of the deregulated genes, we used GeneCodis3 and we considered significant p value (<0.05) for identification of relevant motifs for transcription factors.

Identification of transcription factors binding motif of significantly enriched genes

To get a global view of transcription factor binding sites of the deregulated genes associated with disease progression, we used GeneCodis3, and we considered significant p value (<0.05) for identification of relevant motifs for transcription factors.

Results

Differentially expressed genes in microarray-based gene expression profiling

To identify the differentially expressed genes in aplastic anaemia patients we compared the gene expression profiling of pediatric aplastic anaemia patients compared to healthy controls. The gene were selected as differentially expressed on the basis of p value (<0.05) and log fold change (>2 or <2). Microarray based global gene expression revealed that there were 24 genes that are differentially expressed in case of patients' samples rather than control samples. These genes are down-regulated in aplastic anaemia samples compared to healthy donors. The expression pattern of these genes is depicted in Table 1. Among the entire significantly altered genes, angiotensinogen (AGT) was consistently downregulated among all the samples.

Biological processes of differentially expressed genes

GeneCodis3 analysis of the differentially downregulated genes revealed that various cell signalling pathway, cell proliferation, metabolic pathways, translation, inflammatory and cytokine secretary pathways are significantly altered pathway among paediatric aplastic anaemia cases compared to healthy controls. The significantly altered downregulated genes in those pathways are AGT, FOXQ1, RPS4Y1, RPS4Y2, DGKB and CDCA7. The details of biological processes with the list of genes are depicted in Table 2.

Identification of localization of differentially expressed gene

GeneCodis3 analysis of the differentially downregulated genes revealed the cellular localization of the significantly downregulated genes. Intracellular localization of RPS4Y2, MKI67, RPS4Y1 genes were recorded. Moreover, cytoplasmic expression of DGKB, MKI67 and ribosomal localization of RPS4Y1 and RPS4Y2 are recorded. The details of cellular localization of those genes are depicted in Table 3.

Determination of transcription factor binding sites of the differentially expressed genes

GeneCodis3 analysis of the differentially downregulated genes revealed some transcription factor binding site motifs of the significantly altered genes. This includes fork-head associated transcription factors, diacylglycerol kinase binding factors and ribosomal proteins. The details of transcription factor binding sites are depicted in Table 4.

Discussion

To know about the biology of aplastic anaemia, we should know about the biology of mesenchymal stem cells which enable us to know about the bone marrow microenvironment. This enable us to manipulate human cells and we will be moving into an exciting phase of personalized therapy for bone marrow failure. Till date there was no such microarray based gene expression profiling of mesenchymal stem cells among paediatric aplastic anaemia patients. The microarray based gene expression profiling of mesenchymal stem cells of aplastic anaemia revealed 24 differentially downregulated genes among those patients compared to healthy donors.

Our study revealed that AGT gene was significantly downregulated among all the patients with aplastic anaemia. Angiotensinogen (AGT) is the precursor of the vaso-active peptide, angiotensin II. AGT gene moleculer variant was identified as a significant risk factor and hereditary marker of hypertension [28] which is also associated with cytokine secretion may be related to aplastic anaemia.

In our analysis we have found that ERK1 and ERK2 cascade, PI3K, cell proliferation, cytokine secretion is significantly downregulated among children with aplastic anaemia. It was already reported that ERK activity can be regulated in response to of hematopoietic cytokines and growth factors and it play critical roles in hematopoiesis [29] which justifies our finding. Mesenchymal stem cells play a vital role in haematopoeitic stem cell proliferation; they also

Table 1:Significantly downregulated genes among paediatric patients with aplastic anaemia

SAM PLE 1_log fold change	SAM PLE 2_log fold change	SAM PLE 3_log fold change	SAM PLE 4_log fold change	SAMP LE 5_log fold change	GENE	GENE NAME
-2.144	-2.524	-2.97	-2.841	-2.535	AGT	angiotensinogen
-1.241	-2.025	-2.209	#N/A	#N/A	APOBEC 3B	apolipoprotein B mRNA editing enzyme catalytic subunit 3B
-2.433	#N/A	-2.441	-2.255	-2.35	TTTY14	testis-specific transcript, Y-linked 14 (non-protein coding)
-2.1	#N/A	-2.53	-5.576	-5.346	MEOX2	mesenchyme homeobox 2
-1.788	#N/A	-3.306	-3.3	-3.088	ACTL8	actin like 8
-1.34	-2.402	-2.475	-2.129	#N/A	ZNF853	zinc finger protein 853
-4.927	#N/A	-5.049	-4.988	-5.018	RPS4Y2	ribosomal protein S4, Y-linked 2
-1.4	-2.819	-2.021	-2.307	#N/A	ACKR3	atypical chemokine receptor 3
-4.244	#N/A	-3.341	-2.8	-2.66	USP9Y	ubiquitin specific peptidase 9, Y-linked
-1.175	-2.779	-2.326	-2.284	#N/A	STK24-AS1	STK24 antisense RNA 1
-7.534	#N/A	-6.673	-4.982	-5.907	DDX3Y	DEAD-box helicase 3, Y-linked
-1.035	-2.956	-2.444	-3.421	#N/A	COLEC12	collectin subfamily member 12
-3.347	#N/A	-3.127	-4.316	-4.085	NLGN4Y	neuroligin 4, Y-linked
-6.619	#N/A	-6.066	-5.077	-4.551	EIF1AY	eukaryotic translation initiation factor 1A, Y-linked
-6.605	#N/A	-5.528	-5.443	-5.22	TTTY15	testis-specific transcript, Y-linked 15 (non-protein coding)
-3.877	#N/A	-3.728	-3.073	-3.239	ZFY	zinc finger protein, Y-linked
-5.682	#N/A	-5.841	-5.878	-5.853	RPS4Y1	ribosomal protein S4,Y-linked 1
-2.043	#N/A	-2.452	-4.418	-3.448	KRBOX1	KRAB box domain containing 1
-3.1	-2.091	-2.343	#N/A	-2.027	FOXQ1	forkhead box Q1
-1.048	-2.275	-2.343	-4.192	-2.003	GAS2L3	growth arrest specific 2 like 3
-1.281	-2.809	-3.336	-3.586	-3.243	MKI67	marker of proliferation Ki-67
-1.437	-4.012	-3.921	-3.828	-3.173	TMSB15A	thymosin beta 15a
-1.662	-4.782	-4.223	-4.065	-3.519	CDCA7	cell division cycle associated 7
-2.887	#N/A	-2.896	-2.887	-2.694	DGKB	diacylglycerol kinase beta

Table 2: Significantly enriched biological processes among paediatric patients with aplastic anaemia

Biological processes	P value	Genes
Regulation of cell proliferation	0.0158403	CDCA7, AGT
Cytokine secretion	0.0158892	AGT
Positive regulation of activation of JAK2 kinase activity	0.0169114	AGT
G-protein signaling, coupled to cgmp nucleotide second messenger	0.0171986	AGT
Positive regulation of protein tyrosine kinase activity	0.0208347	AGT
Positive regulation of cellular protein metabolic process	0.0208347	AGT
Positive regulation of cytokine production	0.0230759	AGT
Translation	0.0237124	RPS4Y2, RPS4Y1
Activation of NF-kappa ß-inducing kinase activity	0.024	AGT

Table 3: Cellular localization of significantly enriched genes among paediatric patients with aplastic anaemia

Cellular localization	p value	Genes
Intracellular	0.023189	RPS4Y2, MKI67, RPS4Y1
Cytoplasm	0.024101	DGKB, MKI67
Ribosome	0.04602	RPS4Y2, RPS4Y1

Table 4: Transcription factor binding sites of significantly enriched genes among paediatric patients with aplastic anaemia

Transcription factor binding site	p value	Genes
Forkhead-associated (FHA) domain	0.02199	MKI67
Transcription factor, fork head	0.0277	FOXQ1
Serpin domain	0.019697	AGT
Diacylglycerol kinase, accessory domain	0.00525	DGKB
Diacylglycerol kinase, catalytic domain	0.007575	DGKB
RNA-binding S4	3.24E-06	RPS4Y2, RPS4Y1
Ribosomal protein S4e	1.95E-06	RPS4Y2, RPS4Y1
Angiotensinogen	0.000585	AGT

FIGURE LEGEND

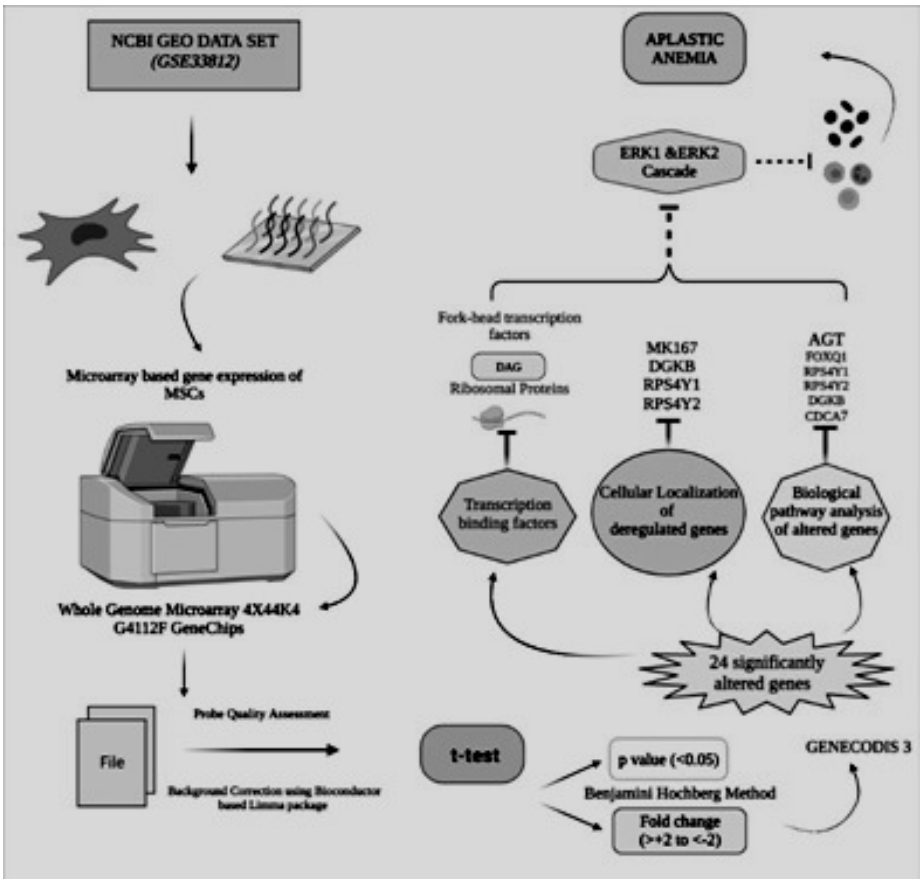


Figure 1: Schematic representation of the role of AGT gene in aplastic anaemia among paediatric patients from GSE33812 GEO dataset

modulate immune responses and maintain an environment supportive of hematopoiesis [30]. It is also well established that cytokine mediate hematopoietic stem cell development [31] and thus downregulation could be relevant for aplastic anaemia. Thus, downregulation of cell proliferation could be relevant for aplastic anaemia.

Advanced knowledge on the transcription factors, in terms of structure, function (expression, degradation, interaction with co-factors and other proteins) and the dynamics of their mode of binding to DNA paved the way for new therapies targeted against transcription factors [32]. In this study we were able to identify various transcription factors (Forkhead-associated domain, fork head transcription factor, serpin domain, diacylglycerol kinase accessory domain, diacylglycerol kinase catalytic domain, RNA binding S4, ribosomal protein S4e, angiotensin) associated with altered gene expression which is important for the therapeutic application.

Children with aplastic anaemia have promising therapeutic outcomes if treated adequately with antibiotics and referred early. Once the genetic nature is confirmed, novel drugs like small molecule inhibitors can be applied and offer hope for the future in the management of these children [33, 34]. Recent study from our lab showed the potential of miRNA as therapeutic target for aplastic anaemia [35]. Thus, it may be an indication that this particular transcription factor or drug may be helpful to use as a therapeutic agent in upcoming days. So, if we alter the gene expression and use transcription factor and novel drug as a therapeutic tool in case of paediatric aplastic anemia patients, this could be helpful for those patients suffering from aplastic anemia.

Acknowledgement:

We thank all the clinicians and researchers who performed the experiments and made accessible online datasets. We thank Chow K et al. of National Chung Hsing University, Taiwan for doing gene

expression profiling of paediatric aplitic anaemia patients and healthy donors and made it accessible in GEO database. The financial support for the study was provided by Department of Biotechnology, Govt. of India [Grant id: BT/PR18640/BIC/101/924/2016 DATED 20.09.2017]. Last but not the least; we are thankful to the Department of Zoology, The University of Burdwan (DST-FIST and PURSE) for the infrastructural support.

SA and PM: Conceived the objectives and performed the analysis, SA and PM: wrote the paper.

Funding: This study was funded by Department of Biotechnology, Govt. of India [Grant id: BT/PR18640/BIC/101/924/2016 DATED 20.09.2017].

Conflict of Interest: None.

Ethical approval: This article does not contain any studies with human participants performed by any of the authors.

References

1. Scott A. Peslak, Timothy Olson, Daria V. Babushok, Diagnosis and Treatment of Aplastic Anemia Curr Treat Options Oncol. ; 18(12): 70. doi:10.1007/s11864-017-0511-z.
2. Young NS, Kaufman DW. The epidemiology of acquired aplastic anemia. Haematologica. 2008; 93(4):489–92. DOI: 10.3324/ aematol. 12855 [PubMed : 18379007]
3. Dufour C, Veys P, Carraro E, Bhatnagar N, Pillon M, Wynn R, et al.Similar outcome of upfront-unrelated and matched sibling stem cell transplantation in idiopathic paediatric aplastic anaemia. Br J Haematol. 2015; 171(4):585–94. doi:10.1111/bjh.13614.
4. Kaufman DW, Kelly JP, Levy M, Shapiro S. The drug etiology of agranulocytosis and aplastic anemia 1991; Oxford University Press, New York.
5. Böttiger LE, Westerholm B. Aplastic anaemia: I. Incidence and aetiology. Acta Med Scand 1972; 192:315–8.
6. Davies SM, Walker DJ. Aplastic anaemia in the Northern Region 1971–1978 and follow- up of long-term survivors. Clin Lab Haematol 1986; 8:307–13.
7. Szklo M, Sensenbrenner L, Markowitz J, Weida S, Warm S, Linet M. Incidence of aplastic anemia in metropolitan Baltimore: a population-based study. Blood 1985; 66:115–9.
8. Issaragrisil S, Sriratanasatavorn C, Piankijagum A, Vannasaeng S, Porapakkham Y, Leaverton PE, et al. Incidence of aplastic anemia in Bangkok. Blood 1991; 77:2166–8.
9. Young NS. Aplastic anemia. Lancet 1995; 346:228–32.
10. Young NS, Calado R, Scheinberg P. Current concepts in the pathophysiology and treatment of aplastic anemia. Blood 2006;108:2509–19.
11. Brodski RA, Jones RJ. Aplastic anaemia. Lancet 2005; 365:1647–56.
12. Yoshida N, Yagasaki H, Hama A, Takahashi Y, Kosaka Y,Kobayashi R, et al. Predicting response to immunosuppressive therapy in childhood aplastic anemia. Haematologica 2011;96:771–774.
13. Timeus F, Crescenzio N, Lorenzati A, et al. Paroxysmal nocturnal hemoglobinuria clones in children with acquired aplastic anemia: a prospective single centre study. Br J Haematol 2010; 150: 483–485.
14. Hubner, B., Merk, S., Rauhut, S., Dugas, M., Haferlach, T., Fuehrer, M., & Borkhardt, A. (2006). Individual Gene Expression Profiling of Bone Marrow CD34 Cells in Acquired Severe Aplastic Anemia (aSAA) in Children. Blood,108(11), 978.
15. Fischer U, Ruckert C, Hubner B, et al. CD34+ gene expression profiling of individual children with very severe aplastic anemia indicates a pathogenic role of integrin receptors and the proapoptotic death ligand TRAIL. Haematologica. 2012;97(9):1304-11.
16. Li J, Yang S, Lu S, Zhao H, Feng J, Li W, Ma F, Ren Q, Liu B, Zhang L, et al. Differential gene expression profile associated with the abnormality of bone marrow mesenchymal stem cells in aplastic anemia. PLoS One. 2012;7:e47764.
17. Kastrinaki MC, Pavlaki K, Batsali AK, Kouvidi E, Mavroudi I, Pontikoglou C, Papadaki HA. Mesenchymal stem cells in immune-mediated bone marrow failure syndromes. Clin Dev Immunol. 2013;2013:265608.
18. Chao YH, Peng CT, Harn HJ, Chan CK, Wu KH. Poor potential of proliferation and differentiation in bone marrow mesenchymal stem cells derived from children with severe aplastic anemia. Ann Hematol. 2010;89:715–723.
19. Hamzic E, Whiting K, Gordon Smith E, Pettengell R. Characterization of bone marrow mesenchymal stromal cells in aplastic anaemia. Br J Haematol. 2015;169:804–813.
20. Fujimaki S, Harigae H, Sugawara T, et al. Decreased expression of transcription factor GATA-2 in haematopoietic stem cells in patients with aplastic anaemia. Br J Haematol. 2001;113:52–7. [PubMed]
21. Chao YH, Wu KH, Chiou SH, et al. Downregulated CXCL12 expression in mesenchymal stem cells associated with severe aplastic anemia in children. Ann Hematol. 2015;94:13 [PubMed]
22. Xu Y, Takahashi Y, Wang Y, et al. Downregulation of GATA-2 and overexpression of adipogenic gene-PPARgamma in mesenchymal stem cells from patients with aplastic anemia. Exp Hematol. 2009;37:1393–9.
23. Kamata M, Okitsu Y, Fujiwara T, et al. GATA2 regulates differentiation of bone marrow-derived mesenchymal stem cells. Haematologica. 2014;99:1686–96.
24. Chow K et al., 2011. Gene expression profiles of bone marrow mesenchymal stem cells in pediatric patients with severe aplastic anemia (Chow K et al., 2011, accession GSE33812).

25. Tabas-Madrid D, Nogales-Cadenas R, Pascual-Montano A: GeneCodis3: a non- redundant and modular enrichment analysis tool for functional genomics. Nucleic Acids Research 2012; doi: 10.1093/nar/gks402.
26. Nogales-Cadenas R, Carmona-Saez P, Vazquez M, Vicente C, Yang X, Tirado F, Carazo JM, Pascual-Montano A: GeneCodis: interpreting gene lists through enrichment analysis and integration of diverse biological information. Nucleic Acids Research 2009; doi: 10.1093/nar/gkp416.
27. Carmona-Saez P, Chagoyen M, Tirado F, Carazo JM, Pascual-Montano A: GENECODIS: A web-based tool for finding significant concurrent annotations in gene lists. Genome Biology 2007 8(1):R3.
28. Cheng JL, Wang AL, Wan J. Association between the M235T polymorphism of the AGT gene and cytokines in patients with hypertension. Exp Ther Med. 2011;3(3):509–512.
29. Geest CR, Coffer PJ. MAPK signaling pathways in the regulation of hematopoiesis. J Leukoc Biol2009;86(2):237-250.
30. Broglie L, Margolis D, Medin JA. Yin and Yang of mesenchymal stem cells and aplastic anemia. World J Stem Cells. 2017;9(12):219–226.
31. Young, N. S., and J. Maciejewski. 1997. The pathophysiology of acquired aplastic anemia. N. Engl. J. Med. 336:1365.
32. Lambert M, Jambon S, Depauw S, David-Cordonnier MH. Targeting Transcription Factors for Cancer Treatment. Molecules. 2018;23(6):1479.
33. Weiss CN, Ito K. A Macro View of MicroRNAs: The Discovery of MicroRNAs and Their Role in Hematopoiesis and Hematologic Disease. Int Rev Cell Mol Biol. 2017; 334:99-175.
34. Guinan EC. Aplastic anemia: management of pediatric patients. Hematology Am Soc Hematol Educ Program. 2005:104-9.
35. Adhikari S, Mandal P. Integrated analysis of global gene and microRNA expression profiling associated with aplastic anaemia. Life Sciences. 2019; 228: 47-52.

Clinico-bacteriological Parameters of Febrile Neutropenia In Pediatric Hematologicalmalignancies: A Hospital-based Prospective Observational Study

***Dr Jasodhara Chaudhuri,*Dr Tamoghna Biswas,*Dr Tapas Kumar Sabui,*Dr Kalyanbrata Mandal, *Dr Archan Sil,** **Dr Sukanta Chatterjee, ***Dr Swapna Chakraborty

**Department of Pediatric Medicine, Medical College Kolkata, Kolkata, India,*

***Department of Pediatric Medicine, KPC Medical College and Hospital, Kolkata*

****Department of Pediatric Medicine, Tripura Medical College and Dr B.R Ambedkar Memorial Teaching Hospital*

Introduction

Fever is a common occurrence in children, however fever in neutropenic children suffering from hematological malignancies can lead to profound morbidity and can even be fatal. When fever occurs in cancer patients having low neutrophil counts due to the disease process or the chemotherapy, overwhelming life threatening infections may occur giving rise todevastating complications.

Febrile neutropenia is one of the commonest oncological emergencies in leukemias and it is very important that the clinician recognizes it, as many signs and symptoms are attenuated due to the low counts. Fever in this case is defined as "a single oral temperature of >=38.3°C (101°F) or a temperature of >=38.^{0}°C (100.4°F) for >=1 h", while neutropenia is defined as a "neutrophil count of <500 cells/mm^{3}, or a count of <1000 cells/mm3 with a predicted decrease to <500 cells/mm3."[1] Early empirical antibiotic therapy is now the accepted intervention in this clinical setting. Multiple antibiotic regimens have been investigated over the years and most evaluations suggest an overall efficacy of about 70%. [2] According to the Infectious Diseases Society of America (IDSA) guidelines, a neutropenic patient with leucocyte counts <500 cells/cmm who becomes febrile has >60% chance of being infected, although recent trends indicate slightly lower rates of microbiologically documented infections (between 30-50%).[3,4] The number of patients at risk of having febrile neutropenia episodes are growing as the duration and intensity of chemotherapeutic regimens are increasing but such patients can be treated successfully with early initiation of empirical antibiotic therapy. Administration of empirical antibiotic therapy is now the standard practice in management of febrile neutropenia but a variety of approaches has been discussed in literature over the past two decades and there is still controversy about selecting an appropriate regimen. So it is necessary to reevaluate the therapeutic options continually due to the changes in the epidemiology of infectious agents and also the emergence of drug resistant strains.[4]

The present study was done to evaluate the clinico-bacteriological correlates of febrile neutropenia in pediatric hematolgical malignancies, with special reference to response to empirical antimicrobial therapies.

Methods

A hospital based, prospective observational study was carried out in the Department of Pediatric Medicine of a tertiary care teaching hospital of Kolkata, Indiaover a period of one year. The study was approved by the Institutional Ethics Committee for Human Research and was conducted in accordance with the principles of the Declaration of Helsinki. Voluntary informed consent was taken from the parents/caregivers of all children included in the study.

Patients were included in the study if they suffered from hematological malignancies (primary or relapsed), were being treated with high dose of chemotherapy and had neutropenia (ANC<500cells/cmm) and fever >38.3^{0}C on at least one occasion in the presence or absence of any clinical evidence of infection. Children aged<1 year or >12 years, those receiving parenteral antibiotics in the past 4 days, those with severe renal or hepatic impairment, and those with evidence of other debilitating diseases were excluded.

Correspondance : Dr Jasodhara Chaudhuri, *Department of Pediatric Medicine, Medical College Kolkata, Kolkata Email : jasodharachaudhuri@gmail.com*

Complete medical history, physical examination, and detailed anthropometric assessment of the included patients were done. Empiric antibiotic therapy was started with ceftazidime and amikacin at the onset of febrile neutropenia, then temperature monitoring was done 4 hourly and noted at the end of 72 hours to mark defervescence of fever. Favorable response to empiric antibiotic therapy was denoted by defervescence of fever. After 72 hours of empiric antibiotic therapy if there was no response, modification of initial empiric antibiotic regimen was done by changing antibiotics to pipercillin-tazobactum and amikacin. Antifungal was added if fever persisted beyond 5 days, addition of vancomycin to the initial antibiotic regime was done if there was culture proven MRSA infection, mucositis, life threatening hypotension or shock.

Blood cultures were sent at the onset of the episode of febrile neutropenia and modification of antibiotics according to the sensitivity pattern. At least two sets of blood culture from two different peripheral sites were taken.

The antibiotic sensitivity testing was done on Muller Hinton agar by Kirby Bauer method. Plates were prepared with Mueller Hinton Agar (M173) for use in the Bauer Kirby method for rapidly growing organisms. For fungal cultures Mueller Hinton Agar was used +2% glucose + 0.5mcg/ml Methylene Blue dye (GMB medium). Only pure cultures were used. Before starting susceptibility test they were confirmed by Gram-staining. 4-5 similar colonies were transferred with a wire, needle or loop to 5 ml Tryptone Soya Broth(M011).They were incubated at 35-37^{0}C for 2-8 hours until light to moderate turbidity developed. The inoculums were diluted or incubated further as necessary so as they attain comparative turbidity. Alternatively, the inoculums were standardized by other appropriate optical method (0.08-0.13 OD turbid suspension at 620 nm yields 105-106 cells/ml). For fungal cultures inoculums were prepared by picking five distinct colonies of approximately 1mm from 24 hours old culture grown on Sabouraud Dextrose Agar (M063) and incubated at 35+-2^{0}C.Colonies were suspended in 5ml of sterile 0.85% saline. The resulting suspensions turbidity was adjusted to yield 1x10^6—5x10^6 cells/ml (i.e. 0.5 Mcfarland standard).The discs were applied using aseptic technique. The discs were incubated immediately at 35+-2^{0}C and were examined after 16-18 hours or longer. For fungal cultures the discs were incubated immediately at 35+-2^{0}C and each plate was examined after 20-24 hours of incubation.The zones showing complete inhibition were measured and the diameters of the zones were recorded to the nearest millimeter using a calibrated instrument. Zone scales PW096,of dimensions 370x65 nm or PW297, a compact (pocket size), of dimensions 200x95 mm were used to measure sizes of zones in the range of 10-40 nm.

The quality control for the antimicrobial susceptibility was done by using standard strains of *Ecoli* (ATCC 25922), S.aureus (ATCC 27853), S.pneumoniae (ATCC 49619) and for the antifungals C.albicans (ATCC 90028).

The initial empirical regimen was continued at least upto 72 hours and the patient was assessed at 72 h of starting the empiric antibiotic therapy. No treatment modification was permitted before 72 h unless a resistant microorganism was found to be occurring in the initial culture report or the patient's condition notably deteriorated. Modification to the antimicrobial regimen was allowed in patients failing to respond to initial therapy (patients with persistent bacteremia or fever beyond 72 h). All patients received antibiotic therapy for a minimum of 7days. At least two afebrile days were required before the initial empiric antibiotic therapy was terminated as per IDSA guidelines. The antibacterial therapy was discontinued in patients without fever for 5 days who were still neutropenic, but clinically well and with no infectious lesions or no laboratory evidence of infection. Otherwise antibiotics were given until recovery from neutropenia or until patients were clinically well. Patients with severe neutropenia (neutrophils < 100 mm3) with unstable vital signs and with severe mucosal lesions received antibiotics throughout the course of neutropenia.

Results

Fifty two children were included in the study after meeting the inclusion criteria but four were lost to follow-up. Forty eight subjects were included in the final analysis.A total number of 164 episodes of febrile neutropenia occurred during the study period.

Of the total 48patients21 were male (43.75%) and

27 female(56.25%). Approximately 33% of the patients belonged to theage groupof 4-6 years, while 27% and 18.7% belonged to the age group of 2-4 years and 6-8 years respectively (Table 1).

Age (years)	Male (%)	Female (%)	Total (%)
0--2	0	0	0
2-4	5 (10.42)	8 (16.67)	13 (27.08)
4-6	8 (16.67)	8 (16.67)	16 (33.33)
6-8	5 (10.42)	4 (8.33)	9 (18.75)
8-10	0	6 (12.5)	6 (12.5)
10-12	3 (6.25)	1 (2.08)	4 (8.33)
Total	21 (43.75)	27 (56.25)	48 (100.00)

Out of 164 episodes of febrile neutropenia, 105 (64.02%) episodes occurred in the consolidation phase of chemotherapy, 49 (29.88%) in the induction phase and10 (6.1%) in the reinduction phase (Table 2). The first episodemostly occurred in the induction phasewhereas second and subsequent episodes mostly occurred in consolidation phase. Most of the deaths occurred in the third and fourth episodes of febrile neutropenia.

Episode	Frequency	Phase of Protocol			Outcome	
		Induction	Consolidation	Reinduction	Survival	Death
1	47	39	8	0	47	0
2	46	9	37	0	44	2
3	42	1	41	0	38	4
4	28	0	18	10	24	4
5	1	0	1	0	1	0
Total	164	49	105	10	154	10

Death occurred most commonly during the consolidation phase (7/10, 70%) of chemotherapy followed by reinduction (2/10, 20%) and induction (1/10, 10%) phases (Figure 1).

Microbiologically documented infection was found in 51 (51/164, 31.1%) cases while 113 (113/164, 68.90%) cases showed no evidence of microbiologically documented infection. Among the documented bacterial infections Staphylococcus aureus(13/51, 25.49%) was the commonest isolated organism followed by Pseudomonas(11/51, 21.57%),Klebsiella(9/51, 17.65%), Escherichia coli(7/51, 13.73%), coagulase negative Staphylococci(7/51, 13.73%), Candida(including both Candida albicans and non-albicans Candida) (3/51, 5.88%) and Acinetobacter(1/51, 1.96%). Majority of Staphylococcus aureusand coagulase negative Staphylococciinfections occurred during the consolidation phase of chemotherapy, while infection with Escherichia coliwas commoner in the induction phase (Figure 2).

Out of164 episodes, a favorable response to initial empirical antibiotics was seen in only 54 (54/164, 32.93%) cases (Figure 3).

Among 110 episodes in which no response to initial empirical antibiotics was found, only 42 cases (42/110, 38.18%) showed a favorable response to an addition of antibiotics, while the rest 68 (68/110, 61.82%) showed no response to the addition (Figure 4).

Change to second line antibiotics was done in 72 episodes. In 50 (50/72, 69.44%) episodes, the change of antibiotics led to a favorable response, while no response was seen in 22 (22/72, 30.56%) cases (Figure 5).

Discussion

The present study was done to evaluate the different parameters associated with febrile neutropenia among the patients with hematological malignancies attending the Department of Pediatrics , Medical College , Kolkata . The variations in age , sex , nutritional status , type of hematological malignancy , the empiric treatment of febrile neutropenia , comparison with culture and sensitivity pattern , duration of treatment , whether the patient responded to empiric antibiotics , whether the patients faced life threatening complications , how many patients died after the episode were all evaluated during the period of the study. At present the mortality in hematological malignancies has decreased to great extent but when associated with febrile neutropenia, the mortality rate increases. Although standard treatment with broad spectrum antibiotics has led to dramatic decline in deaths over the years but in the recent times there has been emergence of multidrug resistant microbiological agents which are posing challenges to the treatment and outcome of febrile neutropenia. Studies are required to investigate the changing milieu of infective organisms and to find out whether the response of these febrile neutropenic patients to

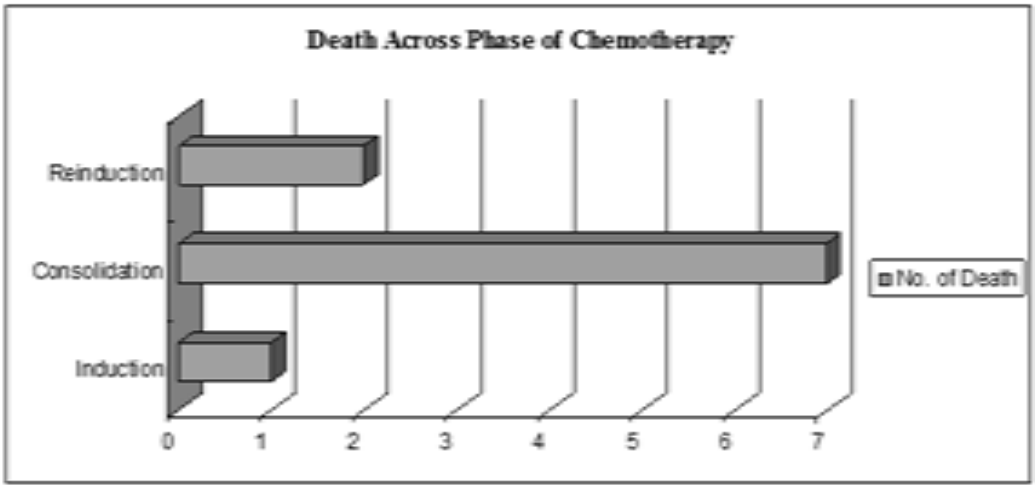


Figure 1

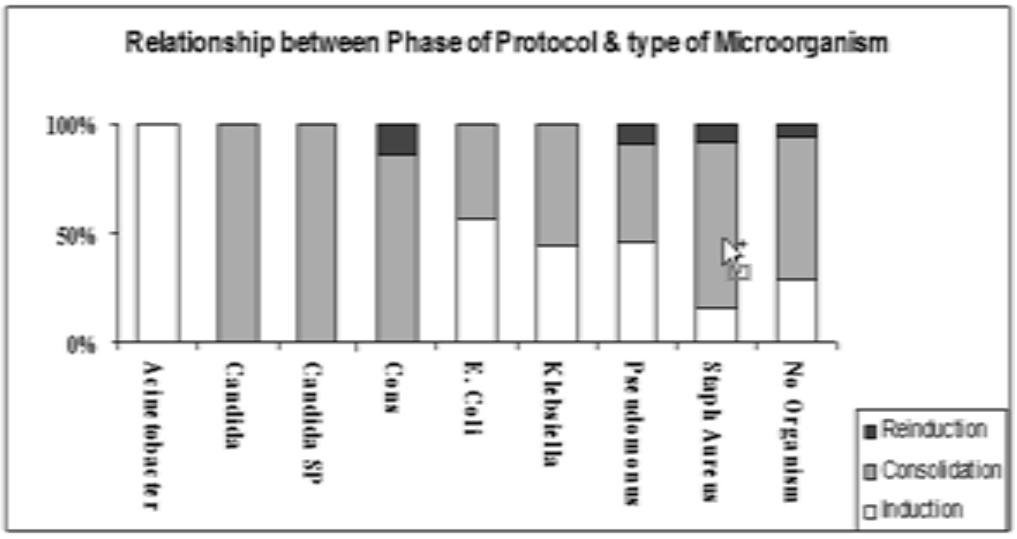


Figure 2

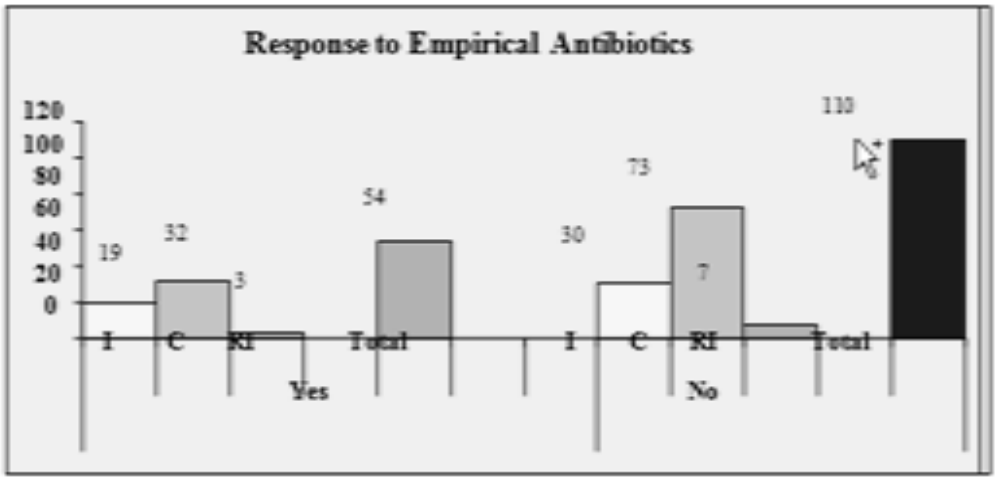


Figure 3

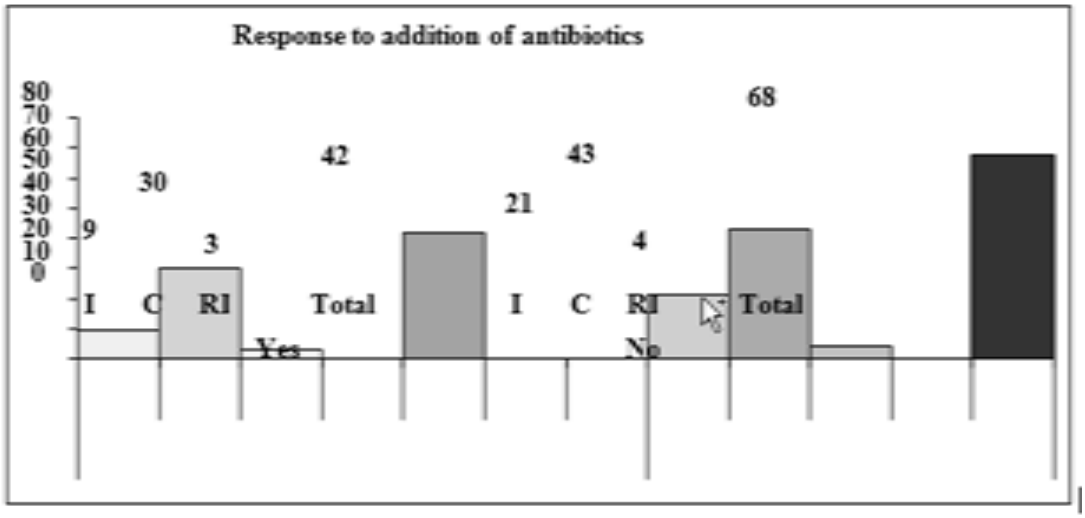


Figure 4

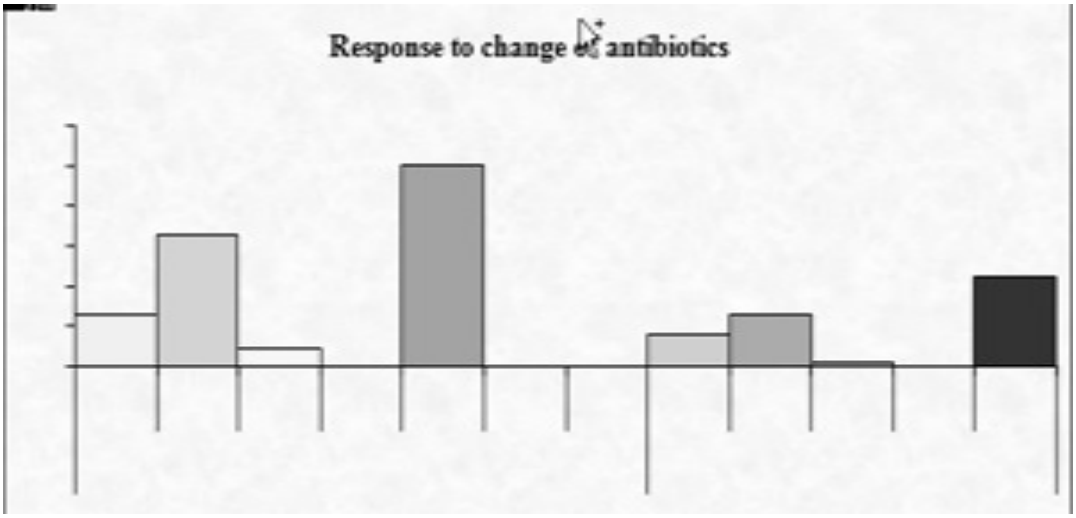


Figure 5

conventional empirical antibiotic therapy was satisfactory or not.

In this study with 52 patients of whom 47 were eligible for statistical analysis, we found 164 episodes of febrile neutropenia and the results that we obtained will be compared to results of previous studies in this section.

Gender Distribution:

In the study by Ramphal et al (1995) [6], they found 45 % male patients and 55 % female patients, in the study by Akova et al (1999) [7] 52% were male and 48% were female, in the study by Palaniyamma et al (2005) [8] it was found that 60 % of the patients were male and 40% were female, Zulfikar et al (2009) [9] showed in their study 68% were male patients and 32 % of the patients were female. So in most of the studies it was found that male preponderance was more.

Our study we found a slight female preponderance as 56 % were females compared to 44 % males.

In the study by Zulfikar ali et al[10] the mean age of children with hematological malignancies was found out to be 5.5 years. In the study by Malcom A. Smith et al [11] the mean age for hematological malignancies was found out to be 4.5 years. In our study we found the mean age to be 5 years that corroborates well with the earlier studies .The age distribution of ALL shows

a major peak at preschool age (between 1 and 5 years of age) with a slow decline towards adolescence.[11]

Malnutrition at diagnosis is associated with significantly more treatment related profound neutropenia. The intensity of chemotherapeutic regimens has to be adapted to the level of available supportive care and patients' nutritional status and tolerance to avoid unacceptable morbidity and mortality. In the study by Israels et al [12] it was found that fifty eight (69%) of 84 patients were malnourished. In our study we found that 50% of the total number of patients were malnourished and malnutrition was mostly prevalent in the age group of 4-6 years followed by 2-4 years .So the high prevalence of malnutrition found in our study corroborates well with other studies.

Consolidation phase of chemotherapy , also known as the intensification phase is most toxic to the patient. In our study we found that 64 % of the total number of episodes of febrile neutropenia are in the consolidation phase, 30% in induction phase and 6% in reinduction phase. This corroborates well with the study by Sung Lilian et al [13] and Ohno R et al [14].

In our study we found that 10 of total 48 patients died and 70 % of the total number of deaths occurred in the consolidation phase , 20 % in the reinduction phase and 10 % in the induction phase. Febrile neutropenia increases the chance of death in patients suffering from hematological malignancies as suggested by Lyman et al.[15]In the study by Basu et al done at University of Rochester , it has been shown that out of 12,446 patients 3,647 patients died, so about 3 % mortality.[16] In the study by Gupta S et al 13 of total 106 episodes of febrile neutropenia resulted in death (12 %).[17] In our study, out of 164 episodes there were 10 deaths ,so, that amounts to 6% of total number of episodes that corroborates well with earlier studies. In the study by Paganin HR et al it has been shown that out of 714 episodes there were 18 deaths (2.5%).[18]According to the study by Santolaya ME et al 14 of 393 episodes of febrile neutropenia (3.6%) died.[19].So the result of our study corroborated well with existing international and national literature.

In our study we have found out that of the total number of 164 episodes, in 54 episodes response to initial empiric antibiotics were favourable proved by defervescence of fever while in 110 episodes the response were not favourable. So in 32 % of the episodes the response to initial empiric antibiotic (ceftazidime-amikacin) therapy was favourable. In the study by Cometta et al (1996) , done with 985 patients , response to ceftazidime and amikacin as initital empiric therapy for febrile neutropenia was found in 52 % cases and the response was 32 % in case of microbiologically documented infections.[20] In the study by Cordonnier et al (1997) done with 353 patients it was found that response of febrile neutropenia episode to ceftazidime as initial empiric therapy was 21 % and after addition of glycopeptide to the empiric regimen the response was 51 %.[21].In the study by Corapcioglu F (2005) it has been shown that the patients with febrile neutropenia who have been treated with ceftazidime and amikacin as empiric antibiotics required more modification of regimen at 72 hours of treatment than the patients who were treated with cefepime alone. [22] In the study by Zinner S et al (1995) it has been shown that of 706 episodes of febrile neutropenia treated empirically with antibiotics response to ceftazidime –amikacin was 35 % while to pipercillin tazobactum the response was 50 %.[23] In the study by K Serefhanoglu et al (2006) it was found that the response to ceftazidime amikacin as initial empirical antibiotic was 34.5 % without modification and 31 % with modification.[24] In the study by Ghaulat et al (2011) done with 25 patients showed 60 % clinical success with empirical therapy with ceftazidime amikacin and 65 % clinical success with pipercillin tazobactum. [25] In the study by Pectasides et al (2006) clinical efficacy of ceftazidime was proved to be 60 % as empiric therapy in febrile neutropenia[26]. In the study by Erman et al (2001) in 208 patients , clinical response to ceftazidime as initial antibiotic therapy was 30 %[27]. So most of the studies in this

context corroborates well with our finding. So, our conclusion is that ceftazidime amikacin can no longer be regarded as a highly effective antibiotic in empiric therapy in patients with febrile neutropenia. We are also analysing the response to addition and change of antibiotics, by addition we meant the addition of glycopeptide like vancomycin if gram positive infection was suspected or other indications like mucositis etc were there after 72 hours of starting the empirical antibiotic therapy and antifungals are to be added if fever persists at day 5 or definite proof of fungal sepsis is present. By change of antibiotics we mean that changing antibiotics from ceftazidime amikacin to pipercillin tazobactum amikacin if there is no response after 72 hours of starting empirical antibiotic therapy. In our study of the 110 cases which did not respond to empiric antibiotic therapy, 42 responded to addition of antibiotics while 68 did not. So, no response in 61 % of the cases with a $p<0.05$. Now of the 72 episodes that required change of antibiotics 50 episodes responded to the change of antibiotics (69%) while 22 did not , so the response of change of antibiotics to pipercillin tazobactum was favourable ($p<0.05$). Earlier studies which suggested vancomycin to be included as a part of the initial empiric regimen for febrile neutropenia are studies by Shenep et al (1988) showing response rates with vancomycin addition - 85%[28], Pico et al (1993) as 48%.[29].In the study by Gary Lyman et al (2010) Vancomycin incorporation with initial empiric regimen did not show any added advantage [30]. In our study the response to pipercillin tazobactum plus amikacin as empiric therapy was 69%.In the study by Favero et al (2001) the response was 53% (196 0f 369).[31] In the study by Yildirim I (2008) the response to pipercillin tazobactum and amikacin among 87 febrile neutropenia episodes was found to be 56.3% [32]. Another study , by Aksoylar S et al (2004) the response to pipercillin amikacin among 57 episodes were found to be 42 % but the overall treatment success rate along with modification was found out to be 90%.[33] The success rate of pipercillin tazobactum was found to be 62 % out of 867 febrile neutropenia episodes in the study by Sanz M A et al.[34] In the study by Hamidah A (2008) out of 150 episodes 67% showed favourable response to pipercillin tazobactum .[35] In their study they have mentioned that pipercillin tazobactum plus amikacin is a regimen with better efficacy than ceftazidime amikacin.

In our study of the total number of episodes in 32 % of the total number of episodes microbiologically documented infections were present while in the remaining 68 % no evidence of microbiological organism was got. In the metaanalysis by Paul et al (2005) they concluded that there has been a pathogen shift in neutropenic patients from gram negative organisms to gram positive organisms which corroborates well with our study.[36] In the study by Ronald Feld (2008) et al detailed discussion have been made on the mileu of microbiological organisms in febrile neutropenia. According to them, in previous EORTC-IDG (IATG) published studies the incidence of bacteremia varied between 32% in 1973 to 22% in 1994. During the same time period, the proportion of Gram-positive pathogens increased. The proportion of bacteremia caused by Gram-negative and Gram-positive changed from 71% and 29% (1973-1978) to 33% and 67% (1992-1994) respectively. The explanation for the change in the pathogens causing bacteremia and also non-bacteremic documented infections is thought to be mainly a result of prophylaxis with cotrimoxazole and fluoroquinolones and probably also due to the increased use of central lines in these patients. More mucositis and use of antacids may also contribute to this. Gram-negative infections (especially bacteremia) generally have a higher mortality then those due to Gram-positive bacteria. Although Gram-negative pathogens may be decreasing in most developed countries the proportion recently (2001-2005) seen in FN in developing countries such as Malaysia, Lebanon etc. remains high, perhaps related to less use of quinolone prophylaxis and central lines, due to their high cost. 1643 (77%) of the patients studied did not have bacteremia and 57 (2.9%) died, while among the 499 with bacteremia 49 (10 %) died. A higher proportion of patients who had

bacteremia also had a higher rate of non-lethal complications (21% versus 13%) in patients with hematological malignancies. 168 bacteremias were caused by single Gram-negative organisms with an 18% mortality rate. E coli, Klebsiella sp. and Pseudomonas aeruginosa occurred frequently enough as a cause of bacteremia for a meaningful analysis, with respective death rates of 18%, 10% and 31 %. Blood cultures were positive in 33 episodes (18.6%), which was lower than previously (30% in 1999 and 28.8% in 1995-1998).[37] Apart from that from the previous studies that we have already discussed Yildirim et al (2008) [32], Gary Lyman et al (2010) [30] , Aksoylar S et al (2004) [33] it was found that the prevalence of bacteremia in febrile neutropenic episodes were found to be about 22 -34% which corroborates well with our study with preponderance towards gram positive organisms.

Analysis of the drug sensitivity pattern of various infective organisms showed most of the organisms were multidrug resistant. In our study Klebsiella, Ecoli, Pseudomonas, Staph Aureus were mostly resistant to cephalosporins except pseudomonas 98% sensitive to ceftazidime. Among the penicillin group of drug, amoxyclav highly resistant to all organisms. Quinolones excepting ciprofloxacin are sensitive to most organisms. Gram positive organisms are highly sensitive to linezolid and vancomycin, and are resistant to cefepime, ceftazidime . Aminoglycosides were found to be moderately sensitive among all groups of organisms. Candida were found to be sensitive to fluconazole and amphotericin both. More or less similar results were seen by V.P Chowdhury et al[38].

In Figure 16 it has been shown that at 72 hours after onset of the febrile neutropenia most of the patients had neutrophil count in the range of 400-499 (27%). 23% of the patients had ANC in the range of 300-399, 22% of the patients had ANC above 500, 21% of the patients had ANC in the range of 200-299.The patients received Granulocytic Colony Stimulating Factor along with empirical antibiotic therapy for the episodes of febrile neutropenia and our finding here corroborated with the finding in the study by Ravaud et al [39]

In the figure 18 it has been shown that most of the patients after 72 hours of onset of the episode show reduction of temperature. At 72 hours of starting the antibiotic 45% of the episodes showed temperature below 100°F , 21% of the episodes showed temperature in the range 100-100.9, 17% showed temperature in the range 101-101.9, 8% showed temperature in the range 102-102.9, 4% showed temperature in the range of 103-103.9, 5% showed temperature in the range of 104-104.9. Study by S.Shamsi et al (2003) yielded similar results.[40] Also the previous studies that we discussed uniformly showed that there has been a reduction in temperature after 72 hours in an episode of febrile neutropenia in majority of the patients with adequate supportive management.

Sites of infection:

Sites of infection could be localised in about 29% of the episodes of febrile neutropenia. Of the sites localised , respiratory system (45% of total number of episodes showing localising symptoms) was found to be involved the maximum number of times followed by urinary tract infection (34%) followed by gastrointestinal tract infection (19%) , while culture positive septicemia was found in 32 % of the total number of episodes. Studies regarding the site of infections by various workers have revealed that lung is the commonest site (Pizzo et al 1984) and infections of gastrointestinal tract were not that common (Elting et al) [24].In a study by Pratupjai Sanboonrat et al (2009) it was found that most common site of infection was oral cavity (29%) followed by respiratory tract infection (24%) followed by urinary tract infection (14.9%) [41]

Duration of febrile neutropenia:

In our study we found that in majority of the cases the duration was 4 days (31%) followed by 3 days in 30 % of the cases as depicted by figure 18.In the study by Hakim H et al (2009) it was shown that the median duration of febrile neutropenia was 3.5 days and the median

duration of hospital stay 5 days, according to their study bacteremia was present in 28% and gram positive infection was more than gram negative infection [42]. Similar results were found in the study by Viscoli et al (2005) which showed the mean duration of febrile neutropenia episode to be 4.5 days. [43] In the study by M Timothy (2011) the mean duration of febrile neutropenia episode was found to be 5.01 days which corroborates well with our study [44]. Also the studies that we have already discussed showed similar results like the studies by Yildirim et al (2008) [32], Gary Lyman et al (2010) [30] .

Response to antibiotics in children with malnutrition:

This figure 20 shows that in presence of malnutrition the response to antibiotics in febrile neutropenia is poor. Non response to antibiotics was mostly in children who had associated malnutrition and it had poorer outcome. 86.36% of the malnourished children failed to show response to antibiotics, hence having poor outcome while only 13.64 % of the well nourished children failed to respond to antibiotics. Therefore, the children without malnutrition had much better response and better outcome. In developing countries, children with cancer are often malnourished at diagnosis. In these children febrile neutropenia management is more challenging as their immune system is already compromised both due to malnutrition and due to cytotoxic chemotherapy.

There has been one definite study in this context, in children with malnutrition who are having febrile neutropenia. This study was done in Malawi by Israels et al (2009) in children who had Burkitt lymphoma and were treated with a local protocol with a limited toxicity. The study was done to evaluate the outcome of febrile neutropenia in children who are already having malnutrition before starting cytotoxic therapy. The study was done with 87 patients and it was found that 69% of them were suffering from malnutrition. In our study we have found that 50 % of the patients were suffering from malnutrition. In that study it was found that malnutrition had definite positive correlation with profound neutropenia and was associated with poor response to antibiotics given empirically for febrile neutropenia. So, the result of this study corroborates well with our study. However the surprising lack of studies in this context warrants further studies on children suffering from hematological malignancies having coexistent malnutrition, particularly in our part of the world.

Summary And Conclusions:

The study was aimed to assess the onset of febrile neutropenia , the response to empiric antibiotics Ceftazidime –amikacin whether any modification to the antibiotic therapy was needed or not , to assess how many episodes of febrile neutropenia were attributable to documented infections and what were the microbiological organisms isolated from those episodes.

52 patients were originally enrolled for the study and ultimately statistical analysis was done with 47 patients and the total number of episodes in these 47 patients were 164.

Of the 47 patients 43(92%) patients had ALL and 4(8%) patients had AML. We in our study got the cases as pre B ALL, we did not get any T cell ALL and a very few number of AML. Of the total patients 44% of the patients are male and 56% of the patients are female. The median age group of the patients was 5.5 years and 33% of the patients belong to the age group 4-6 years, 27% of the patients belong to the age group 2-4 years, 18.7 % of the patients belong to the age group of 6-8 years, 12.5% belong to the age group 8-10 years, 8.3% belong to the age group 10-12 years. Of the total number of patients 50% of the patients suffered from malnutrition.

Of the total episodes of febrile neutropenia, 64 % of the total number of episodes of febrile neutropenia are in the consolidation phase, 30% in induction phase and 6% in reinduction phase. The patients having more than one episodes are having the 1st episode during the induction therapy while the 2nd and the 3rd phases are mostly occuring during the consolidation therapy.

Total number of deaths in the study is 10, out of 164 episodes that amounts to death as outcome in 6.09%. Of the total number of deaths 70%

occurred in consolidation phase, 10% in the induction phase and 20% in reinduction phase.

Out of 164 episodes in 54 cases the response to empirical antibiotics was favorable and in 110 cases it was not showing the intended result. The initial empiric antibiotic used in all cases were ceftazidime and amikacin. Here response to empirical antibiotics is not favourable as 32.9 % only showed positive response and 67.1 % showed negative response. By addition, to the initial empiric antibiotic regimen we mean addition of vancomycin. Antifungal agents are added if fever persisting beyond 5 days. Response to addition of antibiotics to the initial regimen was 38.1% .

72 hours after the initiation of empiric antibiotic therapy if there is no response then initial antibiotic regimen is changed to pipercillin tazobactum and amikacin. In our study we found out that after changing antibiotics 50 out of 72 cases showed favorable response and only in remaining 22 cases there was NO RESPONSE. So change of antibiotics showed favourable response with p value <0.01 and z=3.8.After change of antibiotics , 61.9% in the episodes belonging to induction phase showed favourable response, 76.74% belonging to the episodes in consolidation phase and 80% belonging to the episodes in reinduction phase showed favourable response . Overall , 69.44% of the febrile neutropenia episodes which did not show good response to initial empiric antibiotics showed favourable response to the change of antibiotics.

So from our study we conclude that ceftazidime amikacin is no longer a good option as empiric antibiotic therapy in our set up and pipercillin tazobactum in our set up is acting as a superior antibiotic in combination with an aminoglycoside and the protocol of treatment of febrile neutropenia should be individualized for different institutions according to the local microbiological flora and response of the patients.

In our study of the 164 episodes of febrile episodes , bacterial infection was documented in 32 % of the total cases while 68 % of the total cases showed no bacterial infection. Of the documented bacterial infection highest was found to be Staph aureus(26.4%) that is gram positive followed by gram negative organisms : pseudomonas(20%), klebsiella(16%) Ecoli(13%), CONS(13%), Candida(5%) ,Acinatobacter(2%). In literature, as we have found out, there has been a shift in the microbiological flora in patients with febrile neutropenia who are suffering from infections, from gram negative to gram positive organisms. Probable fungal infection was present in 7% of the cases.

In our study we find that at 72 hours of the episode of febrile neutropenia the absolute neutrophil counts of the majority of the patients were in the range of 400-499(27%) , 23% of the patients had ANC in the range of 300-399, 22% of the patients had ANC above 500, 21% of the patients had ANC in the range of 200-299.

At 72 hours of starting the antibiotic antibiotics 45% of the episodes showed temperature below 100°F , 21% of the episodes showed temperature in the range 100-100.9, 17% showed temperature in the range 101-101.9, 8% showed temperature in the range 102-102.9, 4% showed temperature in the range of 103-103.9, 5% showed temperature in the range of 104-104.9. The results of previous studies yielded similar results.

Localising symptoms were present in 29% of the total episodes of febrile neutropenia of which respiratory tract infection were the most prevalent, 47% of the localizing symptoms were of respiratory tract origin, 28% of the localizing symptoms were urinary tract infections, 15 % of the localizing symptoms were gastrointestinal infections- diarrhea and dysentery. Rest 10% of the infections were attributed to soft tissue infections, oral cavity infections etc.

In our study we found that in majority of the cases the duration was 4 days (31%) followed by 3 days in 30 % of the cases which corroborated well with other major studies in literature.

In our study we found that 50% of the children were suffering from malnutrition. The response to empiric antibiotics were poor when these children suffered from febrile neutropenia episodes and hence their outcome was poorer. 86.36% of the malnourished children failed to show response to antibiotics, hence having poor outcome while only

13.64 % of the well nourished children failed to respond to antibiotics. Therefore, the children without malnutrition had much better response and better outcome.

Our present study highlights many important associations of febrile neutropenia and their outcomes, which will prove to be very useful as febrile neutropenia is a common occurrence in patients suffering from various malignancies and is one of the major causes of morbidity and mortality in malignancy patients. Infectious complications are an important causes of morbidity and mortality in cancer patient especially that receiving cancer chemotherapy. Furthermore neutropenia, fever and infection limit the dose, intensity of antineoplastic chemotherapy in cancer patients, sometimes compelling delays in the treatment of reduced dosages. Early intervention with broad-spectrum intravenous antibiotics has been cornerstone of treatment for over 30 years and has resulted in significant reduction in the mortality. Development of new more flexible antibiotic policies, risk stratification of patients and use of prophylactic antimicrobials are aimed at safe reduction in frequency and length of inpatient admissions with consequent reduction in mortality and morbidity. Increased understanding of problem of febrile neutropenia and recent developments such as the emergence of multidrug resistant organisms led to development of novel treatment strategies.

Empiric treatment with a combination of antibiotics instituted at the onset of fever rather than after identification of the offending pathogen reduced mortality and morbidity in patients, by acting on a broad range of possible pathogens, achieve a bactericidal serum concentration exerts a synergistic effect and prevent emergence of resistant organisms. The epidemiological pattern of bacterial infections continues to evolve globally and locally at the institutional levels, as do patterns of susceptibility and resistance. The global problem of increasing antimicrobial resistance is beginning to limit therapeutic options for the treatment of resistant bacterial infection in patients with neutropenia as well. Unfortunately the development of new drug is not keeping pace with the development of resistance.The past 20 years have yielded many exciting new options for treatment of febrile episodes in the neutropenic cancer pa-tient. Empirical therapy that provides coverage of both gram-negative and gram-positive pathogens is mandatory in light of epidemiologic data suggesting a gradual shift in prevalence from gram-negative to gram-positive organisms in this population. In our study we found out that ceftazidime amikacin is not working as an effective antibiotic in our set up and we need to form institutional protocol for the treatment of febrile neutropenia based on International guidelines like IDSA, depending upon local microbiological flora and antibiotic sensitivity pattern. Also in our study we found out that gram positive organisms were mostly the offending organisms. To prevent infections upgrading the hospital set up should be done to provide a special isolated unit for these children under extreme vigilance. Rigorous hand washing by care givers and stringent asepsis during procedures are also very important. So prevention is always better than cure and when an episode of febrile neutropenia occurs we have to start treating the patient zealously so that we can minimize the mortality and morbidity. Also we need more studies in this aspect from all institutions to understand all aspects of febrile neutropenia better, and ways to prevent and manage the episodes more effectively.

References

1. Hughes WT, Armstrong D, Bodey GP, et al. 2002 guidelines for the use of antimicrobial agents in neutropenic patients with cancer. Clin Infect Dis. 2002 Mar 15;34(6):730-51.)
2. Wade JC, Schimpff SC: Antibiotic therapy for febrile granulocytopenic patients. In: Klastersky J, Staquet MJ (eds) Combination antibiotic therapy in the compromised host. EORTC, vol 9. New York, Raven Press, 1982, pp 125-146.
3. Hughes WT, Armstrong D, Bodey GP, et al. From the Infectious Diseases Society of America. Guidelines for the use of antimicrobial agents in neutropenic patients with unexplained fever. J Infect Dis. 1990 Mar;161(3):381-96. Review. Erratum in: J Infect Dis 1990 Jun;161(6):1316.
4. Hathorn JW, Lyke K. Empirical treatment of febrile neutropenia: evolution of current therapeutic approaches. Clin Infect Dis. 1997 Feb;24 Suppl 2:S256-65.

5. Longo DL , Duffy PL,De Vita VT Jr, et al .The calculation of actual or received dose intensity: a comparison of published methods. J.Clinic.Oncol 1991;9:2042-51.

6. Ramphal R, Gucalp G, Rotstein C, Cimino M, Oblon D.Clinical experience with single agent and combination regimens in the management of infection in the febrile neutropenic patient. Am J Med 1996; 100: 83S-89S.

7. Akova M, Akan H, Korten V, Biberolu K, Hayran M, Ünal S, Kars A, Kansu E.Comparison of meropenem with amikacin plus ceftazidime in the empirical treatment of febrile neutropenia: a prospective randomised multicentre trial in patients without previous prophylactic antibiotics. Int J Antimirob Ag. 1999;13: 15-19.

8 . D.Paliyanamma.A clinical evaluation of antibiotics used in the treatment of febrile neutropenia patients and comparision with the antibiotic sensitivity pattern in cancer patients receiving chemotherapy.Rajiv Gandhi University of Health Sciences, Karnataka, Bangalore.2004

9 . ZulfiqarAliRana, Muhammad Waqar Rabbani, Muhammad Aslam Sheikh, Afsheen Asghar Khan.Outcome of childhood acute lymphoblastic leukaemia after induction therapy—3 years experience at a single paediatric oncology centre.J Ayub Med Coll Abbottabad 2009;21(4).

10. Smith Malcolm A, Chen Timothy , Simon Richard . Age-Specific Incidence of Acute Lymphoblastic Leukemia in U.S. Children: In Utero Initiation Model.J Natnl Cancer Inst.Vol 89.p 1542-44.

11. Gurney JG , Severson RK , Davis S et al. Incidence of cancer in children in the United States. Sex, race and 1 year age specific rates by histologic type .Cancer 1995;75:2186-2195.

12. Trijn Israëls ,Marianne D., Van de Wetering ,et al.Malnutrition and neutropenia in children treated for Burkitt lymphoma in Malawi. Pediatric Blood and Cancer.2009;vol 53 : 47-52.

13. Sung Lilian , Nathan Paul , Lange Beverley et al.Prophylactic Granulocyte Colony-Stimulating Factor and Granulocyte-Macrophage Colony-Stimulating Factor Decrease Febrile Neutropenia After Chemotherapy in Children With Cancer: A Meta-Analysis of Randomized Controlled Trials .Journal of Clinical Oncology.2004; 22: 22-24.

14. R Ohno , S Miyawakee , K Hatake et al.Human urinary macrophage colony-stimulating factor reduces the incidence and duration of febrile neutropenia and shortens the period required to finish three courses of intensive consolidation therapy in acute myeloid leukemia: a double-blind controlled study. Journal of Clinical Oncology.1997;15:2954-68.

15. Lyman Gary ,Michels Shanon , Reynolds Mathew et al.Risk of Mortality in Patients With Cancer Who Experience FebrileNeutropenia.Cancer.2010;116:5556-64.

16. Basu Swati , Fernandez D , Fisher Susan et al.Length of Stay and Mortality Associated With Febrile Neutropenia Among Children With Cancer.Journal Of Clinical Oncology.2005;23;7958-66.

17. Gupta S , Bonilla M , Gamero M et al. Microbiology and mortality of pediatric febrile neutropenia in El Salvador.J Pediatr Hematol Oncol.2011;33:276-80.

18. Paganini HR , Acquirre C , Puppa G et al.A prospective, multicentric scoring system to predict mortality in febrile neutropenic children with cancer.Cancer.2007; 109: 2572-9.

19. Santolaya ME , Alvarez AM , Aviles CL et al. Admission clinical and laboratory factors associated with death in children with cancer during a febrile neutropenic episode. Pediatr Infect Dis.2007;26:794-8.

20. Cometta A, Calandra T, Gaya H et al. Monotherapy with Meropenem versus Combination Therapy with Ceftazidime plus Amikacin as Empiric Therapy for Fever in Granulocytopenic Patients with Cancer. Antimicrobial Agents And Chemotherapy.1996;22:1108-15.

21. Cordonnier C , Herbecht R , Pico JL et al. Cefepime/amikacin versus ceftazidime/amikacin as empirical therapy for febrile episodes in neutropenic patients: a comparative study. The French Cefepime Study Group. Clin Infect Dis.1997;24:41-51.

22. Corapicioglu F , Sarper N.Cefepime versus ceftazidime + amikacin as empirical therapy for febrile neutropenia in children with cancer: a prospective randomized trial of the treatment efficacy and cost. Pediatr Hematol Oncol.2005;22:59-70.

23. Cometta A , Zinner S , Bock R et al. Piperacillin-tazobactam plus amikacin versus ceftazidime plus amikacin as empiric therapy for fever in granulocytopenic patients with cancer. The International Antimicrobial Therapy Cooperative Group of the European Organization for Research and Treatment of Cancer. Antimicrob Agents Chemother.1995;39:445-52.

24. Serefhanoglu K,Ersoy Y , Kuku I et al. Clinical Experience with Three Combination Regimens for the Treatment of High-risk Febrile Neutropenia.Annals Academy of Medicine.2006;35:12-17.

25. Ghalaaut PS , Chaudhury U , Ghalaut VS et al.Piperacillin-tazobactum plus amikacin versus ceftazidime plus amikacin as empirical therapy for Fever in neutropenic patients with hematological malignancies. Indian Journ Hematol Blood Trans.2011;27:131-5.

26. Pectasides D , Fountzillas G , Samonis G et al.Piperacillin/tazobactam monotherapy versus combination ceftazidime plus amikacin for the treatment of febrile neutropenia in patients with cancer. A Hellenic Co-Operative Oncology Group Study. Journal of Clinical Oncology.2006;24:18-37.

27 . Erman M , Akova M, Akan H et al.Comparison of cefepime and ceftazidime in combination with amikacin in the empirical treatment of high-risk patients with febrile neutropenia: a prospective, randomized, multicenter study.Scand J Infect Dis .2001;33:827-31.

28. Shenep JI, Hughes WT. Vancomycin ,Ticarcillin and clavulinic acid and amikacin in the treatment of children with febrile neutropenia.N Engl J Med.1988;319;1053-8

29. Pico J L ,Marie JP,Chiche D et al. Should Vancomycin be used empirically in febrile patients with prolonged and

profound neutropenia? Results of a randomized trial. Eur J Med.1993;2:275-80.

30. Lyman Gary, Kenneth V, Rolston I et al. How We Treat Febrile Neutropenia in Patients Receiving Cancer Chemotherapy.Journal of Oncology Practice.2010;6:149-52.
31. Favero A , Menichetti F, Martino P et al. A Multicenter, Double-Blind, Placebo-Controlled Trial Comparing Piperacillin-Tazobactam with and without Amikacin as Empiric Therapy for Febrile Neutropenia.Clinical Infect Dis.2001;33:1295-1301.
32 . Yildirim I , Aytac S , Ceyhan M et al.Piperacillin/tazobactam plus amikacin versus carbapenem monotherapy as empirical treatment of febrile neutropenia in childhood hematological malignancies. Pediatr Hematol Oncol .2008;26:291-9.
33. Aksoylar S , Cetinqul N , Kantar M et al.Meropenem plus amikacin versus piperacillin-tazobactam plus netilmicin as empiric therapy for high-risk febrile neutropenia in children. Pediatr Hematol Oncol.2004;21:115-23.
34. Sanz MA , Lopez J , Lahuerta J et al.Cefepime plus amikacin versus piperacillin-tazobactam plus amikacin for initial antibiotic therapy in haematology patients with febrile neutropenia: results of an open, randomized, multicentre trial.J Antimicrob Chemother.2002;50:79-88.
35. Hamidah A, Rizal A M, Nordiah A J et al.Piperacillin-tazobactam plus amikacin as an initial empirical therapy of febrile neutropenia in paediatric cancer patients.Singapore Med J 2008;49:26-32.
36 . Mical Paul, Sara Borok, Abigail Fraser et al.Empirical antibiotics against Gram-positive infections for febrile neutropenia: systematic review and meta-analysis of randomized controlled trials. Journal of Antimicrobial Chemotherapy.2005;55:436-444.
37. Feld R.Bloodstream infections in cancer patients with febrile neutropenia.Int J Antimicrob Agents.2008;32:332-38.
38. Choudhury V.P, Tokuhochishi L, Rath GK et al.Etiology of febrile episodes in children with acute lymphocytic leukemia . Ind J.Med.Res.1992;96:12-15.
39. Ravaud A , Chevreau C , Cany el et al. Granulocyte-macrophage colony-stimulating factor in patients with neutropenic fever is potent after low-risk but not after high-risk neutropenic chemotherapy regimens: results of a randomized phase III trial.Journ of Clin Oncol.1998;16:2930-36.
40. T.S.Shamsi,A.Ishaque T.Farzana et al.Febrile Neutropenia in Haematological Disorders:a single center review of Antibiotic policy and the Outcome.2003;11:56-62.
41. Pratupjai Sanboonrat , Su –on Chainansamit,Kanokwan Sriraksa et al. Febrile neutropenia in children with acute leukemia . 2009;33:1-8.
42. Hakim H, Flynn PM, Knapp KM et al.Etiology and clinical course of febrile neutropenia in children with cancer. Journ of Peditr Hematol Oncol.2009;31:623-9.
43. Viscoli C, Varnier O, Machetti M. Infections in patients with febrile neutropenia: epidemiology, microbiology and risk stratification.Clin Infect Dis.2005; 40: 240-45.
44. M Timothy; C Bodkyn. The outcome of febrile neutropenic episodes in paediatric oncology at the Wendy Fitzwilliam Paediatric Hospital. West Indian med. j. 2011; 60 :240-48.

Comparison Between Newer Methods of Surfactant Administration and InSurE in Preterm Newborns with Respiratory Distress Syndrome on Non-invasive Respiratory Support: A Systematic Review and Meta-analysis

Dr Kamirul Islam

**Professor and Head, **Associate Professor, ***PGT(Pediatrics) Department of Paediatrics, Chittaranjan Seva Sadan, Kolkata*

Introduction: Surfactant administration is standard therapy for respiratory distress syndrome in preterm. Aims: A systematic review and meta-analysis of randomised controlled trials was conducted to compare newer methods of surfactant delivery with InSurE technique.

Methods: PubMed, Embase, CENTRAL, different trial registries, conference proceedings and bibliographies of selected articles were searched. Randomised controlled trials that compare any of newer methods of surfactant administration and InSurE in newborns with non-invasive respiratory support, were included in this review. Relative risk and risk difference for different outcome measures were calculated. The χ^2 test for homogeneity and I^2 statistic were used to measure heterogeneity. RevMan version 5.4 was used for data-analysis

Results: Thirteen trials were included in this review with 1642 newborns. Significant reduction of relative risk of death and/or bronchopulmonary dysplasia at 36 weeks post-menstrual age (RR=0.53, 95% CI=0.42-0.65, I^2=0%) and requirement of mechanical ventilation in first 72 hours of life (RR=0.61, 95% CI=0.50-0.74, I^2=0%) with newer methods of surfactant delivery. Significant risk difference was noted for both outcomes- death and/or bronchopulmonary dysplasia (RD=-0.12, 95% CI=-0.16 to -0.08, I^2=69%) and need for mechanical ventilation (RD=-0.12, 95% CI=-0.16 to -0.07, I^2=45%). Significantly less incidence of necrotizing enterocolitis was noted with newer methods of surfactant administration (RR=0.27, 95% CI=0.12-0.64) without any increase in other neonatal morbidity (pulmonary hemorrhage, retinopathy of prematurity etc) or adverse effects.

Conclusion: Newer methods of surfactant delivery were associated with less incidence of death and/or bronchopulmonary dysplasia and lesser requirement of mechanical ventilation without any significant increase in adverse effects/ neonatal morbidity.

Key-words: LISA, MIST, clinical trial, Curosurf, bronchopulmonary dysplasia

Introduction:

Preterm deliveries are increasingly becoming common in developing countries with discovery of modern techniques of in-vitro fertilization.1 The women of Indian subcontinent are also genetically predisposed to an increased risk of delivering preterm babies owing to interplay of different interleukins.2 Respiratory distress syndrome (RDS), the commonest respiratory complication seen in preterm, and is accountable for a major portion of neonatal morbidity and mortality.3 Deficiency of pulmonary surfactants (produced by type II pneumocytes) is responsible for RDS, seen especially in newborns aged <32 weeks. After introduction of surfactant in 1980, it remains the mainstay of treatment of RDS.4 Surfactant acts by reducing the surface tension and thereby preventing alveolar collapse and ventilation-perfusion mismatch. They also help to maintain the functional residual capacity of lungs by preventing alveolar collapse, and thereby help in continuation of continuous positive airway pressure (CPAP) without invasive ventilation. Surfactant is traditionally being administered by InSurE (Intubation, surfactant administration and extubation) approach, which requires endotracheal intubation and hence, may cause epithelial injury leading to bronchopulmonary dysplasia (BPD) in future.5 CPAP and surfactant have now emerged as

Correspondance : Dr Kamirul Islam, Medical Officer, *Burdwan Medical College and Hospital. Email kamirul.islam7@gmail.com*

the standard treatment approach for RDS. As a result some newer techniques of surfactant administration have also invented e.g. Less Invasive Surfactant Administration (LISA), SurE (Surfactant without Endotracheal Tube), Minimally Invasive Surfactant Therapy (MIST) etc.6,7 They use flexible or semi-rigid tubes (like feeding tube, vascular catheter etc according to preference of neonatologists) with or without Magill forceps for administration of surfactant directly into trachea.6,7 The proposed benefits are less lung injury and reduced requirements of mechanical ventilation. These benefits are documented in available literature. The newer methods of surfactant delivery are a promising approach of treatment of preterm newborns, and may become a very useful cost-effective treatment in developing countries like India, where burden of preterm birth is very high. Multiple trials are conducted in different parts of world to evaluate the efficacy of newer methods of surfactant delivery, and hence, we conducted a systematic review and meta-analysis of randomised controlled trials to compare the newer techniques of surfactant administration with the time-tested InSurE technique to accumulate better quality evidences.

Methods:

A literature search was conducted using key-words related to randomized controlled trials comparing pulmonary surfactant delivery by newer methods (LISA/MIST/SurE) with InSurE technique (detailed in Annexure I). The first search was conducted on 1st August, 2021 and second on 1st September, 2021.8 The search was conducted in Embase, PubMed and Cochrane central register of controlled trials (CENTRAL). Few clinical trial registries were also included (clinicaltrials.gov, http://www.who.int/ictrp/en, and ctri.nic.in). We also searched bibliographies of available trials and conference proceedings (especially different national and state conferences of Indian Academy of Pediatrics and National Neonatology Forum of India and their state branches) of last 10 years. Two authors conducted the literature search independently and assessed the eligibility of study. Randomised controlled trials that compare any of newer methods respiratory support and reported outcome as either Death or BPD or requirement of invasive ventilation, were included in this review. Discrepancies, if any, were resolved with consultation of third author. Two authors independently extracted data and compiled it in RevMan5.4 [The Cochrane Collaboration Review Manager 5 (RevMan 5).Version 5.4. Copenhagen: The Cochrane Collaboration, 2020.] and double-checked for accuracy. Discrepancies were again resolved by consulting third author. Two authors independently assessed the methodological qualities of the studies using 'Cochrane risk of bias tool'9 in following domains-

(a) Generation of random sequence

(b) Allocation concealment

(c) Blinding

(d) Incomplete and selective reporting

(e) Other bias

Discrepancies were resolved by discussing with third author. We considered the following as outcome measures-

Primary outcome:

- A composite outcome of either death or BPD at 36 weeks of post-menstrual age
- Requirement of invasive ventilator support in first 3 days of life

Secondary outcome:

- Requirement of 2nd dose of surfactant
- Surfactant reflux
- Adverse events during surfactant administration (Bradycardia/ Desaturation)
- Necrotising enterocolitis(NEC) (=II according to modified Bell's staging)10
- Hemodynamically significant patent ductus arteriosus (HSPDA)
- Retinopathy of prematurity (ROP) (Stage>2)
- Pulmonary hemorrhage
- Pulmonary air leak syndrome
- Intraventricular hemorrhage (IVH) (grade III/ IV)11

Statistical Analysis:

The studies were pooled together using a random effect model to calculate risk ratio with 95% confidence interval (95% CI). In addition risk difference was also calculated for primary outcome measures. For significant risk difference, number needed to treat for an additional beneficial outcome was also calculated. The χ^2 test for homogeneity and I^2 statistic were used to measure heterogeneity. As the χ^2 test has low power, we consider $P<0.1$ as significant. For all other purpose, $P<0.05$ was taken as the level of statistical significance. Sensitivity analysis was only conducted if there is significant heterogeneity. Publication bias was assessed by visually inspecting the funnel plot for asymmetry. RevMan version 5.4 was used for data analysis.

Results: We identified 6449 records through electronic searching and 18 records were found from manual searching. After removal of 1571 duplicate records, we examined the title and abstract of 4896 articles. After inspecting the full-text of 31 articles, 13 articles were included in this review for synthesis of data (for detail, please see figure I). Features of included studies[12-24] are summarized in table II. Majority of them were conducted Middle-East Asia (38.4%). Curosurf was the most commonly used surfactant (84.6%). Studies that do not report outcome of interest, or compared different newer methods of surfactant administration or included newborns with invasive respiratory support were excluded.[25-28] In five studies random sequence generation was not properly mentioned.[14, 16-18, 20] Unclear biases due to allocation concealment was present in few studies. [14, 17-20] Blinding was mentioned in two studies.[23,24] Study protocol was not found for 3 studies[18-20] and these trials were not registered also. Few trials were retrospectively registered.[13,15] Two multicentric trails with no standardization of treatment and logistic regression were found.[16,21] Flexible inclusion criteria were used.[13] Risk of bias is summarized in figure IIa and IIb.

Meta-analysis showed significant reduction of relative risk of death and/or BPD at 36 weeks PMA with newer methods of surfactant delivery (RR=0.53, 95% CI=0.42-0.65) [Figure IIIa and IIIb]. Similarly, relative risk of requirement of mechanical ventilation in first 72 hours of life was also significantly reduced (RR=0.61, 95% CI=0.50-0.74) [Figure Iva and IVb]. As heterogeneity among the studies was low (I^2=0%), we did not conduct any sensitivity analysis. There was significant risk difference for both the composite outcome of death and/or BPD (RD=-0.12, 95% CI=-0.16 to -0.08, I2=69%) and need for mechanical ventilation (RD=-0.12, 95% CI=-0.16 to -0.07, I2=45%). Number needed to treat for beneficial outcome in one patient was 9 (95% CI=6.4-13.0) and 7 (95% CI=5.2-10.4), for the outcome of death and/or BPD and requirement of mechanical ventilation in first 72 hours of life, respectively.

Significantly less incidence of NEC was noted with newer methods of surfactant administration (RR=0.27, 95% CI: 0.12-0.64, P=0.003, I^2=0%). The meta-analysis also demonstrated reduced incidence of pulmonary hemorrhage (RR=0.65), pulmonary air leak syndrome (RR=0.65), ROP (RR=0.79), HSPDA (RR=0.89), and IVH (RR=0.79); but, this reduction was not statistically significant. Non-significant increase of requirement of 2nd dose of surfactant (RR=1.05) and reflux of surfactant (RR=1.62) were also observed. Variable amount of heterogeneity (I2=0-73%) was also noted.

Discussion: In this systematic review, we found that use of newer methods of surfactant delivery decrease the risk of death and/or BPD at 36 week PMA and need of mechanical ventilation. The findings are consistent with majority of previous reports.[29-32] Discrepancy with the findings of More K et al could easily be explained by inclusion of multiple new trials in our review.[15-24, 33] As these methods of surfactant delivery allows to continue CPAP uninterruptedly, loss of functional residual capacity is prevented (by preventing alveolar collapse) and better tissue deposition and incorporation of surfactant could be achieved.[34,35] Lung injury due to mechanical breaths used to deliver surfactant in InSurE technique may also be prevented in newer techniques.[36] Incidence of adverse events

(bradycardia/desaturation), reflux of surfactant, and requirement of 2nd dose of surfactant were comparable between both the groups. But Curosurf was used in majority of our trials. Reflux could better be assessed with bovine surfactants (required amount is larger). Premedication used while administering surfactants by newer techniques might also be responsible for less incidence of bradycardia.16 Reduced incidence of NEC with newer techniques is contrary to previous reports.29 Better tissue oxygenation in newer surfactant delivery techniques could be a possible explanation.37 Similar to the findings of Wu W et al, other neonatal morbidities (e.g. ROP, PDA, pulmonary hemorrhage, IVH and pulmonary air leak syndrome) did not significantly increase with the use of newer techniques. Though a reduced incidence of IVH was reported, they included mechanically ventilated (invasive) newborns in their study.29 Heterogeneity and publication bias is low in this review.

We restricted our analysis to surfactant delivery by flexible/ semi-rigid catheter only. Other modern methods of surfactant delivery (nebulisation, delivery by laryngeal mask airway) could not be checked due to limited literature and difficulty in implementing Indian settings.38,39 The trials mainly included relatively mature preterms with less proportion of extreme prematurity which may interfere with external validity. A recent trial included extremely premature infants may throw light on some unanswered questioned.40 Curosurf was used in maximum number of trials (>80%). Data regarding long-term safety were also unavailable.

Conclusion:

The risk of death and/or BPD and need of mechanical ventilation were decreased with the use of newer techniques of surfactant administration without any significant adverse effect/ associated neonatal morbidity. So, these techniques could be used in our daily practice, but proper training and standardization of delivery technique and premedication should be ensured. Further multi-centric research should be undertaken to overcome the limitations of this study.

Table I: Summary of included studies

Study	Country & Surfactant	Criteria for Surfactant	Newer Methods	InSurE Group	Remarks
Göpel W *et al*[12] (2011)	Germany, Curosurf, 100 mg/kg	Required FiO_2>30%	N=108 GA-27.6±0.8 wk BW-0.98±0.24 kg	N=112 GA-27.5±0.8 wk BW-0.94±0.20 kg	Multi-centric, 2.5-5 Fr catheter, Magill forceps, Premedication in some newborn
Kanmaz HG *et al*[13] (2013)	Turkey, Curosurf, 100 mg/kg	CPAP with FiO_2>40%	N=100 GA-28±2 wk BW-1.09±0.27 kg	N=100 GA-28.3±2 wk BW-1.12±0.27	Single centre, 6 Fr feeding tube
Mirnia K *et al*[14] (2013)	Iran Curosurf 200 mg/kg	FiO2>30%	N=66 GA-29.6±1.7 wk BW-1.34±0.41 kg	N=70 GA-29.6±1.7 wk BW-1.30±0.33 kg	5 Fr Feeding tube, Atropine premedication
Bao Y *et al*[15] (2015)	China Curosurf 200 mg/kg	FiO_2>30% in newborn with CPAP	N=47 GA-29.1±1.5 week BW-1.03±0.22 kg LISA	N=43 GA-29.3±1.6 week BW-1.09±0.20 kg	Single-centre, 16 gauge catheter for surfactant
Mohammadizadeh M *et al*[16] (2015)	Iran Curosurf 200 mg/kg	FiO_2>30% with CPAP or Silverman-Anderson score>5	N=19 GA-30±2 wk BW-1.28±0.22 kg	N=19 GA-31±2 wk BW-1.43±0.27 kg	Multi-centre (13 NICU), 4 Fr catheter and Magill Forceps Premedication

Contd..

Table 1:Summary of included studies (contd.)

Study	Country & Surfactant	Criteria for Surfactant	Newer Methods	InSurE Group	Remarks
Mosayebi Z *et al*[17] (2017)	Iran, Curosurf, 200 mg/kg	CPAP requiring FiO2>40%	N=27 GA-31.9±1.5 wk BW-1.91±0.43 kg	N=26 GA-32.6±1.1 wk BW-1.79±0.55 kg	Single-centre, 5 Fr feeding tube
Choupani R *et al*[18] (2018)	Iran Curosurf 200 mg/kg	FiO2>40% in CPAP	N=52 GA-32.9±2.6 wk BW-1.94±0.55 kg	N=52 GA-33.1±2.3 wk BW-2.07±0.57 kg	5 Fr vascular catheter
Halim A *et al*[19] (2019)	Pakistan Survanta 100 mg/kg	FiO2>40% in CPAP	N=50 GA-31.1 (1.7) wk BW-1.3(0.6) kg	N=50 GA-1.4 (0.4)kg BW-30.9 (1.5) wk	6 Fr feeding tube, Not registered with trial registry
Boskabadi H *et al*[20] (2019)	Iran Curosurf 200 mg/kg	FiO2>40% in CPAP	N=20 GA-29.1±2.6 wk BW-1.28±0.31 kg	N=20 28.2±2.1 wk 1.23±0.22 kg	5 Fr feeding tube, Not registered with trial registry
Jena SR *et al*[1] (2019)	India Neosurf 135 mg/kg	FiO2>30% in CPAP	N=175 GA-31 (4)wk BW-1.63 (0.83) kg	N=175 GA-31 (4)wk BW-1.68 (0.72) kg	16 Fr Angiocath/ 6 Fr feeding tube, Multicentric trial
Gupta BK *et al*[2]	India Curosurf 200 mg/kg	FiO2>30% in NIPPV	N=29 GA- 30.0 ±1.9 wk BW-1.22±0.28 kg	N=29 29.9±1.7 wk BW-1.22±0.32 kg	5 Fr feeding tube with Magill Forceps
Yang G *et al*[23] (2020)	China Curosurf 200 mg/kg	FiO2>40%	N=47 GA-33.7±1.0 wk BW-2.11±0.32 kg	N=50 3GA-4.1±1.3 wk BW-2.22±0.31 kg	6 Fr gastric tube
Pal J *et al*[24]	India Curosurf 100-200 mg/kg	Required FiO2>40% in CPAP	N=78 GA-32.3 (1.7) kg BW-1.40 (0.19) kg	N=78 GA-32.2 (1.5) BW-1.38 (0.18) kg	6 Fr feeding tube Sample size less

GA- Gestational age, BW-Birth weight, NIPPV- Non-invasive positive pressure ventilation, Mean±SD, Median (IQR)

Table 2:Secondary outcomes

Secondary Outcome	Newer Techniques (event/N)	InSurE (event/N)	Effect Estimate Risk Ratio (95% CI)	I^2
Requirement of 2nd dose of surfactant	78/474	75/473	1.05(0.79-1.39)	0%
Surfactant reflux	16/140	10/146	1.62 (0.78-3.36)	0%
Adverse event (Bradycardia and/or Desaturation)	52/412	57/418	0.92 (0.66-1.29)	73%
Pulmonary Hemorrhage	14/357	22/360	0.65 (0.34-1.22)	0%
Pulmonary Air Leak Syndrome	14/438	22/445	0.65 (0.34-1.24)	0%
ROP (Stage>2)	13/373	17/379	0.79 (0.39-1.58)	20%
Hemodynamically significant patent ductus arteriosus	86/575	96/583	0.89 (0.70-1.13)	0%
Intraventricular hemorrhage (grade III/ IV)	29/675	37/678	0.79 (0.50-1.26)	0%
Necrotizing Enterocolitis (Grade≥ II)	5/501	23/511	0.27 (0.12-0.64)*	0%

*Statistically significant

Figure I: Study flow-diagram

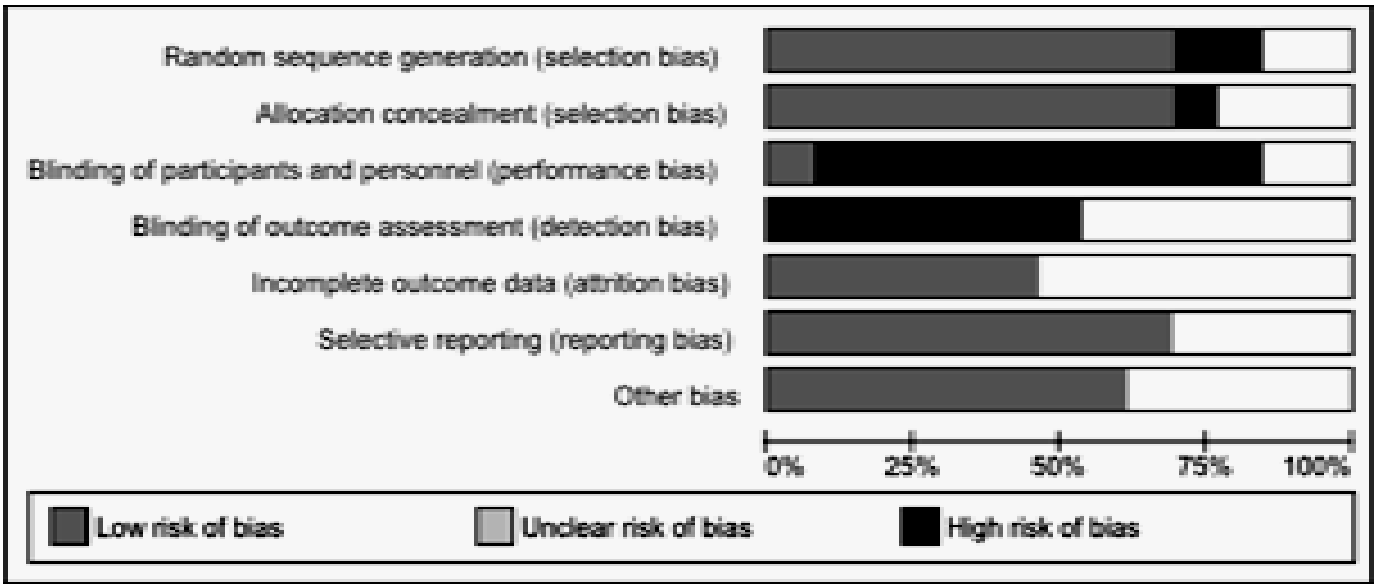


Figure IIa: Risk of bias graph

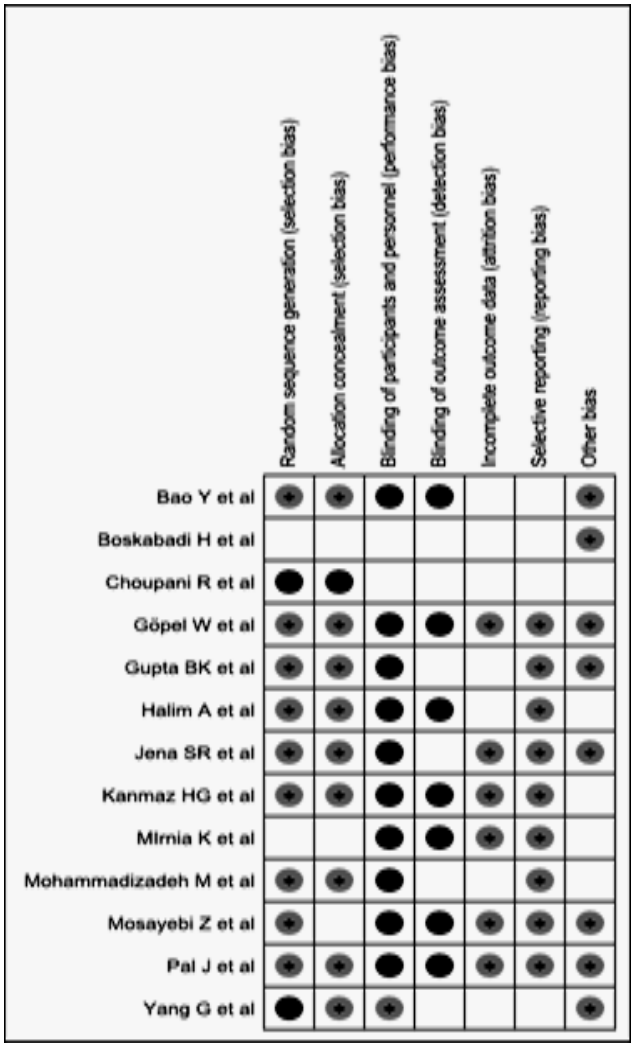


Figure IIb: Risk of bias summary

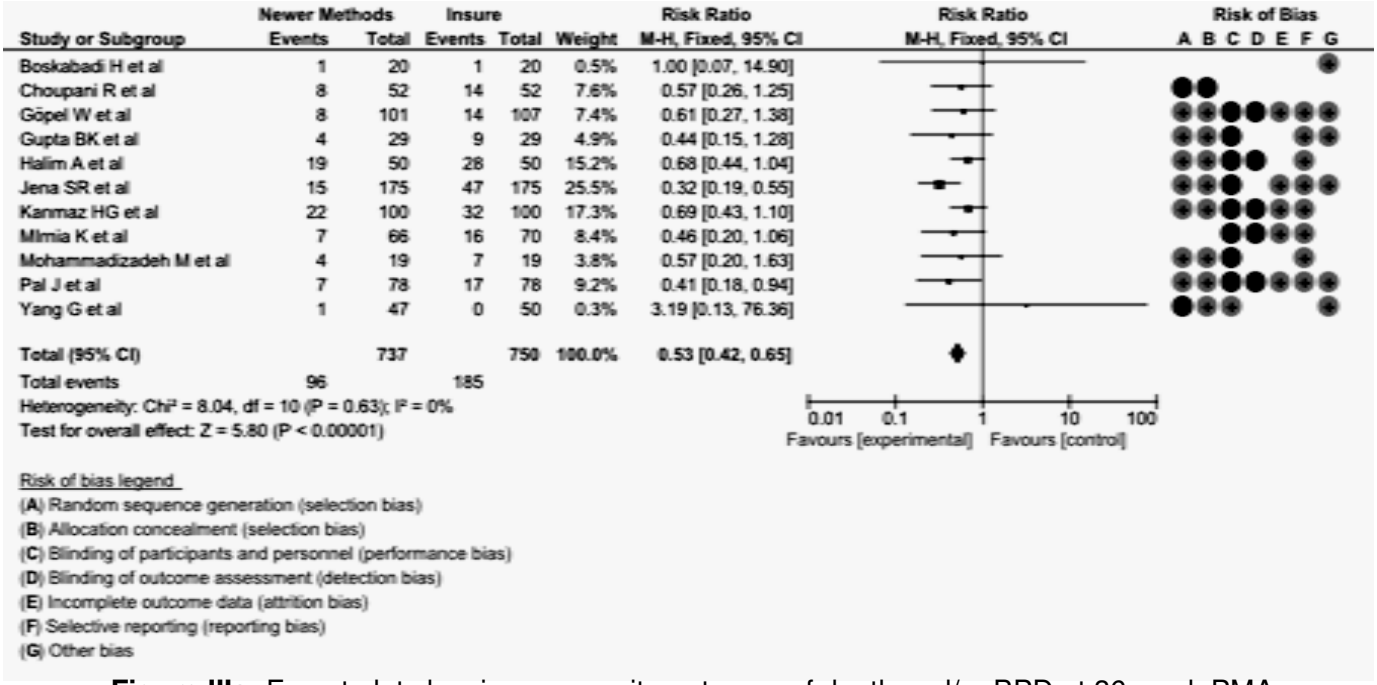


Figure IIIa: Forest plot showing composite outcome of death and/or BPD at 36 week PMA

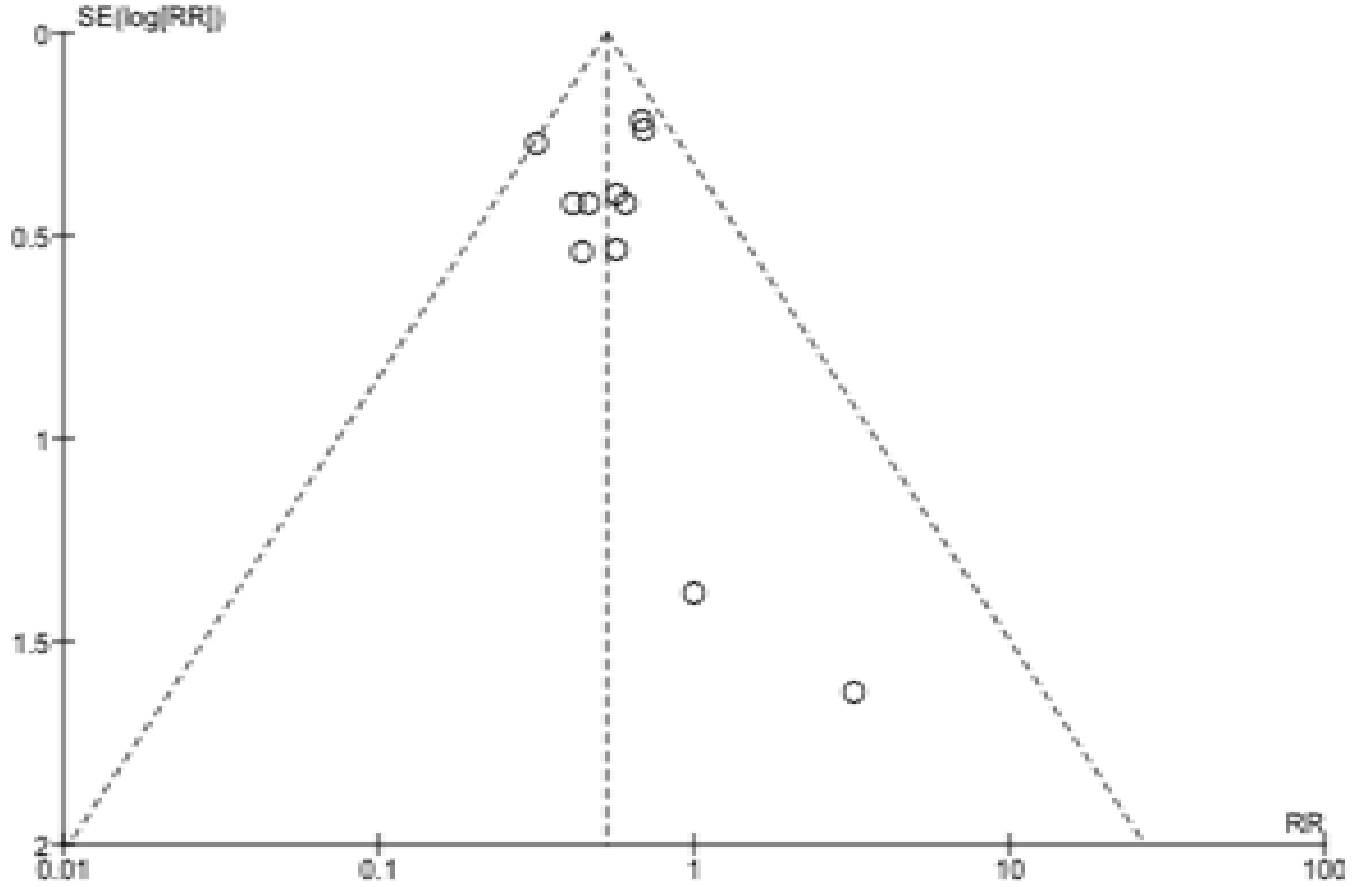


Figure IIIb: Funnel plot for comparison of death and/or BPD

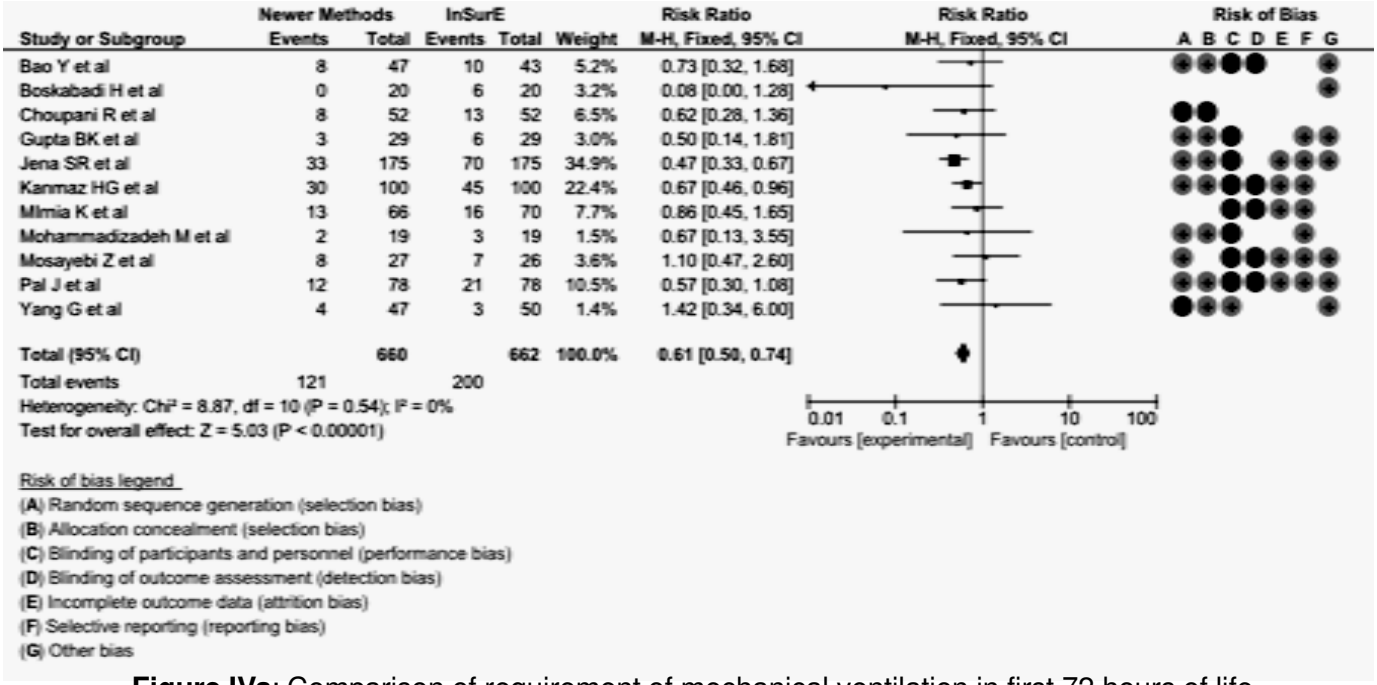


Figure IVa: Comparison of requirement of mechanical ventilation in first 72 hours of life

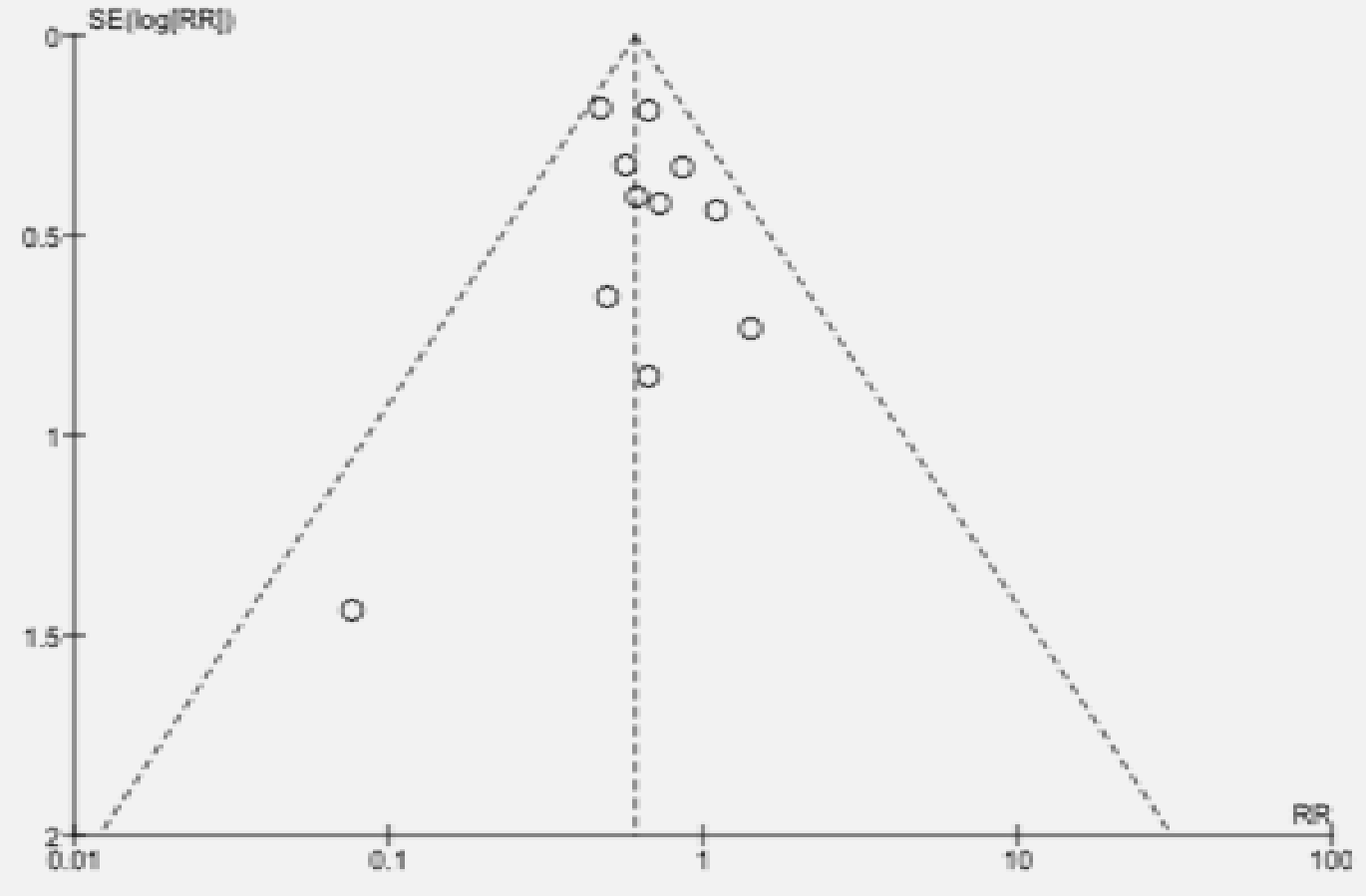


Figure IVb: Funnel plot for comparison of requirement of mechanical ventilation in first 72 hours of life

References

1. Craciunas L, Gallos I, Chu J, et al. Conventional and modern markers of endometrial receptivity: a systematic review and meta-analysis. Hum Reprod Update. 2019;25(2):202-223.
2. Pandey M, Chauhan M, Awasthi S. Interplay of cytokines in preterm birth. Indian J Med Res. 2017;146(3):316-327.
3. Avery ME, Mead J. Surface properties in relation to atelectasis and hyaline membrane disease. AMA Journal of Diseases of Children 1959;97(5 Part 1):517-23.
4. Fujiwara T, Chida S, Watabe Y, Maeta H, Morita T, Abe T.Artifcial surfactant therapy in hyaline membrane disease. Lancet 1980;315:55 9
5. Suresh GK, Soll RG, Goldsmith JP, Karotkin EH. Pharmacologic Adjuncts in Assisted Ventilation of the Neonate. 5th ed. St. Louis:Elsevier Saunders; 2011. p. 375.
6. Herting E, Härtel C, Göpel W. Less invasive surfactant administration (LISA): chances and limitations. Arch Dis Child Fetal Neonatal Ed. 2019;104(6):F655-F659.
7. Shim GH. Update of minimally invasive surfactant therapy. Korean J Pediatr. 2017;60(9):273-281.
8. Higgins JPT, Thomas J, Chandler J, Cumpston M, Li T, Page MJ,et al (editors). Cochrane Handbook for Systematic Reviews of Interventions version 6.0 (updated July 2019). Cochrane, 2019. Available from www.training.cochrane.org/handbook.
9. Higgins JP, Altman DG, Sterne JA, on behalf of the Cochrane Statistical Methods Group and the Cochrane Bias Methods Group. Chapter 8. Assessing risk of bias in included studies. In: Higgins JP, Green S, editor(s). Cochrane Handbook for Systematic Reviews of Interventions Version 5.1.0 (updated March 2011). The Cochrane Collaboration, 2011. Available from handbook.cochrane.org.
10. Papile LA, Burstein J, Burstein R, Koffler H. Incidence and evolution of subependymal and intraventricular hemorrhage: a study of infants with birth weights less than 1,500 gm. J Pediatr. 1978;92(4):529-534.
11. Bell MJ, Ternberg JL, Feigin RD, et al. Neonatal necrotizing enterocolitis. Therapeutic decisions based upon clinical staging. Ann Surg. 1978;187(1):1-7.
12. Göpel W, Kribs A, Ziegler A, Laux R, Hoehn T, Wieg C, et al. German Neonatal Network. Avoidance of mechanical ventilation by surfactant treatment of spontaneously breathing preterm infants: an open-label, randomised, controlled trial (AMV Trial). Lancet 2011;378(9803):1727-34.
13. Kanmaz HG, Erdeve O, Canpolat FE, Mutlu B, Dilmen U. Surfactant administration via thin catheter during spontaneous breathing: randomized controlled trial. Pediatrics. 2013;131(2):e502-e509.
14. Mirnia K, Heidarzadeh M, Hosseini MB, Sadeghnia A, Balila M, Ghojazadeh M. Comparison outcome of surfactant administration via tracheal catheterization during spontaneous breathing with INSURE. Medical Journal of Islamic World Academy of Sciences 2013;21(4):143-8.
15. Bao Y, Zhang G, Wu M, Ma L, Zhu J. A pilot study of less invasive surfactant administration in very preterm infants in a Chinese tertiary center. BMC Pediatr. 2015;15:21.
16. Mohammadizadeh M, Ardestani AG, Sadeghnia AR. Early administration of surfactant via a thin intratracheal catheter in preterm infants with respiratory distress syndrome: Feasibility and outcome. J Res Pharm Pract. 2015;4(1):31-36.
17. Mosayebi Z, Kadivar M, Taheri-Derakhsh N, Nariman S, Mahdi Marash Si, Farsi Z. A randomized trial comparing surfactant administration using InSurE technique and the minimally invasive surfactant therapy in preterm infants (28 to 34 weeks of gestation) with respiratory distress syndrome. Journal of Comprehensive Pediatrics 2017;8(4):e60724.
18. Choupani R, Mashayekhy G, Hmidi M, Kheiri S, Khalili Dehkordi M. A comparative study of the efficacy of surfactant administration through a thin intratracheal catheter and its administration via an endotracheal tube in neonatal respiratory distress syndrome. Iranian Journal of Neonatology 2018;9(4):33-40.
19. Halim A, Shirazi H, Riaz S, Gul SS, Ali W. Less Invasive Surfactant Administration in Preterm Infants with Respiratory Distress Syndrome. J Coll Physicians Surg Pak. 2019;29(3):226-330.
20. Boskabadi H, Maamouri G, Gharaei Jomeh R, Zakerihamidi M. Comparative study of the eJect of the administration of surfactant through a thin endotracheal catheter into trachea during spontaneous breathing with intubation (intubation, surfactant-extubation method). Journal of Clinical Neonatology 2019;8(4):227-31.
21. Jena SR, Bains HS, Pandita A, et al. Surfactant therapy in premature babies: SurE or InSurE. Pediatr Pulmonol. 2019;54(11):1747-1752.
22. Gupta BK, Saha AK, Mukherjee S, Saha B. Minimally invasive surfactant therapy versus InSurE in preterm neonates of 28 to 34 weeks with respiratory distress syndrome on non-invasive positive pressure ventilation-a randomized controlled trial. Eur J Pediatr. 2020;179(8):1287-1293.
23. Yang G, Hei M, Xue Z, Zhao Y, Zhang X, Wang C. Effects of less invasive surfactant administration (LISA) via a gastric tube on the treatment of respiratory distress syndrome in premature infants aged 32 to 36 weeks. Medicine (Baltimore). 2020;99(9):e19216.
24. Pal J, Ghosh T, Konar MC, et al. Comparison of minimally invasive surfactant therapy with InSurE in preterm newborns. Presented at: WBPEDICON,2020; Hooghly.
25. Han T, Liu H, Zhang H, Guo M, Zhang X, Duan Y, et al. Minimally invasive surfactant administration for the treatment of neonatal respiratory distress syndrome: a multicenter randomized study in China. Frontiers in Pediatrics 2020;8(182):1-12.
26. Oncel MY, Arayici S, Uras N, Alyamac-Dizdar E, Sari FN, Karahan S, et al. Nasal continuous positive airway pressure versus nasal intermittent positive-pressure ventilation within the minimally invasive surfactant therapy approach in preterm infants: a randomised controlled trial.

Archives of Disease in Childhood. Fetal Neonatal Edition 2016;101(4):F323-8.

27. Olivier F, Nadeau S, Bélanger S, Julien AS, Massé E, Ali N, et al. Efficacy of minimally invasive surfactant therapy in moderate and late preterm infants: a multicentre randomized control trial. Paediatrics & Child Health 2017;22(3):120-4.
28. Kribs A, Roll C, Göpel W, Wieg C, Groneck P, Laux R, Teig N, et al. NINSAPP Trial Investigators. Nonintubated surfactant application vs conventional therapy in extremely preterm infants a randomized clinical trial. JAMA Pediatrics 2015;169(8):723-30.
29. Abdel-Latif ME, Davis PG, Wheeler KI, De Paoli AG, Dargaville PA. Surfactant therapy via thin catheter in preterm infants with or at risk of respiratory distress syndrome. Cochrane Database Syst Rev. 2021;5(5):CD011672.
30. Aldana-Aguirre JC, Pinto M, Featherstone RM, Kumar M. Less invasive surfactant administration versus intubation for surfactant delivery in preterm infants with respiratory distress syndrome: a systematic review and meta-analysis. Arch Dis Child Fetal Neonatal Ed. 2017;102(1):F17-F23.
31. Göpel W, Kribs A, Härtel C, et al. Less invasive surfactant administration is associated with improved pulmonary outcomes in spontaneously breathing preterm infants. Acta Paediatr. 2015;104(3):241-246.
32. Wu W, Shi Y, Li F, Wen Z, Liu H. Surfactant administration via a thin endotracheal catheter during spontaneous breathing in preterm infants. Pediatr Pulmonol. 2017;52(6):844-854.
33. More K, Sakhuja P, Shah PS. Minimally invasive surfactant administration in preterm infants: a meta-narrative review. JAMA Pediatr 2014;168:901–8
34. Sinclair SE, Chi E, Lin HI, Altemeier WA. Positive end-expiratory pressure alters the severity and spatial heterogeneity of ventilator-induced lung injury: an argument for cyclical airway collapse. J Crit Care. 2009;24(2):206-211.
35. Bohlin K, Bouhafs RK, Jarstrand C, Curstedt T, Blennow M, Robertson B. Spontaneous breathing or mechanical ventilation alters lung compliance and tissue association of exogenous surfactant in preterm newborn rabbits. Pediatr Res. 2005;57(5 Pt 1):624-630.
36. Björklund LJ, Ingimarsson J, Curstedt T, et al. Manual ventilation with a few large breaths at birth compromises the therapeutic effect of subsequent surfactant replacement in immature lambs. Pediatr Res. 1997;42(3):348-355.
37. Meister AL, Doheny KK, Travagli RA. Necrotizing enterocolitis: It's not all in the gut. Exp Biol Med (Maywood). 2020;245(2):85-95.
38. Abdel-Latif ME, Osborn DA. Nebulised surfactant in preterm infants with or at risk of respiratory distress syndrome. Cochrane Database Syst Rev. 2012;10:CD008310.
39. Abdel-Latif ME, Osborn DA. Laryngeal mask airway surfactant administration for prevention of morbidity and mortality in preterm infants with or at risk of respiratory distress syndrome. Cochrane Database Syst Rev. 2011;(7):CD008309.
40. Dargaville PA, Kamlin COF, De Paoli AG, Carlin JB, Orsini F, Soll RF, et al. The OPTIMIST-A trial: evaluation of minimally-invasive surfactant therapy in preterm infants 25–28 weeks gestation. BMC Pediatr 2014; 14:213.

Netherton Syndrome: Multisystem Involvement in Infancy

***Dr Debangshu Baidya, **Dr Balai Chandra Karmakar, ***Prof Kalpana Datta**
**Junior resident, **Associate Professor, ***Professor & Head*
Department of Pediatrics, Medical College Kolkata

Netherton Syndrome (NS) is a rare disorder with autosomal recessive mode of inheritance. It is characterized by the triad of congenital ichthyosiform erythroderma, trichorrhexisinvaginata (bamboo hair), and an atopic dermatitis-like picture with elevated levels of immunoglobulin E (IgE). Infants are usually born with ichthyosiform erythroderma, and typically have immune abnormalities with recurrent and sometimes, life-threatening infections, and hypernatremic dehydration. It may take 18 months for hair shaft changes to appear. We report a 2 months old male infant who has been diagnosed with NS and one of the rare cases so far presenting not only dermatological but with multisystem involvement.

Keywords- *Infancy, Netherton Syndrome, multisystem involvement, psoriatic rash*

Introduction

Netherton syndrome (NS) is a rare genodermatosis with an autosomal recessive pattern of inheritance caused by pathogenic variants in the SPINK5 gene, with an incidence of 1 case per 200,000 newborns [1,2,3]. NS may lead to significant mortality in the first years of life due to potentially fatal complications. Skin and hair defects persist throughout life, but the severity of the disorder usually subsides with age [4].

NS is caused by mutations in the gene SPINK5 on chromosome 5q32, which mainly induce premature stop codons, thereby forming a truncated LEKTI protein and overexpression of three kallikrein (KLK)-related peptidases, KLK5, KLK7, and KLK14. This results in corneodesmosome cleavage thus enhancing the loss of superficial corneocyte layer, abnormal keratinization by enhancing degradation of profilaggrin and lipid processing enzymes (ß-glucocerebrosidase and acid sphingomyelinase) [5], and psoriasis-like inflammation [6].

Case Report

A 2-month-old Muslim male infant born out of non-consanguineous marriagepresented with fever for one month duration, exfoliation of skin since day 4 of birth. He was born at term by LUCS with birth weight 2.5 kg and admitted at SNCU on Day 2 of life due to redness of skin and irritability. Subsequently he developed neonatal jaundice and sepsis, and was treated conservatively.

The skin lesions, initially looked like Seborrheic Dermatitis and was treated with emollients, Hydrocortisone cream and white soft paraffin cream. The infant received a short course of Prednisolone for 5 days which lead to temporary resolution of the lesions but fever, swelling of the body with reappearance of exfoliation of the skin appeared 2 days after discontinuation of stoppage of steroid. Then he developed high grade fever, persistent in nature, with no diurnal variation, not associated with cough and cold, loose stools, crying after feeding or

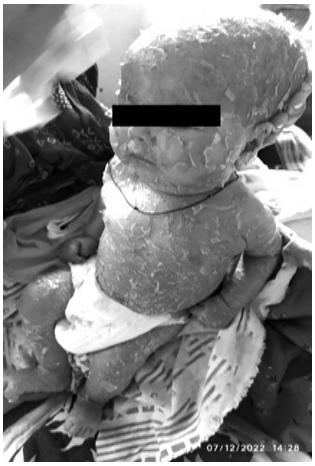


Fig 1: Ichthyosiform Erythroderma in Netherton Syndrome

Correspondance : Prof Kalpna Datta, *Professor, Department of Pediatrics, Medical College Kolkata Email : drkalpanadatta@gmail.com.*

during micturition, or convulsions, but fever did not subside despite treatment. Swelling of the body, with no specific pattern of progression, was not associated with decreased appetite, or decreased urine output, yellowish discolouration of the body or urine, also did not subside after treatment.

As the condition of the patient get deteriorated, he was referred to higher center for evaluation where

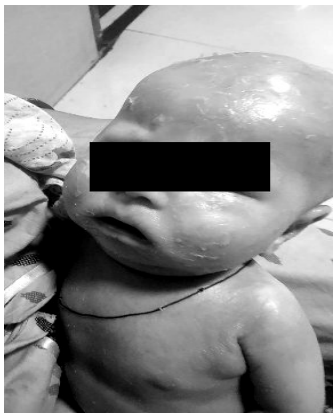
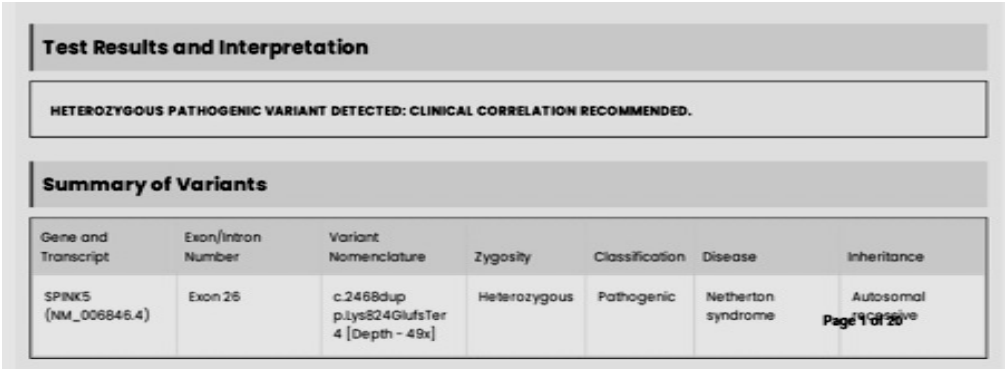


Fig 2: Edema in Netherton Syndrome

he was assessed to have generalized scaly dermatitis. Anthropometry was normal for age. General Examination revealed sparse and brittle scalp hair with near absence of eyebrows and eyelashes. Systemic Examinations revealed no abnormalities. While the initial blood reports revealed anemia (Hb= 8.5), high total leucocyte counts (16,950/cmm, Neutrophil= 60% and Lymphocytes= 36%), no eosinophilia (3%) and high CRP levels (57.6 mg/dl). Fever works up for malaria, dengue, typhoid fever and scrub typhus was negative. The albumin level was low (2.6 g/dl), and along with generalized swelling of the body and falling levels of hemoglobin over the next 3 days (6 g/dl after 3 days) and was treated with albumin and PRBC infusion. USG of the lungs and Echocardiography revealed no abnormalities, but Respiratory Viral Panel was positive for Respiratory Syncytial Virus B. Culture of the CSF study was positive for Enterococcus species, sensitive to Levofloxacin and Vancomycin, and urine culture was positive for E.coli, sensitive to Fosfomycin. The congenital red and scaly rash, atopic dermatitis like presentation, sparse and brittle hair, malnutrition and multiple site infections, prompted us to send immunoglobulin panel, which revealed raised IgE (960 IU/ml) and IgG (644 g/L) raising our suspicion as the case to be Netherton Syndrome. Whole Exome Sequencing reports confirmed the diagnosis as Netherton Syndrome.

Discussion

Netherton Syndrome is not easily diagnosed in infancy, and often confused with atopic dermatitis, psoriatic erythroderma or collodion baby. In those reported during infancy so far, all of them have varied presentation. In a study of 43 babies with Netherton syndrome, half suffered dehydration with high sodium levels in their blood. Two-thirds were underweight, a quarter had cow's milk allergy and a quarter had diarrhoea. Infections were common: two-thirds had skin infections, others had lung, kidney or heart infections and 42% developed septicemia (blood infection). Some unusual hormone problems occurred. Four babies died in the first 9 months, usually with infection but three also had very high sodium levels [7]. In the case of the infant being studied here, there is no evidence of hypernatremia or dehydration, no eosinophilia, and growth of the infant was adequate. He however has fatal multisystem involvement, involving the respiratory infection, sepsis, urinary tract infection and even meningitis, in addition to the skin involvement. The infections were not caused by a single organism, and had delayed response to treatment. The choice of antibiotics was a challenge due to the varying sensitivity patterns. In NS, prompt treatment with appropriate antibiotics is critical for management [8].



Test Results and Interpretation

HETEROZYGOUS PATHOGENIC VARIANT DETECTED: CLINICAL CORRELATION RECOMMENDED.

Summary of Variants

Gene and Transcript	Exon/Intron Number	Variant Nomenclature	Zygosity	Classification	Disease	Inheritance
SPINK5 (NM_006846.4)	Exon 26	c.2468dup p.Lys824GlufsTer 4 [Depth - 49x]	Heterozygous	Pathogenic	Netherton syndrome	Autosomal recessive

Page 1 of 20

Fig 3: Whole Exome Sequencing report of patient suggestive of Netherton Syndrome

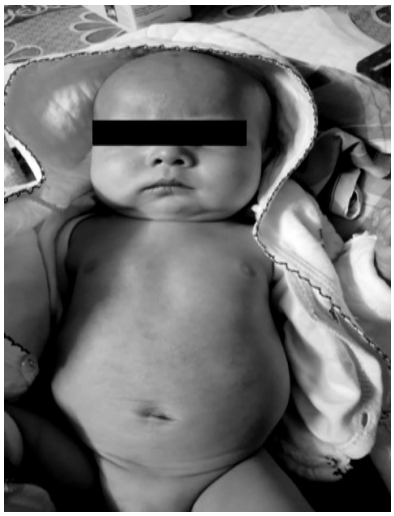


Fig 1: Infant with NS after adequate treatment with antibiotics

The infant studied here suffered from respiratory distress, which was well managed with moist oxygen inhalation via nasal prongs and antibiotics. However, a few cases of NS have led to neonatal respiratory insufficiency and pulmonary hypertension. Macknet et al. [9] reported an infant who received extracorporeal membrane oxygenation treatment for persistent pulmonary hypertension that was probably secondary to bronchopneumonia due to the thickness of the amniotic fluid enclosing exfoliated epidermal cells. Okulu et al. [10] described a newborn who required invasive respiratory support, surfactant and bronchodilators due to respiratory insufficiency and mild pulmonary hypertension.

This infant suffered from hypoalbuminemia inspite of adequate feeding, which was again, treated adequately. They run a risk of dehydration which may lead to severe hypernatremia that needs prompt treatment because it can result in a fatal outcome [11]. Neurologic signs and symptoms could have developed due to the toxic effects of hypernatremia, which were reported in a few cases of NS [12]. Pohl et al. [13] reported a case of acute bilateral renal vein thrombosis as a complication of hemoconcentration. Prompt treatment of the infant in our case had prevented development of such complications.

Conclusion

Netherton Syndrome is rare genetic multisystem disease with varied presentation but no specific treatment modality. The prognosis the infants is guarded as many succumb from severe sepsis or neurological complications due to hypernatremia or severe respiratory distress. Extensive work-up of the cases reported so far with multidisciplinary approach involving pediatricians, dermatologists, immunologist and allergologist is necessary to determine the pathophysiology, which would guide us to formulate a suitable treatment guideline.

References

1. Mallory SB, Krafchik BR. What syndrome is this? Pediatr Dermatol. (1992) 2:157–60. 10.1111/j.1525-1470. 1992.tb01231.x
2. Sun JD, Linden KG. Netherton syndrome: a case report and review of the literature. Int J Dermatol. 2006 Jun;45(6):693–7.
3. Roda Â, Mendonça-Sanches M, Travassos AR, Soares-de-Almeida L, Metze D. Infliximab therapy for Netherton syndrome: a case report. JAAD Case Rep. 2017 Nov;3(6):550–2.
4. Orphanet: Comel Netherton Syndrome (2008). Available online at: https://www.orpha.net/consor/cgi-bin/Disease_Search.php?lng=EN&data_id= 938 & Disease_Disease_Search_ diseaseType= ORPHA & Disease_Disease_Search _diseaseGr oup=634&Disease(s)/group% 20of%20 diseases= Comel-Netherton-syndrome &title= Comel- Netherton-syndrome &search= Disease_Search _Simple (accessed December 10, 2022)
5. M. Kishibe. Physiological and pathological roles of kallikrein-related peptidases in the epidermis. J Dermatol Sci, 95 (2019), pp. 50-55.
6. S. Leclerc-Mercier, C. Bodemer, L. Furio, S. Hadj-Rabia, L. de Peufeilhoux, L. Weibel, et al. Skin biopsy in netherton syndrome: A histological review of a large series and new findings. Am J DermPathol, 38 (2016), pp. 83-91.
7. Bellon et al. Br J Dermatol 2021; 184:532–537.
8. Stryk S, Siegfried EC, Knutsen AP. Selective antibody deficiency to bacterial polysaccharide antigens in patients with Netherton syndrome. Pediatr Dermatol. (1999) 16:19–22. doi: 10.1046/j.1525-1470.1999. 99005.x
9. Macknet CA, Morkos A, Job L, Garberoglio MC, Clark RD, Macknet KD, et al. An infant with Netherton syndrome and persistent pulmonary hypertension requiring extracorporeal membrane oxygenation. Pediatr Dermatol. (2008) 25:368–72. doi: 10.1111/j.1525-1470.2008. 00685.x
10. Okulu E, Tunc G, Erdeve O, Mumcu Y, Atasay B, Ince E, et al. Netherton syndrome: a neonatal case with respiratory insufficiency. Arch Argent Pediatr. (2018) 116: e609–e11. doi: 10.5546/aap.2018.eng.e609
11. Stoll C, Alembik Y, Tchomakov D, Messer J, Heid E, Boehm N, et al. Severe hypernatremic dehydration in an infant with Netherton syndrome. Genet Couns. (2001) 12:237–43.
12. Diociaiuti A, Castiglia D, Fortugno P, Bartuli A, Pascucci M, Zambruno G, et al. Lethal Netherton syndrome due to homozygous p. Arg371X mutation in SPINK5. Pediatr Dermatol. (2013). 30: e65–7. doi: 10.1111/pde.12076
13. Pohl M, Zimmerhackl LB, Hausser I, Ludwig H, Hildebrandt F, Gordjani N, et al. Acute bilateral renal vein thrombosis complicating Netherton syndrome. Eur J Pediatr. (1998) 157:157–60. doi: 10.1007/s004310050789

Orange Urine: The Story of A Boy Who Suffered

***Dr Sankar Narayan Mishra, **Dr Swati Nayek, ***Prof Kaustav Nayek**
**Senior Resident Doctor, **2nd Year Pgt, ***Principal And Professor*
Department of Paediatrics, Burdwan Medical college and Hospital.

Background:

A 5 year old boy from PurbaBardhaman, West Bengal presented with persistent orange coloured urine for 1 month. There were no history of fever, pain abdomen, increased frequency/ urgencynor anyburning sensation during micturition. There was no history of drug or dye or coloured food intake.

Clinical examination:

On examination, the boy had no pallor, no signs of dehydration. On palpation no abdominal tenderness or organomegaly seen.

Blood pressure was : 90/60 mm Hg and

Complete blood count shows: Haemoglobin: 12g%, WBC: 13000/cu.mm, platelets: 4.68 lakhs/cu.mm,

Urine R/E, M/E: pus cells—1 -2/hpf; RBCs—1-2/hpf; epithelial cells—1-2/hpf; protein—trace),

Urine culture showed: no growth after 72 hours of incubation.

USG KUB reveals: no abnormality detected in left and right kidneys and urinary bladder within normal limits.

Serum C3, Aso titre, G6 PD Levels, liver function tests were within normal limits,

Urinary porphobilinogen test was - negative.

After a thorough reading of literature, an association was found between orange coloured urine and citrobacter infection. Citrobactersedlakii produces indole from tryptophan which imparts an orange discolouration to urine.

Urine Culture was repeated with special mention for use of media favourable for growth of Citrobacter. After 72 hours, culture showed growth of Citrobactersedlakii.

Correspondance : Prof Kaustav Nayek, Professor & Principal, *Burdwan Medical College and Hospital. Email kaustav25@yahoo.co.in*

Management:

All intravenous fluids and medications were discontinued and the child was started on syrup cotrimoxazole and the urine became clear after 3 days of oral therapy.

Clinical Description:

A 5 year old boy from PurbaBardhaman, West Bengal presented with history of orange coloured urine for last one month. The boy visited some local physicians and several investigations were done but there was no clue to the diagnosis. At first the family members thought it may be because of dehydration or less fluid intake, so they tried to correct it by giving extra fluids. Eventually urine output increased but orange discolouration of urine persisted. The boy came to us with multiple reports of urine routine and microscopic examination and culture but all were within normal limits. There was no presence of RBC/ Hemoglobin, bile salts or bile pigments found in routine examination of urine. No history of symptoms like fever, urgency, frequency or burning sensation during micturition.

We admitted the patient from our OPD department and routine blood and urine investigations were done.The child was not taking any colouring agent/ coloured food or any dye. The 24 hour urine output was normal(@ 3 ml/kg/hour).

Blood pressure : 90/70 mm Hg. Urine R/E M/E report came next morning and it was normal(Pus cell :1-2, RBC:Nil).And in the mean time we sent a blood sample for G6PD level as the boy was getting several antibiotics that may cause hemolysis but there was no evidence of hemoglobinuria or hematuria.(Other reports—Serum C3 :normal ,CRP:normal, ASO titre:20,LFT-NAD , ICTC-NR, Hb%-12).

Complete blood count: Haemoglobin: 12g%, WBC: 13000/cu.mm, platelets: 4.68 lakhs/cu.mm

Urine R/E, M/E: pus cells—1 -2/hpf; RBCs—1-2/hpf;

epithelial cells—1-2/hpf; protein—trace),

Urine culture: no growth after 72 hours of incubation,

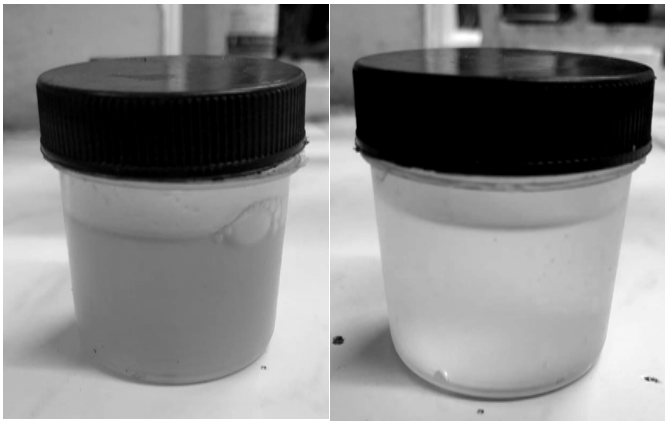
USG KUB: no abnormality detected in left and right kidneys, urinary bladder within normal limits.

Serum C3, Aso titre, G6 PD Levels, liver function tests were within within normal limits,

Urinary porphobilinogen- negative.

After a thorough reading of literature, an association was found between orange coloured urine and citrobacter infection. Citrobactersedlakii produces indole from tryptophan which imparts an orange discolouration to urine.

Urine Culture was repeated with special mention for use of media favourable for growth of Citrobacter. After 72 hours, culture showed growth of Citrobactersedlakii.

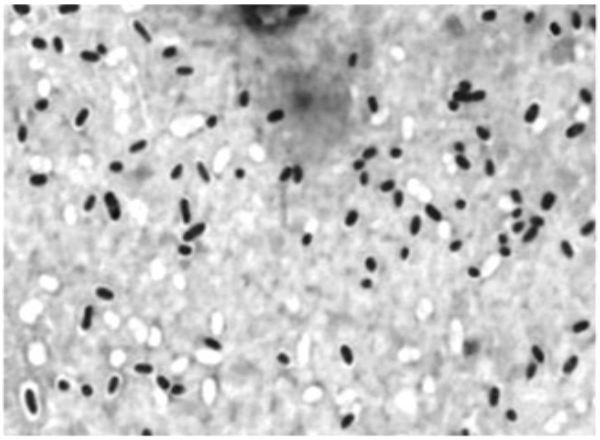


Management and Outcome:

We started the child with syrup co-trimoxazole and after 3 days miracle happened and urine became crystal clear it was an unbelievable experience . . We discharged the patient with 7 days schedule of antibiotic that to be continued for another 3 days. We followed up the patient with USG- KUB and it was normal.

Discussion:

Citrobacter mainly known to Inhabit human and animal intestines, water, soil and sometimes food.This organism rarely colonise in our urinary bladder and causes this kind of infections. The mechanism for this orange discoloration is because of the production of indole from Tryptophan . CitrobacterSedlakii is indole producing Organism and that is the difference from other Citrobacter.



References

1. Doran TI (1999) The role of Citröbacter in clinical disease of children: review. Clin Infect Dis 28:384-394
2. Dyer J, Hayani KC, Janda WM, Schreckenberger pc (1997) Citrobactersedlakii meningitis and brain abscess in a premature infant J ClinMicrobiol 35:2686-2688
3. Borenshtein D, Schauer DB (2006) The genus Citrobacter. Prokaryotes 6:90—98
4. Janda JM, Abbott SL, Cheung WKW, Hanson DF (1994) Biochemical identification of Citrobacter in clinical laboratory: J ClinMicrobiol 32:1850-1854

Large Atrial Septal Defect A Newer Phenotypic Features Of ADAR Gene In Aicardi - Goutières Syndrometype 6

***Prof Joydeep Das, **Dr Pooja Anthwal, ***Dr Vamsi Krishna Bogmala, ****Dr Rahul Singla, *****Dr Smrita Sandhu**

Professor & HOD, **Senior Resident, * Assistant Professor, Dept. of Pediatrics, ****Assistant Professor, Dept. of Radiology, *****Assistant Professor, Dept. of Opthalmology Jagannath Gupta Institute of Medical Science and Hospital*

Introduction

Aicardi-Goutieres syndrome type 6 (AGS 6) is a rare hereditary syndrome of degenerative neuroinflammation of early life characterised by microcephaly, spasticity, ataxia, dystonia and expressive dysarthria (1,2). The gene responsible for AGS 6 is ADAR (OMIM 61510) located in chromosome 1q211. Genotype and phenotype correlation mismatch is not uncommon in dysmorphology as one gene can have multiple phenotypes in AGS(3,4,). In the OMIM database there is a total 9 different types of AGS,out of which only type 9, under the influence of RNU 7 gene(OMIM617876) on chromosome 12p13.31, has cardiac involvement(5). Large atrial septal defect (ASD) described in our case is a newer phenotypic feature of AGS 6 under the influence of ADAR gene on chromosome 1(5). We report a case of AGS 6 confirmed by whole exome sequencing with large 7mm ostium secundum ASD in a 7-year-old Indian boy.

Case report

A 7-year-old boy (Figure 1) presented witha small head circumference (47cm),spasticity, ataxia of all four limbs, dysarthric speech and inability to walk from 18 months of age.

His developmental milestones were delayed, but from 18 months of age he started losing attained skill. He developed progressive poikiloderma of dorsum of hands(Figure 2), feet(Figure 3), elbow,knee and left side of forehead from 2 years of age.

He had a large 7mm ostium secundum ASD operated at 5years of age due to unsatisfactory pulmonary arterial pressure gradient. Skin biopsy findings were suggestive of dyschromatosis symmetrica hereditarian, (6)which means asymptomatic mixtures of hypo and hyperpigmented macules with skin thinning mainly in extensor aspect of distal body parts(Figures 2 and 3). Magnetic resonance imaging (MRI) of brain showed FLAIR hyperintensity in frontal & occipital periventricular and bilateral basal ganglia region(Figure 4) in 1.5T machine.

Fundus photography showed mild microvascular and retinal degeneration in the peripheral retina (Figure 5).

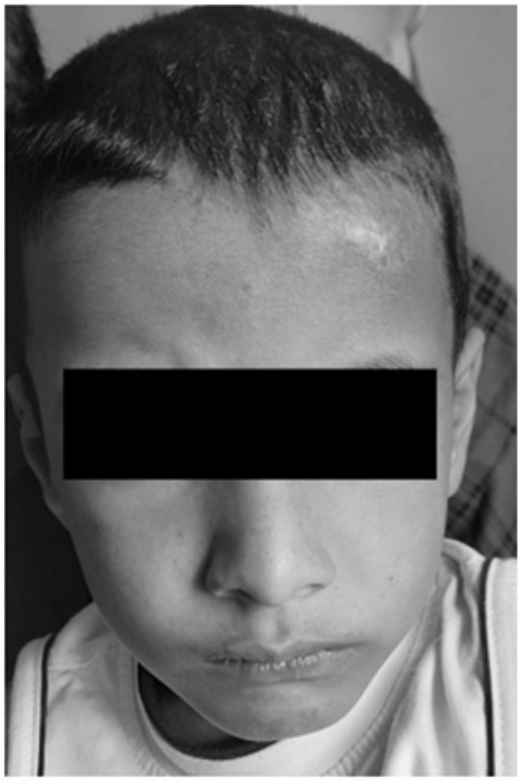


Figure 1: *7-year-old boy*

Correspondance : Prof Joydeep Das, *Professor & HOD, Jagannath Gupta Institute of Medical Science and Hospital Email:jddasjoydeep@gmail.com*

Figure 2: ***Poikiloderma of dorsum of hands***

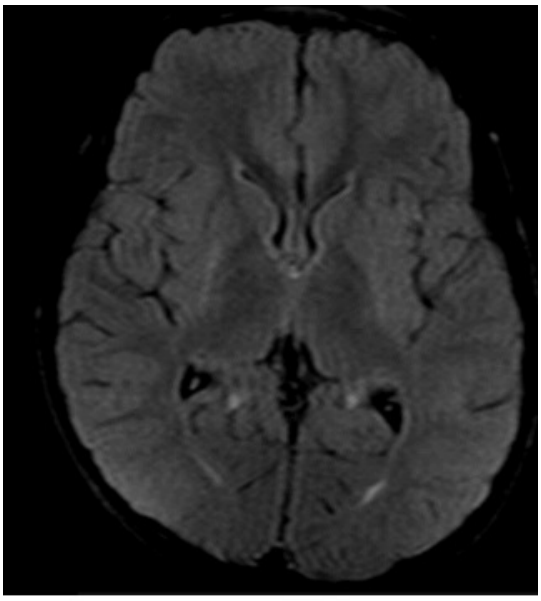


Figure 4: ***MRI of brain***

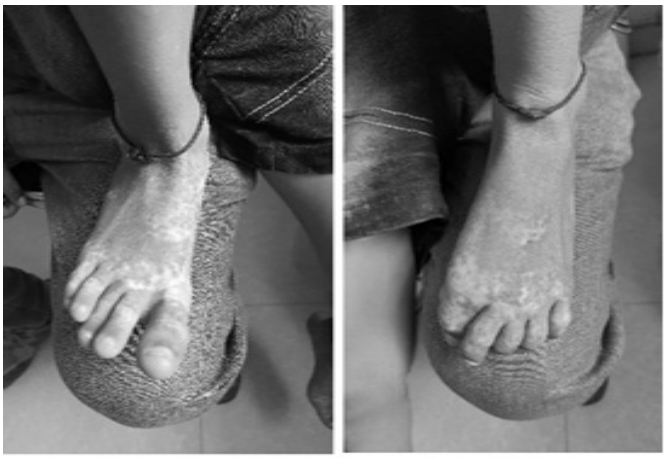


Figure 3: ***Poikiloderma of feet***

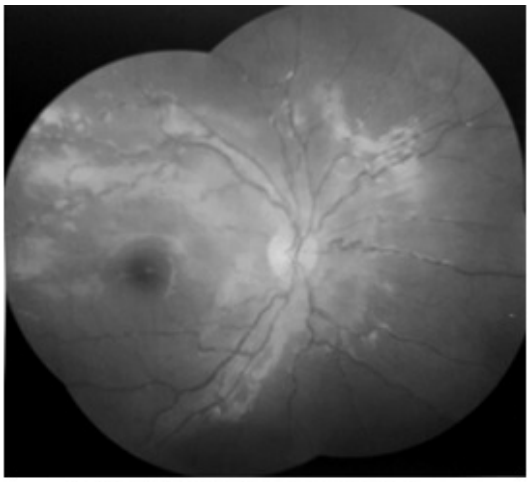


Figure 5: ***Fundus photograph***

Gene	Chromosomal Coordinates	Exon	Variant*	Zygosity	Condition group	Significance (ACMG Classification)	Inheritance
ADAR	chr1:154557322:T:C NM_001111.5	15	c.3641A>G p.Lys1214Arg	Homozygous	Aicardi-Goutieres syndrome 6 [OMIM 615010]	Variant of Uncertain Significance (VUS)	Autosomal Recessive

CSF showed 16 lymphocyte and normal protein which signifies subacute inflammatory process.Whole exome sequencing from 2ml EDTA blood showed presence of ADAR gene on chromosome 1q21.3

Discussion

Jean Aicardi and Francoise Goutieres first described this syndrome in 1984(1).Subsequently over the next four decades many genes and phenotypes were found(7). ADAR gene encodes the enzyme responsible for RNA editing by site-specific deamination of adenosines(7,8). This enzyme destabilizes double-stranded RNA through conversion of adenosine to inosine.Thereby nucleotide content of post transcriptional RNA is changed,hence abnormal proteins are produced in all the body cells mainly in brain and skin(8). Low

levels of melanin in dermal melanocytic vesicle in skin biopsy may be due to faulty post transcriptional RNA(7,8).Latest update of AGS in gene reviews of NIH USA mentioned that under ADAR gene,neuroimaging has various classic and non-classic feature and universally progressive(7). Cardiac involvement is only described in type 9 in OMIM database(5). Here RNU7-1(OMIM 617876) gene is responsible for pericardial effusion and pericarditis(5). But structural heart disease is not described in any phenotypes.

During the process of sequencing one variant of unknown significance (VOUS) (c.3641A>G; p.Lys1214Arg) was found at a depth of 89X. A clear genetic explanation of this variant in this individual could not be found as it is a novel one, not reported earlier in literature.

This case report adds extra phenotypic feature of atrial septal defect of AGS type 6 described in OMIM, may be under the influence of newer genetic variant.

References

1. Aicardi J, Goutières, F. A progressive familial encephalopathy with calcifications of the basal ganglia and chronic cerebrospinal fluid lymphocytosis. Ann Neurol. 1984;15:1.doi:10.1002/ana.410150109
2. Tolmie JL, Shillito P, Hughes-Benzie R, Stephenson JBP. The Aicardi-Goutiéres syndrome (familial, early onset encephalopathy with calcification of the basal ganglia and chronic cerebrospinal fluid lymphocytosis) J Med Genet. 1995;32:881–4. [PMC free article] [PubMed] [Google Scholar]
3. Rice G, Patrick T, Parmar R, Taylor CF, Aeby A, Aicardi J, et al. Clinical and molecular phenotype of Aicardi-Goutieres syndrome. Am J Hum Genet. 2007;81:713–25. [PMC free article] [PubMed] [Google Scholar]
4. Crow, Y. J., Jackson, A. P. et al. Aicardi-Goutieres syndrome displays genetic heterogeneity with one locus (AGS1) on chromosome 3p21. Am. J. Hum. Genet. 67: 213-221, 2000. [PubMed: 10827106, images, related citations] [Full Text]
5. Cassandra L. Kniffin, Phenotypic series,AICARDI-GOUTIERES SYNDROME 6; AGS6, OMIM #615010, -PS225750
6. Amy Chia-Ying Peng, Yi-An Chen, Sheau-Chiou Chao,Dyschromatosissymmetricahereditaria: A retrospective case series and literature review,DermatologicaSinica,Volume 31, Issue 1,2013,Pages 19-24,ISSN 1027-8117,https://doi.org/10.1016/j.dsi.2012.08.005.
7. Aicardi-Goutières Syndrome ,GeneReviews® [Internet].Yanick J Crow, MBBS, BMedSci, MRCP, PhD. Initial Posting: June 29, 2005; Last Update: November 22, 2016.
8. Kondo T, Suzuki T, Mitsuhashi Y, Ito S, Kono M, Komine M, et al. Six novel mutations of the ADAR1 gene in patients with dyschromatosissymmetricahereditaria: histological observation and comparison of genotypes and clinical phenotypes. J Dermatol. (2008) 35:395–406. 10.1111/j.1346-8138.2008.00493.

Congenital Cyanotic Heart Disease ,Microphthalmos And Iris Coloboma:Charge Association;A Case Report

***Dr Nandita Munshi,*Dr Sayan Bera,**Prof Kaustav Nayek, ***Prof Tarak Nath Ghosh**
**, ** Professor & principal, ***ProfessorJunior Resident*
Department of Paediatrics,Burdwan Medical College and Hospital

Background*- CHARGE syndrome is an genetic condition characterized by an association of clinical features. The acronym CHARGE stands for coloboma, heart defect, Choanal atresia, retardation of growth ,genital anomalies and ear malformations and deafness1. This is most commonly associated with CHD7 mutation3,7,10. The updated clinical diagnostic criteria includes 3major and several minor signs and according to these patients are classified as typical/atypical/partial CHARGE syndrome4,5. CLINICAL DESCRIPTION- we present a case of congenital cyanotic heart disease presenting with atypical phenotype of CHARGE syndrome.*

Keywords: *CHARGE,tetralogy of fallot, coloboma, developmental delay*

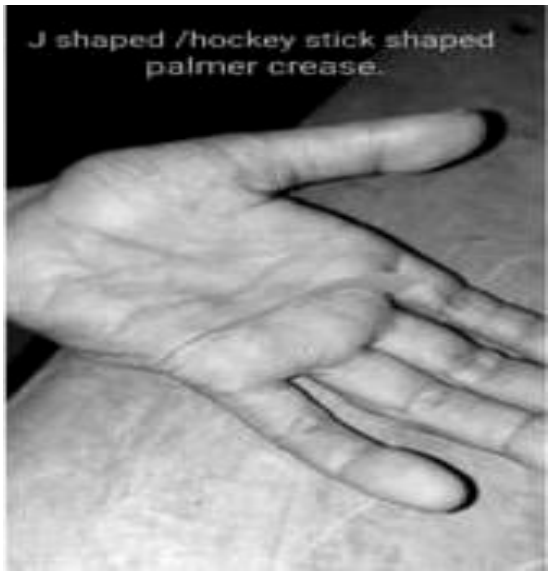
Introduction

CHARGE syndrome is a rare heterogenous syndrome with multiple congenital anomalies and a prevalence of 1 in 10,0006. In addition to features of the acronym (coloboma, heart defect, choanal atresia, retardation o growth, genital abnormality and ear malformation), most children of this spectrum presents with different features as in characteristic facial features, facial nerve palsy, swallowing problem, semicircular canal aplasia/hypoplasia, external and internal ear anomaly, intellectual disability, post- natal growth retardation etc1,2,3,4,5,6,9,10. The most identified cause of CHARGE syndrome is usually sporadic (autosomal dominant sometimes) mutation of CHD7 gene located in 8q12.28,10. Pathologically, this syndrome results from dysblastogenic and dysneurulative process Children with CHARGE syndrome require intensive medical management and multidisciplinary treatment action

Case Report :

A 8 years old boy presented with bluish discolouration of lips, tongue, finger tips for last 5 years and exertional dyspnea for the same duration. This dyspnea and discoloration aggravated during playing and was relived by squatting position. He had repeated episodes of fever and cough from childhood (documents not available). There was no significant past history, he was treated by local doctors for fever and cough previously (no record available), his immunization was as per NIS schedule. The child was product of normal vaginal delivery, he was late preterm with birth weight 1.7kg. Antenatal history was uneventful though his mother missed all the routine scans during her pregnancy. In developmental history, the boy's motor milestones were grossly delayed, he started walking with support at 3years of age and without support at 5 years of age. He started speaking 'mama' 'papa' in late of 4years and could speak a complete sentence during examination. Approximate developmental quotient 56.25 and IQ 36. Physical examination of

Correspondance : Prof Kaustav Nayek, Professor & Principal, *Burdwan Medical College and Hospital. Email kaustav25@yahoo.co.in*



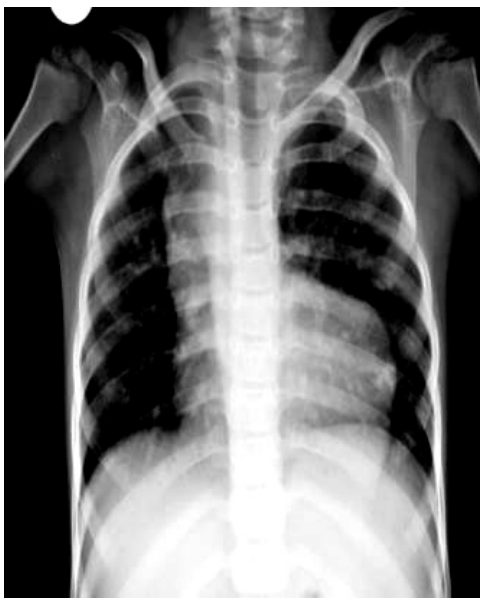


Fig 1. Chest x-ray PA view shows boot shaped heart

the child revealed weight for age and height for age both less than 3rd percentile. The child had dysmorphic facies, microphthalmia, iris coloboma,j-shaped palmar crease and lobeless pinna. Both central and peripheral cyanosis was present along with bilateral grade 3 clubbing. Left testis wasn't palpated in scrotum. All systemic examinations were within normal limit, except CVS. Where we found Tapping apex ,1cm lateral to midclavicular line. There was also systolic blowing murmur in pulmonary area.

Detailed ophthalmological examination showed microcornea, nystagmus, iris and choroidal coloboma. His hematological parameters (CBC,KFT,LFT) were normal. Chest xray showed boot shaped heart. 2D echo revealed 12mm non-restrictive VSD with right to left shunt, RV hypertrophy, overriding of aorta and non- visualization of pulmonary confluence (Fallot physiology with ?pulmonary atresia). ABR test showed mild degree of hearing loss in left ear with prolonged latency of V wave in both ears. Chromosomal analysis revealed 46XY.

The combination of facial, ear and eye anomalies along with growth retardation, decreased intelligent quotient and congenital heart disease were consistent with diagnosis of CHARGE syndrome. The child was referred to DEIC for developmental and speech therapies, started on proper diet, parents were counselled about cyanotic spell and he was referred through proper channel for surgical interventions. Due to monetary crunch Genetic testing could not be performed.

Discussion :

CHARGE association was first proposed by Hall in 1979. Through the years many more associations have been identified. To improve the diagnostic accuracy first Pagon et al1 and later in 2005 Verloes4,5 defined diagnostic criteria with major and minor criteria4,5,9.

Recent revised consensus diagnostic criteria proposes to consider CHARGE syndrome in children with one or two major and several minor criteria. Though the syndrome occurs as constellation, it has been suggested that the terminology should be restricted to coloboma and/or choanal atresia along with three other malformation . Our case had iris coloboma, ear abnormality, heart defect, growth retardation and genital abnormality with absence of choanal atresia, cranial nerve palsy. Hearing was only mildly impaired and hypogonadotropic hypogonadism

was not ruled out though cryptorchidism was present Cranial scans was not performed. So, our patient had two major and 5 minor criteria according to Blake's diagnostic criteria, So clinically he was diagnosed probable CHARGE syndrome. According to Verloes' diagnostic criteria4,5 he had 1major(coloboma) and 3minor criteria (abnormal external ear,heart defect and mental retardation) ;classifying the disease as atypical CHARGE syndrome .

Genetic diagnosis is confirmatory as the CHARGE syndrome acronym doesn't cover all the disorders that may result from this disease. The range of mutations in CHD7 gene results in a broad phenotype causing no single feature universally sufficient to clinically diagnose the disease. Depending on this Bergman et al11 made recommendations of molecular testing and Hale et al included pathogenic CHD7 variant in their proposed major criteria10. Also other syndromes like Di-george and Kabuki syndrome have overlapping features with this disease; genetic testing can help to differentiate these and be useful for future genetic counseling.

Management of this syndrome needs multidisciplinary action including physical and occupational therapy , surgery, interventions by pediatricians, endocrinologists and cardiologists.

In conclusion we presented a case of clinically diagnosed CHARGE syndrome where genetic confirmation was not possible. Nevertheless, by this report we demonstrated different phenotypic features which can lead to diagnosis of CHARGE syndrome so that parental counselling and treatment of associated abnormalities can be initiated.

Blake's criteria[2]	Verloes' Criteria[4,5]
Major Criteria-Coloboma -of iris, retina, choroid, disc; microphthalmia Choanal atresia Cranial nerve (especially VII and VIII) dysfunction Characteristic ear abnormalities	**Major criteria** Coloboma (iris or choroid, with or without microphthalmia) Choanal atresia Hypoplastic semi-circular Canals
Minor Criteria-Genital hypoplasia Developmental delay Cardiovascular malformations Growth deficiency Orofacial cleft Tracheoesophageal fistula Characteristic face	**Minor criteria-** Rhombencephalic dysfunction (brainstem dysfunctions, cranial nerve VII to XII palsies and neurosensory deafness) Hypothalamo-hypophyseal dysfunction (including GH and gonadotrophin deficiencies) Abnormal middle or external ear a Malformation of mediastinal organ (heart, esophagus) Mental retardation
Definite CHARGE: 4 major or 3 major and 3 minor criteria. **Probable/possible CHARGE**: 1 or 2 major and several minor criteria	**Typical** : 3 major, or 2 major and 2 minor criteria **Partia**l: 2 major and 1 minor criteria **Atypical**: 2 major, or 1 major and 3 minor criteria

References

1. Pagon RA, Graham JM, Zonana J, Yong SL. Coloboma, congenital heart disease, and choanal atresia with multiple anomalies: CHARGE association. J Pediatr. 1981;99:223–7
2. Blake KD, Prasad C. CHARGE syndrome. Orphanet J Rare Dis. 2006;1:34.
3. Hall JA, Georgel PT. CHD proteins: A diverse family with strong ties. Biochem Cell Biol. 2007;85:463–76.
4. Verloes A. Updated diagnostic criteria for CHARGE syndrome: A proposal. Am J Med Genet. 2005;133A:306–8.
5. Sanlaville D, Verloes A. CHARGE syndrome: An update. Eur J Hum Genet. 2007;15:389–99.
6. Bedeschi, M.F., Crippa, B.L., Colombo, L. et al. A case series of CHARGE syndrome: identification of key features for a neonatal diagnosis. Ital J Pediatr 46, 2020April;53.
7. Pramudita JJ, Utari A, Winarni TI, Faradz SM. CHARGE Syndrome: An Indonesian Case Report. Journal of Biomedicine and Translational Research [Online]. 2017 Jun;3(1):23-25
8. Williams, M.S. Speculations on the pathogenesis of CHARGE syndrome. Am. J. Med. Genet. 2005; 133A: 318-325.
9. Blake KD, Davenport SL, Hall BD, Hefner MA, Pagon RA, Williams MS, Lin AE, Graham JM. CHARGE association: an update and review for the primary pediatrician. Clin Pediatr (Phila). 1998 Mar;37(3):159-73.

Typhoid Vaccine: Do We Need Boosters ?

***Prof Kripasindhu Chatterjee, **Dr Mandira Roy**
**Professor & HOD, **Assistant Professor*
Deptt of Pediatrics, Santiniketan Medical College, Bolpur

Review of current data and recommendations

Typhoid conjugated vaccine is administered as a single dose schedule as par IAP ACVIP guidelines and World Health Organisation[1,2]. We wanted to analyse any further evidence in support and also whether booster dose is needed.

Immunogenicity and Safety:

Immunogenicity and seroconversion data point to robust data of around 95% with more than 4 fold rise in GMT in different conjugated vaccines in India [3-5] and outside India [6] in age group from 6 months to 45 years which point to good primary uptake.

In an Indian study [3] in 2015, 654 healthy subjects aged 2–45 years in RCT arm and 327 healthy subjects aged 6–23 months received a single dose of Typbar-TCV in an open-label trial (OLT) arm. Both arm showed good seroconversion on day 42, [97% - 98%]. After two years, both RCT and OLT arm showed stable anti-Vi titers (GMT, 82 and 48) and exhibited satisfactory avidity (geometric mean avidity index [GMAI], 60% and 57% respectively. Typbar-TCV induced multiple IgG subclasses and strong booster responses in all ages. No serious vaccine-attributable adverse events were observed.

Duration of protection:

In same Indian study [3] subset of consenting subjects in the RCT and OLT was revaccinated on day 720. Serum anti-Vi IgG responses measured approximately 42 days following second dose showed boostability and immunologic memory. Study continued up to 7 years [7] in 2021 show antibody GMT levels increased significantly on day 762, and remained 32-fold, 14-fold, and 10-fold over baseline at 3, 5 and 7 years respectively among boosted all specimen cohort [ASC] (N = 86) tested by Vacczyme. Among unboosted ASC children (N = 25), GMTs remained 21-fold, 8-fold and 5-fold over baseline at 3, 5 and 7 years, respectively suggesting persistence of immune response. Similar results were obtained in a study in Malawi study[6].

Correspondance : Prof Kripasindhu Chatterjee, *Professor & HOD, Deptt of Pediatrics, Santiniketan Medical College, Email:kschatterjee@gmail.com*

Efficacy

In a controlled human infection model [8] of Salmonella Typhi with 112 participants, about 1 month post-vaccination, participants were challenged orally with 1–5 × 104 colony forming units (CFUs) of S Typhi Quailes strain (a wild-type strain originally isolated from a chronic carrier in Baltimore, MD, USA). Immediately before challenge administration, participants ingested 120 mL of sodium bicarbonate solution to neutralise gastric acid. Following challenge, participants were seen daily for vital sign measurement, blood collection, and general assessment in an outpatient clinic for a 2 week period and reported vaccine efficacies of 54•6% (95% CI 26•8–71•8) for Vi-TT.

A trial in Lalitpur, Nepal,[9] in 2021 with 20 019 children between 9 months to 16 years were randomly assigned to receive TCV or Men A vaccine and followed up for 2 years. The incidence of typhoid fever (cases per 100 000 person-years) was 72 (95% CI 38–123) in the TCV group and 342 (95% CI 262–438) in the Men A group. The protective efficacy of TCV against blood culture-confirmed typhoid fever at 2 years was 79•0% (95% CI 61•9–88•5; p<0•0001). There is no evidence of waning protection over a 2-year period.

In another cluster-randomised trial [10] in 2021 in Bangladesh, with 61756 children aged 9 months to less than 16 years were vaccinated either Vi-TT or

SA 14-14-2 JE vaccine and followed for an average of 17•1 months. Total Vi-TT protection 85% (97•5% CI 76 to 91, p<0•0001). No significant indirect vaccine protection was observed. No serious adverse events were observed.

Navi Mumbai Municipal Corporation[11] launched the world's first public sector TCV introduction. A total of 113,420 children 9 months-14 years old received TCV. No unexpected safety signals were identified.

Both WHO and IAP ACVIP[1,2] recommends single dose of any of Typhoid conjugate vaccine (TCV 25 µg) for infants from 6 months of age and in adults up to 45 years and can be administered with MMR also. Booster dose is not recommended in subsequent years[1]. With availability of more and more evidences on immunogenicity and efficacy and longer term persistence of antibody following single dose, IAP ACVIP reiterates and maintains the same recommendation.

References

1 Kasi SG, Shivananda S, Marathe S, Chatterjee K, Agarwalla S, Dhir SK, Verma S, Shah AK, Srirampur S, Kalyani S, Pemde HK, Balasubramanian S, Parekh BJ, Basavaraja GV, Gupta P. Indian Academy of Pediatrics (IAP) Advisory Committee on Vaccines and Immunization Practices (ACVIP): Recommended Immunization Schedule (2020-21) and Update on Immunization for Children Aged 0 Through 18 Years. Indian Pediatr. 2021 Jan 15;58(1):44-53. doi: 10.1007/s13312-021-2096-7. Epub 2021 Nov 29. PMID: 33257602; PMCID: PMC7840391.

2 World Health Organization. Typhoid vaccines: WHO position paper, March 2018 - Recommendations. Vaccine. 2019 Jan 7;37(2):214-216. doi: 10.1016/j.vaccine.2018.04.022. Epub 2018 Apr 13. PMID: 29661581.

3 Mohan VK et al. Safety and immunogenicity of Typbar-TCV in healthy infants, children, and adults in typhoid endemic areas: a multicenter, 2-cohort, open-label, double-blind, randomized controlled phase 3 study. Clin Infect Dis. 2015 Aug 1;61(3):393-402. doi:10.1093/cid/civ295. PMID: 25870324

4 Kundu R et al. Immunogenicity and Safety of Typhoid Conjugate Vaccine in Healthy Indian Subjects: A Randomized, Active-controlled, Comparative Clinical Trial. Indian Pediatr. 2020 Jul 15;57(7):625-630. PMID: 32727938

5 Subhash Thuluva, Vikram Paradkar, Ramesh Matur, Kishore Turaga & Subba Reddy GV (2022) A multicenter, single-blind, randomized, phase-2/3 study to evaluate immunogenicity and safety of a single intramuscular dose of biological E's Vi-capsular polysaccharide-CRM197 conjugate typhoid vaccine (TyphiBEVTM) in healthy infants, children, and adults in comparison with a licensed comparator, Human Vaccines & Immunotherapeutics, 18:5, DOI: 10.1080/21645515.2022.2043103

6 Nampota-Nkomba N et al. Safety and immunogenicity of a typhoid conjugate vaccine among children aged 9 months to 12 years in Malawi: a nested substudy of a double-blind, randomised controlled trial. Lancet Glob Health. 2022 Sep;10(9):e1326-e1335. doi:10.1016/S2214-109X(22)00275-3. PMID: 35961356; PMCID: PMC9380257

7 Vadrevu KM et al. Persisting antibody responses to Vi polysaccharide-tetanus toxoid conjugate (Typbar TCVR) vaccine up to 7 years following primary vaccination of children < 2 years of age with, or without, a booster vaccination. Vaccine. 2021 Oct 29;39(45):6682-6690. doi: 10.1016/j.vaccine.2021.07.073. Epub 2021 Oct 5. PMID:34625288.

8 Jin C, Gibani MM, Moore M, Juel HB, Jones E, Meiring J, Harris V, Gardner J, Nebykova A, Kerridge SA, Hill J, Thomaides-Brears H, Blohmke CJ, Yu LM, Angus B, Pollard AJ. Efficacy and immunogenicity of a Vi- tetanus toxoid conjugate vaccine in the prevention of typhoid fever using a controlled human infection model of Salmonella Typhi: a randomised controlled, phase 2b trial. Lancet. 2017 Dec 2;390(10111):2472- 2480. doi: 10.1016/S0140-6736(17)32149-9. Epub 2017 Sep 28. PMID: 28965718; PMCID: PMC5720597.

9 Shakya Mila et al. (2021). Efficacy of typhoid conjugate vaccine in Nepal: final results of a phase 3, randomised, controlled trial. The Lancet Global Health. 9. e1561-e1568. 10.1016/S2214-109X(21)00346-6.

10 Qadri F et al. Protection by vaccination of children against typhoid fever with a Vi-tetanus toxoid conjugate vaccine in urban Bangladesh: a cluster-randomised trial. Lancet. 2021 Aug 21;398(10301):675- 684. doi: 10.1016/S0140-6736(21)01124-7. Epub 2021 Aug 9. PMID: 34384540; PMCID: PMC838797

11 Longley AT, Date K, Luby SP, Bhatnagar P, Bentsi-Enchill AD, Goyal V, Shimpi R, Katkar A, Yewale V, Jayaprasad N, Horng L, Kunwar A, Harvey P, Haldar P, Dutta S, Gidudu JF. Evaluation of Vaccine Safety After the First Public Sector Introduction of Typhoid Conjugate Vaccine-Navi Mumbai, India, 2018. Clin Infect Dis. 2021 Aug 16;73(4):e927-e933. doi: 10.1093/cid/ciab059. PMID: 33502453; PMCID: PMC8366822.

WBAP EB Member 2023

Name	Post	Email
DR KALPANA DATTA	PRESEDENT,CIAP EB	drkalpanadatta@gmail.com
DR INDRANIL CHOWDHURY	SECRETARY	indranil.chowdhury1234@gmail.com
DR ASOK KUMAR DATTA	PRESIDENT ELECT	asokdatta31@yahoo.com
DR SUSHMITA BANERJEE	IMM. PAST PRESIDENT	sushmitabanerj@gmail.com
DR DIBYENDU RAYCHAUDHURI	VICE PRESDIENT	dr.dibyenduraychaudhuri@yahoo.com
DR MADHUMITA NANDI	IMM PAST SECRETARY	madhumitabanik@rediffmail.com
DR MIHIR SARKAR	JT. SECRETARY	drmihir09@gmail.com
DR PRIYANKAR PAL	TREASURER	mailme.priyankar@gmail.com
DR KALPANA DATTA	CIAP EB	drkalpanadatta@gmail.com
DR KAUSTAV NAYEK	CIAP EB	kaustav25@yahoo.co.in
DR KRIPASINDHU CHATTERJEE	CIAP EB	kschatterjee@gmail.com
DR ABHIJIT SARKAR	EXECUTIVE MEMBER	dr.sarkar@yahoo.com
DR AGNI SEKHAR SAHA	EXECUTIVE MEMBER	agnisekhar@hotmail.com
DR AMITA SINHA	EXECUTIVE MEMBER	amitasinhamandal@gmail.com
DR ASHIM KUMAR GHOSH	EXECUTIVE MEMBER	akgasn@gmail.com
DR BIPLAB BANERJEE	EXECUTIVE MEMBER	biplabjoy19@gmail.com
DR NILANJAN GHOSH	EXECUTIVE MEMBER	niltughosh@gmail.com
DR RUPA BISWAS	EXECUTIVE MEMBER	drrupa.biswas@gmail.com
DR SAMIK HAZRA	EXECUTIVE MEMBER	samik.hazra@gmail.com
DR SHUBHADEEP DAS	EXECUTIVE MEMBER	shubhadeepnrsdoc@gmail.com
DR SUMANTRA KUMAR RAUT	EXECUTIVE MEMBER	drsuman.raut@gmail.com
DR KAUSTAV NAYEK	Editor-in-Chief, CIAP EB	kaustav25@yahoo.co.in
DR SAMIR RANJAN DAS	Chairperson,Constitution Committee	das.samir2006@gmail.com
DR DEBAJYOTI BURMAN RAY	Chairperson, WBAP Board of Trustee	drdebajyotibroy@gmail.com
DR SWAPAN KUMAR RAY	Chairperson, Scientific Committee	drswapanray@gmail.com
DR VINAY ASAWA	Chairperson, Finance Committee	vinayasawa91@gmail.com
DR INDU SURANA	Chairperson, Community Action Plan	Indu.paed@rediffmail.com
DR ASAMANJA HAJRA	Coordinator, WBW Week	ahajra008@rediffmail.com
DR KAUSIK CHAKRABARTI	Coordinator, Immunisation Committee	drkausik.chc@gmail.com
DR ABUL FAZL A RAHAMAN	SPECIAL INVITEE	afardgp54@yahoo.com
DR ATANU BHADRA	SPECIAL INVITEE	atanu4bhadra@gmail.com
DR CHAMPAK DAS	SPECIAL INVITEE	champakdas23@gmail.com
DR SUBROTO CHAKRABARTTY	SPECIAL INVITEE	chakrabartty.subroto@gmail.com
DR SUKANTA CHATTERJEE	SPECIAL INVITEE	sukantachatterjee@hotmail.com

CHERICOF® 12

Dextromethorphan HBr + Chlorpheniramine Maleate Sustained-Release Suspension (30mg+4mg/5ml)

12 HOURS TOUGH ON DRY COUGH

CHERICOF

Rational & Yummy Cough Formula

CHERICOF Junior

Dextromethorphan Hbr 5mg + Chlorpheniramine Maleate 1mg + Phenylephrine HCL 2.5mg per 5ml Syp.

CHERICOF®-SF

Dextromethorphan Hbr 10mg + Chlorpheniramine Maleate 2mg + Phenylephrine HCL 5mg per 5ml Syp.

Sugar free cough formula

CHERICOF®-LS

Levosalbutamol Sulphate 1mg + Ambroxol HCl 30 mg + Guaiphenesin 50 mg per 5 ml Syp.

CHERICOF®-LS JUNIOR

Levosalbutamol Sulphate 0.5 mg + Ambroxol HCL 15 mg + Guaiphenesin 50 mg per 5ml Syp

Published by Dr Indranil Chowdhury, Hony Secretary, West Bengal Academy of Pediatrics, 15C, Canal Street, Kolkata 700 014 and Printed by Roshni Enterprise, Kolkata