



# IMSA

INTERNATIONAL MEDICAL SCIENCES ACADEMY

January-March 2009

Vol. 22 No. 1

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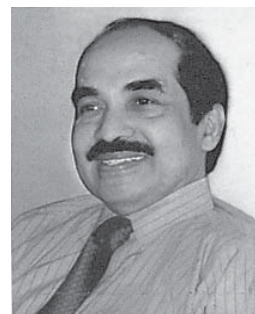
## PRESIDENT'S WRITES

Dear Fellows & Members

The current issue of JIMSA titled "*Recent Advances in Pediatrics*" carries a good coverage of common pediatric problems in depth.

At this juncture let me make a special appeal to all fellows and members to popularise our CME program on a monthly basis covering urban and rural sectors and also to contribute original articles, reviews and case reports, for publication in JIMSA, so that we can make our journal, the very best.

Let me wish all the fellows and members a very happy and prosperous 2009.



*K. Jagadeesan*

Dr. K. Jagadeesan  
President, IMSA

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All fellows and members of IMSA can have access to the site and get information about its objectives, benefits to the fellows/members, chapters and their activities including seminars, refresher courses, rural CME;s etc. and also IMSACON - a regular annual event of international standard; *application form for enrollment as fellow/member can also be downloaded. Fellows - members and even not fellows - members can have access to full text in the quarterly journal - jimsa from July - Sept. 2003 onwards by putting their E-mail address under 'user name' and using the password 'UserJimsa'.*

## IMSA NEWS



**Dr. P. Narasimha Rao**

Ex. President, IMSA World H.Q.

Dear Fellows and Members

You are aware late Dr. P. Narasimha Rao, an international figure both in academic and teaching had been the President of this prestigious organization for more than a decade from 1990 to 2002. He was President of Medical Council of India and Vice Chancellor of various Universities. He had to his credit several outstanding contributions to the medical fraternity till his death. He had been in close association with IMSA since its very inception in 1981. The Academy has flourished tremendously during his tenure as President. Keeping in view his status, services rendered to the mankind and on the insistence of senior Fellows, the Academy has established an International Award in his honour named 'Dr. Pinnamaneni Narasimha Rao International Award', on the lines of Dr. B.C. Roy National Award. Substantial funds are needed for this prestigious award. Initially, the family of Dr. P. Narasimha Rao has contributed a fair amount of money and has also assured to contribute more.

I appeal to all our Fellows and Members to contribute generously for this noble cause in the memory of this dedicated acadamecian - Dr. P. Narasimha Rao. A separate account has been opened for this Award.



**Dr. R.R.Thukral**

Vice President IMSA World H.Q.

(R.R.Thukral)

### IMSA Chapter Activities

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"Approach To Vascular Diseases"

"Ocular Manifestations Of

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"Type II-Diabetes Mellitus

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"Our Experience On Door To Needle Time In Acute Myocardial Infarction

#### CME Delhi Chapter

29/11/2008 Dr.Ashwin Garg

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today'

28/3/2009 Dr.Rajesh Verma

"Interventional Radiology-New Dimensions-

"What a Physician should Know"

Uterine Fibroid Embolization

Joint Replacement- An Overview

Hip and Knee Replacement-'Where are we

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#### Election of Fellows and Members ( Jan-March 2009)

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#### IMSA Fellows/Members Directory 2007

Dear Fellows and Members

International Medical Sciences Academy has published Directory of IMSA Fellows and Members containing information about their mailing addresses, telephone nos. email addresses, wherever available. The Directory was released at the inaugural function of IMSACON 2007 held at Manipal, Karnataka in November, 2007. I shall request you to send a demand draft of Rs. 250 soon to enable us to send to you a copy of the Directory by post. You can also collect in person from IMSA office if you so wish.

Secretary General, IMSA

#### Suggestions to Enhance Image of Medical Profession and Improve Doctor-Patient Relationship

President, Vice President and Trustees of IMSA have stressed that IMSA must engage itself in enhancing the image of Medical Profession by organizing seminars/conferences on various issues relating to medical profession, medico legal, patient — doctor relationship protocol of drug trials and research etc. It was also desired that suggestions be invited from all fellows and members, for improving relationship among doctors and patients.

The Fellows and Members are, therefore, requested to send their suggestions & ways and means to IMSA World Headquarter at New Delhi, for enhancing image of medical profession and improving doctor — patient relationship.

Secretary General, IMSA

### IMSACON-2009 at Chandigarh, Punjab

IMSA is pleased to inform its Fellows and Members that Annual Conference IMSACON 2009 will be held on 24<sup>th</sup> & 25<sup>th</sup> October, 2009, (Saturday-Sunday) in the Auditorium, Sarai building GMCH, Sector -32, Chandigarh, Punjab, India

For further details please contact  
Dr. H.K. Chopra,  
Secretary General, IMSA, WHO, New Delhi.



# JIMSA

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## FROM EDITOR'S DESK

Dear Colleagues

*This special issue "Recent Advances In Paediatrics" comprises of 13 articles, authored by outstanding experts from the field of paediatrics. The issue highlights some of the new concepts in the understanding and management of common childhood disorders: there are new guide lines and new vistas for research for the younger paediatricians.*

*This publication is the outcome of hard work and untiring efforts of Dr. Kamlesh Chopra a senior consultant paediatrician at New Delhi, India. Her team of contributors have done a magnificent job in brilliantly preparing the various manuscripts within the stipulated time. I am confident that this publication will be of immense benefit for the readers of JIMSA. No doubt, a commendable task has been achieved, for which I am extremely grateful to Dr. Kamlesh Chopra and other contributors of this issue.*

*I must acknowledge the assistance rendered by the members of the editorial and advisory boards in the compilation of this issue. I also extend my thanks to the various pharmaceutical firms and other advertisers without whose help this publication would not have been possible.*

**P. D. Gulati**

## JIMSA BEST PUBLISHED ARTICLE AWARDS

Journal of International Medical Sciences Academy has instituted award for **three (3)** best original articles published during the previous 3 years; **guidelines** are as below:

- (1) **Original articles** belonging to any discipline of medicine published in JIMSA during the previous three years.
- (2) Age Limit for the principal author/main researcher should be 45 years and below.
- (3) Number of awards: Three (3) annually, carrying a gold plated medal, citation and cash prize (1st Rs. 3000/-, 2nd Rs. 2000/-, 3rd Rs. 1000/-)
- (4) Awardee should preferably be a fellow/member of IMSA; non-fellows/ non members can also be considered for the award if the original work is outstanding; and if selected for the award will be required to apply for fellowship/membership of IMSA.
- (5) Awardees should preferably plan to receive the award at the annual IMSA conference - IMSACON.

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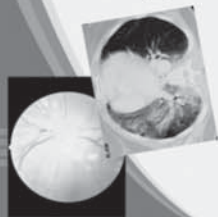


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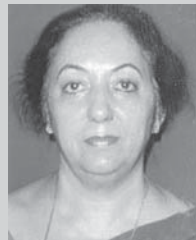
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**OUR GUEST EDITOR**

**Dr. Kamlesh Chopra**, Director Professor was formerly head of the Department of Pediatrics at Maulana Azad Medical College and Lok Nayak Hospital, New Delhi, India. She was the medical director of Himalayan Institute of Medical Sciences, Dehradun. She has 76 publications in national and International journals and has authored 4 books and contributed chapters to several books. She has received various awards for her contributions and achievements. She is presently a visiting consultant in Pediatrics at Indian Spinal Injuries Centre.

**EDITORIAL**

This issue highlights some of the recent developments in pediatrics. Gene therapy is a new pharmacological agent in the armamentarium to treat inherited monogenic and acquired disorders such as cancer, infections and neurodegenerative diseases. The liver has been studied as a target organ because many genetic diseases are amenable to gene replacement therapy in hepatocytes. Liver transplantation is an established mode of therapy in children with fulminant hepatic failure and end stage liver disease due to various causes. Renal transplantation is the most effective treatment of advanced chronic renal failure. The exorbitant cost and lack of awareness of organ donation are the limiting factors for both renal and liver transplantation. Stem cell therapy has the potential to provide therapeutic treatment for some developmental and neurodegenerative diseases in childhood like biliary atresia and leukodystrophies. The availability of recombinant growth hormone has enabled the use of growth hormone in the treatment of short stature due to disorders like Turner syndrome, Prader Willi syndrome, small for gestational age children, chronic renal failure and skeletal dysplasias. One of the most significant developments in the management of childhood ITP has been the acceptance of 'Observation only Approach' due to the recognition that intracranial haemorrhage – the most dreaded complication is rare (0.17% and 0.2% reported in two large series by Lilleyman and Kunhe). In neonatology, there has been a dramatic decrease in mortality and morbidity in hyaline membrane disease. Use of antenatal steroids and surfactant therapy in preterm babies has salvaged many babies. Hypothermia and phenobarbital use in hypoxic ischemic encephalopathy are promising interventions to reduce neuronal damage.

Inhaled glucocorticosteroids are the cornerstone of asthma treatment and various professional bodies have formulated guidelines for the management of asthma. Immunization is one of the most cost effective health intervention to reduce morbidity and mortality from vaccine preventable diseases. Recently many new vaccines have been launched. Rotavirus vaccine is >90% protective after two doses given at 4 weeks interval to babies at 6 weeks of age. Inactivated polio vaccine is safe and seroconversion rates of 90-95% are achieved after 2-3 doses given at 6-8 weeks of age. It can be given to children with symptomatic HIV infection and immunodeficiency. Two types of pneumococcal vaccine are currently available, the 23 valent unconjugated polysaccharide vaccine is recommended for children above two years and the 7 valent conjugated vaccine for infants below 2 years of age. The introduction of PCV-7 in childhood vaccine schedule in the US has led to a dramatic decline in invasive pneumococcal disease. A highly efficacious vaccine against human papilloma virus (HPV)-the causative agent of cervical cancer has recently been launched and is recommended in the age group of 9-26 years. Research is a dynamic process and novel forms of treatment are on the horizon.

**Kamlesh Chopra**

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# URINARY TRACT INFECTIONS: CURRENT MANAGEMENT

R N Srivastava

Division of Nephrology, Department of Pediatrics, Indraprastha Apollo Hospital, New Delhi 110076, India

**Abstract :** Urinary tract infections (UTI) are common in infants and children and recur in 10-30%. UTI are often associated with a structural or functional abnormality of kidney and urinary tract. Vesico ureteric reflux may be detected in about 30% cases with the first UTI. *Escherichia coli* is the most commonly isolated urinary pathogen. Patients with malformations or dysfunction of urinary tract may become infected with other bacterial species that normally have low virulence for urinary tract such as enterococci, *Staphylococcus aureus* or *epidermidis*, *proteus*, *pseudomonas*, and *serratia spp.* UTI occurs when virulent bacteria gain access to the normally sterile urinary tract mostly by the ascending route. Incomplete bladder emptying with residual urine is a major factor in acquiring UTI. Some of the bacterial strains causing such infection may show wide antibiotic resistance. The symptoms of UTI depend on the intensity of inflammation, site of infection and age of the patient. Laboratory evaluation: careful urine analysis and urine culture are crucial. Children with symptomatic UTI should be given antibiotics without delay but after obtaining a proper urine sample. Second and third generation cephalosporins (such as cefixime) given orally or intravenously or amoxicillin + clavum are adequate for empiric therapy For confirmed or suspected pyelonephritis treatment is given for 10- 14 days. Children without symptoms of pyelonephritis are treated for 7-10 days. Further observation and imaging are considered in every case.

Urinary tract infections (UTI) are common (5-10%) in infants and children and recur in 10-30%<sup>1</sup>. The prevalence of UTI in young children having unexplained fever is between 3 to 5% (higher in premature infants than in term infants). After one year of age, the prevalence of UTI in boys decreases to about 2%, whereas in girls it increases to 8%. UTI are often associated with a structural or functional abnormality of kidney and urinary tract. Obstructive anomalies of urinary tract may be present in 2% of girls and 10% of boys with UTI. Vesicoureteric reflux may be detected in about 30% cases with the first UTI<sup>1</sup>. Eradication of UTI is difficult in such situations and recurrence is common. Serious sequelae such as renal scarring, hypertension and chronic renal failure may follow. Urine examination is often neglected in infants and young children with nonspecific symptoms and UTI go undetected. On the other hand, presumed UTIs are inappropriately treated on the basis of wrong interpretation of "routine" urine examination report (showing a few "pus cells" on microscopy).

## CAUSATIVE ORGANISMS

In acute, uncomplicated UTI, the most commonly isolated urinary pathogens are enteric Gram-negative bacteria, especially *Escherichia coli*. Patients with malformations or dysfunction of urinary tract may become infected by other bacterial species that normally have low virulence for urinary tract such as Enterococci, *Staphylococcus aureus* or *epidermidis*, *Proteus*, *Pseudomonas*, and *Serratia spp.* Fungal infection of the urinary tract may occur in pre-term babies and older immunosuppressed patients with an indwelling bladder catheter and those receiving broad spectrum antibiotics. Uropathogens are derived from the fecal flora.

## MECHANISMS OF INFECTION

UTI occurs when virulent bacteria gain access to the normally sterile urinary tract, mostly by the ascending route. Hematogenous spread may occur in neonates who have yet to develop a mature immune system and in children who are immunocompromised. Bacteria reaching the bladder multiply readily unless eliminated by defense mechanisms. Effective voiding washes out the bacteria, but some may be left in the film of urine lining the bladder epithelium. *Incomplete bladder emptying with residual urine is a major factor in recurring UTI.* In the hospital setting, urinary catheters are a major risk factor for acquisition of nosocomial infection, which increases with prolonged duration of catheterization. Some of the bacterial strains causing such infection may show wide antibiotic resistance.

## RECURRENCE OF UTI<sup>3</sup>

Recurrence of UTI is common when underlying anatomical or functional

abnormalities of kidney and urinary tract are present, most common being obstruction and vesicoureteric reflux. Voiding dysfunction is increasingly being recognized as an important cause of recurrent UTI.

### Obstruction

Children with obstructive abnormalities, whether anatomic (posterior urethral valves, ureteropelvic junction obstruction, constipation), neurologic (e.g., myelomeningocele with neurogenic bladder), or functional, are at increased risk of developing UTIs. Stagnant urine is an excellent culture medium for most uropathogens.

### Vesicoureteric reflux.

VUR is very often associated with UTI. Higher grades of VUR constitute the major risk factor for pyelonephritis and renal scarring in infants and young children.

### Uncircumcised infant.

The risk for UTI in uncircumcised boys is 5- 20-fold higher than in those circumcised, with the greatest risk being in boys below 1 year. The presence of preputial folds in uncircumcised boys encourages a high density of bacterial growth and contamination of the urethral opening. Circumcision reduces meatal contamination, thereby decreasing the ascent of bacteria into the bladder. Lack of circumcision puts the infant at a higher risk for having recurrent UTI<sup>2</sup>.

### Dysfunctional voiding.

This term refers to a lack of coordination between the detrusor and the urethral sphincter at the onset of voiding. Ordinarily, the sphincter must relax as the detrusor contracts. Failure of the sphincter to relax causes an obstruction to the outflow of urine (as does the distended rectum in constipated children). These conditions are associated with incomplete emptying of the bladder and significant amounts of residual urine.

## CLINICAL FEATURES

The symptoms of UTI depend not only on the intensity of the inflammatory reaction but also on the site of infection and the age of the patient. During the first year of life pyelonephritis is the most common presentation of UTI. Symptomatic UTI may chiefly involve the upper tract or the lower tract. Infection in a normal urinary tract with no prior instrumentation is considered "uncomplicated," whereas "complicated" infections occur in urinary tracts that have a structural or functional abnormality<sup>1</sup>.

## LOWER URINARY TRACT INFECTION

Majority of children with cystitis present with urgency, frequency and dysuria. Children who have the urge to urinate may have a history of difficulty in initiating the urinary stream. Acute voiding problems are not

synonymous with acute bacterial cystitis as they can occur with vulvitis or balanitis. Occasionally the child may complain of abdominal or suprapubic pain. Fever, if associated, is of low grade. Suprapubic tenderness may be present. Urine may be foul smelling and cloudy. It is important to note that symptoms such as urgency, frequency and dysuria can be caused by any factor or process that gives rise to inflammation in the lower urinary tract. Examples include mechanical irritation (that might result from insertion of foreign bodies, migration of pinworms) and chemical irritation (disinfectants, shampoos).

## **PYELONEPHRITIS**

During the first month of life, the symptoms are non-specific such as vomiting, poor feeding and jaundice, failure to thrive with little or no fever in about 50% cases. Beyond infancy, fever often is the only symptom of acute pyelonephritis. The characteristic features include high fever with chills and rigors, abdominal pain, vomiting and toxic appearance. A neutrophilic leucocytosis is usually present. Children usually cannot complain of loin pain until 4 to 5 years or older.

### **Clinical evaluation**

The height and duration of fever, urinary symptoms, vomiting, recent illnesses, antibiotic intake should be recorded. The following should be carefully evaluated: (1) colour, clarity and smell of urine; (2) age of toilet training; (3) characteristics and frequency of voiding (voiding frequency, urgency, squatting or other holding maneuvers, day time wetting, poor or interrupted stream, dribbling, prolonged voiding, straining during voiding); (4) presence of constipation / soiling; (5) previous UTIs; (6) previous undiagnosed febrile illnesses; (7) family history of frequent UTIs, VUR and other genitourinary abnormalities.

Physical examination includes evaluation of physical development, accurate record of blood pressure and temperature, assessment of suprapubic and costovertebral tenderness, and a search for other sources of fever. External genitalia should be examined for signs of vulvovaginitis, vaginal foreign body and anatomic abnormalities. Lower back should be examined for sinus, pigmentation, lipoma or tufts of hair and if there is suspicion of a neurological abnormality, anal sphincter tone should also be evaluated. UTI and occult bacteremia is a common cause of fever in a "well-appearing infant."

### **Laboratory evaluation**

Careful urinalysis and urine culture are crucial. Urine in the normal bladder is sterile, but may get contaminated by bacteria during the passage from bladder to the sampling container, especially in infants and small children. This often leads to a false diagnosis of UTI and the child is subjected to unnecessary treatment, investigations and follow-up. On the other hand, failure to identify the child with UTI carries a risk of progressive renal damage.

### **Collection of urine specimen**

#### **Clean Voided Bag Samples.**

Culture of a urine specimen obtained by bag is often "false positive". An infant or young child should not receive antibiotics on such a result. If urinalysis from the bag sample suggests UTI, or M/E shows > 5 white blood cells per high-power field (centrifuged specimen), or bacteria on Gram stain of uncentrifuged urine, a fresh urine sample for urinalysis and culture should be collected by invasive means.

#### **Clean catch samples**

Older children can provide clean voided urine mid-stream samples after careful cleansing and minimizing contact with skin.

#### **Suprapubic bladder aspiration**

This procedure is a safe and effective method for obtaining urine specimens in infants and young children. Ultrasound guidance improves the yield.

#### **Bladder Catheterization.**

Bladder catheterization may occasionally be resorted to. The complications

are minimal (urethral trauma and microscopic hematuria) and the risk of introducing infection is very low.

### **Dipstick tests**

Dipstick tests are useful for screening. The strip detects urinary nitrite, which is formed by bacterial reduction of nitrate, (the colour change being proportional to the number of bacteria in urine) and leucocyte esterase. If positive, treatment for UTI can be initiated while awaiting the culture result.

### **Urine microscopy**

A centrifuged sample of unstained urine is examined for the presence of bacteria and leucocytes. WBCs are measured more precisely by microscopy of uncentrifuged urine using a counting chamber; more than 10 leucocytes/ $\mu$ l in a boy and 50 in a girl are abnormal. Neutrophils are present in 80-90% of symptomatic UTI, but a urine sample without WBCs does not exclude UTI. WBCs may also be found in febrile children with infections outside the urinary tract, in inflammatory diseases other than UTI in or near the urinary tract, or due to contamination from the vagina. Phase contrast microscopic examination is greatly superior to characterize cells and formed elements in the urine and is being adopted by modern laboratories.

### **Urine culture**

Urine should be immediately refrigerated at 4°C (and not left at room temperature) until cultured, to prevent growth of contaminating bacteria. This temperature must be maintained during transport. However, the urinary leukocyte count may be altered by refrigeration, possibly affecting interpretation of the urinalysis. The interpretation of culture results depends on the method of urine collection and the clinical background. In urine obtained by suprapubic aspiration, any growth is considered significant. In urine obtained by catheterization, the level of significance is 1000 to 10,000 CFU/ml. Children with 100 to 50,000 organisms should have a fresh urine culture examination. For voided specimens, the cutoff level is 100,000 CFU/ml. In combination with acute localizing symptoms such as marked dysuria and frequency together with pyuria, one positive urine culture can be considered adequate for a diagnosis. For all patients lacking symptoms, a second sample should be obtained before antibacterial treatment is started except when suprapubic aspiration is used.

## **LOCALIZATION OF THE SITE OF INFECTION**

In a typical case of acute pyelonephritis there is little reason to attempt to localize the site of infection. High fever, chills and rigors, vomiting, neutrophilic leucocytosis, and elevated levels of C-reactive protein suggest renal parenchymal involvement. In infants and young children localization is very difficult and unnecessary. When in doubt it is more prudent to regard the UTI as pyelonephritis and manage accordingly. In pyelonephritis DMSA scan shows diminished tracer uptake, which is indicative of parenchymal involvement. The procedure may be employed to determine the site of infection in older children with absent or equivocal features of renal involvement. DMSA scan is not routinely carried out to localize the site of infection.

## **TREATMENT**

The management of children with presumed UTI depends upon a number of factors, including age of the patient, degree of toxicity, presence of vomiting, duration of fever prior to presentation, and the antimicrobial resistance patterns in the community. Children with symptomatic UTI should be given antibiotics without delay, but after obtaining a proper urine sample. Neonates and infants should be hospitalized and provided appropriate supportive care (control of fever, IV fluids).

The choice of antimicrobial therapy is based upon the severity of UTI as assessed clinically. Second- and third-generation cephalosporins (such as cefixime), given orally or intravenously, or amoxicillin+clavum are adequate for empiric therapy. Aminoglycosides are used in inpatients.

Quinolones are effective and bacterial resistance is uncommon but these are not recommended as initial agents. First generation cephalosporins and trimethoprim-sulfamethoxazole may be used in older children with lower UTI. For confirmed or suspected pyelonephritis, treatment is given for 10 to 14 days. Children without symptoms of pyelonephritis are treated for 7-10 days. Shorter courses of therapy are not recommended in children. A repeat urine culture is obtained if fever does not abate within 48 hours. Further observation UTI with urinary tract abnormalities and imaging are considered in every case.

## IMAGING MODALITIES<sup>6,7</sup>

Imaging of kidney and urinary tract is necessary to exclude any anomaly of the kidney and urinary tract, exclude obstruction and detect VUR<sup>1,5</sup>. Plain X-ray film of abdomen and I.V.P. are rarely performed.

### Ultrasonography

Renal ultrasonography detects the location, and size of the kidney, dilatation of the upper urinary tract, severe loss of renal parenchyma, and any major bladder anomaly. The procedure is inadequate for detecting VUR.

### Voiding cystourethrogram

VCUG is required to look for VUR and any abnormality of bladder and urethra. The initial procedure is the contrast VCUG, which provides clear anatomic resolution and is necessary for grading VUR. Strict aseptic precautions are necessary and an antibiotic cover is provided.

### Renal scintigraphy

DMSA or Mercurioacetylglucine (MAG3) are the agents commonly used for scanning to detect parenchymal involvement. DMSA is injected intravenously, and uptake by the kidney is measured two to four hours later. An area of decreased uptake represents an area of pyelonephritis or scarring. In children with UTI and VU reflux, the kidney having reflux is most at risk of both congenital and acquired renal damage, and this risk increases with the severity of reflux.

### Recommendations for imaging<sup>5</sup>

1. Ultrasonography should be performed in infants and young children and whenever pyelonephritis is suspected. Documenting the absence of an anomaly is also important.
2. VCUG is indicated in all children with UTI who are less than two years of age and in any child with recurrent episodes of UTI regardless of sonographic findings. In older children with a first febrile UTI, VCUG should be obtained if the ultrasound demonstrates significant abnormality.
3. DMSA scanning should be carried out in children below 2 years and preferably below 5 years to detect renal scarring. In occasional instances DMSA scanning may be performed if the diagnosis of pyelonephritis is uncertain due to equivocal urinalysis or culture results.

## MANAGEMENT: SPECIAL CONSIDERATIONS

Obstructive abnormalities are appropriately treated. Grade 1 – 4 VUR is managed conservatively with antibacterial prophylaxis (using nitrofurantoin or cotrimoxazole) and UTI surveillance. Careful evaluation of various problems is made before deciding to undertake a surgical procedure (ureteric reimplantation or intravesical injection of Deflux) for grade 5 VUR or bilateral VUR. The benefits of chemoprophylaxis or surgical intervention are unproven. However, until clear evidence is available existing recommendations should be followed<sup>8</sup>.

### Recurrent UTI in the absence of structural anomaly and VUR

Recurrent UTI in the absence of demonstrable anatomic abnormality of kidney and urinary tract raise the suspicion of an underlying voiding dysfunction<sup>9</sup>. A detailed history of voiding pattern is obtained particularly about frequency, urgency, dribbling, holding maneuvers and constipation. Frequency, urgency and urge incontinence suggest dysfunctional voiding as occurs with idiopathic detrusor instability or urge syndrome. A voiding diary over a 3-4 day-period, noting the volume of urine output, fluid intake and involuntary wetting is very useful in evaluation. A careful neurological examination is done and perianal sensations tested. Any evidence of spinal and sacral anomalies (lipoma, dimple, tuft of hair) is

looked for and perineum is examined for ectopic ureter, epispadias, vaginal pooling and labial adhesion in girls. Ultrasonographic evaluation is done and the bladder capacity and volume of post void residual urine (more than 2 ml/kg or 25 ml is abnormal) recorded. The presence of neurogenic bladder is carefully excluded. Clinical and ultrasound evaluation is sufficient in a majority of cases but occasionally uroflowmetry and urodynamic studies are required.

### Voiding disorders<sup>10,11,12</sup>

Voiding disorders involve an abnormality during the *bladder filling or evacuation*. These are distinguished on urodynamic studies. A small capacity, hypertonic bladder or detrusor instability characterise filling phase defect. In evacuation defect detrusor sphincter dyssynergia may be a prominent feature.

### Symptomatic Management

Treatment of constipation, a liberal fluid intake and regular complete voiding are most important. In children with detrusor instability anticholinergic agents such as oxybutynin or tolterodine may be useful. Patients with dysfunctional voiding and having large post residual volumes, it is important to lower intravesical pressures with clean intermittent catheterisation. Motivational therapy and behaviour modification measures are undertaken as necessary.

### Recurrent UTI with no obvious abnormality<sup>13</sup>

In some children with recurrent UTI no structural or functional abnormality appears to be present. It is important to appreciate that asymptomatic bacteriuria does not require antibiotic therapy. For symptomatic recurrent UTI, prophylactic antibiotic administration has been employed, using a single dose of nitrofurantoin or cotrimoxazole given at bedtime. Nonspecific measures including use of vitamin A, probiotics and cranberry juice have been tried and may be beneficial in individual cases. Lactobacilli adhere to mucus and form a biosurfactant barrier that could prevent attachment of uropathogens to epithelial receptors and may modulate the host immune system<sup>14</sup>. Their usefulness is currently being examined. UTI surveillance is, however, important since UTI may occur despite instituting non specific measures.

## CONCLUSIONS

Urinary tract infections are common in infants and children and may lead to serious complications. UTI must be suspected, confirmed by careful urine examination and adequately treated. Imaging procedures of kidney and urinary tract should be considered in every case of UTI. Besides structural abnormalities and vesicoureteric reflux, voiding dysfunction should be looked for and appropriately managed.

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## RECENT ADVANCES IN THE MANAGEMENT OF STEROID SENSITIVE IDIOPATHIC NEPHROTIC SYNDROME

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**Abstract :** Nephrotic syndrome is one of the commonest renal problems encountered in day to day nephrology practice. About 90% children with idiopathic nephrotic syndrome have 'minimal lesion' (MCD) on renal histological examination and respond promptly to corticosteroid therapy with remission of proteinuria. Hence children without any atypical features are not subjected to biopsy and managed as presumed MCD. The ability to achieve a remission is the single most important step that helps in preventing the onset of chronic renal failure and progression to end stage renal disease (ESRD). A small proportion of patients who are steroid resistant are also at risk for various complications and renal insufficiency. Prednisone continues to be the sheet anchor of therapy. Based on the response to steroid treatment these patients can be classified on follow up into steroid response categories. Alternative agents are recommended for children who are frequent relapsers, steroid dependent and steroid resistant. These children should be managed in consultation with a pediatric nephrologist. These children need to be monitored for side-effects of the drugs as well as complications of the disease.

### INTRODUCTION

Nephrotic syndrome is one of the commonest renal problems encountered in day to day nephrology practice. In children it has a reported incidence of 2 per 100,000 per year and a cumulative prevalence of 16 per 100,000<sup>1</sup>. Its prevalence is strongly influenced by ethnic factors and it is more common in blacks and Asians as compared to Caucasians.

About 90% children with idiopathic nephrotic syndrome have 'minimal lesion' on renal histological examination and respond promptly to corticosteroid therapy with remission of proteinuria. Approximately three-fourths of these patients have one or more relapses that require repeated treatment with corticosteroids. Such patients are at high risk of corticosteroid toxicity, frequent serious infections and other complications. A small proportion of patients who are steroid resistant are also at risk for similar complications and renal insufficiency.

### DEFINITION

Nephrotic syndrome is characterized by heavy proteinuria, hypoalbuminemia, which is usually associated with hyperlipidemia and edema. A urine albumin > 40 mg/m<sup>2</sup> per hr (on a 6-12 hr sample) in children is considered as nephrotic range proteinuria. Alternatively a protein/creatinine (mg/mg) ratio > 2 is indicative of nephrotic range proteinuria<sup>2</sup>. Hypoproteinemia is defined as serum albumin < 2.5 g/dl and hyperlipidemia is considered to be present when serum cholesterol > 200 mg/dl. In most instances, the finding of 3+/4+ proteinuria (on dipstick or boiling test) is adequate for defining nephrotic range proteinuria. Precise quantitative assessment of proteinuria is not essential and a 24-hr urine protein measurement is not required for the diagnosis of nephrotic syndrome.

### HISTOPATHOLOGY

In children minimal change disease (MCD) is the commonest cause followed closely by focal glomerulosclerosis (FSGS). In our study

the distribution of histopathological types was significantly different in different age groups. MCD was the commonest histopathological type in children younger than 8 years while MPGN was the commonest type between 8 -12 years and between 12-16 years<sup>3</sup>. Although MCD remains the commonest cause of idiopathic nephrotic syndrome in children, there have been reports of increasing prevalence of FSGS in both children and adult patients with idiopathic nephrotic syndrome<sup>3</sup>.

### GENETICS

There is no gene that has been localised for the vast majority of children with steroid responsive nephrotic syndrome.. Mutations of NPHS1, NPHS2, ACTN4 and WTI genes are responsible for severe forms of SRNS in childhood, progressing to end-stage renal failure<sup>3</sup>. Positional cloning has revealed defects in these 4 different genes as monogenic causes of SRNS in familial cases (Table 2)<sup>4</sup>. However no gene has been localised for the vast majority of children with steroid sensitive nephrotic syndrome. We evaluated the associations of the HLA class II associations in Indian children with INS and studied the correlation with the severity of the clinical course in terms of the steroid response categories. We observed that the allele DR-b1\*150X was a marker of steroid resistance.

### INVESTIGATIONS

Screening investigations to be carried out at the initial episode are:

- Urinalysis, complete blood count, blood levels of total protein, albumin, cholesterol, triglycerides, urea and creatinine
- If persistent microscopic or gross hematuria: blood levels of antistreptolysin O, C<sub>3</sub>
- Evaluate for underlying illness, if clinically suspected (*e.g.*, antinuclear antibodies for systemic lupus erythematosus)
- Urine culture, if urinary tract infections are clinically suspected
- X-ray chest, Mantoux test

- g) Hepatitis B surface antigen
- f) Kidney biopsy : All children with idiopathic nephrotic syndrome are managed as presumed MCD. Kidney biopsy is recommended in children in the following situations: (i) age of onset of NS less than 1 year (ii) no response to 4 weeks of standard prednisolone therapy i.e who are steroid resistant (iii) children with 2 or more unusual clinical features (hypertension, gross hematuria) and /or laboratory abnormalities (abnormal renal functions)<sup>4</sup>.

## MANAGEMENT

It has been demonstrated that the adequacy of initial therapy effects the subsequent course of illness<sup>5</sup>. Moreover the ability to achieve a remission is the single most important step that helps in preventing the onset of chronic renal failure and progression to end stage renal disease (ESRD), especially in case of Focal Segmental Glomerulosclerosis (FSGS) and Membranoproliferative Glomerulonephritis (MPGN). Thus the economic benefits of timely and appropriate management of patients with NS are enormous. This is especially true for developing countries like ours where treatment for ESRD is neither easily available nor affordable for the vast majority. Appropriate therapy helps in minimizing side effects besides decreasing referrals to tertiary care centers.

### **Prednisone**

MCD is the commonest cause of nephrotic syndrome in children. Children without any atypical features are not subjected to biopsy and managed as presumed MCD.

It is believed that MCD, mesangial proliferative glomerulonephritis (MesPGN) and focal segmental glomerulosclerosis (FSGS) are different ends of the same spectrum of disease. All these entities are treated by a common protocol for steroids and cyclophosphamide<sup>2</sup>.

#### **(a) Treatment of Initial Episode**

Adequate treatment of the episode is extremely important. Current evidence suggests that treatment of the initial episode influences the subsequent course of the illness<sup>2</sup>. The intensity of initial treatment may decrease the rate of subsequent relapses. It is necessary to treat infections before starting treatment with prednisolone. The management consists of 6 weeks daily prednisolone therapy in doses of 60mg/m<sup>2</sup>/day followed by 6 weeks of 40mg/m<sup>2</sup>/alternate day for the next 6 weeks<sup>6</sup>. The prednisone is then tapered over the next 2-3 months (not exceeding a total duration of 7 months). We have shown that slow tapering of prednisone results in a lower relapse rate as compared to abrupt stoppage, after the initial 12 weeks in the management of first episode of nephrotic syndrome<sup>7</sup>.

#### **(b) Treatment of Relapse**

The patient should be examined for infections, which are treated before initiating corticosteroid therapy. Prednisolone is administered in a dose of 2 mg/kg/day (single or two divided doses) until urine protein is trace or nil for 3 consecutive days, or for two weeks. Subsequently, prednisolone is given in a dose of 1.5 mg/kg on alternate days for 4 weeks, and then discontinued<sup>8</sup>. The usual duration of treatment for a relapse is thus 5-6 weeks. Prolongation of therapy is not necessary for patients with infrequent relapses (see below). In case the patient is not in remission despite two weeks

treatment with daily prednisolone, such treatment might be extended for two more weeks.

Based on the response to steroid treatment these patients can be classified on follow up into steroid response categories (as per ISKDC guidelines); infrequent relapsers (IFR) children with less than 2 relapses over 6 months, frequent relapsers (FR) children with 2 or more relapses over 6 months, steroid dependent (SD) - children with 2 consecutive relapses within 2 weeks of stoppage or tapering of steroid, initial non responders (INR) - children with no response to steroid therapy for 4 weeks and subsequent non responders (SNR) - children who responded to steroid initially but secondarily become steroid resistant. Of the 116 children of idiopathic nephrotic syndrome with follow up of more than 6 months in our series who could be categorized into a definitive category, infrequent relapsers constituted the majority (37.9%) followed by FR (21.6%), SD (18.1%), INR (17.3%) and SNR (5.1%)<sup>9</sup>.

### **Alternative agents**

For patients who are FR and SD, a variety of therapeutic modalities have been tried<sup>5-10</sup>.

- (A) **Cyclophosphamide** Treatment with cyclophosphamide may be considered in patients showing, i) significant steroid toxicity, ii) severe relapses with hypovolemia or thrombosis and iii) poor compliance or follow up. Cyclophosphamide (2mg/kg/d) and chlorambucil (0.5mg/kg) have an established role in prolonging remission in FR (given for 8 weeks) and SD (given for 12 weeks). Intravenous pulse cyclophosphamide in monthly doses of 500mg/m<sup>2</sup> is an alternative modality. In our study the overall response rate of 49% was comparable to that reported previously. Furthermore the response was observed at 40% lesser cumulative dose<sup>11</sup>. An additional advantage of the IVCP regimen was a better tolerance and hence a better compliance. We have found this modality to be efficacious in steroid resistant MCD and FSGS.
- (B) **Levamisole**: Levamisole has a weak steroid sparing effect and is useful in milder cases. The other alternatives available include Levamisole (2.5mg/kg/alternate day for 3 months). A longer duration of therapy of 6-18 months concurrent with alternate day prednisolone has been found to be reducing the relapse rate of SDNS<sup>6</sup>.
- (C) **Long term low dose prednisolone** in a dose of 0.25mg/kg/day for 18 months have been found to be beneficial in FR group.
- (D) **Cyclosporine**: This given in a daily dose of 5mg/kg is another therapeutic alternative. Cyclosporine is recommended for patients that continue to relapse despite a course of cyclophosphamide. It has a response of 80%. However it has a number of side-effects including hirsutism, gingival hypertrophy, besides nephrotoxicity. However it is advisable to monitor serum levels of these agents periodically and perform kidney biopsies annually if therapy is prolonged beyond a year. In addition these patients have a potential of becoming cyclosporine dependent.
- (E) **Tacrolimus** Tacrolimus is a calcineurin inhibitor that is more potent in cytokine suppression than cyclosporine. The main mechanism of action of tacrolimus is through the inhibition

of IL-2 dependent T-cell activation, a process occurring during the early phase of T-cell activation Tacrolimus is newer calcineurin inhibitor that has far less cosmetic side-effects. It has been found to be an efficacious agent in steroid resistant nephrotic syndrome and can be used in children who are frequent relapsers or steroid dependent who continue to relapse despite other therapies. The advantages of using these drugs should be balanced against their potential nephrotoxicity. Hence it is advisable to monitor serum levels of these agents periodically and perform kidney biopsies annually if therapy is prolonged beyond a year. Most SD children can be maintained in remission with cyclosporine or tacrolimus, but relapses usually occur when the therapy is stopped.

- (F) **Mycophenolate Mofetil (MMF)** is another newer agent that has been used in children with FRNS and SDNS. It is a prodrug of mycophenolic acid (MPA) which is formed by hydrolysis. MPA is a potent, selective, uncompetitive, and reversible inhibitor of inosine monophosphate dehydrogenase. The latter is an enzyme required for de novo purine synthesis. MPA inhibits B and T-lymphocyte proliferation, as these cells are critically dependant upon de novo purine synthesis for their proliferation whereas other cell types can utilize salvage pathways for purine synthesis. It has response rate of 25% in children with FRNS whereas in SDNS these children relapse after stoppage of therapy. It is a useful agent in children who become cyclosporine dependent, as CsA which is potentially nephrotoxic, can be weaned off under cover of MMF. Pefloxacin, an antibiotic with immunomodulatory effect can also be used in dose of 25mg/kg/day both in relapsing and resistant cases<sup>12</sup>. A metanalysis revealed that cyclophosphamide was the only agent whose effect persisted beyond the stoppage of therapy.
- (G) **Rituxumab** is an anti CD 20 monoclonal antibody that has been found to be very effective in these children with steroid dependent nephrotic syndrome. The exact mechanism of action of rituxumab in idiopathic NS is not known. The following effects have been found that may possibly explain its efficacy viz. down regulation of  $\alpha$ -cell receptors, shedding of CD 23 cells and apoptosis of CD20<sup>+</sup> cells, general regulatory effects on the cell cycle, and increases in MHC II and adhesion molecules LFA-1 and LFA-3 (lymphocyte function – associated antigen). It has been found that 2 doses given at an interval of 2 weeks can lead to sustained remission in these children.

### Supportive care

This forms an important aspect of managing children with nephrotic syndrome.

**Diet** A balanced diet adequate in protein and calories is recommended. The child should receive 1.5-2 g/kg of proteins. Patients with persistent proteinuria are prone to malnutrition and should receive 2-2.5 g/kg of protein daily<sup>6</sup>. Not more than 30% calories should be derived from fat and saturated fats avoided. Carbohydrates are best given in complex forms (starch and maltodextrin). Salt restriction is not necessary in most patients with steroid responsive nephrotic syndrome. A modest reduction (1-2 g per day) is advised in the presence of marked edema. Salt should not be added to salads and fruits; snacks containing high salt are avoided during these periods.

Corticosteroids stimulate the appetite, and advice should be given about ensuring physical activity and preventing excessive weight gain.

**Edema control.** This is an integral part of supportive care. Treatment with corticosteroids usually leads to diuresis within 48-72 hours. Diuretics are thus avoided unless edema is significant and should not be used in children with diarrhea, vomiting or hypovolemia. In moderate or persistent edema, furosemide is administered in a dose of 1-3 mg/kg per day. Additional treatment with potassium sparing diuretics (e.g., spironolactone) is not required if furosemide is used in this dose for less than one week. Patients requiring higher doses and prolonged duration of treatment with furosemide should receive amiloride or spironolactone (dose 2-4 mg/kg daily). Blood pressure should be monitored frequently. A gradual reduction of edema, over one week, is preferred<sup>6</sup>. Edema not responding to the above therapy should be managed in a hospital under close supervision. For refractory edema, a combination of diuretics and albumin infusion may be used. Infusion of albumin is followed by administration of furosemide in a dose of 1-2 mg/kg intravenously. Though infusion of albumin results in increased urine output, the effect is not sustained, especially in patients with steroid resistant nephrotic syndrome. Albumin infusions are usually administered on alternate days to allow fluid shifts to occur and prevent fluid overload. Patients receiving albumin should be carefully observed for respiratory distress and congestive heart failure. Refractory ascites interfering with respiration or associated with breaks in the skin may be removed by repeated tapping.

## COMPLICATIONS

### Infections

These remain an important complication of children with nephrotic syndrome, especially in developing countries like ours. Besides being the commonest cause of mortality, infections result in significant morbidity. They may also be responsible for non-response to steroids or induce a relapse in a child who has already attained remission. A knowledge of the spectrum of infections is important not only from the therapeutic point of view, but also for planning preventive strategies like pneumococcal vaccination and prophylactic antibiotics. Evaluation of all patients both admitted as well as those being followed up on an outpatient basis reveals that urinary tract infections were the commonest (13.7%), followed by pulmonary tuberculosis (10.4%), peritonitis (9.1%), skin infections (5.2%), recurrent upper respiratory infections (5.2%), lower respiratory tract infections (3.9%) and pyomeningitis (0.6%)<sup>12</sup>.

### Thrombotic Complications

Children with nephrotic syndrome are at risk for venous and rarely, arterial thrombosis. Reduced intravascular volume and other abnormalities predispose to thrombus formation. Diuretics should be used judiciously. Puncture of deep vessels should not be done. Renal vein thrombosis is suspected in a patient with oligoanuria, hematuria or flank pain especially following an episode of dehydration. Ultrasound examination of the abdomen might show large kidneys and thrombi in renal veins. Femoral arterial thrombosis may occasionally occur. Deep vein thrombosis of calf veins is less common in children but may lead to pulmonary embolism. Saggital sinus and cortical venous thrombosis may follow episodes of diarrhea and present with convulsions, vomiting, altered sensorium and neurological deficits. Doppler studies and cranial CT scan are useful

in confirming the diagnosis. Patients with thrombotic complications require urgent treatment under the supervision of a specialist. The treatment is supportive and consists of early mobilization, treatment of sepsis, prompt treatment of dehydration and cautious use of anticoagulants.

#### **Hypertension**

This may be noted at the onset of nephrotic syndrome or occur due to steroid toxicity. Therapy may be initiated with ACE inhibitors, calcium channel or beta adrenergic blockers.

#### **Hypovolemia**

This complication can occur due to unsupervised use of diuretics especially if accompanied by septicemia, diarrhea or vomiting. The diagnosis is suggested by the presence of hypotension, tachycardia, cold extremities and poor capillary refill. Blood levels of urea and uric acid are elevated. Some children might complain of moderate to severe abdominal pain. A rapid infusion of normal saline or plasma in a dose of 15-20 ml/kg, or albumin 1g/kg is essential. The blood pressure should be monitored carefully. Albumin should be used with caution if the child is hypertensive because of the risk of pulmonary edema. Once adequate hydration is achieved, but the child remains oliguric, a single dose of frusemide (1-2 mg/kg intravenously) may be given. In case no urine is passed despite these measures, the diagnosis of acute renal failure is suspected.

#### **Metabolic bone disease**

There is recent evidence that children with idiopathic nephrotic syndrome (INS) are at risk for metabolic bone disease (MBD) due to biochemical derangements caused by the renal disease, as well as steroid therapy. The risk is greater in those who receive higher doses of steroids (FR, SD and INR). Prophylactic calcium and vitamin D supplements help in minimizing the risk of low bone density in these children.

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## **LITERATURE REVIEW**

### ***Laparoscopic Kidney retrieval in donor with an extended criterion: Assessing the safety and outcome***

*Arvind R. Ganpule, Mahesh R. Desai and Shashikant Mishra. Ann Natl Acad. Med Sci (India), 44 (3): 165-175, 2008.*

There is an ever increasing need to accommodate deserving transplant recipients coupled with the relative paucity of active cadaver transplant programme. The terms extended donor criterion and marginal donors are confusing, they may simply mean usage of suboptimal quality cadaver renal grafts, non heart beating donors and living donors with some acceptable medical risks. We have identified old age, hypertension, diabetes mellitus, obesity, and anatomical anomalies such as multiple vessels and double ureters as extended indications for laparoscopic donor nephrectomy (LDN) as we feel these donors require consideration surgical/medical expertise and acumen to attain good results.

The term marginal kidney donor/extended donor criterion is not clearly defined. In the article we assess the safety and outcome of laparoscopic donor nephrectomy (LDN) in donors with an extended criterion. A retrospective analysis of our database was done to assess the outcome of extended donor. Analysis was done between normal donors (group 1) and donors with extended criterion (group 2). The parameters analyzed were pre and post operative serum creatinine in donors and recipients, serum creatinine at day 1, 7, 30 days and 1 year in recipients, operative time, warm ischemia time, analgesia requirement in donors and impact of extended criteria on recipient outcome. Group I and II recipients had comparable creatinine at 1 year. Donors with BMI more than 30 kg/m<sup>2</sup> required extended hospital stay. Recipient's creatinine, with single vs. multiple vessel donors was comparable at 1 year. Donors with urolithiasis had good recipient outcome. The donor creatinine was comparable in extended and non extended indication donors. In our study, LDN was found to be safe, feasible and efficacious in donors with extended indications such as old age, BMI more than 30, multiple vessels and anatomical anomalies. Recipient outcome for donors with normal vs. extended criteria was comparable at one year follow up. All donors with extended criterion had normal post operative creatinine levels at two years follow up; long term follow up would be of interest.

## KIDNEY TRANSPLANTATION IN CHILDREN

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**Abstract :** There are various modalities to treat children with ESRD i.e. hemodialysis, chronic ambulatory peritoneal dialysis and renal transplant. Satisfactory rehabilitation of uremic children can be achieved by renal transplantation, with dialysis only bridging the period of terminal insufficiency, until transplantation becomes possible. With recent advances in medical technology and availability of expertise, transplantation has become the gold standard for management of children with chronic kidney disease. Currently it is recommended that all children with chronic kidney disease should be worked up for a pre-emptive kidney transplant (transplant without prior dialysis). All potential pediatric recipients of transplants should receive standard immunizations at least 6 weeks prior to transplantation. The immunosuppressive protocol consists of induction antibody treatment (referred to as quadruple immunosuppression) followed by CNI, MMF/ azathioprine and prednisone. The major limiting factor for this protocol in our country is the affordability. The long term outcome is excellent. In the Swedish study of children, which evaluated long term outcomes in children who received cyclosporine, prednisolone, and azathioprine, 5 and 10 year allograft survival rates were 77 and 66 percent, respectively. Patient survival is better in pediatric renal transplant recipients than adults.

### INTRODUCTION

The point prevalence of end stage renal disease (ESRD) in the pediatric population is 55 per million-child population<sup>1</sup>. There are various modalities to treat children with ESRD i.e. hemodialysis, chronic ambulatory peritoneal dialysis and renal transplant. Satisfactory rehabilitation of uremic children can be achieved by renal transplantation, with dialysis only bridging the period of terminal insufficiency, until transplantation becomes possible<sup>2</sup>.

### INDICATIONS

With recent advances in medical technology and availability of expertise, transplantation has become the gold standard for management of children with chronic kidney disease. Currently it is recommended that all children with chronic kidney disease should be worked up for a pre-emptive kidney transplant (transplant without prior dialysis). In developing countries, considerations of cost remains a major impediment in its widespread use<sup>3</sup>.

### CONTRAINDICATIONS

Children with acute or chronic active infection and those with malignancy are not generally candidates for kidney transplantation. Most centers consider transplantation in a child who has been disease free for 2 years following treatment of cancer.

Transplantation is also contraindicated in any child or family with a history or high likelihood of noncompliance with a prescribed medication regimen. Active systemic lupus erythematosus and Goodpasture disease are also contraindications to transplantation because these processes can damage an allograft. The success rate of renal transplantation in very young children, especially those younger than 1 year, is significantly less than that in older children. Therefore, carefully evaluate all alternatives for treatment of ESRD. Generally, continuous ambulatory peritoneal dialysis (CAPD) is the preferred method of treatment of children younger than 1 year. However, CAPD may not be possible because of peritoneal scarring. Hemodialysis is difficult in very small children. In such persons, transplantation may be the best option.

### LABORATORY EVALUATION

- Twenty-four-hour urine collection for creatinine clearance
- Complete blood cell count
- Basic metabolic panel
- Coagulation evaluation
- Viral titers (HBV, HCV, HIV, EBV, HSV)
- Panel reactive antibody

### IMAGING EVALUATION

- Chest radiography
- Imaging of the kidneys and renal vessels
- In the past, this included intravenous pyelography and angiography.

However, current radiologic techniques provide suitable imaging of the entire urinary tract with 3-dimensional computed tomography (3D CT) scanning or magnetic resonance angiography (MRA) using gadolinium to demonstrate the vascular anatomy.

### IMMUNIZATIONS

All potential pediatric recipients of transplants should receive standard immunizations at least 6 weeks prior to transplantation .

Special attention needs to be given to the live-attenuated vaccines, including varicella and measles-mumps-rubella (MMR), because the risk of active disease following vaccination is increased in children who are immunocompromised. Similarly, inactivated polio vaccine (IPV) is indicated, rather than oral polio vaccine (OPV). Family and household contacts also should receive MMR and varicella vaccine as indicated.

Although the HBV vaccine is routinely administered to all children now, nonimmunized children still must be immunized with this vaccine. The recommended dose schedule is at 0, 1, 2, and 6 months. Periodically monitor the antibody level after transplantation and administer a booster to those with low titers (<10 mIU/mL) .

It is suggested that routinely an annual influenza vaccine should be given to children with transplants and their family and/or household contacts.

## OPERATIVE PROCEDURE

In larger children, as in adults, the renal allograft is placed in the iliac fossa outside the peritoneal cavity. A curved lower abdominal (Gibson) incision is made in either lower quadrant, and the iliac vessels are exposed. The renal artery is anastomosed either to the external iliac or the internal iliac artery.

In past decades when allograft survival rates were significantly lower, use of the internal iliac artery for a first kidney transplant was more common because this left the external iliac artery available for subsequent grafting. Currently, most kidney transplants are performed using the external iliac artery for blood supply. The renal vein is anastomosed to the external iliac vein. With the kidney perfused, the ureter is anastomosed to the bladder, using an extravesical technique that avoids opening of the bladder.

Infants and small children require modification of the standard surgical approach because of their size. Although transplantation of a small kidney into a young child can be performed using the approach and technique described above, most children receive an adult-sized kidney transplant. Through a midline incision, the peritoneal cavity is entered and the great vessels are exposed. The renal vessels are then anastomosed to the abdominal aorta and inferior vena cava. The common iliac artery and vein can also be used, depending on the size of the kidney and the recipient. The ureter is anastomosed to the bladder as described above.

Approximately 1 in 4 children presenting for transplantation have ESRD from urologic abnormalities. A small but significant proportion of these children have abnormal urine storage function due to a neurogenic bladder, lower urinary tract obstruction, reflux, or a congenital anomaly of the bladder or urethra (eg, exstrophy, posterior urethral valves). In addition, some children may have lost their bladders as a consequence of malignancy, radiation, or scarring from chronic infection. Despite these challenges, kidney transplantation can be successful in these patients.

Several reports describe drainage of a kidney transplant ureter to an augmented bladder, an incontinent urinary conduit, or a continent urinary reservoir with allograft survival comparable to that in children with normal bladders (Hatch, 1993). Such recipients are at increased risk of urine infections; therefore, closely monitor them. When necessary, clean intermittent catheterization has been successfully used in these patients to drain the urine.

## MEDICAL THERAPY

Modulation of the normal immune response mechanisms is a vital prerequisite to successful organ transplantation. The cascade of immunologic events triggered by the presence of foreign antigens can be interrupted or diminished at several key points.

### Antibodies

Many antilymphocyte antibodies have been used in transplantation. Polyclonal antibodies provide a relatively less specific impairment of lymphocyte activity. Monoclonal antibodies (ie, muromonab-CD3, daclizumab, basiliximab) provide more specific inhibition of lymphocyte function. Antibodies are used for induction (temporary use immediately following transplantation, while other immunosuppressive agents are adjusted) and for treatment of acute rejection.

- *Names* - Antithymocyte globulin (eg, Thymoglobulin, Atgam), muromonab-CD3 (Orthoclone OKT3), daclizumab (Zenapax), basiliximab (Simulect)

- *Method of action* - In general, antibodies used in immunosuppression interfere with the function of T lymphocytes; lysis of lymphocytes with a resulting lymphopenia caused by some agents; some antibodies cover or impair function of cell surface markers necessary for recognition and processing of foreign antigens in the cascade of the immune response; others may result in an increase of suppressor T lymphocytes

- *Dose* - Varies according to center and use
- *Antithymocyte globulin (Atgam), dose* - 10-15 mg/kg/d IV administered once daily for 5-14 days; many centers vary the duration, discontinuing the antibody when cyclosporine or tacrolimus levels are consistently within therapeutic range

- **Daclizumab dose** - 1 mg/kg IV on the day of transplant and repeated at weeks 2, 4, 6, and 8 following transplantation; some centers use only 2 doses in adults and older pediatric recipients (teenagers)

- **Basiliximab dose:** Children aged 2-15 years - 12 mg/m<sup>2</sup> IV administered within 2 hours prior to transplantation and repeated 4 days following transplantation

Patients older than 15 years - 20 mg IV administered within 2 hours prior to transplantation and repeated 4 days following it.

### Corticosteroids

Methylprednisolone dose - 10 mg/kg IV immediately prior to transplant with relatively rapid conversion to prednisone and tapering of dose over 12 weeks to baseline dose of 0.3 mg/kg/d PO. *Adverse effects* are hirsutism, acne, hypercholesterolemia, hyperlipidemia, avascular necrosis of the hip, glucose intolerance, growth retardation, gastritis, gastric ulcer, obesity, cataracts, impaired wound healing and mood alteration. In an effort to increase growth of pediatric recipients of kidney transplants and to avoid adverse effects, some centers taper and ultimately discontinue corticosteroids within 1 year of transplantation. Others have used steroid free protocols along with aggressive induction and maintenance therapy.

### Antimetabolites

These are useful adjunctive agents in any immunosuppressive protocol.

#### Mycophenolate mofetil, Azathioprine

- *Method of action:* Inhibition of cell proliferation. Mycophenolate mofetil blocks inosine monophosphate dehydrogenase, an enzyme necessary for purine synthesis specifically in lymphocytes. This provides more specific immunosuppression for transplantation than azathioprine. Azathioprine impedes purine synthesis, thus impairing cell division and proliferation.

- *Dosage:* Mycophenolate mofetil, dose- 1200 mg/m<sup>2</sup>/d PO divided in 2 doses. Azathioprine, dose - 1-2 mg/kg PO once daily.

### Calcineurin inhibitors

These constitute the sheet anchor of most modern day immunosuppressive protocols.

- *Method of action* - They block production of or action of IL-2 or other cytokines
- *Dosage* - Cyclosporine, 8-10mg/kg/d PO divided twice daily; dose adjusted to maintain trough whole blood level of 325-

400 ng/mL or a 2-hour peak level of 1,000-1,200 ng/mL; absorption and metabolism vary considerably; therefore, adjust dose individually; very young children and those with rapid metabolism of the drug may require 3 doses per day to maintain adequate trough level

- **Tacrolimus:** 0.2-0.3 mg/kg/d PO divided twice daily; dose adjusted to maintain trough whole blood level of 9-12 ng/mL in first 3 months following transplantation
- **Sirolimus:** 2 mg/m<sup>2</sup> PO once daily, administered 4 hours following cyclosporine; dose adjusted to maintain trough whole blood level of 8-15 ng/mL; simultaneous ingestion of fat may decrease absorption; patients should take sirolimus consistently either with or without food; it should not be taken with grapefruit juice (impairs absorption).

#### **Adverse effects of immunosuppressants**

- **Cyclosporine** - Hypertension, nephrotoxicity, hirsutism, gingival hyperplasia, neuropathy, increased susceptibility to infections, increased risk of malignancy
- **Tacrolimus** - Nephrotoxicity, neurotoxicity, hyperglycemia, hyperkalemia, increased susceptibility to infections, increased risk of malignancy
- **Sirolimus** - Hypercholesterolemia, hyperlipemia, hypertension, rash, increased susceptibility to infections, increased risk of malignancy, interstitial pneumonitis

#### **Current Immunosuppressive Protocols**

Data in children from the NAPRTCS study suggests that antibody induction therapy gives better results in pediatric transplantation<sup>4</sup>. Most centres have started using induction antibody treatment (referred to as quadruple immunosuppression) the major limiting factor being the affordability. At SGPGIMS, which has emerged as one of the largest pediatric transplant centres in our country, so far performed more than 100 kidney transplants in children. In our initial experience with 39 kidney transplants, a triple drug regimen of Csa, Aza and Prednisone was the cornerstone of immunosuppression in this public sector hospital<sup>5</sup>. We also observed that discontinuation of Csa was a major reason for poorer long term graft survivals<sup>5</sup>. There has been a dramatic increase in the use of tacrolimus instead of cyclosporine in view of the cosmetic advantages and superior potency. It has been estimated that treating 100 recipients with tacrolimus instead of cyclosporine for the first year after transplantation avoid 12 patients having acute rejection and two losing their graft but causes an extra five patients to develop insulin dependent diabetes. In our country too, use of tacrolimus is gaining popularity in pediatric transplants. We were amongst the first to use tacrolimus based protocol in children in our country. In our series of the last 30 children at SGPGIMS, we changed over to Tacrolimus, MMF and Prednisone as the standard immunosuppressive protocol. The major limitation to its widespread use remain the easy availability of drug level estimation. In contrast quadruple immunosuppression using antibody induction with tacrolimus has been the norm at leading private sector hospitals (Kher et al. personal communication) Excellent rehabilitation was observed with most children with functioning grafts, attending their school or college normally, doing well in both curricular and extracurricular activities<sup>8</sup>.

## **FOLLOW-UP CARE**

### **Laboratory studies**

- Complete blood cell count: CBC is performed to detect leukopenia (potential adverse effect of some immunosuppressive agents), leukocytosis (evidence of infection), and anemia.
- Serum electrolytes and liver enzyme tests: These tests are performed to monitor K<sup>+</sup>, PO<sub>4</sub> (hypophosphatemia common following successful kidney transplantation), and liver enzymes (hepatotoxicity from immunosuppressive medications).
- **CNI trough levels:** Because absorption and metabolism of cyclosporine and, to a lesser degree, tacrolimus can vary considerably, periodically measuring trough drug levels is important.

### **Imaging studies**

- **Ultrasonography:** By far the most useful imaging technique following transplantation, ultrasonography allows rapid visualization of the kidney, the collecting system, and the vessels. Color Doppler ultrasonography detects abnormalities of blood flow, including kinking of the artery or vein and thrombosis. Ultrasonography also aids in the detection of obstruction (hydronephrosis), lymphocele, urine or blood leakage (perinephric fluid collection), and kidney stones (rare).

Over the last decade there has been tremendous improvement in patient and graft survival. However these children remain at risk of numerous complications. The important *long-term complications* are as follows: (A.) Infections; (B.) Acute rejection; (C.) Chronic rejection; (D.) Renal artery stenosis; (E.) Malignancy; (F.) Growth; (G.) Graft loss; (H.) Patient death.

## **RENAL ALLOGRAFT SURVIVAL**

The outcome of renal transplantation in children has improved over the last several decades, a period which coincides with the introduction and widespread use of cyclosporine and other immunosuppressive agents<sup>11</sup>. In addition to the efficacy of newer immunosuppressive regimens, other general factors implicated in the success or failure of a renal allograft in children include<sup>12</sup>:

- (1.) source of donor kidney living-related donors;
- (2.) HLA compatibility;
- (3.) age of the donor and recipient;
- (4.) presence of preformed anti-HLA antibodies (sensitization);
- (5.) prolonged cold ischemia time;
- (6.) ethnicity of the recipient;
- (7.) history of focal glomerulosclerosis;
- (8.) acute rejection episodes;

The most common etiology of allograft loss among children and adolescents is rejection. Acute and chronic rejection caused almost half of the kidney transplant losses reported by members of the NAPRTCS<sup>11</sup>. This was true for both initial and second kidney transplant failures. Other causes in order of decreasing frequency include technical complications including thrombosis, death with a functioning kidney, recurrent or de novo kidney diseases, primary nonfunction, noncompliance with immunosuppression, and others. In our series the 1-year patient and graft survival was 89%. Three year patient and graft survival was 70%. Kaplan Meier revealed actuarial graft survival at 5 years of 50%. Twelve children discontinued CsA after 1 year post transplant and 5 of these had a

graft loss. Graft losses were significantly greater in patients who discontinued CsA (5/12 vs 2/22) as compared to those who continued CsA<sup>3</sup>.

## PATIENT SURVIVAL

Patient survival is better in pediatric renal transplant recipients than adults. In most pediatric series, mortality rates have decreased in recent years<sup>13</sup>. In data from the North American Registry, seven-year patient survival exceeded 90 percent in all recipients of living related or cadaveric donors, other than 0- to 1-year-old recipients of cadaveric donor kidneys. Patient survival is higher for living related graft recipients than for cadaveric graft recipients. Patient survival is significantly lower for very young (<1y) recipients<sup>11</sup>. Infant recipients of cadaveric kidneys have the highest mortality rate. The mortality is higher for very young children, and may vary with the underlying renal disease. As an example, the outcome after renal transplantation may be worse in children with lupus nephritis.

## GROWTH

Most children have an improvement in statural growth after successful renal transplantation; however, complete catch-up growth is infrequent<sup>14</sup>. The most important factors limiting growth after renal transplantation are renal graft function and corticosteroid therapy: Catch-up growth is only observed with normal or nearly normal function of the allograft. Growth is impaired when the dose of corticosteroids is above 5 mg/m<sup>2</sup> per day. These agents interact with the growth hormone insulin-like growth hormone axis, and are therefore associated with decreased levels of growth hormone and insulin-like growth hormone activity. Children who receive living-related donor (LRD) grafts appear to have better growth compared to those who receive cadaveric (CAD) donor grafts<sup>15</sup>. Growth hormone can be used safely in children who are more than 1 year post transplant and have stable graft function. Recently there has been a tendency to use steroid free protocols/ steroid minimization. In our anecdotal experience they have resulted in significant gain in

height without any increased rejection rate.

Thus renal transplant has revolutionized the therapy of children with end stage kidney disease. From what once considered an experimental procedure, it has over the last 3 decades evolved as the gold standard for therapy of these children.

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## LITERATURE REVIEW

### *Recovery of renal function after 90 days on dialysis: implications for transplantation in patients with potentially reversible causes of renal failure*

*Siddiqui S et al Clin Transplant 2008; 22: 136-140. ýý 2008 Blackwell Munksgaard*

Late recovery of renal function in patients requiring dialysis is a well recognized but an uncommon phenomenon. Moves to increase the number of live donor transplants and the recognition that early transplantation is associated with better graft survival; it is possible that patients who are going to recover renal function may be transplanted unnecessarily. Prospective survey of patients receiving dialysis for more than 90 d in south west Scotland from 1 January 1994 to 31 December 2005. Routine measurement of residual renal function by combined urea and creatinine clearance allowed us to detect late recovery whenever this occurred. Eight of 202 (4%) patients recovered sufficient renal function to stop dialysing after 90-d treatment. The likely cause of the renal failure in five of these patients was atheroembolism. One with atherosclerotic renovascular disease had been stented and would have received a live related renal transplant had his sister not had second thoughts about the procedure. **Conclusion:** It may be sensible to postpone transplantation in patients with certain types of renal failure, perhaps particularly patients with renovascular disease who have recently undergone a failed revascularization procedure.

## RECENT ADVANCES IN THE MANAGEMENT OF HYALINE MEMBRANE DISEASE

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**Abstract :** Hyaline membrane disease (HMD), also known as respiratory distress syndrome (RDS) is the most common cause of respiratory distress in preterm neonates. The greatest risk factor is low gestational age and the disease is due to inadequate pulmonary surfactant. The structurally immature and surfactant-deficient lungs have decreased compliance and a tendency to atelectasis. An eosinophilic membrane composed of a fibrinous matrix of materials from the blood and cellular debris (the hyaline membrane) lines the visible airspaces hence the term HMD. Clinical features include tachypnea, grunting and retractions in a preterm neonate usually beginning immediately or within few hours after birth. The incidence of HMD has markedly decreased by prenatal treatment with glucocorticoids. The development of surfactant is one of the great success stories in neonatal care because the therapy specifically treats the surfactant deficiency and changes the pathophysiology and outcome of HMD. Benefits of early use of CPAP are fast emerging in the management of hyaline membrane disease. Despite substantial improvements in the management of HMD, prevention is still the ultimate goal.

### INTRODUCTION

Hyaline membrane disease (HMD), also known as respiratory distress syndrome (RDS) is the commonest cause of respiratory distress in preterm neonates. It occurs after the onset of breathing in neonates with insufficiency of pulmonary surfactant. The clinical diagnosis is often made in preterm infants with respiratory difficulty that includes tachypnea, retractions, nasal flaring and need for oxygen all presenting within 4-6 hours after birth. There is a reticulogranular chest X ray appearance as a result of widespread atelectasis. Pathophysiologically, this condition is characterized by non-compliant (stiff) lungs, which become atelectatic at end-expiration. Histologically, hyaline membrane formation occurs in advance stage, lining the terminal airways.

Various advances that have resulted in decrease in incidence and reduction in morbidity and mortality in neonates with HMD include

1. Use of antenatal steroids to enhance pulmonary maturity
2. Appropriate resuscitation with immediate use of continuous positive airway pressure (CPAP) for alveolar recruitment
3. Early administration of surfactant
4. Using gentle modes of ventilation to minimize damage to the immature lungs.

These therapies have also resulted in the survival of extremely premature infants. With survival of these extremely preterm infants, there is an associated increase in the morbidities, which include patent ductus arteriosus (PDA), intraventricular hemorrhage (IVH), necrotizing enterocolitis (NEC), septicemia, retinopathy of prematurity (ROP), bronchopulmonary dysplasia (BPD), periventricular leukomalacia (PVL) with associated neurodevelopmental and audiovisual handicaps. Direct attention to anticipate and minimizing these complications and to prevent premature delivery whenever possible are the strategic goals of its management.

### EPIDEMIOLOGY

HMD typically affects infants <35 weeks gestational age (GA) but

may affect older infants who have delayed lung maturation. Low GA is the greatest risk factor for HMD, and its incidence varies inversely with birth weight among AGA infants (Table 1)<sup>1</sup>. This is due to deficiency of the surfactant in these neonates which leads to decreased compliance of the lungs. The maturation of surfactant synthesis reflects the decreasing incidence of HMD with an increase in gestational age. The incidence of HMD is reported to be 6.8-14.1% of preterm live births in our country<sup>2</sup>. HMD is the commonest indication of ventilation in neonates in India<sup>3</sup>. The reported survival of babies ventilated for HMD has varied from 25% to 64% in our country<sup>2</sup>. The lung epithelium of preterm infants is leakier than term infants, increasing the likelihood of protein passage on to the alveolar surface, where it inhibits surfactant function. Moreover preterm infants are more prone to asphyxia, hypothermia, hypoxia and hypotension, all of which impair surfactant synthesis or increase the leakiness of alveolar capillaries. Various other factors which influence the risk of HMD among preterm infants are discussed below.

Table 1.: Incidence of HMD by Gestational Age

Gestational Age	Incidence of HMD
501-750	86%
751-1,000	79%
1,001-1,250	48%
1,251-1,500	27%

The disease occurs more commonly in males than females and is more common in white than in non-white infants<sup>4</sup> with an increased risk of death in males<sup>5</sup>. The delayed maturation of Lecithin Sphingomyelin ratio and the late appearance of phosphatidylglycerol<sup>6</sup> which are androgen induced<sup>7</sup> explain the increased incidence in males. The incidence of HMD in preterm African infants < 32 weeks GA has been shown to be 40% as compared to 75% in Caucasian preterm infants<sup>8</sup>. Allelic variation in the surfactant protein A gene has been reported between American whites and Nigerian blacks<sup>9</sup>. The incidence of HMD is higher for infants born by caesarean section without labor at any gestational age than infants delivered vaginally<sup>10</sup>. The timing of caesarean section is also important: the need for

mechanical ventilation being 120 times higher after elective cesarean section at 37 to 38 weeks as compared with 39-41 weeks<sup>10</sup>. When the effect of gestational age is removed, the occurrence of HMD is more in infants of mothers with gestational or insulin dependent diabetes. This is due to delay in the maturation of alveolar type II cells and decreased proportion of saturated phosphatidylcholine in the surfactant. Improvements in maternal diabetic control during pregnancy have now facilitated the delay in delivery until the 39<sup>th</sup>-40<sup>th</sup> week of gestation and HMD now occurs in < 1% of patients. Babies who are depressed at birth are at an increased risk of developing HMD, due to the ischemic damage to pulmonary capillaries as a result of fetal asphyxia leading to a leakage of protein fluid out of the damaged capillaries. This leakage of fluid leads to inactivation of the surfactant. Also, the hypoxemia and acidemia predispose to pulmonary hypertension and hypoperfusion, leading to a right to left shunt and reduction of surfactant synthesis by inhibition of synthetic enzymes. The contributions of genetic variations (polymorphisms and mutations) to the pathogenesis of HMD have been shown by molecular biology techniques. Congenital alveolar proteinosis, a disorder in which there is deficiency of Surfactant protein B resulting in lethal respiratory failure has been described in various families<sup>11</sup> and the inheritance is autosomal recessive. Partial deficiency of SP-B, which may be compatible with survival, has been reported. Other factors which contribute to occurrence and progression of HMD have been enumerated in Table 2.

Table 2.: Risk factors of HMD

<b>Increased Risk</b>	<b>Decreased Risk</b>
Prematurity	Chronic intra-uterine stress
Male Gender	Prolonged rupture of membranes
Familial predisposition	Maternal hypertension or toxemia
Cesarean section without labor	IUGR/SGA
Perinatal asphyxia	Antenatal glucocorticoids
Caucasian race	maternal use of narcotics/cocaine
Infant of diabetic mother	Tocolytic agents
Chorioamnionitis	Hemolytic disease of the newborn
Non-Immune hydrops fetalis	
Hypothyroidism	
Second of Twin	
Hypothermia	
Early clamping of cords	

Maternal conditions which compromise fetal growth may be associated with a decreased risk of HMD, and include pregnancy induced hypertension, chronic hypertension, sub acute placental abruption, narcotic addiction, smoking and alcohol ingestion. Heroin can mature surfactant synthesizing systems and the effect of cocaine is unclear.

## **PATHOLOGY**

The lungs on autopsy are edematous, congested and are diffusely atelectatic. There is reduced distensibility and lungs are easily collapsible. The initial histological findings in non-treated neonates with HMD include alveolar epithelial cell necrosis which develops within 30 minutes after birth. The epithelial cells become detached from the basement membrane and small patches of hyaline membrane are formed on the denuded areas (Fig 1). The peripheral air spaces are collapsed, but more proximal respiratory bronchioles which are lined with necrotic epithelium and hyaline membranes are over distended. The hyaline membranes, composed of plasma exudation products and associated with damaged capillaries appear within 3 hours after birth<sup>12</sup>. Hyaline membranes are eosinophilic on staining with haematoxylin and eosin and contain nuclear debris. Studies in

animals have shown neutrophil accumulation in the air spaces and capillaries within few hours, which may contribute to lung injury. Circulating neutrophil counts are lower in infants with HMD and the neutrophil count is inversely correlated with severity of HMD. The recovery phase is characterized by the regeneration of alveolar cells particularly type II cells with the resultant increase in surfactant activity.

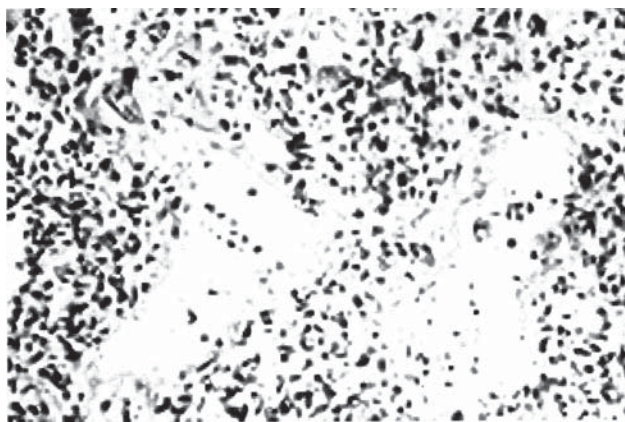


Fig. 2.: Histologic appearance of lungs in an infant with respiratory distress syndrome note the marked atelectasis and so called hyaline membranes lining the dilated alveolar ducts

## **PATHOPHYSIOLOGY**

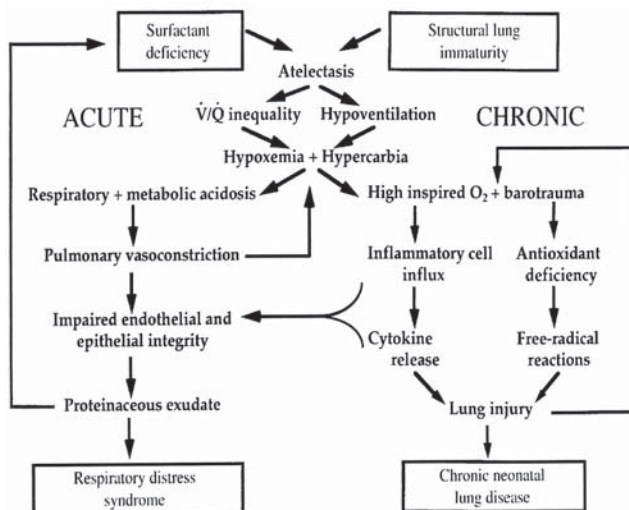
The lungs are non compliant initially with low functional residual capacity (FRC) which is the amount of air inside the lungs at end-expiration. Once surfactant begins to appear, the compliance improves and returns to values of 1-2 ml/cm/H<sub>2</sub>O by 6 to 7 days of age. The functional residual capacity as analyzed by nitrogen washout may be decreased to 3 ml/kg in cases of severe disease, whereas it may be at a normal level of 25-30 ml/kg in recovering babies. Babies with HMD have a low tidal volume because of low FRC and to compensate, they have to increase their respiratory rate. However, the increase in minute ventilation due to an increase in respiratory rate is not sustained, resulting in alveolar underventilation and carbondioxide retention. Pressure - volume loops on lungs dying of HMD have a characteristic pattern<sup>13</sup>. There is a small increase in volume for a given increase in pressure during inflation and during deflation, the change in volume follows a track almost same as inflation, whereas in normal lungs there is retention of air until lower pressures are reached. As the pressure reaches zero, very little or no air is retained in the surfactant-less alveoli, corresponding to the low FRC measured in vivo. There is an increase in the expiratory resistance as a result of the closure of the airway prior to the expiratory grunt and also due to the presence of an endotracheal tube.

The time constant gives a measure of the time available for the gas to leave the lung during expiration, which is accepted to take 3 time constants. It is equal to the product of compliance and airway resistance.

$$T_c(0.1 \text{ sec}) = \text{Compliance (0.001L/cm H}_2\text{O)} \times \text{Resistance (100 cm H}_2\text{O/L/sec)}$$

It is markedly short in cases of severe HMD. In babies with less stiff lungs, the time constant will be longer, and if the baby breathes rapidly, this will result in gas being retained in his lungs when the next expiration starts.

Expiratory grunt occurs classically in babies with HMD as a result of baby attempting to sustain an FRC by delaying the escape of air from lungs during expiration. This occurs due to two mechanisms, firstly the diaphragm continues to contract during expiration, thus retaining gas within the alveoli and secondly, by contracting the constrictor muscles of the larynx, an attempt is made to close the upper airway as in Valsalva manoeuvre. Since the abdominal muscles contract at the same time as the laryngeal muscles relax, there is an explosive exhalation of air which is the characteristic grunt. The preterm infant is born with poor reserves of surfactant which gradually disappears and results in progressive deterioration as the neonate struggles to maintain ventilation in stiff, surfactant deficient lungs. The lungs become non-compliant and atelectatic until surfactant begins to appear from 36-48 hours of age. The leakage of proteins such as fibrin into the alveolar space further inactivates surfactant. Thus, the deficiency of the surfactant together with the decreased lung compliance leads to alveolar hypoventilation and ventilation perfusion (V/Q) mismatch. This mismatch represents the true intrapulmonary right to left shunt, when pulmonary capillary blood passes through the lung without coming into contact with a ventilated alveolus. Thus, the severity of hypoxemia in babies with HMD is directly related to the size of the open poorly ventilated compartment. The low V/Q ratio produces hypoxic vasoconstriction and there is alteration in the right to left shunt with changes in oxygen concentration. Right to left shunt may also occur across PDA and foramen ovale during the first 48-72 hours in babies with HMD in the presence of high pulmonary pressures. There is also increased carbon dioxide in arterial blood (PaCO<sub>2</sub>) due to hypoventilation secondary to atelectasis, decreased tidal volume and decreased dead space. The relative role of surfactant deficiency and pulmonary hypo perfusion in the overall clinical picture of HMD vary somewhat with each patient. The natural history is invariably altered these days due to the often-combined use of exogenous surfactant therapy and mechanical ventilation (Fig 2).



**Fig. 2 Pathophysiology of Hyaline Membrane Disease**  
Schematic representation of the complex series of acute and chronic events that lead to neonatal respiratory distress syndrome and the accompanying lung injury secondary to therapeutic intervention in these infants

## CLINICAL FEATURES

The neonate is almost always a preterm and symptoms usually appear within minutes after birth, although they may present several hours after birth. Prominent symptoms include rapid and labored breathing with a characteristic grunt, subcostal and intercostal retractions, oxygen requirement which gradually increases over 24-48 hours, nasal flaring, cyanosis and oliguria. Retractions are prominent and are due to the collapse of the compliant rib cage on inspiration as the infant tries to generate high inspiratory pressures to expand the poorly compliant lungs. Downe's score<sup>14</sup> (Table 3) is used for monitoring the progress of respiratory symptoms in these babies. It consists of 5 components and each is assigned a score of 0, 1 or 2. A cumulative Downe's score of 4 and above indicates the need for ventilation. Additional clinical features include pallor due to anemia or peripheral vasoconstriction and hypotension. Hypotension is usually the result of hypoxia and acidaemia leading to decrease in the blood supply of various organs causing an increase in anaerobic metabolism, worsening acidosis, renal failure and NEC. The work of breathing progressively worsens over the next 24-36 hours in the absence of treatment with exogenous surfactant. Recovery usually starts by 3<sup>rd</sup> day of life with the onset of diuresis. Surfactant therapy greatly shortens the duration and severity of the disease. If the disease is severe enough, as is the case in extremely low birth weight infant, to require assisted ventilation or is complicated by the development of air leaks, significant shunting across PDA, recovery may be delayed by days to weeks. The presence of apneic episodes at an early stage of the disease is an ominous sign that could reflect thermal instability, electrolyte imbalance or sepsis but is often a sign of hypoxemia and respiratory failure.

**Table 3.: Assessment of Downe's Score**

Parameters assessed	0	1	2
Respiratory rate	<60/min	60-80/min	>80/min
Retractions	No retractions	Mild retractions	Moderate Retractions
Cyanosis	Nil	In room air	In FiO <sub>2</sub> ≤0.4
Air Entry	Bilaterally equal	Decreased Unilaterally	Decreased or Absent Bilaterally
Grunting	No Grunting	Audible with stethoscope	Audible with naked ear



**Fig. 3 Chest X-ray of a preterm neonate showing Air bronchogram**

## IMAGING STUDIES

Chest radiographs of a neonate with HMD shows bilateral diffuse reticular granular or ground-glass appearances, air bronchograms, and poor lung expansion (Fig 3). The prominent air bronchogram represent aerated bronchioles superimposed on a background of collapsed alveoli. The appearance can be very variable, from a slight

granularity to lungs that are so opaque that it is difficult to distinguish between lung and cardiac silhouette. Chest X ray appearance also depends upon the phase of the respiratory cycle, being worse during the expiratory phase. The cardiac silhouette may be normal or enlarged. Cardiomegaly may be the result of prenatal asphyxia, maternal diabetes, PDA, an associated congenital heart anomaly, or simply poor lung expansion. These findings may be altered with either early surfactant therapy or a PDA or with mechanical ventilation. The radiological findings of RDS cannot be differentiated reliably from those of pneumonia.

Echocardiographic evaluation is performed in selected infants to assist in diagnosing PDA and in determining the direction and degree of shunting on Doppler study. It is also useful in diagnosing pulmonary hypertension, assessing cardiac function, and excluding structural heart disease.

## PREVENTION

The most effective way to decrease the incidence of HMD would be by preventing prematurity as HMD occurs predominantly in preterm neonates. This can be done by use of tocolytics, early diagnosis and treatment of infections and cervical cerclage. Presently the two major approaches for prevention include predicting risk of HMD by testing amniotic fluid, antenatal administration of glucocorticoids to accelerate fetal lung maturation and use of prophylactic surfactant at birth.

## PRENATAL PREDICTION

Prenatal estimation of lecithin sphingomyelin ratio (L/S), phosphatidylglycerol (PG), surfactant proteins A and B, lamellar body counts and foam stability tests in amniotic fluid samples have been used to predict the occurrence of HMD. The L/S ratio reflects the secretory activity of the lung, which is greatly accelerated at 35 weeks. The incidence of HMD is only 0.5% for an L/S ratio of 2 or more but 100% for an L/S ratio of less than 1<sup>15</sup>. The presence of PG at 1% of total phospholipids indicates an extremely low risk for HMD. However, in certain pregnancies, characterized by diabetes and Rhesus isoimmunization, L/S ratio is not reliable<sup>16</sup>.

## ANTENATAL GLUCOCORTICOIDS

### ADMINISTRATION

Antenatal steroids cause induction of enzymes for surfactant synthesis and the genes for the production of surfactant proteins A, B, C and D. They also improve the quality of surfactant produced. Both dexamethasone and betamethasone have been used antenatally for prevention of HMD as they cross the placenta without being degraded. In 1972, Liggins and Howie published the results of the first randomized controlled trial (RCT) evaluating the effects of a single course of antenatal corticosteroids (ACS)<sup>17</sup>. In women who had been in spontaneous preterm labour, ACS reduced the risk of RDS and early neonatal mortality. Over the ensuing 20 years, multiple clinical investigations continued to document the effectiveness of ANCS on fetal lung maturation. Despite these findings, use of this therapy remained low in the United States (only 8.5% to 18% of women delivering preterm infants weighing <1,500 g), which prompted a 1994 NIH Consensus Development Conference on the Effect of Corticosteroids for Fetal Maturation on Perinatal Outcomes<sup>18</sup> Table 4. The panel reviewed the results of clinical trials from 1972 to 1993. A meta-analysis of 15 published studies employing a single course of ANCS demonstrated consistent evidence of beneficial effects on neonatal outcome for infants born at less than 34 weeks' gestation<sup>19</sup>. There was a 50%

Table 4.: NIH Consensus Recommendations for the Use of Antenatal Corticosteroids

<b>All fetuses between 24 and 34 weeks gestation at risk of preterm delivery should be considered candidates.</b>
<b>Administration should not be altered by fetal race or gender or by availability of surfactant therapy.</b>
<b>Patients eligible for therapy by tocolytics also should be eligible for treatment.</b>
<b>Treatment should be given unless immediate delivery is anticipated.</b>
<b>In preterm premature rupture of membranes at less than 30- 32 weeks gestation in the absence of clinical chorioamnionitis, treatment is recommended.</b>
<b>In complicated pregnancies where delivery prior to 34 weeks gestation is likely, treatment is recommended unless there is evidence that corticosteroids will have an adverse effect on the mother or the delivery is imminent.</b>

reduction in overall incidence of RDS in the treated groups for infants born 24 hours to 7 days after treatment. In addition, there was a significant reduction in mortality (40%) and the development of intraventricular hemorrhage (IVH) (62%) among these infants. The data also indicated a reduction in RDS mortality and IVH among infants treated less than 24 hours before delivery. This resulted in NIH consensus panel recommendations which are highlighted in Table 4. A 2006 cochrane review of 2 RCTs involving more than 4269 infants, reports a reduced risk of neonatal death, RDS, and IVH with a single course of ACS, and a strong trend towards a reduced risk of abnormal neurodevelopmental outcome on long-term follow-up of the children.

Because approximately 50% of women given a course of ACS remain undelivered 7 to 14 days later, it has been suggested that women, who remain undelivered after a single course of ACS may benefit from receiving additional courses of ACS. Over recent years, several large clinical RCTs have been initiated to study the effects of repeat (weekly/biweekly) courses of ACS. If found to be beneficial in reducing serious neonatal morbidity, repeat courses of ACS will find their way into standard care in much less time than the twenty years that were required for single course ACS. In a study from India, it was found that a single course of antenatal betamethasone was as efficacious as multiple courses, with respect to prevention of neonatal morbidity<sup>20</sup>. Multiple antenatal betamethasone courses had long-term adverse effects on infant weight and length growth, but not on OFC and neurodevelopment.

Treatment consists of two doses of betamethasone 12 mg given intramuscularly 24 hours apart or 4 doses of dexamethasone 6 mg given 12 hours apart. Meta-analysis of trials comparing betamethasone with dexamethasone revealed that although both agents decrease the frequency of RDS only betamethasone decreased neonatal mortality.

## PROPHYLACTIC SURFACTANT

Human pulmonary surfactant consists of 90% lipids and 10% proteins. Surfactant has been studied for treatment of HMD since 1960s however the earliest successful use of surfactant in preterm neonates was reported by Fujiwara in 1980<sup>21</sup>. Surfactant can be used either prophylactically or as a treatment. A prophylactic, or preventive surfactant strategy is defined as intubation and surfactant administration to infants at high risk of developing respiratory distress syndrome for the primary purpose of giving surfactant rather than treatment of respiratory distress syndrome. This has been performed in clinical studies as surfactant administration before the onset of

respiratory symptoms or efforts, before initial resuscitation efforts, or, most commonly, after initial resuscitation but within 10 to 30 minutes after birth. This contrasts with a rescue surfactant strategy, in which surfactant is given to preterm infants with established respiratory distress syndrome. Rescue surfactant is most often administered within the first 12 hours after birth when specified threshold criteria for respiratory distress syndrome are met. Early rescue was defined as surfactant treatment within 1 to 2 hours of birth, and late rescue was defined as surfactant treatment 2 or more hours after birth. Cochrane database review published in 2001 concluded that prophylactic administration of natural surfactant resulted in a significant reduction in pneumothorax, mortality and the combined outcome of mortality and CLD in neonates less than 30 weeks of gestation<sup>22</sup>. The use of prophylactic surfactant for infants born at greater than 32 weeks of gestation would result in unnecessary treatment in a large number of infants particularly if the mother has received antenatal steroids.

**MANAGEMENT**

Management of infants with respiratory distress syndrome includes application of general supportive measures supplemented by surfactant treatment and measures of controlled assisted ventilation.

**General supportive measures**

These include appropriate resuscitation of preterm infants followed by transportation to the NICU in a thermo neutral environment. Early CPAP or surfactant should be considered in preterm infants depending upon the unit policy and all neonates who require intubation should be ventilated in the transport incubator. The infants should be nursed in a thermo neutral environment to maintain oxygen consumption at minimal levels. Monitoring of blood pressure, blood gases, electrolytes, calcium and glucose are needed in babies with HMD. These blood samples may be obtained by placing an umbilical arterial catheter. Appropriate attention should be made to the fluid and electrolyte status of the neonate guided by the change in baby’s weight and monitoring serum electrolyte status. Early administration of intravenous lipid and amino acids along with dextrose should be encouraged until an adequate intake of calories and protein is reached.

**Surfactant Therapy**

Surfactant given as early rescue therapy improves the outcome in babies with established RDS, resulting in reduction in pneumothorax, mortality and the combined outcome of mortality and BPD. Both natural and artificial surfactants are available and among natural surfactants bovine and porcine are commonly used. The various available surfactant preparations have been summarized in table 5. Surfactant-administration strategies have been based on manufacturer guidelines for individual surfactants. Type of surfactant, dose, frequency of administration, and treatment procedures have been modeled after research protocols (table 5). Furthermore, repeated doses of surfactants given at intervals for predetermined indications have decreased mortality and morbidity compared with placebo or single surfactant doses.

Surfactant has been administered through an endotracheal tube located in the trachea of infants either by bolus or infusion through an adaptor port on the proximal end of the endotracheal tube (Fig 4). Because data are conflicting and limited, the optimal method of surfactant administration in preterm infants has yet to be clearly proven although clinical experience and results of animal studies have shown that rapid instillation is more effective than slow

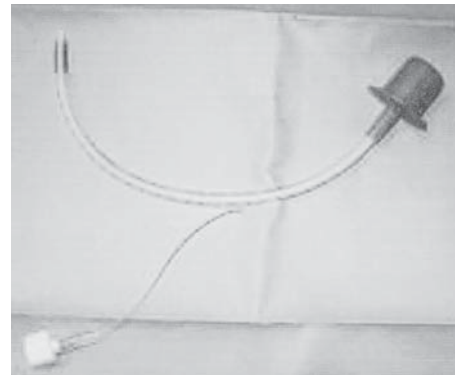


Fig. 4: Endotracheal tube with a side port for administering surfactant

Table 5.: Various surfactant preparations available in India

Name of Surfactant	Dose	Volume per vial	Content in mg per ml	Approximate Cost Rupees/vial
Survanta	4 ml/kg	8 ml	25	13000
Curosurf	2.5 ml/kg 1st dose repeat dose 1.25 ml/kg	2.5 ml	80	10000
Neosurf	5 ml/kg	3 ml 5 ml	27	3 ml vial 5000 5 ml vial 7000

instillation<sup>23</sup>. Valls-i-Soler and colleagues showed that use of a dual lumen tube rather than disconnection from the ventilator led to fewer dosing problems<sup>24</sup>. The surfactant usually disseminates homogeneously, particularly if large rather than small doses are used. There was no difference in clinical outcomes when 2 fractional doses of surfactant were given in 2 body positions compared with 4 fractional doses given in 4 positions<sup>25</sup>. Aerosolized surfactant preparations and continuous positive airway pressure–aided delivery of pharyngeal surfactant theoretically could allow administration without intubation; these preparations and route of delivery have yet to be proven effective. Treatments with animal-derived surfactants have several advantages over first-generation protein- free synthetic surfactants. Some infants do not respond favorably to surfactant therapy. Factors that lead to an unfavorable response include the presence of PDA, cardiogenic shock or PPHN, air leaks and systemic hypotension. The need for a high oxygen concentration and ventilation pressures during the early stages of the disease have been identified as risk factors for an inadequate response.

**Ventilation strategies**

There is still uncertainty among clinicians whether the primary aim in the early hours of a preterm infant’s life should be to avoid intubation and provide support with nasal CPAP or to intubate electively to provide surfactant therapy before determining what other respiratory support is then appropriate. Whichever approach is preferred, many preterm infants require a period of artificial ventilation during their initial stabilization after birth. When ventilating preterm infants after birth, large volume lung inflations, as indicated by excessive chest wall movement, should be avoided. Although measured peak inflation pressure does not correlate well with volume delivered when respiratory mechanics are changing, monitoring of pressure may help to provide consistent inflations and to avoid unnecessarily high pressures. If positive-pressure ventilation is required during initial stabilization, an initial inflation pressure of

20 to 25 cm H<sub>2</sub>O is adequate for most preterm infants. If a prompt improvement in heart rate or chest movement is not obtained, higher pressures may be needed. There is insufficient information about the value of PEEP during resuscitation. If ongoing ventilation is considered necessary, however, PEEP should be employed as soon as practicable. PEEP can be delivered in the labor room with T piece resuscitator till the baby can be shifted to transport incubator. Various devices are available for administration of PEEP which includes nasal prongs and masks (Fig 5) In the COIN trial published in 2008, it was seen that in infants born at 25-to-28-weeks' gestation, early nasal CPAP did not significantly reduce the rate of death or bronchopulmonary dysplasia, as compared with intubation<sup>26</sup>. Even though the CPAP group had more incidence of pneumothorax, fewer infants received oxygen at 28 days, and they had fewer days of ventilation.

## KEY MESSAGES

1. Hyaline Membrane Disease is a disorder of preterm neonates
2. Presenting symptoms include tachypnea, retractions, grunting and increasing oxygen requirements occurring immediately or within few hours of birth
3. Antenatal treatment with glucocorticoids decreases the incidence and severity of the disease
4. Early surfactant therapy should be given as soon as possible in neonates with symptoms suggestive of the disease and prophylactically in neonates less than 28 weeks

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## DRUG PROFILE

### COLISTIMETHATE SODIUM

**Mode of action:** Colistimethate sodium is a cyclic polypeptide antibiotic derived from *Bacillus polymyxa* var. *Colistin* and belongs to the polymyxin group. Work by damaging the cell membrane and is selective for Gram-negative bacteria that have a hydrophobic outer membrane. Microbiology: Commonly susceptible species *Pseudomonas aeruginosa* acinetobacter species citrobacter species, *Escherichia coli* neisseria species proteus species anaerobes all gram-positive organisms. Pharmacokinetics: In healthy volunteers given a bolus injection of 150 mg (2 million units approx.), peak serum levels of 18 mg/L are observed 10 minutes after injection. When given by nebulisation, absorption is variable. Protein binding is low. The steady-state volume of distribution in cystic fibrosis patients is 0.09 L/kg. Colistimethate sodium undergoes conversion to its base (Colistin) in vivo. The main route of elimination after parenteral administration is by renal excretion with 40% of a parenteral dose recovered in the urine within 8 hours and around 80% in 24 hours dose reduction is required in renal impairment to prevent accumulation. After intravenous administration to healthy adults, the elimination half-life is around 1.5 hours. In a study in a cystic fibrosis patients given a single 30-minute intravenous infusion, the elimination half-life was 3.4±1.4 hours.

**Indications:** Serious infections caused by Gram-negative bacteria by inhalation of *Pseudomonas aeruginosa* lung infection in patients with cystic fibrosis. **Dosage and method of administration:** The drug is given as a 50 ml intravenous infusion over a period of 30 minutes. Minimum of 5 days treatment is generally recommended. For the treatment should be continued for up to 12 days. **For the adult up to 60 kg :** 50,000 units/kg/day to a maximum of 75,000 units/kg/day. Given at approximately 8 hour intervals. For over 60 kg: subject 1-2 million units three times a day. The maximum dose is 6 million units in 24 hours. Serum level estimations are recommended impairment, neonates and cystic fibrosis patients. Levels of 10-15 mg/L colistimethate sodium should be adequate for most infections. For children <2

years: 500,000-1 million units twice daily, for children >2 years and adults: 1-2 million units twice daily. **Reconstitution for Inhalation** the required amount of powder is dissolved, preferably, in 2-4 mL of 0.9% sodium chloride solution and poured into the nebuliser. **CONTRAINDICATIONS :** In patients with known hypersensitivity to colistimethate sodium (colistin) or to polymyxin B and in patients with myasthenia gravis. **Drug Interactions:**

**Table :** Dosage Adjustment in Renal Impairment

Grade	Creatinine Clearance (mL/min)	Over 60 kg Bodyweight
Mild	20-50	1-2 million units every 8 hours
Moderate	10-20	1 million units every 12-18 hours
severe	<10	1 million units every 18-24 hours

Concomitant use of colistimethate sodium with aminoglycoside (increased risk of nephrotoxicity) neuromuscular blocking drugs and either colistimethate sodium crosses the placental barrier and hence should be used during pregnancy only if the potential benefit justifies the potential risk to the foetus, the drug is secreted in breast milk.

**Undesirable effects :** Adverse events may be related to the age, renal function and condition of the patient. In cystic fibrosis patients, neurological events occur in up to 27% of patients. These patients treated with the recommended dosage limits, nephrotoxicity appears to be rare (less than 1%). Hypersensitivity reactions, including skin rash and drug fever, have been reported. Inhalation may induce coughing or bronchospasm.

## RECENT ADVANCES IN THE MANAGEMENT OF HYPOXIC ISCHEMIC ENCEPHALOPATHY

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**Abstract :** Perinatal asphyxia is an important cause of both neonatal deaths and devastating sequelae amongst survivors. Conventional neuroprotection treatments have not been very useful in improving outcomes. There have been newer strategies such as hypothermia, phenobarbitone, magnesium sulphate and allopurinol which seem to hold some promise for improving outcomes of neonates with hypoxic ischemic encephalopathy.

Perinatal asphyxia accounts for about 20% of neonatal deaths. Hypoxic ischemic encephalopathy (HIE) is an important cause of morbidity and mortality in term neonates. It is also an important cause of cerebral palsy and mental retardation. The pathogenesis of HIE is a result of cascade of multiple biochemical processes. Our current understanding of the cellular mechanisms of HIE indicate neuronal damage is consequent to several mechanisms that occur in response to hypoxia and ischemia to the neuronal cell. These include release of oxygen free radicals, calcium influx into the cell and presence of glutamate, which facilitates calcium influx into the cell consequent to cell energy failure<sup>1</sup>. The result of these responses is either cell necrosis due to primary energy cell failure or delayed neuronal death due to secondary energy cell failure and apoptosis. The interval between the primary and secondary energy failure phases offers a 'therapeutic window' during which time treatments can be applied to reduce brain injury. The exact duration of this therapeutic window is not known, but animal experiments suggest that it could be about 6 hours<sup>2</sup>.

### CURRENT STANDARD MANAGEMENT

In asphyxiated neonates it is important that effective resuscitation is carried out to establish oxygenation and circulation. There is sufficient evidence to indicate that room air is as effective as 100% oxygen in neonatal resuscitation and results in lower mortality and potentially generate less oxygen free radicals. Standard management practices include maintaining ventilation, perfusion, fluid and electrolyte balance and normal acid-base status. The conventional practices for neuroprotection in neonates with HIE aimed at reducing cerebral edema - hyperventilation or use of decongestive agents, and seizure control have not resulted in improved outcome of neonates with HIE. Investigators were prompted to use oxygen radical scavengers, calcium channel blockers and glutamate antagonists to ameliorate the damage due to primary energy cell failure and necrosis. However, in animal experiments their benefit has been demonstrated when animals were pre-treated with these agents before the hypoxic insult. However, these modalities are not of great benefit in the clinical situation. Promising interventions have been hypothermia, phenobarbital and drugs such as allopurinol.

#### **Phenobarbital.**

Phenobarbital has been a subject of investigation in perinatal asphyxia

for several years. Hall et al conducted a randomized controlled study to study the effect of 40mg/kg phenobarbital given within few hours of birth in babies with severe asphyxia. The phenobarbital group had a 27% lower incidence of seizures and improved neurodevelopmental outcome at 3 years<sup>4</sup>. In a more recent study Singh et al<sup>5</sup> conducted an RCT in neonates > 34 weeks with HIE in the first six hours of life to study the effect of phenobarbital 20 mg/kg IV on death or abnormal neurologic examination at discharge. There was a significant reduction in seizures in the phenobarbital group (8%) compared to the controls (40%). There was no significant difference in mortality or neurologic abnormality at discharge. However, it is still not clear who are the neonates who should get phenobarbitone, when and at what dosage?

#### **Hypothermia.**

Hypothermia during experimental ischemia has been shown to have long lasting neuroprotection. The exact mechanism for its beneficial effects is not known, but it is believed that cooling affects all pathways that leads to cell death. Reduction of cerebral metabolic rate by cooling of the head has demonstrated substantial neuronal cell recovery. Both experimental and clinical trials have demonstrated recovery of cellular energy functions after head cooling and consequently better long term outcomes in animals or infants treated by head cooling. Hypothermia also decreases permeability of the blood-brain barrier and recovery of electrophysiologic function after cerebral ischemia. A meta-analysis by Schulzke et al<sup>6</sup> reviews all randomized clinical trials that have used either head or whole body cooling in asphyxiated neonates. They identified 5 clinical trials that enrolled 552 neonates. These trials considered infants as asphyxiated if one of the following were present: 10 minute apgar was  $\leq 5$ , pH  $\leq 7.1$  within one hour of life or ongoing resuscitation at 10 minutes of life. One of the studies used selective head cooling while the remaining 4 used total body cooling. The target cooling ranged from 32.5-35.5°C. The analysis revealed a relative risk (RR) of 0.78 (95%CI 0.66-0.92) for death or disability in favor of hypothermia on pooling data from 3 trials. When mortality was assessed, pooled data from all 5 trials showed a benefit in favor of therapeutic cooling (RR 0.75; 95% CI 0.59,0.96). When neuro-developmental abnormality at >18 months was assessed, a significant benefit was seen in favor of cooling (RR 0.72; 95% CI 0.52, 0.98). There was no benefit with respect to visual or hearing disabilities. Significant adverse events reported included sinus



## RECENT ADVANCES IN CHILDHOOD ITP

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**Abstract :** Immune thrombocytopenic purpura (ITP) is a disorder mediated by antiplatelet antibodies and characterized by accelerated destruction of platelets and impaired platelet production. The result is thrombocytopenia of varying degrees. Clinically recognized cases are typically associated with marked, isolated thrombocytopenia, mucocutaneous bleeding, and, rarely, more severe hemorrhage (ie, intracranial hemorrhage). Recent advances in our understanding of the specific pathways involved in immune-mediated platelet destruction and the significance of suboptimal thrombopoiesis have led to the development and investigation of new therapeutic agents. This article discusses the current knowledge on pathophysiology and new information on pharmacologic approaches, tolerability, toxicity, and efficacy data for established and novel investigational therapies for patients with this relatively common disorder of childhood.

The clinical signs and symptoms of idiopathic immune thrombocytopenic purpura (ITP) are caused by an increased rate of premature platelet destruction which occurs preferentially in the spleen, liver, bone marrow and lung. It was long suspected that immune thrombocytopenic purpura is mediated by autoantibodies, since transient thrombocytopenia occurs in neonates born to affected women, and this suspicion was confirmed on the basis of the development of transient thrombocytopenia in healthy recipients after the passive transfer of plasma, including IgG-rich fractions, from patients with immune thrombocytopenic purpura. The thrombocytopenia of ITP is mainly attributed to the early destruction of platelets by the activated reticuloendothelial system, following their sensitization by antiplatelet glycoprotein autoantibodies<sup>1,2</sup>. Other mechanisms such as complement mediated lysis<sup>3</sup>, ineffective thrombopoiesis<sup>4</sup>, or direct T-cell cytotoxicity also contribute<sup>5</sup>. The severity of thrombocytopenia thus reflects the balance between platelet production by megakaryocytes and the accelerated clearance of sensitized platelets. This article focuses on the recent insights in the pathophysiology and the management of ITP in children.

### PLATELET ANTIGEN AND AUTOANTIBODY

Platelets coated with IgG autoantibodies undergo accelerated clearance through Fc receptors that are expressed by tissue macrophages, predominantly in the spleen and liver. A compensatory increase in platelet production occurs in most patients. In others, platelet production appears to be impaired, as a result of either intramedullary destruction of antibody-coated platelets by macrophages or the inhibition of megakaryopoiesis<sup>6</sup>. The level of thrombopoietin is not increased<sup>7</sup>, reflecting the presence of the normal megakaryocyte mass. The first antigen to be identified was recognized on the basis of the failure of immune thrombocytopenic purpura antibodies to bind to platelets that were genetically deficient in the glycoprotein IIb/IIIa complex<sup>6</sup>. Antibodies that react with glycoproteins Ib/IX, Ia/IIa, IV, V and diverse other platelet determinants have since been identified<sup>8</sup>, and the presence of antibodies against multiple antigens is typical<sup>9</sup>. The destruction of platelets within antigen-presenting cells — presumably, although not necessarily, initiated by antibody — may generate a succession of neoantigens, resulting in sufficient antibody production to cause thrombocytopenia.

### MECHANISM OF IMMUNE DESTRUCTION

The rapid destruction of platelets is due either to autoantibodies that bind via the antigenic site or immune complexes via Fc receptors on platelets. These opsonized cells are rapidly removed by cells of the mononuclear phagocytic system. The quantity of antibodies correlates with the severity of thrombocytopenia. Phagocytosis of platelets has been demonstrated by in vivo studies using reticuloendothelial blockade with monoclonal anti-Fc receptor antibodies. The factors that initiate autoantibody production are unknown. Most patients have antibodies against several platelet-surface glycoproteins at the time the disease becomes clinically evident. Here, glycoprotein IIb/IIIa is recognized by autoantibody (orange, inset), whereas antibodies that recognize the glycoprotein Ib/IX complex have not been generated at this stage. Antibody-coated platelets bind to antigen-presenting cells (macrophages or dendritic cells) through Fc receptors and are then internalized and degraded. Antigen-presenting cells not only degrade glycoprotein IIb/IIIa, thereby amplifying the initial immune response, but also may generate cryptic epitopes from other platelet glycoproteins. Activated antigen-presenting cells express these novel peptides on the cell surface along with co-stimulatory help (represented in part by the interaction between CD40 on the activated macrophages and CD154 on the T cell) and the relevant cytokines that facilitate the proliferation of the initiating CD4-positive T-cell clones. B-cell immunoglobulin receptors that recognize additional platelet antigens are thereby also induced to proliferate and synthesize anti-glycoprotein Ib/IX antibodies in addition to amplifying the production of anti-glycoprotein IIb/IIIa antibodies by B-cell clone 1. Naturally occurring antibodies against glycoprotein IIb/IIIa show clonal restriction in light-chain use<sup>8,9</sup>, and antibodies derived from phage-display libraries show highly constrained VH gene use. Sequencing of the antigen-combining regions of these antibodies suggests that they originate from a limited number of B-cell clones by antigen-driven affinity selection and somatic mutation. Some patients with immune thrombocytopenic purpura often have increased numbers of HLA-DR+ T cells, increased numbers of soluble interleukin-2 receptors, and a cytokine profile suggesting the activation of precursor helper T and type 1 helper T cells. In these patients, T cells stimulate the synthesis of antibody after exposure to fragments of glycoprotein IIb/IIIa but not after exposure to native proteins. The derivation of these cryptic epitopes

in vivo and the reason for sustained T-cell activation are unknown. *Dysfunctional cellular immunity* is considered important in ITP pathophysiology<sup>5</sup>. Several studies have found evidence supporting a T helper 0 (Th0)/Th1 polarization of the immune response in ITP<sup>10</sup>, whereas others have yielded inconsistent or opposing results. Other studies<sup>11</sup> have shown the presence of activated platelet-specific auto reactive T cells that recognize and respond to autologous platelet antigens and drive the generation of platelet reactive autoantibodies by B cells in the peripheral blood of ITP patients. The autoimmune process is believed to be seated in the spleen; memory platelet-specific T cells are released into the peripheral circulation<sup>12</sup>.

*Eradication of Helicobacter pylori infection* has been variably associated with a platelet response in patients with ITP. Responses occur in approximately half of ITP patients infected with this bacterium, more frequently in Japan and Italy than in other countries. For those with severe ITP (platelet count  $< 30 \times 10^9/L$ ) and a long duration of disease, eradication therapy seems to be less effective. Despite extensive efforts, distinctive clinical features and factors predicting the response to eradication therapy have not been consistently identified. There is no established mechanism to explain how H pylori could be implicated in the pathogenesis of an immune-mediated platelet destruction. Several theories have been proposed to explain the platelet response to anti-H pylori therapy, including molecular mimicry, platelet aggregation, and the induction of a Th1 phenotype that favours the onset and/or persistence of ITP. The role of bacterium-related factors, such as the CagA (cytotoxin-associated gene A) protein, are still under investigation. Eradication therapy is simple and inexpensive, with limited toxicity and the advantage of avoiding long-term immunosuppressive treatment for those who respond. Although the evidence and follow-up are limited, it appears reasonable to routinely screen patients with ITP for H pylori, particularly in those populations with a high background prevalence of H pylori infection.

## TREATMENT OF ITP

Many new therapies are now in early stages of development for the treatment of ITP. These new treatments have diverse mechanisms of action, aimed at amelioration of platelet destruction and enhancing platelet production. This progress has resulted from a sustained commitment to improving our understanding of megakaryopoiesis, thrombopoiesis, and autoimmunity, and a further elucidation of the mechanism of action of traditional therapies. Additional work is required to optimize efficacy and response rates, although given the natural heterogeneity in diseased pathophysiology among patients who have a diagnosis of ITP, it is unlikely that any single treatment

will be universally applicable. Further detailing of the pathologic factors underlying the variation among patients in clinical course and treatment response is required to enable further progress in drug development and will improve selection of the most appropriate management strategies for patients who have ITP.

### “Observation Only” Approach

One of the most significant developments in management of acute and chronic ITP over last two decades has been wide acceptance of “Observation Only Approach.” Factors which have led to increasing acceptance of this approach include:

- a) Recognition that intracranial hemorrhage (ICH) - the most feared clinical feature (or complication) of ITP is extremely rare.
- b) Better understanding of natural history of ITP.
- c) Absence of significant bleeding even in patients with severe thrombocytopenia (platelet count  $< 20 \times 10^3/mm^3$ ),
- d) Publication (and acceptance) of guidelines for treatment of ITP in children and adults.

Most of the recent treatment guidelines suggest taking into account the bleeding symptoms rather than low platelet counts alone for making decisions regarding drug therapy in acute ITP. Children with mild clinical disease can be managed with watchful waiting. Drug treatment should be reserved for children with moderate bleeding symptoms and platelet count  $< 20 \times 10^3/mm^3$  or children with severe bleeds. The fear of ICH and high morbidity and mortality associated with it prompts most physicians to treat ITP. The risk of ICH was 0.9% of 1693 children included in various series reviewed by George and co-workers<sup>13</sup>. Even this figure is considered an overestimate as most series reviewed by them originated from academic centres

which are likely to have referred cases. Lilleyman estimated a much lower incidence of 0.2% based on UK data. A similar incidence of 0.17% has recently been reported in a series of 1742 newly diagnosed children with ITP<sup>14</sup>. Over the last decade, two surveys conducted in UK included 425 and 304 children respectively. In both these surveys, severe thrombocytopenia (platelet count  $< 20 \times 10^3/mm^3$ ) was observed in 84% and 89% children. However, severe bleeding occurred in 3% and 5% cases only. Even moderate bleeding symptoms were observed in 21% and 22 % cases<sup>15,16</sup>. Chandra et al have also reported similar observations in children with acute or chronic ITP during periods of severe thrombocytopenia. Most of the cases in their series also had no or only skin bleeds during severe thrombocytopenia<sup>17</sup>. These data suggest that most children with ITP have minor or moderate bleeding manifestations even when they have severe thrombocytopenia. Natural history of acute ITP in children is more clearly defined now. George and co-workers reviewed 12 series including 1597 cases. Complete remission rate of 74% was observed by them<sup>13</sup>. Kuhne and co-workers reported remission rates of 68%, 73 % and 66% in children with acute ITP receiving no treatment, intravenous immunoglobulins (IVIg) and corticosteroids respectively. These observations highlight excellent outcome of ITP in children irrespective of initial treatment<sup>18</sup>. The predictors of early response are identified and scoring system to that effect has recently been reported using abrupt onset, preceding viral infection, wet purpura, platelet count  $< 5 \times 10^3/mm^3$ , age under 10 years and male gender as predictors<sup>19</sup>. Development of scoring system for various bleeding manifestations and severity of ITP has made assigning the severity to a given case more objective which helps in decision making regarding need for therapeutic intervention<sup>15,20</sup>. Over the years guidelines for management of ITP have been brought out by American Society of Hematology, British Society of Hematology and British Committee on Standards in Hematology<sup>13,15,21</sup>. As a result of these new developments, there is wider acceptance of Observation Only Approach for management of ITP in children. UK surveys clearly recorded this change as 60% cases were

treated in the first survey while in the survey performed after wider circulation of guidelines showed the decline in number of cases receiving treatment to 37% only<sup>15,16</sup>. A survey from Nordic countries has shown that only 57% cases were given platelet – enhancing therapy<sup>22</sup>.

### ADVANCES IN DRUG THERAPY

IVIG has been used for treating ITP since 1981<sup>23</sup>. Transient blockade of Fc receptor of macrophages in the reticuloendothelial system, particularly in spleen, is believed to play a major role in prompt increase in platelet count after IVIG infusion. Other mechanisms through which IVIG acts include inhibition of antibody binding to

platelets due to presence of anti-idiotypic antibodies in IVIG preparations and decreased antibody production through suppression of B lymphocytes. In addition, clearance of red cells has been demonstrated after IVIG infusion which is supposed to have at least a minor role in Fc blockade<sup>24,26</sup>. The latter mechanism forms the basis of use of anti-D in treatment of ITP. In a series by Blanchette and co-workers, the rate of platelet response was faster in children treated with IVIG compared to those who were not treated with drugs<sup>27</sup>. In their second study, they compared two IVIG treatment regimens (1 g/kg on 2 consecutive days and 0.8 g/kg once), oral prednisone (4 mg/kg per day for 7 days with tapering and discontinuation by day 21), and for the subset of children who were

blood group rhesus (D) positive, IV anti-D (25mg/kg on 2 consecutive days). The key findings from this second randomized trial in children who had newly diagnosed ITP and platelet counts less than  $20 \times 10^9/L$  were (1) a single dose of IVIG (0.8 g/kg) was as effective as the larger dose of IVIG 1 g/kg for 2 days in raising the platelet count and (2) both IVIG regimens were superior to IV anti-D administered as 25 mg/kg for 2 days for the clinically important endpoint of time (number of days) to achieve a platelet count greater than or equal to  $20 \times 10^9/L$ <sup>28</sup>. Currently this dose is recommended as higher doses do not offer advantage and are more often associated with side effects<sup>29</sup>. Rapidity of rise of platelet count and thus shortening of duration of severe thrombocytopenia (with its favourable effect on patient, parents and physicians) with use of IVIG is most important factor for its wide use as initial management of ITP. A recent meta-analysis comparing rapidity of platelet rise with IVIG and corticosteroids has demonstrated that patients are more likely to have platelet count  $>20 \times 10^3/mm^3$  with IVIG use than with corticosteroids at 24, 48 and 72 hours. This difference may hold clinical importance<sup>30</sup>. Rapidity of platelet rise is similar when anti-D is used in higher doses<sup>28</sup>.

**Intravenous Anti- D (i.v anti-D):** Salama and co workers demonstrated reversal of thrombocytopenia in patients who had ITP and were rhesus (D) positive with administration of i.v anti-D<sup>31</sup>. The mechanism of action of anti-D involves anti-D coating of red cells of rhesus (D) positive patients. These antibody coated red cells are preferentially removed by RES, thus sparing the platelets<sup>26,32,33</sup>. Studies to look for other possible mechanisms through which anti-D might act have failed to demonstrate any effect of anti-D on humoral and cellular arms of immune system<sup>33</sup>. After anti-D administration, patients become Coomb's positive

and have laboratory evidence of hemolysis. Study by Scaradavou and co-workers highlighted that platelet responses are better in children than in adults and responders tended to respond to re-treatment with iv anti-D. They also noted that conventional doses were ineffective in splenectomized patients<sup>32</sup>. A recent study on children with ITP compared the efficacy of IVIG and two different doses of anti-D. A clear superiority of 75mg/kg dose of anti-D was observed compared to 50mg/kg dose with respect to number of cases with platelet count  $>20 \times 10^3/mm^3$  at 24 hours of therapy. While 50mg/kg of anti-D was found to be inferior to 0.8 gm/kg of IVIG, efficacy of 75mg/kg dose of anti-D was equivalent to 0.8 gm/kg of IVIG<sup>34</sup>. Short term side effects of anti-D include fever, chills, nausea and vomiting and are observed to be more frequent with 75mg/kg dose. These side effects are probably related to pro-inflammatory cytokine release after anti-D administration. As no clinically significant increase in fall of Hb is noted with 50 or 75mg/kg anti-D, the latter (single) dose can now be recommended as standard dosing for treatment of ITP in children who are rhesus (D) positive<sup>29</sup>. It should be remembered that this form of therapy is ineffective in D negative patients. In patients with significant anemia, use of anti-D should be avoided. Abrupt severe hemolysis has been reported after i.v. therapy, majority of these cases were in adults<sup>35</sup>. It is important to advise the patients to be watchful for any change in the colour of urine. Rapidity of platelet rise and ease of administration (slow i.v push in contrast to infusion of IVIG) are important appealing factors for increasing clinical use of anti-D, especially as OPD administration. Survey conducted in USA, four years apart have observed increased use of anti-D from 10% to 33%<sup>36</sup>. another factor which would promote the increasing use of anti-D as a first line therapy is the rising cost of IVIG both at national and international level which would decrease the usage of IVIG in the developing countries.

**Corticosteroids:** Prednisolone at conventional dose of 2mg/kg ( $60mg/m^2$ ) increases the platelet count slowly and hence cannot be usually recommended in children with very low platelet count or clinically significant bleeding. For this reason, high dose oral or parenteral corticosteroids have been used. Using oral high dose methyl-prednisolone (M-PDN), Ozsoylu and co-workers demonstrated efficacy equivalent to IVIG<sup>37</sup>. Intravenous M-PDN was used by Van Hoff and Ritchey at 30mg/kg for three days. With this dose, median time to achieve platelet count  $>20 \times 10^3/mm^3$  was 24 hours, an observation similar to IVIG or anti-D use<sup>38</sup>. This schedule is currently more commonly used. Coraco et al's experience with short-course oral prednisone (4 mg/kg per day x 4 days without tapering) is complementary. Eighty-three percent of children who had acute ITP and platelet counts less than  $20 \times 10^9/L$  achieved a platelet count above  $20 \times 10^9/L$  within 48 hours of starting corticosteroid therapy. Reviewing all the studies using high dose oral steroids for shorter duration it can be concluded that a clinically significant increment in platelet count can be achieved rapidly in the majority of children who have acute ITP after the administration of high-doses of corticosteroids (approximately 4 mg/kg per day of prednisone or an equivalent corticosteroid preparation) administered orally or parenterally. It seems wise to use high-dose corticosteroid regimens for as short a period of time as is necessary to achieve a clinically meaningful endpoint (eg, cessation of bleeding or achievement of a platelet count  $> 20 \times 10^9/L$ ). This approach

minimizes the predictable, and sometimes serious, adverse effects of long-term corticosteroid therapy. Use of corticosteroids other than prednisolone or M-PDN deserves mention. Oral pulsed high dose dexamethasone (HDD) was initially used for chronic ITP in adults by Anderson<sup>39</sup>. He demonstrated 100% response rate. Subsequent studies in adults and children showed a moderate success only<sup>40,41</sup>. More recently, this form of therapy has been used in newly diagnosed children and adults with ITP. This Italian study by Mazzucconi and co-workers<sup>42</sup> modified the initial dosing schedule used by Anderson. Instead of giving HDD at monthly interval, they used four courses of HDD (each course using dexamethasone 20mg/m<sup>2</sup> for 4 consecutive days) at two weeks interval, thus completing the therapy in 2 months time. A complete response rate of 64.5% and overall response rate of more than 85% was recorded. Response was similar in adults and children. 87% responders enjoyed long term response with a median of 8 months (range 4-24 months) without relapse or need for any therapy. Therapy with HDD was described as well tolerated. Compared to IVIG and i.v anti-D, therapy with dexamethasone is very economical.

**Rituximab:** Use of rituximab for ITP and other immune cytopenias is relatively new. Rituximab is human murine (chimeric) monoclonal antibody that depletes B cells from the blood, lymph node and bone marrow by targeting CD20 which is expressed on the surface of premature and mature B lymphocytes. It was particularly developed for treatment of Non-Hodgkin lymphoma. Central role of B cells in autoimmunity and selective depletion of B cells by rituximab provided a case for exploring its use in the treatment of autoimmune diseases<sup>43,44</sup>. Initial success with rituximab has led to its wider use in immune cytopenias including ITP, immune hemolytic anemia and Evan's syndrome. Mechanism of action of rituximab in ITP involves much more than mere B cell depletion. In fact, levels of autoantibodies are not always significantly affected by rituximab. Taylor and Lindorfer have recently suggested another mechanism of action of rituximab in autoimmune diseases- the immune complex decoy theory. They hypothesized that as rituximab-opsonized B cells will be recognized by monocytes and macrophages and these effector cells will be diverted away from interaction with autoimmune antibody complexes<sup>45</sup>. Another theory was put forward by Stasi and co-workers to explain a rapid response in ITP. The auto-antibodies in ITP are driven by T cell dependent mechanisms and the normalization of these autoreactive T cell responses may be underlying mechanism of action of rituximab in

ITP<sup>46</sup>. Over the past few years, rituximab has emerged as an important alternative treatment for ITP. The treatment regimen used most frequently was 375 mg/m<sup>2</sup> administered weekly for 4 weeks. A pediatric series by Bennet et al reported data including 36 patients, ages 2.6 to 18.3 years, six of whom had Evans's syndrome. Responses, defined as a platelet count greater than 50 x 10<sup>9</sup>/L during 4 consecutive weeks starting in weeks 9 to 12 after 4 weekly doses of rituximab (375 mg/m<sup>2</sup> per dose), were observed in 31% of cases (CI, 16% to 48%). Data on over 100 children treated for ITP with rituximab has been reviewed by Garvey<sup>43</sup>. In various series complete response rate has varied between 32-68%. Additional 8-27% cases have enjoyed partial remission. The responses are described as durable with longest disease free interval of 3.2 years (ongoing). No differences have been observed between adults and children<sup>47-49</sup>.

## NEWER APPROACHES TO TREATMENT OF ITP

The advances in understanding the pathogenesis of ITP have been in two fields: (a.) Demonstration that platelet production is impaired in some patients with ITP. (b.) Better elucidation of mechanism of production of antiplatelet antibodies.

It is believed that platelet production is increased in patients with ITP, the platelet count being determined by a balance between platelet production and rate of platelet destruction. At least in some patients with ITP, thrombopoiesis is inadequate to offset rapid destruction, actually platelet production may be decreased in some of them. Circulating or tissue bound thrombopoietin (TPO) levels are normal or slightly increased in ITP in contrast to the increased TPO levels observed in cases with thrombocytopenia due to impaired platelet production<sup>36,50</sup>. Therapy with corticosteroids and IVIG also does not increase TPO levels<sup>51</sup>. Studies by Chang and co-workers demonstrated that in-vitro proliferation of megakaryocytes is impaired when they are incubated with plasma from patients with ITP containing antiplatelet antibodies, particularly anti-GPIIb antibodies<sup>52</sup>. Observations of Chang and co-workers and other studies have led to use of TPO and other drugs in ITP which increase platelet production. On the other hand studies in last decade have improved our understanding of how antiplatelet autoantibodies are produced and lead to platelet destruction. It is now believed that B lymphocyte activation in ITP and other autoimmune disorders is T cell-dependent. CD40 is a cell surface receptor that belongs to the tumor necrosis factor-receptor family, and that was first identified and functionally characterized on B lymphocytes. CD40-ligand (CD40L/CD154), a member of the TNF superfamily, is a cell membrane molecule expressed on activated CD4<sup>+</sup> T lymphocytes and is essential for activation of B lymphocytes. It is speculated that platelet-associated CD154 is competent to induce the CD40-dependent proliferation of B lymphocytes. Studies have shown increased expression of platelet-associated CD40L/CD154 in ITP patients<sup>53,54</sup>. These observations have led to development of antibodies interfering with this arm of immune dysregulation.

### Drugs Increasing Platelet Production

Megakaryopoiesis is controlled by signalling through the TPO receptor (c-Mpl) present on megakaryocyte and platelet surface. Studies have evaluated polyethylene glycol-conjugated form of TPO (PEG-megakaryocyte growth and development factor (PEG-MGDF), a recombinant TPO. This led to increased platelet counts in four out of five patients with ITP<sup>55,56</sup>. However, PEG-MGDF was found to be immunogenic and induced production of anti-TPO antibodies leading to severe thrombocytopenia in some recipients<sup>57</sup>. Following these reports, further research on this compound was stopped. The second generation agents include TPO peptide mimetics, TPO non-peptide mimetics and TPO agonist antibodies<sup>58</sup>.

Studies with two of these compounds i.e.: AMG 531 (Romiplostim) and Eltrombopag are in late stages of a positive signal for clinical usage. These are c-Mpl peptide agonists that share no sequence homology with native TPO.

**AMG 531:** In a study by Bussel and co-workers, escalating doses of 0.2 to 10mg/kg weekly subcutaneously for 6 weeks were administered to 41 patients. In phase I of the study, platelet count above 50 x 10<sup>3</sup>/mm<sup>3</sup> was achieved in 7 out of 12 patients. Increase in platelet count

was dose dependent. In phase II, 10 of 16 patients receiving 10mg/kg weekly for six weeks showed platelet count above  $50 \times 10^3/\text{mm}^3$ . No major adverse events were noticed<sup>59</sup>. Newland and co-workers have also reported similar observations. Eight of their 11 patients receiving doses  $>1.0$  mg/kg showed increase in platelet count<sup>60</sup>. Reversible marrow fibrosis has been documented in some patients receiving AMG 531<sup>29</sup>.

**Eltrombopag:** Eltrombopag (SB-497115) is orally administered agent. It is a small-molecule, non-peptide thrombopoietin-receptor agonist. This drug initiates thrombopoietin-receptor signalling by interaction with trans-membrane domain of receptor, thereby inducing proliferation and differentiation of cells in megakaryocytic lineage. In a study by Bussel and co-workers, patients with chronic ITP were administered 30, 50 and 75 mg of eltrombopag daily. By day 15, more than 80 % patients receiving 50 or 75 mg of eltrombopag had an increased platelet count. TPO levels remained within normal range<sup>61</sup>.

**IL-11:** IL-11 stimulates megakaryopoiesis in vitro. A recombinant human interleukin-11 (rhu IL-11, oprelvekin), has been used in cancer patients having thrombocytopenia. Wilde and Faulds have reviewed its clinical use. The drug increases megakaryocyte size and ploidy. The recommended adult dosage of subcutaneous oprelvekin is 50 mg/kg once daily, administered until the platelet count is  $\geq 50,000/\text{mm}^3$ . Three placebo-controlled trials involving patients with cancer (mostly breast cancer) undergoing dose-intensive cancer chemotherapy, with or without autologous bone marrow transplantation (n=75 to 82), have been conducted. Compared with placebo, oprelvekin 50 mg/kg/day was associated with significantly fewer patients requiring platelet transfusions and a trend towards a lower median number of platelet transfusions<sup>62</sup>. Bussel and co-workers used rhuIL-11 in adults with refractory ITP. At 50 $\mu\text{g}/\text{kg}$  dose, rhuIL-11 failed to increase the platelet counts in these patients<sup>63</sup>. There are other thrombopoietic agents under development that seem promising in trials in normal volunteers, such as AKR-501. Further study will reveal the potential of these agents for long-term maintenance therapy, and the usefulness of these agents in children who have ITP.

## OTHER IMMUNOMODULATORY DRUGS

T cell mediated B lymphocyte activation in patients with ITP forms the basis of use of these compounds. Kuwana and co-workers conducted a dose escalating trial of *humanized monoclonal antibody to CD 154* (IDEC-131/E6040) in patients with refractory ITP. No rise in platelet count was observed with 1, 2 or 5 mg/kg dose. Three patients treated with 10 mg/kg dose showed an increase in platelet count<sup>64</sup>. Another study by Patel and co-workers has demonstrated 24% overall response rate using 5-20 mg/kg dose of this monoclonal antibody<sup>65</sup>. *Specific Inhibitors of Phagocyte-Mediated Consumption of Platelets:* the thrombocytopenia in ITP results at least in part from an interaction between platelet surface-bound immunoglobulin and Fc $\gamma$ RIII receptors on macrophages. Specific antibodies of Fc $\gamma$ RIII have therefore been designed as potential therapy to prevent platelet clearance. Early trials with a murine anti-human Fc $\gamma$ RIII antibody demonstrated the feasibility of this approach, but treatment could not be repeated as a result of universal development of human antimouse antibodies. A humanized antibody, GMA-161, has been developed and used in low dose in four adults who had chronic refractory ITP. In these

initial studies, responses were fairly short-lived. Further study is required to demonstrate the applicability of specific Fc $\gamma$ RIII targeting in the treatment of ITP and other autoimmune disorders. An inhibitor of syk kinase (R788) that targets the Fc $\gamma$ RI signaling pathway is also in early trial with promising results<sup>66</sup>.

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#### ETHICAL GUIDELINES FOR BIOMEDICAL RESEARCH

The need for uniform ethical guidelines for research on human subjects is universally recognised. It has acquired a new sense of urgency as the critical issues in the area of biogenetic research involving human subjects have become acute. Apart from the mandatory clinical trails on new drugs, a number of diagnostic procedures, therapeutic interventions and prevention measures including the use of vaccines, are being introduced which involve human subjects. Further the advent of new medical devices and radio-active materials and therapeutic benefits of recombinant DNA products have added a new dimension to the ethical issues that need to be considered before evaluating these for their efficacy, utility and safety.

Any research using the human beings as subjects shall bear in

mind the following principles of : i) essentiality, (ii) voluntariness, informed consent, (iii) non exploitation, (iv) privacy and confidentiality, (v) precaution and risk minimisation, (vi) professional competence, (vii) accountability & transparency, (viii) maximisation of public interest and distributive justice (ix) institutional arrangements (x) public domain (xi) totality of responsibility and (xii) compliance.

Recent advances in the field of Assisted Reproductive technologies, organ transplantation, Human genome analysis, and gene therapy promise unquestionable benefits to mankind. At the same time, they raise many questions of law and ethics, stimulating public interest and concern.

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## LIVER TRANSPLANTATION IN CHILDREN

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**Abstract:** Liver transplantation is now an established mode of therapy in children with fulminant hepatic failure and end stage liver disease due to various causes. The indications have evolved over the last few years to include various metabolic disorders. A thorough pre transplant evaluation followed by pre-emptive identification and management of anticipated complications is essential for the success of a liver transplant. Low socio economic and education levels and insufficient social assistance have a considerable impact on the practicality of a transplant taking place in India. The liver transplant programme in India has come a long way over the past 10 years with patient survival rates comparable to the best centers in the world. The improvements in surgical and medical expertise have contributed in a big way to this achievement.

### INTRODUCTION

Paediatric liver transplantation (LT) is now an established therapeutic procedure for children with fulminant hepatic failure and end stage liver disease due to diverse etiologies. The survival has improved significantly over the last decade with pediatric recipients faring better than adults<sup>1</sup>. Initially this therapeutic modality was available only in developed world but over the past decade pediatric liver transplantation has become established in India with survival rates comparable to that of established centers in the West<sup>2,3</sup>. The medical and surgical expertise gained over the past few years has allowed the application of liver transplantation to even very young infants with excellent results.

### THE NEED FOR PEDIATRIC LIVER TRANSPLANTATION

In the West approximately 2-3 pediatric liver transplants are performed per million of population per year. At this rate, around two to three thousand children need liver transplantation in India annually. Out of 2436 hepatobiliary referrals to our centre in last 10 years, 256 children satisfied the criteria for liver transplant. As in the West, extrahepatic biliary atresia (EHBA) was the commonest indication with over 60 percent of children requiring LT (Table 1). In India however, most children with EHBA are either diagnosed late or have not undergone the Kasai procedure at all. At another tertiary care center, nearly 70 percent babies with EHBA required LT based on internationally accepted criteria<sup>2</sup>. Internationally, the donor pool for children has been extended by the use of cadaveric cut-down, split and living-related transplantation to match the increasing pool of potential liver transplant recipients<sup>3</sup>. In India, however, majority of liver transplants are living related liver transplants<sup>4,5</sup>.

### INDICATIONS FOR LIVER TRANSPLANTATION

The indications for a transplant have increased over the last few years to include various metabolic disorders (Table 2). While biliary atresia continues to be the commonest indication for a pediatric liver transplant world over, more and more children with diverse etiologies are being offered this life saving modality with successful results.

#### Chronic liver failure

**Chronic liver failure** secondary to cholestatic liver disease is the most common indication for liver transplant in children. The single most common cause for chronic liver failure in infancy and childhood is **biliary atresia**. The Kasai procedure is successful in one-half of all patients and

**Table 1:** Liver transplant indications at Indraprastha Apollo Hospital, New Delhi (1/10/1997 to 24/10/08)

Total Hepatobiliary referrals 2436	
Criteria for transplant satisfied 256 (12.5%)	
Neonatal cholestasis syndrome	156
Fulminant hepatic failure	44
Cryptogenic	27
Wilson's disease	07
Hepatoblastoma	05
PFIC	06
Tyrosinemia	04
Hepatocellular carcinoma	03
Budd Chiari syndrome	02
Crigler Najjar syndrome	01

**Table 2 Indications for Liver transplantation in children; Chronic liver failure**

#### A) Cholestatic liver disease

Biliary atresia  
Idiopathic neonatal hepatitis  
Progressive familial intrahepatic cholestasis  
Bile duct hypoplasia

#### B) Metabolic liver disease

Wilson disease  
Tyrosinemia type I  
Glycogen storage disorder type IV  
Cystic fibrosis

#### C) Chronic hepatitis

Autoimmune  
Idiopathic  
Cryptogenic cirrhosis  
Post viral (Hepatitis B, C)  
Fibropolycystic liver disease ± Caroli's disease

#### Acute Liver failure

##### A) Fulminant hepatic failure

Viral hepatitis  
Acetaminophen poisoning

##### B) Metabolic liver disease

Fatty acid oxidation defects  
Neonatal hemochromatosis  
Tyrosinemia type I  
Wilson disease

#### Inborn error of metabolism

Crigler-Najjar syndrome type I  
Organic acidemias  
Primary hyperoxaluria  
Urea cycle defects  
Hepatic Tumors  
Benign tumors

Unresectable malignant tumors with no extra-hepatic spread

if jaundice is fully relieved, most children will grow and develop normally but by age of 10 years about 50% of these patients need a transplant for chronic liver failure<sup>6</sup>. While 21 % of all hepatobiliary referrals at our centre were of neonatal cholestasis, nearly 20% of these had biliary atresia. Of all the children who received the transplant, 29 % had a diagnosis of biliary atresia. The outcome of cholestatic liver diseases like Progressive Familial Intrahepatic Cholestasis (PFIC) is more variable. Liver transplant is indicated if **decompensated cirrhosis** and/or **intractable portal hypertension** develop, if malnutrition and growth failure are unresponsive to nutritional support or if there is intractable pruritis that is resistant to maximum medical therapy or biliary diversion. Ten percent of children who underwent LT at our centre had diagnosis of PFIC.

#### **Metabolic disorders**

Wilson disease is a rare indication of liver transplant in childhood. Early diagnosis and therapy with penicillamine should be curative, but many children present with established cirrhosis. Liver transplant is indicated in children who present with advanced liver disease, progressive hepatic disease despite medical therapy or fulminant hepatic failure. The clinical presentation of Tyrosinemia type-I includes both acute and chronic liver disease and multiorgan failure. It constitutes an important metabolic indication for liver transplant both in the infantile and older age groups. Metabolic disorders constituted less than 5% of children requiring LT at our centre in last 10 years. Early identification of these disorders is essential before severe extra hepatic manifestations ensue. A number of inborn errors of metabolism are secondary to hepatic enzyme deficiencies that lead to severe extra hepatic disease while the liver function remains normal. Crigler Najjar syndrome type-I, primary hyperoxaluria are some of the examples of such disorders. A liver transplant is required in these cases to prevent or reverse extra hepatic disease. The timing of transplant in these cases depends on the rate of progression of the disease, the quality of life of the affected child and the development of severe irreversible extra hepatic disease.

#### **Acute liver failure**

Acute liver failure (ALF) is a rare condition in children, although it is associated with significant mortality without transplantation. Kelly et al found that 50% of acute liver failure in pediatric patients was due to an infection with more than half of these diseases being non-A-non-B-non-C-hepatitis<sup>7</sup>. In India, Hepatitis A is the commonest cause of ALF in children<sup>8</sup>. Metabolic diseases like Tyrosinemia type-I and Wilson disease can also present as acute liver failure, especially in the first year of life. The criteria from King's College London are widely used to assess for LT and comprise of 4 variables: leukocyte count >9000/ cu mm, bilirubin 13.8 mg/dl, age below 2 years and INR 4. If one of these variables appears in a child with acute liver failure mortality rises dramatically to 76%. In the case of the appearance of 2 variables mortality rises to 93% and with the presence of 4 variables mortality is 100%. ALF is the second most common indication among the children who required the transplant at our centre. While ALF was the presentation in 4 % of children referred to our centre during the last ten years, nearly 50 % of these satisfied the Kings College criteria for a transplant.

### **PRE-TRANSPLANT ASSESSMENT**

The aims of assessment for liver transplantation are to confirm the diagnosis and severity of disease, define the patient's general medical status, arrange interim supportive care and assess socioeconomic and educational status of the family. Assessment is carried out by a multi-disciplinary team and involves the patient and their families. The ability of the child's family to comply with instructions and follow-up plans are relevant factors which must be considered in the transplant assessment process. Hepatic function is assessed by measurement of albumin and prothrombin time (synthetic function) and bilirubin, transaminases, alkaline phosphatase and gamma

glutamyl transpeptidase. The vascular anatomy is delineated by Doppler ultrasonography and/or MR or conventional angiography. A nutritional, developmental, cardiac and dental assessment is performed in all children. Serological examination is performed to assess immunity to viral pathogens.

### **PREPARATION FOR LIVER TRANSPLANTATION**

The important aspects of the preparation are nutritional rehabilitation, immunization, treatment of hepatic complications and counseling.

*Nutritional rehabilitation:* Recent data suggests that preoperative nutritional status is an important factor affecting outcome post transplant<sup>10</sup>. Modular feeds which allow protein, carbohydrate and fat content to be individually prescribed for each child are recommended.

*Immunization:* It is essential to make sure that routine immunizations are complete. If necessary, immunization for MMR and varicella should be brought forward. However, in children undergoing emergency liver transplants, completing the immunization with live vaccines is not possible<sup>11</sup>.

*Treatment of hepatic complications:* Ascites and fluid retention is managed by restricted sodium and fluid intake and the use of diuretic therapy. Bleeding varices are treated with intravenous somatostatin or octreotide, endoscopic band ligation or transjugular intrahepatic portal shunts. It is preferable to employ band ligation instead of sclerotherapy because of the potential risk of portal vein thrombosis and ulceration. Hepatic encephalopathy is treated by low protein diet and oral lactulose. The role of branched chain amino acids remains controversial and use of extracorporeal liver assist devices as a bridge to transplantation is not yet fully established<sup>12</sup>.

*Counseling:* Education and counseling of the family and the child is of paramount importance to sustain them through the stressful procedure, the prolonged post-operative period and the life-long immunosuppressive therapy with its attendant risks and side effects

### **ICU CARE**

In the immediate post operative period the main issues revolve around monitoring graft function (PT/INR, PTT and other liver function tests) in addition to maintenance of hemodynamic parameters, fluid balance and oxygenation to ensure adequate blood flow to the liver graft. Strict aseptic precautions must be followed in caring for transplant patients. Duration of mechanical ventilation in transplant recipient depends upon age and preoperative condition of the patient. A child with FHF may require a longer duration of ventilation depending on the neurological state. On the other hand, patients with normal pulmonary function preoperatively may require short term or no ventilation. Infants and small children are more likely to need postoperative ventilation. Ventilatory management aims at avoiding the respiratory complications of atelectasis, effusions and pneumonia. Some smaller patients may require a higher end expiratory pressure to compensate for a distended abdomen pushing onto the diaphragm. In a single centre series the mean time to extubation was  $11.1 \pm 15$  hours and the mean duration of ICU stay was  $7.2 \pm 5.5$  days<sup>13</sup>. Pain control is commonly achieved by opioid infusions titrated to effect. Judicious use of muscle relaxants might be needed. Atracurium (a non-depolarizing muscle relaxant metabolized by non enzymatic hydrolysis (Hoffman elimination) can be safely used. General principles of respiratory care apply in all mechanically ventilated patients. Close monitoring, early detection and treatment of complications remains the key to success in addition to supportive management by the liver transplant team.

### **COMPLICATIONS**

Primary non-function is rare among living related liver transplant recipients.

It must however be closely monitored for the first 24 hours (rising transaminases, profound hypoglycemia and acidosis, coagulopathy, oliguria) requiring emergency retransplantation. Acute rejection occurs in first few weeks after transplantation (average 23 days after transplant in children) characterized by fever, increased bilirubin and liver enzyme levels and encephalopathy. Five to twenty percent patients will have vascular occlusion with accompanying graft loss. Vena cava, portal vein and more commonly hepatic artery may get occluded by intramural thrombus, or less commonly by extrinsic compression or vessel kinking. Vessels smaller than 3 mm diameter tend to have higher incidence of arterial thrombosis. Signs and symptoms consist of FHF, increased transaminase and bilirubin levels with worsening coagulopathy. Portal thrombosis presents with fulminant necrosis and intestinal edema with ascitis. Doppler ultrasonography and CT angiography are useful tools in early identification of vascular occlusion<sup>14</sup>. Early identification and management of bile leak and postoperative bleeding requires close monitoring.

### IMMUNOSUPPRESSION

The usual immunosuppressive regimen consists of calcineurin inhibitors cyclosporine or tacrolimus and prednisolone along with mycophenolate mofetil (MMF). Tacrolimus based immunosuppression is preferred as it has been associated with less acute rejection and better long-term graft survival rates<sup>14</sup>.

### INDIAN SCENARIO

Till a few years back, in the absence of LT facilities in India, a patient with liver failure had only two options, certain death or travel abroad for a transplant. Receiving a liver transplant in the West was not only a costly exercise but also entailed a long waiting period as being a foreigner, he/she would get a low priority on the cadaver waiting lists which were understandably biased to favor the state health entitled native population. The development of liver transplant programme in India led to an overall improvement in level of care in allied specialties (anesthesia, critical care, blood bank, radiology, histopathology) on one hand and on the other helped save precious foreign exchange<sup>15,16</sup>. Our experience over the last 10 years has shown that it is possible to establish a programme but there are certain unique hurdles that need to be overcome<sup>15,16</sup>. The social evaluation in our setting is extremely important. Low socioeconomic and educational levels along with insufficient social assistance have a considerable impact on practicality of a transplant taking place in India. Most patients are referred late for a transplant, quite often when they are not transplantable (Table 3). The sub optimal management of the patients in the pre transplant phase results in a sizeable number being unfit for transplantation<sup>15,17,18</sup>. Cost is still a formidable problem for most patients and absence of medical insurance and state funding further aggravates the problem. A pediatric LT at our center costs between 12 to 15 lakhs, which is roughly one-tenth of what it would cost in the West. Despite these huge difference in costs, contributions from philanthropic organizations and medical insurance are required if LT is to become available to the vast majority of patients in India. There is a clear bias against the girl child with most families hesitating to spend on transplantation for a girl (Table 4). There is a paucity of reliable cadaver organ supply primarily due to low awareness about organ donation. With an appropriate brain death law already in place, sufficient public and professional education is needed to develop cadaver donation. More awareness and acceptability towards organ donation in our country is required so that cadaver organs are available for patients in urgent need for a transplant. Till then, living related liver transplantation is the only way forward.

### THE WATERSHED

Presently the patient survival rates in India are comparable to the best centers in the world<sup>4</sup>. Over the last decade, 28 transplants have been

Table-3: Liver transplantation – Assessment details

Unfit	134
Neonatal cholestasis syndrome	104
Infection	74
Malnutrition	104
FHF	34
Ataxia	01
Sepsis	16
Multi organ failure	17

Table 4: Liver transplantations (1/10/97 to 24/10/08)

Fit for transplant	122
Refused (Girls 64, Boys 15)	79
Economic factor	70
Willing	51
Cadaver only	23
LRLT	28
Transplants performed	28*
Cadaver	02
LRLT	26
* One re-transplant	

#### Longest follow up 10 year

performed at our center. The year 2006 was water shed in terms of the learning curve being re written. We have recorded 100% survival rates in all fourteen pediatric liver transplants since then. The surgical expertise gained over the last few years combined with the advancements made and lessons learnt in the medical management have contributed in a big way to this achievement. Liver transplants in infants are now being performed with results comparable to those of children more than one year of age.

Despite these success stories, there is still a lack of awareness on existing liver transplant facilities in India even within the medical fraternity. It is the faith and support of the medical fraternity and the public at large that can help our endeavors to make LT a practically feasible and economically viable treatment modality in India.

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# STEM CELL TRANSPLANTATION IN PEDIATRICS

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**Abstract :** Pediatric diseases are unique in that there is a preponderance of genetic and metabolic conditions which afflict this age group. There are aggressive malignancies not amenable to or which have failed conventional therapy. Stem cell therapy technology has evolved to make treatment possible for these difficult disorders. Stem cell treatment can be broadly divided into two groups, Hematopoietic stem transplantation (HSCT) and Stem cell therapy. Bone marrow- or peripheral blood-derived allogeneic hematopoietic stem cell transplantation from an HLA-identical sibling or matched unrelated donor can cure children with thalassemia major, severe aplastic anemia and leukemias. There are several centers which perform HSCT in India, they have been performed for both malignant and non malignant diseases. Post transplant complications occur in both the acute setting with acute graft versus host disease, sinusoidal obstructive syndrome and infections causing early mortality and morbidity. Late complications include chronic graft versus host disease and infections as well as the sequelae of treatment. Advancements in understanding transplant biology and the newer immunosuppressive drugs, have helped to reduce and treat graft versus host disease, routine prophylaxis, better early diagnostic markers for viral infections and immunizations have helped in prompt identification, treatment and prevention of infections. Better chimerism techniques have enhanced post transplant monitoring for early identification of relapse and rejection so that intervention can be delivered. For diseases like thalassemia major and aplastic anemia if transplants are planned, the patient and donor workup needs to be performed early as the transplant outcome is better prior to receiving multiple transfusions. Stem cell therapy is the forefront of regenerative medicine and has been utilized for degenerative disorders. Newer applications of stem cell therapy in congenital and developmental pediatric diseases holds promise for many incurable disorders.

## INTRODUCTION

Pediatric diseases differ from adult conditions in that many genetic and metabolic conditions which are either incompatible with a normal lifespan or cause serious developmental sequelae afflict children. Certain aggressive malignancies also occur only in young children and adolescents; these are frequently not amenable to or have failed conventional therapy. These difficult hitherto untreatable, life threatening conditions have led to the identification of a field of treatment using stem cell therapy. The concept of stem cell therapy has been with us for sometime, now the technology has evolved to make treatment possible for these and many other pediatric disorders. Stem cell treatment can be broadly divided into two groups. (1.) **Hematopoietic stem transplantation:** the biology, indications, dosage and complications have been well defined. (2.) **Stem cell therapy:** which is a promising, evolving new modality and the last frontier of a new field of medicine. This is however not yet fully elucidated. We will be discussing these two entities separately in this article.

## HEMATOPOIETIC STEM CELL TRANSPLANTATION (HSCT)

Hematopoietic stem cell transplantation is a standard procedure in which progenitor cells that have the capacity to reconstitute normal bone marrow function, are transplanted into the patient. This procedure may utilize hematopoietic stem cells from the same patient or from a donor. Hematopoietic stem cell transplantation was started more than 50 years ago. The first successful hematopoietic stem cell transplants were conducted in the 1960s for primary immunodeficiency diseases and leukemia. These early attempts suffered from high morbidity and mortality. This was due to toxicity related to the conditioning (or preparative) regimen given for the bone marrow transplantation, post transplant infectious complications and a complication unique to HSCT, due to immunological effects of the donor cells on the host body called graft versus-host-disease (GVHD).

Subsequent advances in transplant clinical care and transplant biology

have focused on improvement of conditioning regimens that are tailor made for optimum results in different diseases. The standardization of prophylaxis and awareness of common infections and an understanding of the immune mechanisms associated with the adverse effects (GVHD) and the antitumoral effects of the engrafted hematopoietic stem cells have all led to reduction in transplant mortality and morbidity.

### Sources of stem cells

First, a source of stem cells must be identified for the patient, the sources of hematopoietic stem cells for transplantation are conventional bone marrow, cytokine mobilized peripheral blood stem cells (PBST) and umbilical cord blood hematopoietic stem cells. CD34 (+) is a cell surface marker on hematopoietic stem cells which is used to identify and enumerate these cells. The decision about choice of stem cell origin for the patient is based on multiple factors such as host disease, donor characteristics and most importantly HLA match. Each of these sources of cells has specific advantages and disadvantages, which are taken into account for making the selection (Table 1).

**Table 1:** Summary of the characteristics of hematopoietic sources - bone marrow, peripheral blood stem cells and umbilical cord blood.

Property	Bone marrow	Peripheral blood stem cells	Umbilical cord blood
Collection of stem cells	Needs general anesthesia for donor	Needs granulocyte colony stimulating factor for donor	Collection at time of delivery
HLA matching	HLA match class I and Class II	HLA match class I and Class II	Mismatch of 1-2 HLA-A,B-DR
Engraftment time	14- 18 days	9-11 days	20 days
Risk of acute GVHD	Equal to peripheral blood	Equal to bone marrow	Least
Risk of chronic GVHD	Lower than peripheral blood	Highest	Least
Immunotherapy	Feasible	Feasible	Not feasible
Time to unrelated donor identification, donor attrition donor	Unrelated donor search, requires database, funds and time for search. Donors may refuse	Unrelated donor search, requires database, funds and time for search. Donors may refuse	Cord blood units stored and typed. But small units, expense and logistics of starting bank

GVHD= graft versus host disease

HLA = human leucocyte antigen

### Types of hematopoietic stem cell transplantation

The major types of hematopoietic stem cell transplantation (HSCT) are classified by the source of progenitor cells used in the transplant. Stem cells from the host are called autologous stem cells. *Autologous stem*

cells are usually peripheral blood stem cells, but may be bone marrow cells, stem cells from a donor are allogeneic stem cells, these may be peripheral blood, bone marrow or donated umbilical cord blood. Allogeneic stem cell transplantation has been made possible by the identification and study of Histocompatibility antigens the Human Leukocyte Antigen system. These are antigens which are expressed on the surface of most cells in the body including leucocytes. These antigens are also known as the major histocompatibility complex (MHC) and occupy the short arm of chromosome 6. This genetic region has been divided into chromosomal classes which are matched to find the most closely matched or best suited donor. Class I and II matching are prerequisites to selecting an allogeneic donor. Class I region is made up of *HLA-A*, *HLA-B* and *HLA-C* and Class II is made up of *HLA-DR*, *HLA-DP* and *HLA-DQ* genes. Traditionally, the critical loci for HLA matching are *HLA-A*, *B*, and *DR*. The roles of *HLA-C* and *HLA-DQ* have recently gained importance and are now also being considered while selecting a donor. HLA typing is performed by serology and molecular techniques, this is a rapidly expanding field, with identification of mismatches which are acceptable and those that may result in later severe complications such as graft versus host disease or graft rejection. Killer cell immunoglobulin-like receptor (KIR) typing is now being performed and correlated with GVHD, to study the role of alloreactive natural killer cells. A completely matched sibling donor is considered the ideal donor. However every patient does not have an HLA matched sibling, hence unrelated donor transplant in which the donor is completely matched or has a single mismatch can be performed. In certain circumstances e.g. relapsed leukemia, a greater donor mismatch is tolerated and may even be beneficial.

Another source of hematopoietic stem cells are those collected from the umbilical cord blood. The use of cord blood transplantation has rapidly increased because of ease of collection, prompt availability, absence of donation risk, less GVHD due to increased tolerance to HLA-mismatch<sup>1</sup>. Cord blood banking has become important, these cord blood units are HLA typed and stem cells are quantified. They are available to prospective patients. Use of cord blood as a source is constrained by the quantity of cells available in each cord blood unit. The use of multiple cord blood transplants, in which multiple cord blood units from different donors has expanded their use to larger sized patients and has shortened the time to engraftment<sup>2</sup>. However cord blood banks that store the cord blood only for the donors own use in the future, have limited utility<sup>3</sup>.

*Patients without an HLA matched sibling can try for an unrelated donor search in the voluntary bone marrow donor registries.* However for patients belonging to ethnic minorities, particularly Asians the probability of finding an HLA-matched donor is remote, due to their unique HLA alleles. The time taken to search and donor attrition (refusals, inability to donate etc.) have added further hurdles to expanding this process in India. *Most Indian patients cannot afford the cost of an unrelated marrow search, or develop serious complications before transplantation due to the prolonged time taken to complete such a search.*

The transplant team selects the stem cell source based on availability, dose of stem cells needed for the patient, disease, benefit of graft versus leukemia effect and degree of HLA mismatch and need for rapid engraftment of blood cells. If more than one donor is available then the most compatible donor is chosen by evaluating donor sex, cytomegalovirus (CMV) status, degree of blood group mismatch etc.

Autologous transplantation is a technique of giving high dose chemotherapy in which the hematopoietic system is rescued by returning the patient's own stem cells. This is most useful in chemosensitive

hematopoietic and solid malignancies where the higher than conventional dose therapy is able to eliminate the residual malignant disease, and prevent severe toxicity to the patient who would without the rescue have suffered profound ablation of the bone marrow. The host's peripheral blood stem cells must be collected after conventional treatment to control the disease and mobilized by cytokine granulocyte colony stimulating factors. Every effort to ensure that the stem cell product is free of tumor is done since the major complication after autologous transplant is relapse of the original disease. This procedure does not have the immunological complications seen with allogeneic transplants and immunosuppression is not required as the reconstituted immune system is that of the original host.

### Indications

Pediatric hematopoietic stem cell transplants are performed for *malignant and non malignant diseases*. Common malignant diseases for which transplant is indicated are *advanced solid tumors* and *high risk leukemia* patients when a HLA matched sibling donor is available e.g. acute myeloid leukemia, Philadelphia positive Acute Lymphoblastic leukemia (ALL), Juvenile myelomonocytic leukemia (JMML), Infants acute lymphoblastic leukemia (ALL) with 11q23 rearrangement and chronic myeloid leukemia (CML) in children. Patients who have graft versus host disease (GVHD) experience improved relapse-free survival. Children with acute promyelocytic anemia t(15;17), and those with inv(16) and t(8;21) fare well with chemotherapy these patients are not treated with up-front transplant regimens<sup>4,6</sup>. Even in CML and Ph positive ALL with better chemotherapy and newer agents like imatinib and dasatinib some children may do well even without transplant.

Other children for whom hematopoietic stem cell transplantation may be a good option include those who have experienced induction failure or early relapse within 18 months of diagnosis<sup>7</sup>. In children with relapsed leukemias, the response rates to transplant vary with time to relapse, response to the relapse chemotherapy protocol and type of relapse (see Table 2a and b).

**Table 2 a** Indications for Autologous transplantation in pediatric patients

Malignant disorders	Non malignant disorders
Neuroblastoma	Trials for collagen vascular and severe autoimmune disorders
Relapsed lymphomas	
Germ cell tumors	
Certain indications for-	
Chronic myeloid leukemia,	
Acute myeloid leukemia	
Acute lymphoblastic leukemia	
In clinical trials for advanced malignancies e.g. Brain tumors, other pediatric solid tumors.	

**Table 2b** Indications for Allogeneic hematopoietic transplantation in Pediatric patients

Malignant Conditions	Non malignant conditions
Acute myeloid leukemia	Hemoglobinopathy- e.g. Thalassemias, sickle cell disease
Acute lymphoblastic leukemia	Aplastic anemia, Fanconi anemia
Infant Acute lymphoblastic leukemia	Storage disorders
Philadelphia positive Acute lymphoblastic leukemia	e.g. Adrenoleukodystrophy
Juvenile chronic myelo monocytic leukemia	Hurler syndrome
Myelodysplastic and myeloproliferative disorders e.g. Chronic Myeloid Leukemia	Krabbe disease
In clinical trials for advanced malignancies-	Primary Immunodeficiency diseases
e.g. Relapsed lymphomas.	Macrophage and granulocyte disorders
	Kostmans, hemophagocytic syndromes
	Osteopetrosis

Transplantation for *non malignant conditions* are performed for *hemoglobinopathies* e.g. *thalassemia major*; *aplastic anemia* and a variety of immunodeficiencies and genetic disorders. Since the transplants entail correction of gene defect or supply of absent hematopoietic stem cells/ substrate only allogeneic transplants can be performed for these conditions (see table 2b). Children who have received multiple transfusions (Thalassemia major and aplastic anemia) are at risk for rejection and other serious complications of transplant. Hence early referral for matched sibling related transplant is of benefit. In children with immunodeficiencies and genetic disorders the conditions needs to be identified promptly and the children transplanted from a healthy sibling or by using maternal haplo-identical stem cells to save the life of the child and prevent serious morbidity. Severe auto immune diseases such as systemic lupus erythematosus have also been successfully auto-transplanted.

**Transplantation Procedure**

**a) Conditioning**

The initial step for transplantation of hematopoietic stem cells involves selection of the conditioning regimen. This is the name given to the combination of chemotherapy, immunosuppressants, radiation therapy or radio labeled monoclonal antibodies given prior to infusion of the hematopoietic stem cells. The role of conditioning is to make space in the bone marrow for the new stem cells, remove the pathologic cells from the host and provide immune suppression to prevent rejection and graft versus host disease. This conditioning varies in the drugs chosen, doses of chemotherapy and inclusion or exclusion of radiotherapy dependent on the disease entity for which transplantation is being performed.

**b) Collection of stem cells**

- (i) *Harvesting bone marrow*: Stem cells are obtained from the bone marrow by repeated aspirations of the posterior iliac crests of the donor under general anesthesia. Collection of the required adequate cell dose can be a difficult procedure in small sibling donors. The collections can be of unprimed bone marrow or primed with granulocyte colony stimulating factors (G-CSF).
- (ii) *Peripheral blood stem cell collection*: The bone marrow stem cells can be mobilized into the peripheral blood and collected via leukocytapheresis. Along with increasing the number of cells, G-CSF also causes the release of proteases that degrade the proteins that anchor the stem cells to the marrow stroma, causing their release into the peripheral blood. Autologous stem cells are collected post recovery after a cycle of chemotherapy with hematopoietic growth factors like G-CSF. In allogeneic transplant the healthy matched donor is given 4-5 days of granulocyte colony stimulating factor (G-CSF) 10 µg/kg/day and stem cells are collected by apheresis technique on a cell separator machine. Peripheral blood stem cells can be cryopreserved for later infusion or given to the patient on the same day. Peripheral blood stem cells have more T cells than bone marrow and consequently there is an increased risk of chronic GVHD. Peripheral blood stem cells speedily engraft compared to other stem cell sources. However it is technically difficult to collect peripheral stem cells from small children
- (iii) *Umbilical cord blood*: Collection of Umbilical cord blood (UCB) is performed at the time of delivery, usually after delivery of the placenta. It must be performed after cleaning the cord, using aseptic technique a needle is inserted into the umbilical cord and blood is withdrawn. Manipulating the placenta to increase the yield is contraindicated as it may lead to contamination with maternal blood.

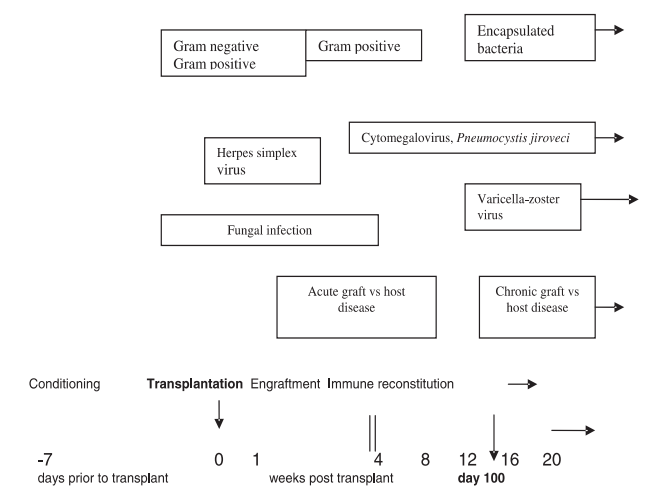
**c) Infusion of stem cells**

The mononuclear cell count and CD34(+) count in the peripheral blood determines the timing of collection. After collection flow cytometric enumeration of cells is performed. Although the minimum number required for engraftment is considered to be  $1 \times 10^6$  cells per kilogram of body weight, the preferred number is  $2-2.5 \times 10^6$  cells/kg or more. The stem cell infusion is given like a blood transfusion with monitoring and hydration. The CD34 and stem cells home to the bone marrow. The engraftment of hematopoietic cells occurs within 8-20 days depending on stem cell source and dose (table 1).

**COMPLICATIONS**

Hematopoietic stem cell transplantation (HSCT) related complications are classified into early and late depending on their timing. Early complications occur in the first 100 days of transplant. Usually post 100 days the patient if stable, is discharged from the hospital and continues on immuno-suppression for six months to one year (table 3).

**Table 3** Timeline of hematopoietic stem cell transplantation and common complications and infections post transplantation



**Early complications**

- a) *Acute graft versus host disease (aGVHD)* is a common complication of allogeneic transplantation and occurs within the first 100 days after the procedure. It is an immune response of donor T lymphocytes against host cells. The skin, gastrointestinal tract, and liver are the target organs involved<sup>8</sup>. Severe acute (grade IV) graft versus host disease is a life threatening disease. Every protocol usually uses cyclosporine and methotrexate for prophylaxis, for treatment of graft versus host disease corticosteroids, tacrolimus, mycophenolate mofetil (MMF) and several newer monoclonal antibodies have been used. The severity of GVHD is inversely related to the risk of relapse and strategies to reduce GVHD may increase relapse rates. New strategies are being developed to separate these effects and to decrease the incidence and severity of GVHD without increasing the risk of relapse.
- b) *Mucositis* is one of the most common adverse effects of transplantation, it is due to the conditioning regimen and varies in degree with drugs used and prior host related factors. This may be mild or severe enough to prevent oral intake, compromise nutrition and cause pain and bleeding. It is usually managed

symptomatically with narcotics and topical anesthetics.

- c) *Hemorrhagic cystitis* is a disorder that manifests as dysuria and hematuria. Hematuria may be microscopic or may be gross. This disorder usually occurs during the immediate post transplant period but may occur later. Cyclophosphamide and other drugs have been implicated and bladder protectant (MESNA) and hyperhydration are routinely used to reduce this complication. Late onset hemorrhage is associated with infections such as adenovirus or BK virus.
- d) *Sinusoidal obstructive syndrome (SOS)* is a potentially fatal syndrome of tender hepatomegaly, direct hyperbilirubinemia, ascites, and weight gain. SOS is caused by damage to the sinusoidal endothelium, which results in sinusoidal obstruction. This entity occurs within the first 20 days of hematopoietic stem cell transplantation, and preexisting liver disease and genetic polymorphisms which alter drug metabolism may increase its risk. SOS has an overall mortality rate of as much as 50%. No standard effective therapy is currently available. Defibrotide is an investigational agent which has shown favorable response rates.
- e) *Transplantation related infections* Life-threatening bacterial, fungal, and viral infections are common in patients undergoing hematopoietic stem cell transplantation. The patients are at risk due to prolonged neutropenia, use of immunosuppressants e.g. cyclosporine, steroids etc. and immunodeficiency associated with GVHD. Bacterial sepsis occurs early in the course of transplantation, whereas viral infections by cytomegalovirus and other viruses usually occur after engraftment. Fungal infections may occur after 7-10 days of onset of neutropenia and the patient is at risk till engraftment. Early recognition and prompt treatment are essential, prophylaxis for common infections is routinely given till immunosuppression is ongoing, this becomes prolonged in patients being treated for chronic graft versus host disease.
- f) *Graft rejection* is a serious complication, this occurs more commonly in transplantation for non-hematological disorders and multi-transfused allo-immunized patients. Chimerism monitoring is required to monitor for stable engraftment and identify graft rejection. Short tandem repeats/variable number tandem repeats (STRs/VNTRs) are the best tool for chimerism monitoring.

### **Late Effects**

- a) *Chronic graft versus host disease* is characterized by an auto-immune phenomenon like that seen in scleroderma, Sjögren syndrome and lupus. This results from thymic injury during conditioning, resulting in loss of negative selection of autoreactive T cells, and the alloreactivity of mature post thymic donor T lymphocytes. Immunosuppression with drugs like corticosteroids, tacrolimus, and mycophenolate mofetil are the mainstay of treatment.
- b) *Endocrine effects* in children such as impaired growth and hypothyroidism may be found.
- c) *Pulmonary effects* include restrictive and chronic obstructive lung disease. Conditioning regimens, infections, and GVHD are important risk factors. Bronchiolitis obliterans is a specific form of obstructive lung disease seen in hematopoietic stem cell transplant recipients and this has a fatality rate of 50%.
- d) *Neurocognitive effects*-Lower intelligence quotient (IQ) scores, fatigue, memory problems and even developmental delay have

been reported, these occur frequently in children who have received cranial radiation either as part of their oncological therapy or as part of their hematopoietic stem cell transplantation conditioning.

- e) *Immune effects*-Host immunity is suppressed for months to years after hematopoietic stem cell transplantation. This is due to severe neutopenia and lymphopenia due to the myeloablative conditioning, acute GVHD that further suppresses host immunity, and the use of immuno-suppressants to prevent or treat GVHD. These immune effects should be considered because these patients are prone to serious infections long after the completion of the stem cell transplant. There is consensus that re-vaccination is required to boost vaccine-acquired immunity. The repeat vaccination is required approximately one year after allogeneic stem cell transplantation, if there is no active chronic GVHD and the patient is off immunosuppressive therapy for at least 6 months. Live vaccines should never be given prior to one year post cessation of immuno-suppressants. Early vaccinations may not result in an appropriate immune response.

### **BONE MARROW TRANSPLANT SCENARIO IN INDIA**

The initial HSCT were performed in India in Tata Hospital, Mumbai and Christian Medical College (CMC) Vellore, with this initial success, several other centers around the country now have facilities for performing hematopoietic stem cell transplantation. Indian data for all pediatric transplants is unavailable but a total of approximately 1540 HSCT have been performed in adult and pediatric patients. The maximal allogeneic transplant experience is at CMC Vellore<sup>9</sup>.

At our center, Department of Hematology AIIMS we have performed allogeneic transplants for a variety of conditions, predominantly for aplastic anemia, leukemia, thalassemia major and myelodysplastic syndrome in adults and children. The HSCT have been performed safely with excellent outcomes. We did not experience any 30-day mortality and 100-day mortality was 2.5%. We have ascertained the safety of performing allogeneic HSCT in single. non-high efficiency particulate air (HEPA) filter rooms<sup>10,11</sup>. We have performed 20 pediatric HLA matched sibling transplants -Aplastic anemia 10, Thalassemia major 6, E Beta Thalassemia 1, relapsed Acute lymphoblastic leukemia 2, Chronic myeloid leukemia-1. Median age 9.5 years (1-17 years), male: female 16:4. Median time till neutrophil engraftment was 10 days (range 8-21). Day 100 mortality was nil, Kaplan Meier survival 90% with follow up ranging 4-54 months (unpublished data). Deaths occurred in 3 patients-child with aplastic anemia transplanted with fungal sinusitis died of chronic graft versus host disease and fungal sepsis at ten months, another with aplastic anemia suffered graft rejection and meningitis, the child of relapsed acute lymphoblastic leukemia suffered a second relapse one year later. Our infection risks are similar to those reported from other centers<sup>12</sup>.

### **CONCLUSION**

A major impediment to hematopoietic stem cell transplantation (HSCT) is the availability of a genetically matched donor. Smaller family size limits the options of sibling donors and because the Indian population has the presence of novel HLA alleles and unique haplotypes (HLA-A\*0211, B\*2707, A\*26-B\*08-DRB1\*03), many Indians who have these rare HLA alleles will find difficulty finding an unrelated optimally matched donor from foreign bone marrow registries. These limitations can be overcome by developing unrelated

volunteer marrow donor registries in India<sup>13</sup>. Also by improving technology and establishing the facilities required to perform matched and unrelated transplants we will be able to increase the number of children who can benefit from this potentially life saving procedure<sup>9,14</sup>.

## 2. STEM CELL THERAPY

Stem cell biology is currently one of the most exciting areas of biochemical research having the potential to provide therapeutic treatment for developmental and degenerative disorders. Advances in stem cell biology and the discovery of pluripotent stem cells have made the prospect of cell therapy and tissue regeneration a clinical reality. Cell therapies hold great promise to repair, restore, replace or regenerate affected organs<sup>15</sup>.

Although **embryonic stem cells** isolated from the inner cell mass (ICM) of the blastocyst or fetal gonadal tissue are the 'ultimate' stem cell, ethical issues and the potential danger of teratoma formation have limited the development of these cells as therapeutic options. However, hematopoietic adult stem cells have been used therapeutically for many years in malignant hematological diseases and are currently the best characterized stem cells. Therefore these adult stem cells are now at the forefront of therapeutic research and currently the most widely used cells in clinical trials. Below are details of promising clinical work being done presently at our center and other leading hospitals in India.

### **Congenital malformations**

Stem cells with the potential to transform into healthy cells and repair damaged cells may prove beneficial in various congenital malformations. Stem cells are being tried in selected congenital malformations for which the present treatment options are either limited or not available when the irreversible changes have already taken place at an early stage. These include extra hepatic biliary atresia (EHBA) and spina bifida with neurological deficiency.

Current work at AIIMS is being done in the field of EHBA, this is a condition in which outflow biliary channels from the liver are congenitally blocked, thus predisposing to cirrhotic liver and hepatocellular failure. The goal of stem cell therapy is to repair damaged tissue that has lost the property to heal itself. This can be accomplished by transplanting stem cells into the damaged area and directing them to grow new and healthy tissue. Autologous bone marrow stem cells are a type of adult stem cells, a multipotent unit still capable of differentiating into specialized cells. In this study, histopathology could be done at 6 months after the stem cell infusion, in the three patients who were alive with adequate follow-up. A comparative improvement in fibrosis was seen in all patients. Stem cells were used in spina bifida with neurological deficiency with an aim to repair the damaged neurons and improve the existing neurological deficits. Recovery of central nervous system disorders is hindered by the limited ability to regenerate lost cells, replace damaged myelin, and re-establish functional neural connections. Both hematopoietic stem cells and marrow stromal cells have been shown to have the potential to restore the injured spinal cord and promote functional recovery in mice<sup>16,17</sup>. This positive effect was most pronounced using mesenchymal stem cells. Progressive complete functional motor recovery with evident nervous tissue regeneration has been achieved following administration of bone marrow stromal cells in traumatic central spinal cord cavities of adult rats with chronic paraplegia due to a previous injury to the spinal cord. In our experience with favorable responses in 50% of cases, it is hoped that stem cells may prove

beneficial to improve the neurological deficits associated with cases of spina bifida. To conclude, stem cell therapy may prove beneficial in cases of liver cirrhosis due to congenital anomalies by reversing or delaying the onset of end-stage liver disease and decreasing the need for liver transplantation and immuno-suppression. The initial use of stem cells in meningocele has shown promising results. However, long term evaluation with randomized controlled trials is essential to draw conclusions. Major concerns remain: what prompts stem cells to assume specific functions and which factors would dictate them to stop multiplication once the aim is achieved.

### **Pediatric leukodystrophies**

This group comprises of diseases that manifest in childhood with deficiencies in myelin production or maintenance; these may be due to hereditary defects in one or more genes critical to the initiation of myelination, as in Pelizaeus-Merzbacher Disease, or to enzymatic deficiencies with aberrant substrate accumulation-related dysfunction, as in the lysosomal storage disorders. In the light of the wide range of disorders to which congenital hypomyelination and/or postnatal demyelination may contribute, and the relative homogeneity of central oligodendrocytes and their progenitors, the pediatric leukodystrophies are attractive targets for cell-based therapeutic strategies. As a result, glial progenitor cells (GPCs), which can give rise to new myelinogenic oligodendrocytes, have become the focus of great interest as potential therapeutic vectors for the restoration of myelin to the hypomyelinated or dysmyelinated childhood central nervous system. In addition, by distributing themselves throughout the deficient host neuraxis after perinatal allograft, and giving rise to astrocytes as well as oligodendrocytes, the glial progenitors appear to have great potential for rectifying enzymatic deficiencies<sup>18</sup>.

### **Multicystic dysplastic kidneys**

The incidence of embryological development disorders of the kidney and urinary tract varies from 0.3–0.8% in live born infants. Embryologically, when the ureteric bud makes contact with the metanephrogenic mesenchyme, a series of inductive signals are exchanged. As the uretric bud grows and branches, its tips contact fresh stem cells and induce them into the nephrogenic pathway. There is therefore a gradient of developmental age in a fetal kidney with the outermost cortex composed of stem cells that are not yet committed to differentiation, and the inner most region contains maturing nephrons and supporting stromal cells. Thus multicystic kidney disease (MCKD) results from incomplete development of the kidney. Recruitment of stem cells to the kidney to elicit repair and the function of dedifferentiation of resident renal cells has been studied and recognized. Stem cells whether recruited to the kidney from a distant organ or delivered to the kidney after *ex vivo* expansion of an isolated stem cell population, may contribute to repair via the production of specific cytokines, chemokines or growth factors, transdifferentiation into specific renal cell types or by cell fusion. Reports have suggested that bone marrow derived stem cells in the kidney can transdifferentiate into tubular epithelial cells, mesangial cells, glomerular endothelial cells and even podocytes. However, the lineage of bone marrow derived cells that appear in the kidney in response to damage is unclear, and their ability to elicit transdifferentiation is controversial because the possibility of cell fusion has not been eliminated. Some authors have concluded that though recruitment of bone marrow derived cells does occur, the repair of renal tissue is predominantly elicited via proliferation of endogenous renal cells<sup>19,21</sup>.

### Ocular surface disorders

The ocular surface comprises of the cornea, the conjunctiva and the limbus which is the transitional zone between the two. The cornea is the transparent tissue which consists of epithelium, stroma and endothelium. The main function of the epithelium is to provide a smooth and transparent surface.

Corneal limbus is known to be the source of corneal epithelial stem cells that are important as a regenerative source for epithelial cells. Limbal stem cells may become partially or totally depleted, resulting in varying degrees of stem cell deficiency with resulting abnormalities in the corneal surface. Limbal stem cell deficiency (LSCD) of any cause may lead to poor corneal epithelialization, persistent epithelial defects, corneal vascularization, corneal scarring, and so-called conjunctivalization of the cornea. These problems lead to decreased vision, ocular discomfort, pain and an unstable ocular surface.

Though there has been a significant improvement in the management of LSCD over the last 10-15 years, with penetrating keratoplasty [PK] and the use of artificial tears, patients with LSCD continue to have a poor prognosis. Now with improved microsurgical techniques and an understanding of the role of limbal stem cells and improvement in immunosuppressive therapies the treatment and the outcome of LSCD has improved. Partial stem cell deficiency can be managed by removing abnormal epithelium along with the transplantation of human amniotic membrane, to resurface cells derived from the remaining intact limbal epithelium. The same can also be combined with amniotic membrane transplantation (AMT).

In case of total stem-cell deficiency, autologous limbus from the opposite normal eye or homologous limbus from living related or cadaveric donors can be transplanted (directly or after *in vitro* expansion) to the affected eye. Transplanted limbal cells will require long-term systemic immunosuppression. At AIIMS we have performed over 35 cases in the pediatric age group and LVPEI, Hyderabad has conducted more than 600 cases in both adults and children. Significant improvement in visual acuity and ocular surface stability has been noticed in patients who have received cultured limbal stem cells along with amniotic membrane<sup>22,23</sup>.

### Muscular Dystrophy

Muscular dystrophy (MD) refers to a group of genetic, hereditary muscle diseases that cause progressive muscle weakness. Muscular dystrophies are characterized by progressive skeletal muscle weakness, defects in muscle proteins and the death of muscle cells and tissue. The best known type of MD is Duchenne muscular dystrophy (DMD). Duchenne muscular dystrophy is inherited in an X-linked recessive pattern, it is caused by mutation of the gene for the dystrophin protein and characterized by an elevated serum creatinine kinase and progressive muscle weakness starting in childhood and progressing to respiratory muscle failure and death. There is no treatment for this disorder.

Stem cell therapy has been tried for this fatal condition, one approach has been transplantation of muscle precursor cells (myoblasts) which can produce dystrophin protein. However, clinical trials have revealed that the transplanted human myoblasts are rapidly lost. Another very promising approach is to genetically modify the stem cells and to upregulate the dystrophin protein. This strategy introduces U7 RNA to induce exon 51 skipping. Skipping this exon places the mRNA back in frame so that a truncated, but functional, dystrophin protein missing only exon 51 is transcribed.

*Additional research is needed to be performed to substantially enhance*

*our understanding of the mechanisms underlying this effect and may lead to the improvement of gene and cell therapy strategies for DMD.*

The promising developments represented by these new approaches maintain the hope for DMD patients and their families for future autologous stem cell therapies<sup>24</sup>.

### CONCLUSION

Stem cells are being evaluated for many pediatric conditions such as osteogenesis imperfecta, Hirschsprung's disease, dilated cardiomyopathy and acute brain injury<sup>25</sup>. However considerable practical hurdles must be overcome prior to the broad application of stem cell therapies, including the issues related to the sourcing of material, safety, storage, tracking and standardization.

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## APPROACH TO INBORN ERRORS OF METABOLISM

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**Abstract :** Inborn errors of metabolisms (IEMs) are inherited disorders occurring due to a block in body's biochemical pathways because of deficiency of an enzyme or its cofactor. These errors lead to symptoms due to either accumulation of the substrate or conversion to a toxic metabolite or deficiency of the end product. The IEMs are collectively not uncommon in our country. They must be considered in the differential diagnosis of all sick infants, as prevention of morbidity (due to irreparable brain damage) and death is possible only by early detection and prompt treatment. The burden on the family can be reduced by prenatal diagnosis. This article describes an approach for testing a sick neonate, and gives a glimpse of newborn screening for such disorders, diagnostic algorithms for a suspected case of IEM are given.

### INTRODUCTION

Inborn errors of metabolism (IEMs) are genetic disorders occurring due to lack of an enzyme in the metabolic cascade in our body<sup>1</sup>. This disrupts a biochemical pathway, leading to symptoms and signs due to accumulation of toxic metabolites prior to the block or deficiency of the end product beyond the metabolic block. Nearly all the inborn errors of metabolism are inherited recessively, mostly autosomal and some X-linked. They are individually rare but collectively numerous<sup>2</sup>. They are increasingly being recognized because of the advancement in diagnostic facilities like blood gas analysis and automated biochemical tests, and the reduction in mortality related to infections and perinatal asphyxia<sup>3</sup>. Other factors that lead to an increased frequency of IEMs in our country are a large population, high birth rate, and custom of consanguineous marriages among many communities<sup>4</sup>. Limited studies have been published on the prevalence of IEMs in India<sup>5,6,7</sup>. At Sir Ganga Ram Hospital, a study of 393 cases suspected to have an inborn errors of metabolism resulted in specific diagnosis in 56 (6.7%) cases<sup>8</sup>. Organic acidemias were the commonest disorder followed by urea cycle disorder, galactosemia and other aminoacid disorders.

### CLASSIFICATION OF INBORN ERRORS OF METABOLISM

IEMs are classified in many ways.

- I) **Clinically**, they are broadly three groups<sup>9</sup>. The enzyme defects occurring in the metabolism of amino acids, carbohydrates, and fatty acids or in the mitochondria energy metabolism are referred to as '*small molecule disorders*'. Clinical manifestations of these disorders are severe and non-specific, and occur early in life. In contrast, *larger molecule or storage disorders* the symptoms develop gradually due to accumulation of substrates, thus resulting in progressive organomegaly and coarse body features. A third group is that of other *miscellaneous* disorders of metals (Wilson and Menkes disease), lipoproteins, congenital adrenal hyperplasia and hyperinsulinism.
- II) **Pathophysiologically**, the IEMs are classified into three groups:
  - i) **Intoxication type:** The group includes disorders of intermediary metabolism that lead to an acute or progressive intoxication from the accumulation of toxic compounds

proximal to the metabolic block. Most organic acidurias (MSUD, MMA etc), urea cycle disorders, aminoacidopathies (tyrosinemia, homocystinuria etc), sugar intolerances (galactosemia etc), metal intoxication (Wilson, Menkes etc) and porphyrias are included in this group. Common features in all of them are a) a symptom free interval (no interference with embryo/fetal development), b) clinical signs of 'intoxication' and c) provocation for acute metabolic crisis in catabolic situations like fever, intercurrent illness and fasting.

- ii) **Energy deficiency type:** These disorders occur due to at least partly to a deficiency in energy production or utilization within liver, myocardium, muscle, brain or other tissues. Important groups included are mitochondrial respiratory chain disorders, fatty acid oxidation and ketone body synthesis.
- iii) **Storage type or 'complex molecules' disorders:** This group involves cellular organelles and includes diseases that disturb the synthesis or catabolism of complex molecules. Lysosomal storage disorders, peroxisomal disorders and disorders of intracellular trafficking and processing such as congenital disorders of glycosylation (CDG) are included in this group. Symptoms are permanent, progressive, independent of intercurrent events and unrelated to food intake.

The focus of this article is on the 'small molecule disorders' or the 'intoxication and energy deficiency' type of disorders, which comprise classical IEMs in the neonatal and infantile period. These disorders are dynamic, fluctuating with the patient's metabolic state and require early diagnosis for therapeutic intervention to prevent the irreparable brain damage.

### APPROACH TO INBORN ERRORS OF METABOLISM

There are *two approaches* to screening for inborn errors of metabolism. The first is the *testing of suspected cases* in order to establish diagnosis in a sick infant. The second approach is to *screen all newborns* for selected group of disorders. The latter is an established practice in the developed countries, but has yet to take off in India. All countries need to establish diagnostic facilities for IEMs, because a majority of them are treatable. Once these facilities are set up and strong socio-economic and health infrastructure has been built up, newborn screening should be initiated. Newborn screening project has been initiated in five cities in India, sponsored by the Indian Council of Medical Research.

## SCREENING OF SICK NEONATES

Enormous strides have been made in the past three decades in the recognition and understanding of metabolic illnesses. Progress on the reverse process - identifying specific metabolic disorders on the basis of signs and symptoms of disease - has been slower. The newborn appears to have a limited repertoire of ways to respond to insult, with the result that the clinical features of many inherited metabolic diseases are superficially indistinguishable from that of the commoner acquired conditions, such as infections and intoxications<sup>10</sup>. Thus, a high index of suspicion is required in order to identify a sick neonate with an IEM. The diagnosis of IEM should be considered along with that of other common illnesses, and should not be a diagnosis of exclusion. **Pointers** to the presence of IEM are<sup>10</sup>:

- History of acute deterioration* after a period of normalcy after birth. This is so because symptoms are caused by postnatal accumulation of toxic metabolites, which were getting cleared in- utero by the placenta and maternal metabolism ('Intoxication' type). Examples are urea cycle defects, organic aciduria and galactosemia. However, it is important to note that this period of normalcy may not always be present and obscured by other problems like perinatal asphyxia.
- A family history* of consanguinity or similar illness in a sibling.
- Progressive illness* showing non-specific signs of cerebral dysfunction such as poor sucking, limpness, vomiting, irritability, respiratory distress, lip smacking movements and hypothermia.
- Unusual odors* may offer an invaluable aid to the diagnosis. In Maple syrup urine disease (MSUD), there is a characteristic odor of maple syrup or burnt sugar. Performing a DNPH test in the urine of such a case can make the diagnosis fairly easy.
- Some IEM are associated with *concomitant sepsis and neutropenia* like organic aciduria and galactosemia (*E. coli* sepsis).

Initial *laboratory investigations* (listed in Table 1) should be carried out as soon as possible. Some tests like blood ammonia and lactate are considered crucial for diagnosis of IEMs, and thus should be available to every neonatal care unit. Specialized investigations are done depending upon the clinical features and initial investigations. MRI brain is increasingly being recognised as an important modality for diagnosis of some IEMs such as molybdenum cofactor defects where there are extensive changes suggestive of the diagnosis. A simplified algorithm for diagnosis of Inborn errors of metabolism in an acutely sick neonate and infant below 1 year of age is given in Figure 1.

Figure 1: Initial approach to a sick neonate/infant

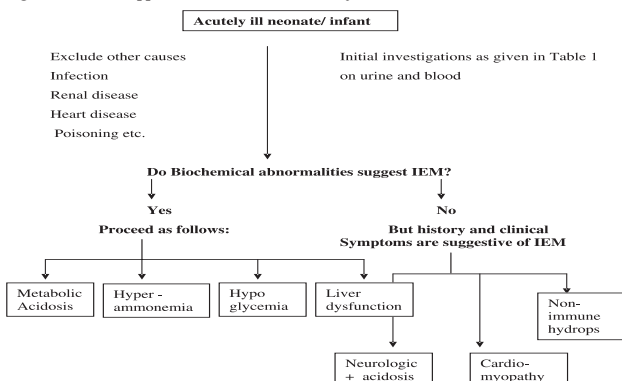


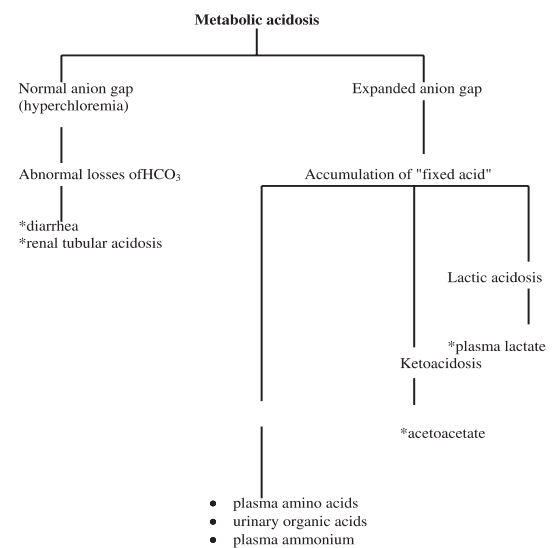
Table 1: Initial investigations in a suspected case of IEM.

<b>A. Initial investigations</b>	<b>Blood:</b> Hemoglobin, white blood count, platelets Blood gases and plasma electrolytes (anion gap (Na+K)-(HCO <sub>3</sub> +Cl)= normal<20 nmol/L) Glucose Calcium, Magnesium Blood urea nitrogen, creatinine Liver function tests (Prothrombin time, liver enzymes, albumin etc.) Ammonia Lactate <b>Urine:</b> Specific gravity, pH Proteins Sugar, Reducing substances (Benedicts and uristix) Ketones, Ketoacids (DNPH) Sulfites Microscopy
<b>B. Specialized investigations</b>	<b>Blood:</b> Amino acid analysis, quantitative Galactosemia screening test Carnitine, total and free (Tandem Mass Spectrometry) Plasma for storage at -20°C: 2-5 ml <b>Urine:</b> Organic acids (GC-MS) Succinyl acetone Urine for storage at -20°C: 10-20 ml <b>Neuro imaging:</b> CT/ MRI brain

Modified from Clarke JTR (10).

- Metabolic acidosis** is an important finding and narrows down the diagnosis to a few disorders including all the organic acidurias and primary lactic acidurias. A diagnostic algorithm is given in Figure 2. The common organic acidurias prevalent in our country are methyl malonic aciduria, propionic aciduria, multiple carboxylase deficiency and maple syrup urine disease (MSUD)<sup>8</sup>.

Figure 2: Flow chart for Metabolic acidosis

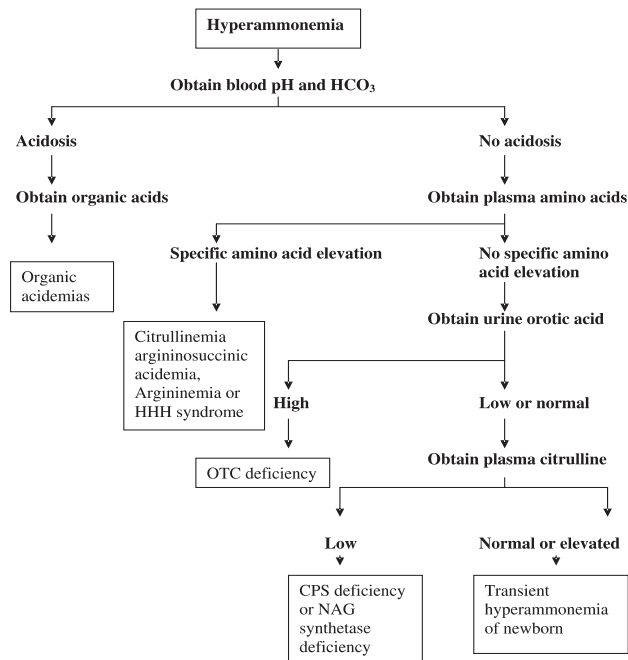


Clarke JTR (10)

- Hyperammonemia** is a very useful clue, and often associated with encephalopathy and seizures. Urea cycle disorders occur without metabolic acidosis. These babies are sick and need immediate treatment with sodium benzoate or phenyl butyrate if available. Raised ammonia in presence of metabolic acidosis suggests organic aciduria, which can be diagnosed

using approach given in Figure 3. Other causes of raised ammonia are lysinuric protein intolerance, liver failure and ‘congenital hyperinsulinism with hyperammonemia’.

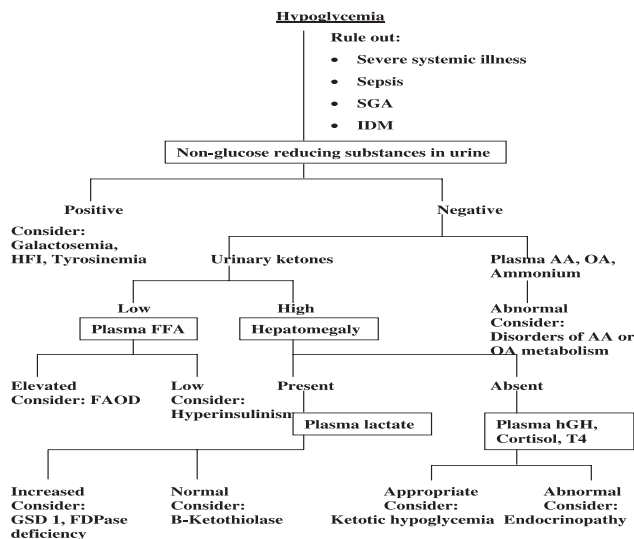
Figure 3: Algorithm for diagnosis of hyperammonemia:



Modified from Nelson's Textbook of Pediatrics (11)

3. **Blood glucose** is one of the first investigations performed on a sick neonate and thus hypoglycemia is commonly encountered. When common causes of hypoglycemia like maternal diabetes, small for gestational age baby and sepsis are ruled out, one can follow the algorithm given in figure 4 to make a diagnosis of IEM.

Figure 4: Flow chart for diagnosis of hypoglycemia



Clarke JTR (10)

- Neurologic dysfunction** occurs most commonly in newborns in form of lethargy, coma, hypotonia, poor reflexes and seizures. This encephalopathy may or may not be associated with acidosis. While the disorders related to acidosis are given above, the IEMs associated with *encephalopathy without acidosis* include: (i) Urea cycle disorders; (ii) Maple syrup urine disease; (iii) Homocystinuria; (iv) Pyridoxine dependent seizures and other neurotransmitter disorders; (v) Xanthine-sulfite oxidase deficiency/ molybdenum cofactor defects; (vi) Non-ketotic hyperglycinemia; (vii) Electron transport chain disorder or Pyruvate dehydrogenase deficiency; (viii) Fatty acid oxidation defects; (ix) Peroxisomal disorders like Zellweger syndrome.
- Hepatic involvement** manifesting as jaundice, hepatocellular dysfunction and hypoglycemia, is a common presentation of many inborn errors of metabolism. The main disorders here are *amino acidopathies* – hepatorenal tyrosinemia, disorders of carbohydrate metabolism – hereditary fructose intolerance (HFI) and glycogen storage diseases (GSD), fatty acid oxidation defects – Medium chain acyl CoA dehydrogenase (MCAD), long chain acyl-CoA dehydrogenase (LCAD), carnitine palmitoyl transferase II deficiency (CPT II) and carnitine –acylcarnitine translocase deficiency (CAT)<sup>10</sup>. These disorders are diagnosed by tandem mass spectrometry. Mitochondrial energy metabolism – cytochrome C oxidase deficiency and mitochondrial depletion syndromes also present with hepatic symptoms.
- Non-immune hydrops** may be due to IEMs and these should be suspected after exclusion of anemia and hemolysis and in the presence of significant organomegaly. Main IEMs to be looked for are GM1 Gangliosidosis, Gaucher Disease, Niemann-Pick disease, Sialidosis, Galactosialidosis, I-cell disease, Morquio (MPS IV) and Sly disease (MPS VII).
- Cardiac involvement** occurs as rhythm disturbances, shock like symptoms, cardiomegaly or cardiomyopathy leading to cardiac failure<sup>2</sup>. Some IEMs such as glycogen storage disease type 2 (Pompe disease), fatty acid oxidation defects – MCAD, LCAD, CPT I and II, CAT deficiency, tyrosinemia type 1, CDG type Ia, Barth syndrome and mitochondrial respiratory chain disorders may present with cardiac symptoms.

## COLLECTION, STORAGE AND TRANSPORT OF SAMPLES

1. **Urine** : Collect 10-15 ml of urine, preferably first morning specimen. Tests include

- Routine biochemical tests, protein, glucose, reducing substances, pH.
- Chemical tests like DNPH, Ferric Chloride test, Sulfite dipstick test etc.
- Amino acid chromatography and quantitative estimation.
- Organic acids (collect on filter paper)

2. **Blood**

- For ammonia estimation, a free flowing fresh sample should be drawn in vial and transported directly to lab on ice as soon as possible. This is critical, as ammonia tends to rise falsely if sample is not processed immediately. For lactate and pyruvate, a fluoride vial is used and same precautions as for ammonia are to be taken.
- Collect in EDTA if plasma is required for testing or for storage.
- For galactosemia and tyrosinemia testing, heparinised blood is required.
- For enzyme assays on blood leukocytes, blood sample should be collected in EDTA vial.
- For Tandem Mass Spectrometry, take fresh blood on filter paper, or 1 ml of blood in heparin or EDTA vial and put on filter paper.

Every suspect case of IEM who is very sick should have following samples stored for diagnostic purpose and to carry out prenatal diagnosis in the family's future.

1. DNA - collect blood in EDTA tube. All gene studies require DNA sample. DNA to be stored at 4 –8 degree Celsius.
2. Plasma to be stored at –20 degree Celsius.
3. Urine to be stored at –20 degree Celsius.
3. **Other body tissues** – skin, liver, muscle, bone biopsy, kidney etc. may be utilized later for diagnostic purpose and therefore should be stored in culture medium for enzyme assays and DNA studies. Transport of urine and blood samples on WhatmanO no. 903 filter paper, and DNA can be done in room air while plasma samples and blood for enzyme assays need to be cooled during transportation.

### THE THERAPY, GENETIC COUNSELLING AND PRENATAL DIAGNOSIS

Management of inborn errors of metabolism is out of scope of this article. Books on IEM may be consulted (1,2, 9,10,11).

It is essential to make a diagnosis in a given suspected case of IEM for genetic counseling and prenatal diagnosis in the couple's subsequent pregnancies. This holds importance in our country since the burden of these disorders is high in view of the costly treatment and lack of government assistance. Blood and urine sample should be stored in anticipation of an adverse outcome as the diagnosis can be made on these samples later.

Prenatal diagnosis can be offered after an accurate diagnosis by DNA technology or enzyme assay on chorionic villi or cultured amniocytes, as there is a 25% risk of recurrence in all the autosomal and X-linked recessive disorders.

### NEWBORN SCREENING FOR INBORN ERRORS OF METABOLISM

A novel idea struck Robert Guthrie in 1961 and the world of the newborn changed for ever. He successfully performed a test on dried blood spots on filter paper for phenylketonuria (12). Medical science took a major leap forward and newborn screening emerged as a preventive health program to prevent mental retardation. Newborn screening is mandatory in many developed countries as part of their public health programs. The time has come to introduce newborn screening programs in India.

WHO task force committee defines newborn screening as a "public health program aimed at the early identification of conditions for which early and timely interventions can lead to the elimination or reduction of associated mortality, morbidity and disabilities. It involves the following components : screening, short-term follow-up, diagnosis, treatment/management, and evaluation" (13). Thus, it is not just diagnosis but also about treatment to prevent disabilities and reduce the burden of such illnesses. Neonatal screening service involves an integrated effort from many professionals such as nurses, laboratory technicians, clinical biochemists, pediatricians, neuropsychiatrists, social workers, obstetricians, medical geneticists, but some one is required to coordinate all these activities.

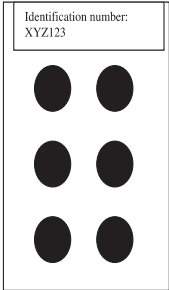
Since the introduction of screening for phenylketonuria, many other disorders have been added for analysis of dried blood spots on filter paper. Newborn screening for congenital hypothyroidism was introduced in 1974 by Dussault et al (14). Later, with the advent of newer technologies, several changes in the newborn screening were

incorporated for detection of many other in-born errors of metabolism, notably by TMS. Presently as many as 30 disorders amongst the 600 odd IEMs are detectable using the filter paper methodology (15).

### PRINCIPLES AND CRITERIA FOR NEWBORN SCREENING

Screening is the application of a test to people who are as yet asymptomatic, with the purpose of classifying them with respect to their likelihood of having a particular disease. Since the tests are carried out on normal individuals, this raises certain ethical issues. The WHO (1968) had outlined certain criteria for a screening program based on principles formulated by Wilson and Junger (16). These are:

1. Clinically and biochemically well-defined disorder.
2. Known incidence in the population
3. Disorder associated with significant morbidity or mortality
4. Effective treatment available
5. Period before onset during which interventions improves outcome
6. Ethical, safe, simple and robust screening test
7. Cost- effectiveness of screening is established

ANALYTES		METHODS
Amino acids	<div style="border: 1px solid black; padding: 5px; text-align: center;">           Identification number:            XYZ123   </div>	BIA
Organic acids		RIA
Acyl carnitines		ELISA
Carbohydrates		DELFLIA
Hormones		FIA
Lipoproteins		Enzymatic
Antibodies		Electrophoretic
Enzymes		TLC
Hemoglobins		PC
Metals		HPLC
DNA		MS/FABS
At least 150 analytes		TMS
		DNA Technology
		(including microchips approach)

Abbreviations: BIA, bacterial inhibition assay; RIA, Radioimmunoassay; ELISA, Enzyme linked immunosorbent assay; DELFLIA, Delayed Europium-linked Fluorescence Immunoassay; TLC, Thin layer chromatography; MS/FABS, Mass Spectrometry/Fast Atom Bombardment Mass Spectrometry; TMS, Tandem Mass Spectrometry

### Newborn Screening Methodology:

The newborn screening methods have evolved over the last 4 decades from the basic bacterial inhibition assay used for phenylketonuria by Robert Guthrie to the more sophisticated tandem mass spectrometry today. Methods such as ELISA and Time resolved fluorometry are being used in many countries for newborn screening because of its low cost and rapid analysis of multiple samples. Conditions included in Newborn screening programs are given in Table 2 (17):

The various analytes tested for screening of above disorders and the evolving methodologies from time to time are enlisted in the Figure 5 (17).

**Table 2:** Disorders detectable by newborn screening

Common disorders
Phenylketonuria
Endocrine
Congenital Hypothyroidism
Congenital Adrenal Hyperplasia (21 hydroxylase deficiency)
G-6-phosphate dehydrogenase deficiency
Galactosemia
MSUD
Biotinidase deficiency
Homocystinuria
Tyrosinemia
Cystic fibrosis
MCAD
Alpha-1 antitrypsin deficiency
Other disorders
Infections
HIV
Toxoplasmosis
Tumors
Neuroblastoma
Ambiental toxicity
Lead exposure
Sickle cell anemia
DMD
Hypercholesterolemia

## Tandem Mass Spectrometry

Tandem Mass Spectrometry has gained immense popularity and is used as a standard method for newborn screening in many centres all around the world. This is because of its simplicity, rapidity and ability to detect a number of disorders in one sample. It has its own controversy because of the ability to screen some disorders also, which do not have any treatment at present.

Mass spectrometers are an analytical tool used for measuring the molecular weight (MW) of a given sample, be it blood, urine, water etc (18). They are used in industry and academia for both routine and research purposes. Most commonly they are employed in biotechnology, pharmaceutical, environmental and geological fields and also in clinical field such as neonatal screening. Mass spectrometers can be divided into three fundamental parts, namely the 'ionisation' source, the 'analyzer', and the 'detector' (19). The sample once introduced into the ionization source gets ionized which are separated according to their mass to charge ratios (m/z). The separated ions are detected and this signal sent to a data system. The Tandem (MS-MS or TMS) mass spectrometers are instruments that have more than one analyzer and so can be used for structural and sequencing studies. The TMS is extremely useful as it gives accurate results in terms of detecting and quantifying biomolecules.

Tandem mass spectrometry expands neonatal screening to several disorders in amino acid, fatty acid oxidation and organic acid metabolism, more than 60 in number. TMS can improve the detection of PKU also. A high sensitivity (95.4%) and specificity (99.7%) is reported with a combined prevalence of 1:2336 (in Germany) (20). Treacy et al argue that after diagnosis using TMS, response to treatment was complete in 12% of IEMs, there was no response in 34%, and in more than half (54%) there was a partial response, which justifies some intervention (21). Thus, TMS provides an early diagnosis and facilitates early intervention, so that 'partial benefits'

can become 'substantial benefits'. Further, once an IEM is diagnosed in a family, a prenatal diagnosis can be performed to prevent birth of a child if it's a disorder, which is not treatable. This perhaps has more relevance in our country with little resources and health care system not being completely state funded.

## INDIAN SCENARIO

India has progressed immensely in the field of medical technology and health care. Therefore, this is the time to implement a newborn-screening program. Pilot studies are required to determine the prevalence of the disorders in different parts of the country before starting a national program.

## CONCLUSION

It is the fundamental right of a human being to live a disease free life as far as possible. The newborn screening systems can help in achieving this goal. Let us benefit from the medical advancements and give our newborns a disease free life.

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## RECENT ADVANCES – VACCINOLOGY IN CHILDREN

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**Abstract :** The field of vaccinology is highly dynamic. Newer vaccines as well as vaccine technologies are constantly being developed. Novel technologies such as chimerivax and virosomes have led to more efficacious vaccines. Combination vaccines and ready to use preparations offer better compliance and ease of administration. Newer vaccine adjuvants lead to increased vaccine immunogenicity. Vaccines against diseases with significant morbidity and mortality such as rota virus diarrhoea, viral hepatitis A, cervical cancer due to human papilloma virus, Japanese encephalitis, conjugated pneumococcal and conjugated typhoid vaccines have recently been approved. Lastly, vaccines under development such as those against malaria, dengue and HIV are in various stages of development. Future in vaccinology lies in the development of DNA vaccines, therapeutic vaccines and vaccines against non-infectious diseases such as malignancies and autoimmune disorders. The following review details recent updates in the field of vaccinology with special reference to the pediatric population

### INTRODUCTION

For many years people have noted that prior contact protected against future infections. Because of the enormous development in the field of immunologic research, most children today receive protection from the common childhood illnesses in the form of “vaccines”. The science of vaccines is a highly dynamic and cost-effective branch with a need to update oneself constantly on the recent developments in the subject. This review details recent updates in vaccine technology, newer vaccines and the future of vaccinology.

### NEWER VACCINE TECHNOLOGIES

#### CHIMERIVAX TECHNOLOGY

This is a novel approach for rapid development of a molecularly-defined, live, attenuated vaccines. The technology (ChimeriVax) is applicable to the development of vaccines against flaviviruses, and products against Japanese encephalitis, West Nile and dengue are undergoing clinical trials, respectively. ChimeriVax vaccines utilize the safe and effective vaccine against the prototype flavivirus -yellow fever 17D- as a live vector. Infectious clone technology is used to replace the genes encoding the pre-membrane (prM) and envelope (E) protein of yellow fever 17D vaccine with the corresponding genes of the target virus (e.g. West Nile). The resulting chimeric virus contains the antigens responsible for protection against the target virus but retains the replication efficiency of yellow fever 17D<sup>1</sup>.

#### VIROSOMAL TECHNOLOGY

This novel technology has been used in developing vaccines against hepatitis A, influenza and malaria. Out of these the virosomal hepatitis A vaccine is commercially available. The hepatitis A virosomes are spherical vesicles made of unilamellar phospholipid bilayers atleast 100 times smaller than the particles in aluminium adjuvanted vaccines. Purified haemagglutinin (HA) and neuraminidase (NA) influenza surface glycoproteins, isolated from the influenza A/Singapore 6/86 (H1N1) virus strain, are intercalated into this phospholipid bilayer. The final step in the production of the virosomes is the adsorption of the formalin-inactivated, highly purified HAV virions of the RG-SB strain onto the virosome surface<sup>2,3</sup>.

It has been shown that the influenza virus HA component of the virosome enables binding to immunocomponent cells such as macrophages leading to endocytosis. Within the endosome, the virus

antigen is proteolysed to antigenic peptides. Thereafter, the antigen containing endosomes join with major histocompatibility class II (MHC II) molecules. The resulting MHC II-antigen complex is transported to the cell surface where it initiates both humoral and cell-mediated immune response. This natural process of antigen presentation enables effective HAV antigen processing and the stimulation of protective immune responses without inducing the non-specific inflammatory response characteristic of aluminium-salt based vaccines<sup>3</sup>.

### COMBINATION VACCINES

With the introduction of increasing number of newer vaccines to prevent childhood diseases combination vaccines are a solution to the problem of increased number of injections during hospital visits. A combination vaccine consists of two or more immunogens combined in a single preparation. This is in contrast to simultaneous vaccination in which there are concurrent but physically separate injections. Combination vaccines commonly used are diphtheria and tetanus alone (DT or dT) or with pertussis vaccine (DPT), inactivated polio vaccine (IPV) and MMR vaccine. Newer penta or hexavalent vaccines also include HiB and/or hepatitis B in the above combination. The advantages of combination vaccines include reduced number of injections with consequent reduction in parental anxiety, pain to the vaccinee and risk of needle stick injury to the vaccinator. There is higher compliance, reduced number of visits, reduced need for storage space, packaging, handling, transportation, less documentation and logistics.

Some of the *drawbacks* of combination vaccines include chemical interference and immunologic interference between the constituent antigens. Consequently, vaccines should not be combined at the time of administration and only authorised combination vaccine used.

### READY TO USE PREPARATIONS

Many vaccines are now available in single-dose pre-filled syringes thus eliminating the need for reconstitution and hence causing minimal contamination and risk of infection.

### RECENT ADVANCES IN VACCINE ADJUVANTS

Adjuvants are substances that boost the immunogenicity of vaccines. Adjuvants can be broadly separated into two classes based on their

principal mechanisms of action: vaccine delivery systems and immunostimulatory adjuvants. Vaccine-delivery systems generally are particulate (e.g., emulsions, microparticles, iscoms, and liposomes) and function mainly to target associated antigens into antigen-presenting cells. In contrast, immunostimulatory adjuvants are derived predominantly from pathogens and often represent pathogen-associated molecular patterns (e.g., lipopolysaccharide, monophosphoryl lipid A, CpGDNA) which activate cells of the innate immune system. The discovery of more potent adjuvants may allow the development of prophylactic and therapeutic vaccines against cancers and chronic infectious diseases. In addition, new adjuvants may also allow vaccines to be delivered mucosally<sup>4</sup>.

## NEWER VACCINES

### *Rotavirus vaccine*

Globally, rotavirus is the single most important etiologic agent of severe diarrhea in infants and young children, with 114 million cases, 25-55% of all hospital admissions for diarrhea and more than 610,000 deaths per year<sup>5,6</sup>. In India, 6-45% of all childhood diarrhea requiring hospitalisation is due to rotavirus. Serotypes G1, G2, G3, P8, P6 and P4 account for 65-70% of rotavirus infections in India<sup>7</sup>. It has been reported that, after the first rotavirus infection, 88% children are protected against severe gastroenteritis and following a second infection, virtually all children are protected against severe diarrhea and most are protected against any rotavirus disease<sup>8</sup>. Hence an attenuated vaccine that simulates natural infection, and is administered in two doses, should induce protective immunity and prevent severe diarrhea and its complications<sup>9</sup>. Rotavirus diarrhea causes significant mortality and morbidity. Its consequences are more severe in the underprivileged. Moreover, in developed countries it was observed that even with improved sanitation the prevalence of rotavirus diarrhea did not decrease. Hence the vaccine will be a useful addition to the national immunisation programme.

Currently, two live viral oral vaccines are licensed and marketed worldwide, Rotarix<sup>TM</sup> and Rotateq<sup>TM</sup>. Rotarix<sup>TM</sup> is a monovalent attenuated human rotavirus vaccine derived from the strains 89-12. Rotateq<sup>TM</sup> is a human bovine reassortant vaccine. Large phase 3 double-blind placebo controlled trials from USA, Europe and Latin America have shown 85-98% efficacy against severe rotavirus gastroenteritis and 42-59% efficacy against hospitalisation due to diarrhea of any cause. Both vaccines are safe with no increased risk of intussusception<sup>10, 11, 12</sup>. Studies show no interference between rotavirus vaccines and other childhood vaccines including IPV, pneumococcal, Hib, DTaP and Hep B<sup>12</sup>. There is no reduction in efficacy when simultaneously administered with OPV<sup>13</sup>. The first dose of Rotarix<sup>TM</sup> is administered between 6-12 weeks of age and interval between two doses is at least 4 weeks. The two dose schedule should be completed by 16 weeks and no later than 24 weeks of age. It is available as lyophilised vaccine to be reconstituted with liquid diluent prior to administration. The Rotateq<sup>TM</sup> is given as three oral doses at 2, 4 and 6 months of age with the 1<sup>st</sup> dose between 6-12 weeks and interval between doses being 4-8 weeks. Vaccination should not be initiated after 12 weeks of age. It is available as a liquid preparation mixed with buffer with no need for prior reconstitution.

### *Human Papillomavirus Vaccine*

Every year 500,000 new cases of cervical cancer are detected and 350,000 succumb annually to this disease<sup>14</sup>. It is well documented

that HPV is the causative agent of cervical cancer with types 16 and 18 accounting for 70% of the cases of invasive cervical cancer globally<sup>14</sup>. These serotypes have also been implicated in anal, vulvar, vaginal, penile and oropharyngeal cancers<sup>15</sup>. Serotypes 6 and 11 cause anogenital warts<sup>11</sup>. Cervical cancer is an important cause of death in Indian women with 132,000 new cases annually with 74,000 deaths<sup>16</sup>. Until recently, cytology based screening programmes (using pap smears) were the main tools to prevent cervical cancers which are capable of detecting upto 80% of cervical cancers in developed countries. However, these are difficult to implement in developing countries with consequent higher mortality. HPV vaccines address a critical public health need and will be an important element of a cervical cancer control strategy.

As of January 2008 two HPV vaccines have been licensed for use in many countries. One is a quadrivalent vaccine containing serotypes 6, 11, 16 and 18 while the second is a bivalent vaccine with serotypes 16 and 18. The vaccines are sterile liquid suspensions prepared from highly purified virus like particles (VLPs) of the recombinant major capsid (L1) protein. The L1 proteins are produced by separate fragmentation in recombinant *Saccharomyces cerviciae* and self assembled in VLPs. The VLPs for each type are purified and adsorbed in aluminium containing adjuvants. The vaccine is available in 0.5 ml single dose ready to use syringes. It is indicated in females between 9-26 years for the prevention of cervical cancer, genital warts, cervical adenocarcinoma in situ, cervical intraepithelial neoplasia grades 1,2,3, vulvar intraepithelial neoplasia grades 2,3 and vaginal intraepithelial neoplasia grades 2,3. The vaccine is administered as three separate intramuscular doses at 0, 2 and 6 months.

Published analysis restricted to females who had not been infected with vaccine related HPV types before vaccination have shown that:

- Both vaccines induce high levels of serum antibodies against HPV types 16 and 18.
- The quadrivalent vaccine had an efficacy of more than 96% in preventing high grade precancerous lesions of the cervix, vagina and vulva and genital warts arising from HPV types 6, 11, 16 and 18 in completed phase 3 clinical trials<sup>17</sup>.
- The bivalent vaccine has an efficacy of more than 90% in preventing high grade cervical lesions due to types 16 and 18 in interim results of phase 3 clinical trials and an efficacy of 75% in preventing persistent infection due to types 16 and 18<sup>18</sup>.
- Although the duration of protection is not yet known there is evidence of protection for at least 6 years after vaccination with both vaccines. Studies are evaluating long term efficacy<sup>19</sup>.

Local side effects were pain, swelling and erythema. No serious vaccine related adverse effects were noted. Recent studies have indicated that both vaccines may provide partial protection against other oncogenic HPV types that are genetically related to HPV 16 and 18. Data on efficacy of the vaccine in preventing diseases in males are not yet available.

In conclusion both the HPV vaccines are safe and efficacious and should be given to all females in the prescribed age group prior to sexual debut.

### *Inactivated Polio Vaccine*

Polio cases have decreased by over 99% since 1988, from an estimated 350,000 cases then to 1997 cases in 2007. This is result of the global effort to eradicate polio. Persistent pockets of polio transmission in northern India, northern Nigeria and the border between Pakistan and Afghanistan are the current focus of the polio eradication initiative. However, in an increasing number of polio free countries the risk of vaccine associated paralytic poliomyelitis (VAPP) is greater than the risk with importation or laboratory handling

of wild polio virus. Some of these countries have introduced IPV as a safe and effective alternative for routine immunisation by using one of the two approaches: replacement of OPV by IPV and introduction of a sequential IPV/OPV schedule.

All currently available IPV are enhanced potency vaccines (eIPV) that contain 40, 8 and 32 D units of types 1, 2 and 3 respectively. It is highly immunogenic and seroconversion rates of 95% and 92% are achieved when two doses are given starting at 2 months of age, at 2 months interval and when 3 doses are given starting at 6 weeks of age and given at 4 weeks interval respectively<sup>20</sup>. It can be given in combination with DTwP and HiB without impairing seroconversion and increasing side effects. IPV also has excellent herd effect<sup>21</sup>. It is a very safe vaccine and is being considered for use in India by the government.

### **Hepatitis A Virosomal Vaccine**

At least 1.5 million clinical cases of hepatitis A occur worldwide each year. The morbidity of hepatitis A is greatest in susceptible adults who are at higher risk of hospitalisation and death. Infected children usually are either asymptomatic or have milder disease but are important sources of infection. As living conditions improve, there is an epidemiologic shift from high to transitional endemicity in many countries which results in more people not previously exposed to HAV and therefore lacking natural immunity. Without natural immunity the risk of outbreaks is increased adding to health costs and making universal HAV immunisation worthwhile.

The successful propagation of the HAV in human derived cell-line culture in vitro in 1979<sup>22,23</sup> paved the way for development of inactivated vaccine. Similar to other small viruses or subunit antigen, inactivated HAV is poorly immunogenic on their own and need some form of immunostimulation in order to be effective as a vaccine. Adsorption to aluminium salt was the only adjuvant for many years<sup>24</sup>. Although widely accepted as safe and effective, aluminium salt based adjuvants have non-specific actions and cause local side effects such as pain and swelling. A new approach has been the development of an aluminium free virosome adjuvanted HAV vaccine. Both types of vaccine are highly immunogenic<sup>25</sup>. Virosomal vaccines have side effects and elicit both cell-mediated and humoral immune responses<sup>26</sup>. In a comparative trial the virosomal vaccine, the aluminium adsorbed formulation and the soluble HAV antigen (no adjuvant), all administered intramuscularly, were assessed for immunogenicity and tolerability. All recipients (100%) of the virosomal vaccine were seroprotected after 14 days compared with 71% of those receiving the aluminium adsorbed antigen and 32% of those receiving the soluble antigen. HAV antibody titres were higher in the virosome group and the virosomal vaccine was better tolerated than the other two formulations.

The virosomal vaccine is available in 0.5ml, single-dose, pre-filled syringes for intramuscular use to be given in two doses 6 months apart. It induces protective antibody level within 10 days of primary vaccination<sup>27</sup> and provides seroprotection for upto 20 years<sup>28</sup>. The two types of vaccine are interchangeable<sup>29</sup>. It can be administered with other vaccines and prophylactic medicines<sup>30</sup>.

### **Conjugated Pneumococcal Vaccine**

*Streptococcus pneumoniae* causes significant morbidity in children less than 2 years of age. It causes over 15-50% of community acquired pneumonia, 30-50% of acute suppurative otitis media (ASOM), significant proportion of bacteremia and meningitis and 30% of mortality due to pneumonia worldwide<sup>31-33</sup>. Studies in patients with

invasive pneumococcal disease (IPD) indicate that serotypes 6, 1, 19, 14, 4, 5, 45, 12, 7, 23 are the most prevalent serotypes with 1 and 5 accounting for 30% IPD<sup>34</sup>.

The earlier *pneumococcal 23 valent*, unconjugated polysaccharide vaccine was a T-cell independent vaccine. It was poorly immunogenic in less than 2 years of age, had low immune memory, did not reduce nasopharyngeal carriage and did not induce herd immunity. It has at most 70% efficacy in high risk population against IPD and does not protect against non-bacteremic pneumonia and ASOM. Not more than 2 lifetime doses are recommended as subsequent doses cause hyporesponsiveness<sup>35</sup>. The *heptavalent pneumococcal conjugated vaccine* (PCV) counters the problems of low immunogenicity of the polysaccharide vaccine in children less than 2 years of age<sup>33</sup>. It contains serotypes 4, 6B, 9V, 14, 18C, 19F and 23 attached to a protein carrier. It covers 85% serotypes causing IPD. Trials show reduction in IPD by 95% and that of X-ray proven pneumonia by 30% in those vaccinated<sup>36</sup>. Efficacy in ASOM is 8%<sup>32</sup>. It has significant herd effect by reduction in nasopharyngeal carriage<sup>37</sup>. There are concerns about serotype replacement with non-vaccine serotypes increasing their share of disease burden with increase in vaccination coverage<sup>38</sup>.

Primary vaccination schedule consists of 3 doses at 2, 4 and 6 months with a booster at 12-15 months.

### **Conjugated Typhoid Vaccine**

Typhoid fever has been reported from all parts of the globe with 16 million cases being reported annually with 600,000 deaths<sup>39</sup>. Until recently typhoid was considered a disease of children above 3-5 years since it presented with atypical clinical features in infants and young children which made it difficult to diagnose. However, a number of studies have shown a high incidence of the disease in less than 2 years ranging from 27-35%<sup>40,41</sup>.

The currently available *Vi polysaccharide vaccine*, though safe and efficacious, is not immunogenic in less than 2 years of age. Hence this age group remains largely unprotected. Moreover, revaccination is required every 2-3 years. The newly introduced conjugated *Vi polysaccharide typhoid vaccine* can be used in infants from 3 months of age with upto 90% protection with longer lasting protection due to immune memory<sup>42,43</sup>. The vaccine is available as a single-dose 0.5ml glass vial for intramuscular use. One dose contains 5 $\mu$ g Vi polysaccharide of *S. Typhi* conjugated to 5 $\mu$ g of tetanus toxoid protein in isotonic saline.

### **Japanese Encephalitis Vaccine**

Japanese encephalitis (JE), a mosquito-borne arboviral infection, is the leading cause of viral encephalitis in Asia<sup>44-46</sup>. Approximately 50,000 sporadic and epidemic cases of JE are reported annually from the People's Republic of China (PRC), Korea, Japan, Southeast Asia, the Indian subcontinent, and parts of Oceania. Infection leads to overt severe encephalitis in only 1 of 20 to 1,000 cases with a fatal outcome in 25% of cases and residual neuropsychiatric sequelae in 30% of cases<sup>44, 47</sup>. In areas where JE is endemic, annual incidence ranges from 1 to 10 per 10,000<sup>44</sup>. Children less than 15 years of age are principally affected. Seroprevalence studies indicate nearly universal exposure by adulthood. In developed countries of Asia and in areas where children are protected by immunization, a secondary increase in JE incidence has been observed in the elderly<sup>49</sup>.

An *inactivated mouse brain derived vaccine* has been mainly used for protection against Japanese encephalitis among travellers and residents of endemic areas. The vaccine has an overall efficacy of

91%<sup>48</sup> with a moderate frequency of local (20%) and mild systemic (10%) side effects<sup>48, 50, 51</sup>. The neural tissue substrate of the vaccine has raised concerns about the possibility of vaccine-related neurologic side effects<sup>(52)</sup>. Protective levels of neutralizing antibody persist for at least 2 years in vaccinees who have completed a three-dose primary series. The full duration of protection is unknown, therefore, definitive recommendations cannot be given on the timing of booster doses. Booster doses of 1.0 mL (0.5 mL for children less than 3 years of age) may be administered after 2 years.

A new Japanese Encephalitis vaccine is undergoing phase III clinical trial. It is manufactured using the Chimerivax technology and as per the trial results it met and exceeded its immunogenicity end point of the trial. The vaccine at present is undergoing pediatric trials in Asia as children are the main target of the disease in endemic regions.

## VACCINES UNDER DEVELOPMENT

1. Malaria Vaccine
2. HIV Vaccine
3. Dengue vaccine

## FUTURE TRENDS IN VACCINOLOGY

1. Development of DNA vaccines
2. Development of therapeutic vaccines.
3. Newer vaccines against non-infectious diseases

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# PHARMACOLOGICAL MANAGEMENT OF ASTHMA

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**Abstract :** A critical evidence based review of the present knowledge has resulted in development of consensus guidelines for management of Asthma by various institutions like GINA, BTS and IAP. These guidelines have now started focusing on 'control' of asthma. The 'grades' of asthma have been redefined in a simpler and more practical manner. Grade I is now named Intermittent Asthma thereby indicating that preventive therapy is not required in contrast to other three grades of asthma which have been called mild, moderate and severe persistent asthma, thereby implying that preventive pharmacological therapy is required for these grades. It is now recognized that increasing the dose of inhaled corticosteroids (ICS) to beyond 400-800 mcg per day budesonide (or its equivalent) does not give dose related additional benefit and safety issues become important at higher doses. Hence, add on therapy should be the next step. Studies on effect of ICS on growth indicate that moderate doses do not have any significant effect on final height of the child even after years of use. Similarly ICS do not result in higher incidence of bone fractures while more than four courses of oral steroids at any time do. Theophyllines are acknowledged to provide both reliever and preventer effect. However, the effects are modest and side effects of theophyllines are significantly higher. A strategy to use formoterol and budesonide combination inhaler for both reliever and preventive therapy has been found to be useful in some trials. Guidelines for stepping down therapy once control is achieved have been laid down recently. Overall, reducing the dose of ICS to low dose levels takes priority over withdrawing add on therapies.

Asthma has a significant impact on individuals, their families, and society. Although there is no cure for asthma, appropriate management that includes a partnership between the physician and the patient/family most often results in the achievement of control.

The **goals** for successful management of asthma are to:

- Achieve and maintain control of symptoms
- Maintain normal activity levels, including exercise
- Maintain pulmonary function as close to normal as possible
- Prevent asthma exacerbations
- Avoid adverse effects from asthma medications
- Prevent asthma mortality

An overall concept for asthma management is oriented around the focus on asthma control. Achieving and maintaining asthma control is emphasized as the goal of asthma treatment. Before the treatment starts the patient has to be assigned into one of the following categories depending on severity of symptoms:

## Intermittent

- Symptoms less than once a week
- Brief exacerbations
- Nocturnal symptoms not more than twice a month
- FEV1 or PEF 80% predicted or more
- PEF or FEV1 variability <20%

## Mild persistent

- Symptoms more than once a week but less than once a day
- Exacerbations may affect activity and sleep
- Nocturnal symptoms more than twice a month
- FEV1 or PEF ≥80% predicted
- PEF or FEV1 variability <20–30%

## Moderate persistent

- Symptoms daily
- Exacerbations may affect activity and sleep
- Nocturnal symptoms more than once a week
- Daily use of inhaled short-acting  $\beta_2$ -agonist
- FEV1 or PEF 60–80% predicted
- PEF or FEV1 variability >30%

## Severe persistent

- Symptoms daily

- Frequent exacerbations
- Frequent nocturnal asthma symptoms
- Limitation of physical activities
- FEV1 or PEF 60% predicted
- PEF or FEV1 variability >30%

**Asthma control** - The patient's level of asthma control and current treatment determine the selection of pharmacologic treatment (Table 1). Like if asthma is not controlled in a patient on the current treatment regimen, which he is taking, then treatment should be stepped up until control is achieved. If control has been maintained for at least three months, treatment can be stepped down with the aim of establishing the lowest step and dose of treatment that maintains control. If asthma is partly controlled, an increase in treatment should be considered, subject to whether more effective options are available (e.g. increased dose or an additional treatment), safety and cost of possible treatment options, and the patient's satisfaction with the level of control achieved. Medications to treat asthma can be classified as controllers or relievers.

LEVELS OF ASTHMA CONTROL			
Characteristic	Controlled (All of the following)	Partly Controlled (Any measure present in any week)	Uncontrolled
Daytime symptoms	None (twice or less/week)	More than twice/week	Three or more features of partly controlled asthma present in any week
Limitations of activities	None	Any	
Nocturnal symptoms/awakening	None	Any	
Need for reliever/rescue treatment	None (twice or less/week)	More than twice/week	
Lung function (PEF or FEV1) <sup>†</sup>	Normal	< 80% predicted or personal best (if known)	
Exacerbations	None	One or more/year <sup>*</sup>	One in any week <sup>‡</sup>

\* Any exacerbation should prompt review of maintenance treatment to ensure that it is adequate  
<sup>†</sup> By definition, an exacerbation in any week makes that an uncontrolled asthma week.  
<sup>‡</sup> Lung function is not a reliable test for children 5 years and younger.

**Controllers** are medications taken daily on a long-term basis to keep asthma under clinical control, they act chiefly through their anti-inflammatory effects.

- Inhaled and systemic glucocorticosteroids, (Inhaled glucocorticosteroids are the most effective controller medications currently available)
- Leukotriene modifiers,
- Long-acting inhaled beta 2-agonists in combination with inhaled glucocorticosteroids,
- Sustained-release theophylline,
- Cromones,
- Anti-IgE, and
- Systemic steroid-sparing therapies.

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**Relievers** are medications used on as-needed basis that have quick onset of action and reverse bronchoconstriction and relieve its symptoms.

- Rapid-acting inhaled beta 2-agonists,
- Inhaled anticholinergics,
- Short-acting theophylline,
- Short-acting oral beta agonists.

**Inhaled therapy** is the *cornerstone of asthma treatment* for children of all ages. It's seen that all children can be taught to effectively use inhaled therapy. Different age groups require different inhalers for effective therapy, so the choice of inhaler must be individualized (Table 2). In general, a metered-dose inhaler (MDI) with spacer is preferable to nebulizer therapy due to its greater convenience, more effective lung deposition, lower risk of side effects, and lower cost of the therapy. Therapy with nebulizers has imprecise dosing, is expensive, and is time consuming to use and care for, and require maintenance. They are mainly reserved for children who cannot use other inhaler devices. In severe acute asthma exacerbations a nebulizer is often used, although an MDI with a spacer is equally effective

**Table 2 : Choosing an inhaler device for children with asthma**

Age Group	Preferred Device	Alternate Device
Younger than 4 years	Pressurized metered-dose inhaler <i>plus</i> dedicated spacer with face mask	Nebulizer with face mask
4 – 6 years	Pressurized metered-dose inhaler <i>plus</i> dedicated spacer with mouthpiece	Nebulizer with mouthpiece
Older than 6 years	Dry powder inhaler, or breath-actuated pressurized metered-dose inhaler, or pressurized metered-dose inhaler with spacer and mouthpiece	Nebulizer with mouthpiece

## STEP WISE MANAGEMENT OF ASTHMA

**Step 1: Mild Intermittent Asthma-** This requires short-term bronchodilator therapy Options are:

- Inhaled short-acting  $\beta_2$  agonists
- Inhaled ipratropium bromide
- $\beta_2$  agonist tablets or syrup
- Theophyllines.

Short-acting inhaled  $\beta_2$  agonists work more quickly and/or with fewer side effects than the alternatives and its seen that using short acting  $\beta_2$  agonists on SOS basis is at least as good as regular (four times daily) administration Various studies have correlated the need for use of short acting  $\beta_2$  agonist with asthma control. Good asthma control is associated with little or no need for short-acting  $\beta_2$  agonist.

Using two or more canisters of  $\beta_2$  agonists per month or >10-12 puffs per day is a marker of poorly controlled asthma that puts individuals at risk of fatal or near-fatal asthma<sup>2</sup>

**Step 2: Introduction of Regular Preventer Therapy-Indications:** Inhaled steroids should be considered for adults, children aged 5-12 years and children under the age of five with any of the following features<sup>20</sup>:

- Using inhaled  $\beta_2$  agonists three times a week or more
- Symptomatic three times a week or more
- Waking one night a week.

In addition, inhaled steroids should be considered in adults and children aged 5-12 who has had an exacerbation of asthma requiring

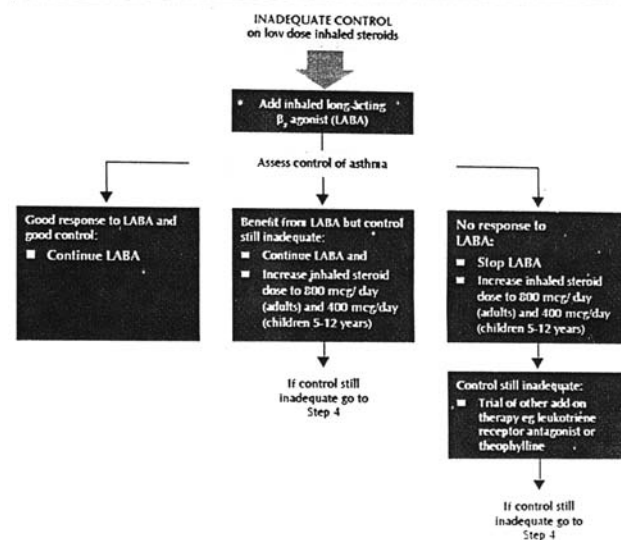
oral corticosteroids in the last two years. Inhaled steroids are the most effective preventer drugs for adults and older children for achieving good control on asthma and there is enough evidence demonstrating that, at recommended doses, they are also safe and effective in infants and younger children with asthma. About the starting dose there have been two schools of thought; one follows step up and other step down protocol. In mild to moderate asthma, starting at very high doses of inhaled steroids and stepping down confers no benefit<sup>51</sup>, so starting dose of inhaled steroids should be appropriate to the severity of disease and we should titrate the dose of inhaled steroid to the lowest dose at which effective control of asthma is maintained. On frequency of administrations its been seen that most current inhaled steroids are slightly more effective when taken twice rather than once daily, but may be used once daily in some patients with milder disease.

The most important determinant of appropriate dosing is the clinician's judgment of the patient's response to therapy. The clinician must monitor the patient's response in terms of clinical control and adjust the dose accordingly. Once control of asthma is achieved, the dose of medication should be carefully titrated to the minimum dose required to maintain control, thus reducing the potential for adverse effects. (Table3) Fig.1.

**Estimated equipotent daily doses of inhaled Glucocorticosteroids for children**

Drug	Low Daily Dose ( $\mu$ g)	Medium Daily Dose ( $\mu$ g)	High Daily Dose ( $\mu$ g) <sup>†</sup>
Beclomethasone dipropionate	100 - 200	>200 - 400	>400
Budesonide*	100 - 200	>200 - 400	>400
Ciclesonide*	80 - 160	>160 - 320	>320
Flunisolide	500 - 750	>750 - 1250	>1250
Fluticasone	100 - 200	>200 - 500	>500
Mometasone furoate*	100 - 200	>200 - 400	>400
Triamcinolone acetonide	400 - 800	>800 - 1200	>1200

<sup>†</sup> Comparisons based upon efficacy data  
<sup>‡</sup> Patients considered for high daily doses except for short periods should be referred to a specialist for assessment to consider alternative combinations of controllers. Maximum recommended doses are arbitrary but with prolonged use are associated with increased risk of systemic side effects  
\* Approved for once-daily dosing in mild patients.



## Safety of Inhaled Steroids

**A. Growth:** Its one of the key questions asked by parents when the child is being started on steroids as controller medication. Few important aspects about growth and ICS are that<sup>5</sup>

- Uncontrolled or severe asthma adversely affects growth and final adult height
- No long-term controlled studies have reported any statistically or clinically significant adverse effects on growth of 100-200  $\mu$ g/day- of inhaled glucocorticosteroids
- Growth retardation may be seen with all inhaled glucocorticosteroids when a high dose is administered

- Growth retardation in both short- and medium-term studies is dose dependent
- Important differences seem to exist between the growth-retarding effects of various inhaled glucocorticosteroids and inhalers
- Different age groups seem to differ in their susceptibility to the growth-retarding effects of inhaled glucocorticosteroids; children aged 4–10 yrs are more susceptible than adolescents
- Glucocorticosteroid-induced changes in growth rate during the first year of treatment appear to be temporary
- Children with asthma treated with inhaled glucocorticosteroids attain normal adult height (predicted from family members) but at a later age

**B.) Bone:** The clinically relevant adverse effects of inhaled glucocorticosteroids on bones in children are osteoporosis and fracture. Several cross-sectional and longitudinal epidemiologic studies have assessed the effects of long-term inhaled glucocorticosteroid treatment on these outcomes<sup>6,7</sup>. The summary of these studies are

- No studies have reported any statistically significant increased risk of fractures in children taking inhaled glucocorticosteroids
- Oral or systemic glucocorticosteroid use increases the risk of fracture
- The risk of fracture increases along with the number of treatments, with a 32% increase after four courses
- Use of inhaled glucocorticosteroids reduces the need for systemic courses
- Controlled longitudinal studies of 2–5 yrs duration and several cross-sectional studies found no adverse effects of inhaled glucocorticosteroid treatment on bone mineral density
- No prospective studies have followed children on inhaled glucocorticosteroid treatment until peak bone mineral density has been reached

**C. Hypothalamic- pituitary-adrenal (HPA) axis abnormalities** -It is seen that treatment with inhaled glucocorticosteroid doses of less than 200 ug budesonide or equivalent daily is normally not associated with any significant suppression of the HPA axis in children. At higher doses, small changes in HPA axis function can be detected with sensitive methods. However, in few studies adrenal crisis has been reported in children treated with excessively high doses of inhaled glucocorticosteroids.

**D. Cataracts.** Inhaled glucocorticosteroids have not been associated with an increased occurrence of cataract in children

**E. Central nervous system effects.** Few isolated case reports have shown that inhaled steroids are associated with CNS adverse effects however no increase in such effects has been found in two long-term controlled trials of inhaled budesonide involving more than 10,000 treatment years<sup>8,9</sup>

**F. Oral candidiasis, hoarseness, and bruising.** Clinical thrush is seldom a problem in children treated with inhaled or systemic glucocorticosteroids and this side effect may seem to be related to use of antibiotics, high daily doses, dose frequency and inhaler device. Giving the medication via spacers has been shown to reduce the incidence of oral candidiasis, and along with this mouth rinsing was found to be beneficial. In a study the occurrence of hoarseness or other noticeable voice changes during budesonide treatment is similar to placebo. Treatment with an average daily dose of 500-ug budesonide for 3 to 6 years is not associated with an increased tendency to bruise.

#### Other Prevent Therapies

**A. LTRA :** These Provide clinical benefit in children aged >5 yrs at all levels of severity but less than that of low-dose inhaled glucocorticosteroids. Leukotriene modifiers provide partial protection against exercise-induced bronchoconstriction within hours

**B. LABA :** Addition of long acting inhaled beta 2-agonists to a daily regimen of inhaled glucocorticosteroids (> 5 year of age)

- Improves symptom scores
- Decreases nocturnal asthma
- Improves lung function
- Decreases the use of rapid-acting inhaled  $\beta_2$ - agonists
- Reduces the number of exacerbations and achieves clinical

control of asthma in more patients, more rapidly, and at a lower dose of inhaled glucocorticosteroids than inhaled glucocorticosteroids given alone

**C. Theophyllines:** Have been shown to be effective as monotherapy and as add-on treatment to inhaled or oral glucocorticosteroids in children aged >5 yrs, though adverse effects are common.

- It is significantly more effective than placebo at controlling day and night symptoms and improves lung function
- Marginal protective effect against exercise induced bronchoconstriction
- Reduces the maintenance glucocorticosteroid dose
- Efficacy of theophylline is less than that of low-dose inhaled glucocorticosteroids
- Sustained-release products are preferable for maintenance therapy, since they enable twice-daily dosing
- Plasma theophylline level is not necessary in otherwise healthy children when doses less than 10 mg/kg/day are used
- Adverse effects are reduced if treatment is initiated with daily doses ~ 5 mg/kg/day and then gradually increased to 10 mg/kg/day

**D. Doxophyllin (7-(1,3-dioxalan-2-ylmethyl) theophylline):** is a novel xanthine bronchodilator, which differs from theophylline in that it contains a dioxalane group in position 7. Similar to theophylline, its mechanism of action is related to the inhibition of phosphodiesterase activities, but in contrast it appears to have decreased affinities towards adenosine A1 and A2 receptors, which may account for its better safety profile- CNS side effects are claimed to be less.

**E. Cromones:** Sodium cromoglycate and nedocromil sodium- these products have a limited role in the long-term treatment of asthma in children. Nedocromil sodium has been shown to reduce exacerbations, but its effect on other asthma outcomes is not superior to placebo. There are some studies which suggest there use in exercise / cold induced asthma<sup>11</sup>.

#### Step 3: Initial Add-On Therapy- Some Patients With Asthma May Not Be Adequately Controlled At Step 2.

But before we add a new therapy we should: (a) recheck compliance; (b) inhaler technique and (c) eliminate trigger factors

No exact dose of inhaled steroid can be specified at which to add another therapy But in general in adult patients taking inhaled steroids at doses of 200-800 mcg/day and in children taking inhaled steroids at a dose of 400 mcg/day add on therapy may be of value:

- (1.) Inhaled long-acting  $\beta_2$  agonist (LABA), – It has been shown in studies that the first choice as add-on therapy to inhaled steroids in adults and children (5-12 years) is an inhaled long-acting  $\beta_2$  agonist, which should be added before going above a dose of 400 mcg BDP or equivalent per day and certainly before going above 800 mcg BDP. If for instance it is found that asthma control remains suboptimal after the addition of an inhaled long-acting  $\beta_2$  agonist then the dose of inhaled steroids should be increased to 800 mcg/day in adults or 400 mcg/day in children (5-12 years), if not already on these doses.
- (2.) Leukotriene receptor antagonists may provide improvement in lung function, a decrease in exacerbations, and an improvement in symptoms if added at this stage.
- (3.) Theophyllines may improve lung function and symptoms, but side effects are more
- (4.) Slow-release  $\beta_2$  agonist tablets may also improve lung function and symptoms, but side effects are more. Addition of short-acting anticholinergics is of no value

#### COMBINATION INHALERS

No difference in efficacy was noticed whether ICS and LABA are

given in combination or via different inhalers. Main advantage is of guaranteeing that the long-acting  $\beta_2$  agonist is not taken without inhaled steroid. Further using one inhaler instead of two separate ones is more convenient. A strategy to use formoterol and budesonide combination inhaler for both reliever and preventive therapy has been found to be useful in some trials<sup>12</sup>.

#### **Step 4: Poor Control On Moderate Dose Of Inhaled Steroid + Addon Therapy Addition of Fourth Drug -**

Is indicated in a very small group of whose patients asthma is not adequately controlled on a combination of short acting  $\beta_2$  agonist as required, inhaled steroid (800 mcg daily), and an additional drug, usually a long-acting  $\beta_2$  agonist. There are very few trials available to guide therapy in this group. The available options are:

- Increasing inhaled steroids to 2000 mcg/day (adults) or 800 mcg/day (children 5-12 years)
- Leukotriene receptor antagonists
- Theophyllines
- Slow release  $\beta_2$  agonist tablets, though caution needs to be used in patients already on long-acting  $\beta_2$  agonists

Due to lack of controlled trials it is very difficult to tell which of these is best but side effects seems to be more with theophyllines and  $\beta_2$  agonist tablets.

#### **Step 5: Continuous or Frequent Use of Oral Steroids**

For the very small number of patients not controlled at step 4, we can use daily steroid tablets in the lowest dose to provide control.

### **STERIOD SPARING MEDICATION**

The aim of treatment is to control the asthma using the lowest possible steroid dose or, if possible, to stop long term steroid tablets completely. There are following available medications:

- In adults, the recommended method of eliminating or reducing the dose of steroid tablets is inhaled steroids, at doses of up to 2,000 mcg/day, if required.
- In children aged 5-12 years consider very carefully before going above an inhaled steroid dose of 800 mcg/day.
- Some studies have shown a role for a trial of treatment with long-acting  $\beta_2$  agonists, Leukotriene receptor antagonists, and theophyllines for about six weeks. They should be stopped if no improvement in steroid dose, symptoms or lung function is detected.
- Immunosuppressants (methotrexate, cyclosporin and oral gold) decrease long term steroid tablet requirements, but all have significant side effects.
- Continuous subcutaneous terbutaline infusion has been reported to be beneficial in severe asthma but efficacy and safety have not been assessed in RCTs.
- Anti-TNF alpha therapy has been investigated in severe asthma but these studies are too small and too short term to allow recommendation of anti-TNF therapy outside the context of a controlled clinical trial
- ANTI IgE - Anti-IgE (omalizumab) is a treatment option limited to patients with elevated serum levels of IgE. Its current indication is for patients with severe allergic asthma who are uncontrolled on high doses of inhaled glucocorticosteroids (although the dose of concurrent treatment has varied in different studies). Cost is prohibitively high.
- IVIG -The use of intravenous Immunoglobulin is not recommended.

### **STEPPING DOWN THERAPY**

- When inhaled glucocorticosteroids alone are being used in medium-to-high doses, a 50% reduction in dose should be attempted at 3-month intervals
- Where control is achieved at a low dose of inhaled glucocorticosteroids alone, in most patients treatment may be switched to once-daily dosing
- When asthma is controlled with a combination of inhaled glucocorticosteroid and long-acting  $\beta_2$ -agonist: The preferred

approach is to begin by reducing the dose of inhaled glucocorticosteroid by ~50% while continuing the long-acting  $\beta_2$ -agonist. If control is maintained, further reductions in the glucocorticosteroid dose should be attempted until a low dose is reached, when the long-acting  $\beta_2$ -agonist may be stopped

- When asthma is controlled with inhaled glucocorticosteroids in combination with controllers other than long-acting  $\beta_2$  - agonists, dose of inhaled glucocorticosteroids should be reduced by 50% until a low dose of inhaled glucocorticosteroids is reached, then the combination treatment stopped as described previously
- Controller treatment may be stopped if the patient's asthma remains controlled on the lowest dose of controller and no recurrence of symptoms occur for 1 yr

#### **Oral Prednisolone for Pre-school children with Acute Viral induced wheezing**

Attacks of wheezing induced by upper respiratory viral infections are common in preschool children between the ages of 10 months and 6 years. A short course of oral prednisolone is widely used to treat preschool children with wheezing who present to a hospital, but there is conflicting evidence<sup>13,14,15</sup> regarding its efficacy in this age group. In a recent study it was concluded that in preschool children presenting to a hospital with mild-to-moderate wheezing associated with a viral infection, oral Prednisolone was not superior to placebo<sup>16-18</sup>.

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# NON-PHARMACOLOGICAL MANAGEMENT OF ASTHMA

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**Abstract :** Several forms of non pharmacological therapies , alternative and complementary medicine have been popular in various parts of the world for treatment of asthma .Most such interventions are used as preventive therapies .Many of them have been evaluated in recent times using the modern scientific tools and consensus guidelines have been issued regarding their use. Among the non-pharmacological therapies for primary prevention of asthma no effect has been seen with domestic aeroallergen avoidance, food allergen avoidance, modified milk formulae, modified weaning, nutritional supplements during pregnancy, dietary probiotics in pregnancy, immunotherapy and immunization. However, avoidance of tobacco smoking by parents and encouragement of breast-feeding are advised. Recent reviews also do not favor complementary and alternative medicine interventions like acupuncture, air ionisers, herbal and traditional chinese medicine and homeopathy

Asthma is a chronic inflammatory disorder of the airways in which many cells and cellular elements play a role. The chronic inflammation is associated with airway hyper responsiveness that leads to recurrent episodes of wheezing, breathlessness, chest tightness, and coughing, particularly at night or in the early morning. These episodes are usually associated with widespread, but variable, airflow obstruction within the lung that is often reversible either spontaneously or with treatment. Asthma is a common medical ailment, which affects the pediatric age group patients; there are various guidelines for management of asthma, which are periodically revised and form the basis for current understanding and management of asthma in children. Asthma management can be divided into 2 broad subgroups:

1. Non Pharmacological Management; 2. Pharmacological Management

## NON-PHARMACOLOGICAL MANAGEMENT OF ASTHMA

There is a common thinking amongst care takers and patients that there are numerous environmental, dietary triggers of asthma and that avoiding these triggers will improve asthma and reduce the requirement for pharmacotherapy. Although effective medications for managing asthma exist within conventional western medicine (e.g., inhaled corticosteroids and bronchodilators), some parents turn to complementary and alternative medical (CAM) therapies to treat their children's asthma. Failure to address a patient, parent or a care takers concern about these issues may compromise concordance with recommended pharmacotherapy.

There are 2 types of **Prophylaxis**

- **Primary Prophylaxis** - interventions introduced before the onset of disease and designed to reduce its incidence.
- **Secondary Prophylaxis** - interventions introduced after the onset of disease to reduce its impact.

### PRIMARY PROPHYLAXIS

The evidence for these strategies is based predominantly on observational studies, though some have been tested using experimental methods. Many of the studies are multifaceted and it can be difficult to disentangle the effects of one exposure or intervention from another.

#### 1. Aeroallergen and food allergen avoidance

It is seen that exposure to high levels of house dust mite allergen in early life is associated with an increased sensitization to house dust mite by three to seven years of age. Sensitization to house dust mite is an important risk factor for the development of asthma and a few studies have suggested

that high early house dust mite exposure increases the risk of subsequent asthma while others have shown no effect on either allergic sensitization or symptoms of allergic diseases<sup>1,2</sup>. It is also said that sensitization to foods like eggs precedes the development of aero allergy in children and subsequent asthma<sup>3</sup>. However it has been shown that the food allergen avoidance in pregnancy and postnatally has not been shown to prevent the later development of asthma<sup>4</sup>. In the absence of any evidence of benefit and given the potential for adverse effects, maternal food allergen avoidance during pregnancy and lactation is not recommended as a strategy for preventing childhood asthma and in the absence of consistent evidence of benefit from domestic aeroallergen avoidance it is not possible to recommend it as a strategy for primary prevention of childhood asthma.

#### 2. Breast feeding

A review of observational studies on the allergy preventive benefits of breast-feeding reveals that it is effective for all infants irrespective of allergy, heredity. The preventive effect of breast-feeding for allergy was more pronounced in high-risk infants provided they are breast fed for at least four months<sup>5</sup>. However, not all the studies have demonstrated benefit and in a large birth cohort it was seen that there was no protective effect against atopy and asthma and maybe even an increase in risk<sup>6,7</sup>. Breast-feeding should be encouraged for its multiple benefits, and it may also have a potential protective effect in relation to early asthma.

#### 3. Modified Infant Milk Formulae

There are many trials of modified milk formulae but none have included sufficiently long follow up to establish whether there is any impact on asthma<sup>8</sup>. In the absence of any evidence of benefit from the use of modified infant milk formulae it is not possible to recommend it as a strategy for preventing childhood asthma.

#### 4. Weaning

Early introduction of allergenic foods into the infant diet predisposes the infant to subsequent development of allergy and atopic eczema. However, in one study, it was seen that late introduction of egg was associated with a non-significant increase in pre-school wheezing<sup>9</sup>. In the absence of evidence on outcomes in relation to asthma no recommendations on modified weaning can be made.

#### 5. Nutritional supplementation in pregnancy - fish oils (n-3 pufas)

Its seen that western diets have a low intake of n-3 PUFAs with a corresponding increase in intake of n-6 PUFAs and this change in diet has been associated with increasing rates of allergic disease and

asthma. However, there are no good studies to support this<sup>10</sup>. In the absence of any evidence of benefit from the use of fish oil supplementation in pregnancy it is not possible to recommend it as a strategy for preventing childhood asthma.

## 6. Microbial Exposure

The well known “hygiene hypothesis” of asthma suggests that early exposure to microbial products would switch off allergic responses thereby preventing allergic diseases such as asthma. The hypothesis was supported by some epidemiological studies, which compared large populations who have or have not had such exposure<sup>11,12</sup>. In a double blind placebo controlled trial of the probiotic lactobacillus GG was given to mothers and it was seen that it resulted in a reduced incidence of atopic eczema in their children but had no effect on IgE antibody or allergic skin test responses<sup>13</sup>. There is insufficient evidence to indicate that the use of dietary probiotics in pregnancy reduces the incidence of childhood asthma.

## 7. Avoidance of tobacco smoke and other air pollutants

In various clinical trials and studies no evidence has been found to support a link between exposures to environmental tobacco smoke (ETS) or other air pollutants and the induction of allergy. However, it is seen that there is an increased risk of infant wheezing with maternal smoking during pregnancy which adversely affects infant lung function<sup>14</sup> and there is ample evidence, which suggests that early life ETS exposure is associated with later persistent asthma<sup>15,16</sup>. Parents and parents-to-be should be advised of the many adverse effects which smoking has on their children including increased wheezing in infancy and increased risk of persistent asthma.

## 8. Immunisation

In keeping with the “microbial exposure hypothesis” some studies have suggested that there is an association between tuberculin responsiveness and subsequent reduced prevalence of allergy, suggesting a protective effect of BCG<sup>17</sup>. However investigation of the effects of any other childhood immunization suggests that at worst there is no influence on subsequent allergic disease and maybe some protective effect against the development of asthma<sup>18</sup>. All childhood immunizations should proceed normally as there is no evidence of an adverse effect on the incidence of asthma.

## SECONDARY PROPHYLAXIS

### 1. (a) House Dust Mite Avoidance

It has been seen that increased allergen exposure in sensitized individuals is associated with an increase in asthma symptoms, bronchial hyper-responsiveness and deterioration in lung function; however, evidence that reducing allergen exposure can reduce morbidity and/or mortality in asthma, is not clear. Cochrane reviews on house dust mite control measures in a normal domestic environment have concluded that chemical and physical methods aimed at reducing exposure to house dust mite allergens cannot be recommended.

### (b) Other Allergens

Animal allergens, particularly of cat have been observed to be potent provokers of asthma symptoms. But the reported effects of removal of pets from homes are paradoxical, with either no benefit for asthma or a potential for continued high exposure to induce a degree of tolerance. It is seen that individual aeroallergen avoidance strategies have shown that single intervention has limited or no benefit. A multi

faceted approach is more likely to be effective if it addresses all the indoor asthma triggers. A systematic review of this topic concluded that more research is required.

## 2. Smoking

Direct or passive exposure to cigarette smoke negatively affects quality of life, lung function of the child and increase the need for rescue medications for acute episodes of asthma and long-term control with inhaled steroids. There are two studies, which have demonstrated that there was reduction in childhood asthma severity when parents stopped smoking. Along with this fact it is seen that uptake of smoking in teenagers increases the risks of persisting asthma<sup>19,20</sup>. One study showed a doubling of risk for the development of asthma over six years in 14-year-old children who started to smoke in teenage<sup>21</sup>. Parents with asthma should be advised about the dangers of smoking to themselves and their children with asthma and offered appropriate support to stop smoking.

## 3. Air Pollution

Pollutants can increase the response of patients with asthma to allergen inhalation. Research suggest that air pollution may provoke acute asthma attacks or aggravates existing chronic asthma although the effects of this are very much less than those with infection or allergen exposure<sup>22</sup>.

## 4. Immunotherapy

Two modes of administration of immunotherapy have been studied

**a) Subcutaneous immunotherapy** – There have been trials of immunotherapy in which subcutaneous injection of increasing doses of allergen extracts was given and they have consistently demonstrated beneficial effects compared with placebo in the management of allergic asthma. Allergens that were included in trial were house dust mite, grass pollen, tree pollen, cat and dog allergen and moulds. Cochrane reviews were done and it has concluded that immunotherapy reduces asthma symptoms, the use of asthma medications and improves bronchial hyper-reactivity. The most recent review included 36 trials with house dust mite, 20 with pollen, 10 with animal allergens, two with cladosporium mould, one with latex and six with multiple allergens<sup>23</sup>. However evidence comparing the roles of immunotherapy and pharmacotherapy in the management of asthma is lacking. Immunotherapy can be considered in patients with asthma where a clinically significant allergen cannot be avoided. The potential for severe allergic reactions to the therapy must be fully discussed with patients

### b) Sublingual immunotherapy

Recently there has been increasing interest in the use of sublingual immunotherapy, as it is associated with fewer adverse reactions than subcutaneous immunotherapy. A systematic review done suggested there were some benefits for asthma control but the magnitude of the effect was small. Thus further randomized controlled trials are required. Sublingual immunotherapy cannot currently be recommended for the treatment of asthma in routine practice.

## 5. Dietary Manipulation

### Electrolytes

Various electrolytes were studied for their implication in asthma management. Of these two important and most widely studied are sodium and magnesium. *Increasing dietary sodium* has been implicated in the geographical variations in asthma mortality and high sodium intake is associated with increased bronchial hyper-responsiveness in various studies. A systematic review was done of

intervention studies reducing salt intake, which revealed that there was only minimal effects, and concluded that dietary salt reduction could not be recommended in the management of asthma<sup>24</sup>.

*Low magnesium intakes* have been associated with a higher prevalence of asthma with increasing intake resulting in reduced bronchial hyper-responsiveness and higher lung function. Magnesium plays a beneficial role in the treatment of asthma through bronchial smooth muscle relaxation thus it is used as IV preparation in acute asthma management. The researches of oral supplementation are limited and more trials are required<sup>25,26</sup>.

#### ◆ Fish Oils/Lipids

Its suggested that supplementing the diet with omega n-3 fatty acids, which are most commonly found in fish oils, might reduce the inflammation associated with asthma. However Cochrane review of nine randomized controlled trials concluded that there was insufficient evidence to recommend fish oil supplementation for the treatment of asthma<sup>27</sup>.

#### ◆ Weight Reduction In Obese Patients

Several studies done have reported an association between increasing body mass index and its effects on symptoms of asthma in patients<sup>28,29</sup>. A randomized parallel group study has shown improved asthma control following weight reduction in obese patients with asthma<sup>30</sup>. Weight reduction is recommended in obese patients with asthma to promote general health and to improve asthma control.

#### ◆ Probiotics

Studies have suggested a role of imbalance in gut flora and higher risk of development of allergy<sup>31</sup>. The use of probiotics in the treatment of established allergic disease has been investigated and the study focused on asthma, finding a decrease in eosinophilia but no effect on clinical parameters<sup>32,33</sup>. In the absence of evidence of benefit, it is not possible to recommend the use of probiotics in the management of asthma.

## IMMUNISATIONS

There are studies that have concluded that high vaccination coverage has no significant impact on any allergic outcome or asthma<sup>34,35</sup>. However there is a suggestion that the higher the vaccine coverage the greater the protection against the development of allergy in the first years of life. There is some discussion about whether BCG immunization may confer protection against allergy and asthma. The use of BCG, with or without allergen, as a means to switch off allergic immune responses has been investigated but results are not very clear and this is an area that requires further investigation<sup>36</sup>.

There has been earlier concern that influenza vaccination might aggravate respiratory symptoms in patients of asthma, though any such effect would be outweighed by the benefits of the vaccination. However research done in children have suggested that immunization with the vaccine does not exacerbate asthma<sup>37</sup> but has a small beneficial effect on quality of life in children with asthma.

Immunizations should be administered independent of any considerations related to asthma. High-dose inhaled steroids may attenuate responses to vaccines

## IVY LEAF

Ivy leaf, the dried leaf of *Hedera helix* L., is believed to have bronchodilating, spasmolytic, and antibacterial effects. Ivy leaf has been used for the treatment of upper respiratory tract infections and coughs<sup>38</sup>. A review of three small German-language trials, including crossover, double-blind trials, examining the efficacy of ivy leaf cough drops, suppositories, or syrup in children who had asthma found

ivy leaf in all forms to improve respiratory function<sup>38</sup>. Only one adverse event (AE) of ivy treatment, exacerbation of existing atopic dermatitis, was reported in one of these trials. Although this preliminary evidence suggests that ivy leaf may improve airway resistance in children who have asthma, larger RCTs and additional safety data, are needed before ivy supplements can be recommended

## COMPLEMENTARY AND ALTERNATIVE MEDICINE IN ASTHMA

### (I) Acupuncture

A Cochrane review of 21 trials on acupuncture highlighted many methodological problems with the studies. The review concluded that there was no evidence for a clinically valuable benefit for acupuncture and no significant benefits in relation to lung function

### (II) Air Ionisers

Ionisers are widely being promoted as being of benefit for patients with asthma. But a Cochrane review of five studies using negative ion generators and one with a positive ion generator found that there was no evidence of benefit in reducing symptoms in patients with asthma<sup>39</sup>.

### (III) Breathing Exercises Including Yoga And The Buteyko Breathing Technique

The basic principle of yoga and Buteyko breathing technique are to control hyperventilation by lowering respiratory frequency. A Cochrane review of breathing exercises found that there was no change in routine measures of lung function after these techniques<sup>40</sup>. However one study showed a small reduction in airway responsiveness to histamine utilizing pranayama, a form of yoga breathing exercise<sup>41</sup>. The Buteyko breathing technique focuses specially on control of hyperventilation and any reduction on pCO<sub>2</sub> levels. Some clinical trials have suggested that there were benefits in terms of reduced symptoms and bronchodilator usage but no effect on lung function<sup>42-43</sup>. Buteyko breathing technique may be considered to help patients to control the symptoms of asthma.

### (IV) Herbal And Traditional Chinese Medicine (TCM)

TCM herbal remedies include mixtures of several herbs that have different physiologic effects. Huntley and Ernst conducted a systematic review of 17 RCTs investigating the effectiveness of herbal medicines in asthma, six of which evaluated the effects of TCM<sup>44</sup>. It was found that all of the TCM trials were of poor methodological quality, as assessed by the Jadad scale, which evaluates the quality of clinical trials (i.e., lack of blinding or sufficient information about randomization, dropouts, or AEs). In the literature there are various reported side effects associated with TCM preparations from China that have been contaminated with toxins (i.e., heavy metals such as arsenic or mercury) or adulterated with prescription medications such as glucocorticosteroids<sup>45</sup>. Given the poor quality of data on effectiveness and the substantial concerns about product variability and adulteration, TCM herbs are not recommended in the treatment of pediatric asthma

### (V) Homeopathy

In the Cochrane review it identified that only three methodologically sound randomized controlled trials are there out of which two have reported some positive effects. But the studies did not employ individualized homeopathy, which is the essence of this approach to treatment<sup>46</sup>. In a recent trial of homeopathy in childhood asthma, which was placebo controlled and appropriately powered it was

revealed that there was no benefit over conventional treatment in primary care <sup>47</sup>.

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## RECENT TRENDS IN USE OF GROWTH HORMONE THERAPY

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**Abstract :** Until the mid 1980's, growth hormone (GH) therapy was only prescribed to treat children with severe growth hormone deficiency (GHD). Today, however, with abundance of recombinant human GH (rhGH), it is used to treat a wide range of conditions. rhGH can be used to treat short stature from GH deficiency (GHD), insufficiency and other disorders leading to poor growth. Currently it is also used for patients with chronic renal failure (CRF), Turner syndrome (TS), Prader Willi Syndrome (PWS), small for gestational age (SGA) without catch up growth by 2 years, Idiopathic short stature (ISS) and some Dysmorphic syndromes with short stature. With GH therapy many children can achieve adult height better than the anticipated based on their pretreatment growth pattern.

Human growth hormone (hGH) or somatotropin is a single chain of polypeptides comprising of 191 amino acids that circulates either complexed to a binding protein or in the unbound (free) state. At all ages, fetal through adult, GH is secreted in an intermittent, pulsatile pattern largely as a result of reciprocal interactions of two hypothalamic peptides i.e. Growth hormone releasing hormone (GHRH) and somatostatin or Somatostatin release inhibiting factor (SRIF), Fig 1

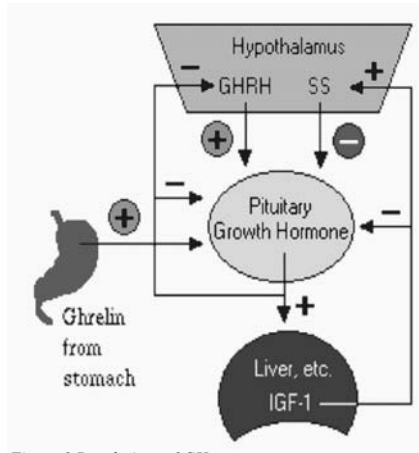


Figure 1 Regulation of GH

Growth hormone interacts with its receptor to generate IGF-1 (insulin like growth factor), the main mediator of GH action, in the liver and in most other tissues, including epiphyses. This receptor (the extra cellular domain of which is identical with the circulating GH binding protein) must link up with a second molecule through a two-site GH bridge. This molecular complex comprising of two receptors and one hGH molecule permits IGF-1 generation.

Many of the effects of GH are mediated by IGF-1, which circulates in the plasma, bound to one of a series of binding proteins, called IGF-BPs. These proteins circulate and modify IGF-1 action, either as stimulators or as inhibitors. IGF-BP-3 is the major circulating form of the binding protein. This complex system subserves the process of growth. At puberty the pulsatile release of GH is increased 2-3 fold, predominantly by increased amounts of GH released at each secretory episode. Along with increasing amounts of sex steroid hormones, this accounts for most of the pubertal growth spurt following which the secretion of GH returns towards

prepubertal values. Secretion of GH may be mediated by input from higher centers, allowing for modification of growth rate by environmental and emotional factors. The process of growth also depends on adequate nutrition, normal bone structure and biochemistry, normal thyroxine levels and other endocrine secretions, as well as general health. Disruption of normal growth may therefore be an indication of many pathologies.

After the establishment of the National Pituitary Agency in 1961, pituitary derived GH was used till mid 80s, when its use was prohibited with the emergence of Creutzfeldt Jacob disease in 1985. Around the same time recombinant human GH which was ready for use by the late 80s (1978) was approved by the FDA and introduced for the treatment of GHD in 1985. Subsequently it was found to be beneficial in patients with chronic renal failure and Turner Syndrome, a decade later (1995) FDA approved its use for these disorders. Currently GH therapy has been approved by FDA for additional conditions; i.e. Prader Willi Syndrome – PWS (2000), Small for gestational age – SGA without catch up growth by 2 years (2001), Idiopathic short stature – ISS, (2003) and some of the Dysmorphic syndromes with short stature.

### GROWTH HORMONE DEFICIENCY

The classical indication for GH treatment is growth hormone deficiency, irrespective of the underlying cause which leads to the GH deficient state. From a clinical prospective; GHD can be subdivided on the basis of etiology into 2 categories: organic and isolated idiopathic GHD.

Variability in the diagnosis of GH deficiency remains a clinical challenge and is related to the continuum between severe GHD, insufficiency and normalcy<sup>1-3</sup>. Marked variability in GH assays in the tests used, arbitrary cut offs to define GHD based on stimulation tests have been some of the problems in arriving at the diagnosis of GHD. Prior to proceeding with the investigative evaluation, careful clinical history, clinical and auxologic examination, the relationship among chronologic age, height age, bone age and height evaluation in relation to the relevant population based charts and midparent based target height is important.

The diagnosis of GHD is a challenge in the absence of the classic phenotype. A significant proportion of short, slowly growing children have no overtly obvious clinical features. A three step approach to diagnosis is:

- 1) Comprehensive clinical and auxological assessment to differentiate non-endocrine and non-GHD states, and select patients most likely to have GHD;
- 2) biochemical investigations of the HP-GH axis in carefully selected patients and
- 3) neuroimaging to define pituitary

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morphology<sup>4</sup>.

Investigations of the HP-GH axis should be undertaken in a centre with expertise in pediatric endocrinology. There is no single test to assess the HP-GH axis but peak GH responses are more reproducible with arginine or pyridostigmine which stimulate GH releasing hormone secretion and control endogenous somatostatin tone. The agents most commonly used for the GH provocation test are clonidine and insulin. Insulin is the gold standard for GH provocation test. In addition to GH levels following provocation, the GH-dependent peptides IGF-1 (and IGF-BP3 if available) should be measured because low levels of all strongly support a diagnosis of GHD, although normal levels would not exclude a diagnosis. Levels of IGF-1 and IGF-BP-3 should be interpreted against age, gender and pubertal stage matched normal ranges. Acute and chronic malnutrition, intercurrent illness or liver disease may all affect IGF-1 levels and complicate interpretation in the context of possible GHD.

rhGH in children with GHD provides physiological replacement<sup>5-8</sup>. Titrating the dose of rhGH to maintain IGF-1 levels in the normal range while normalizing growth can be considered to approximate physiological replacement. Younger age at the beginning of treatment, longer duration of treatment, smaller height deficit at start of treatment and greater catch up in height in the first year of treatment are an advantage for final height. The dosage used for GHD is 23-39ug/kg/day, or 0.7-1.0mg/m<sup>2</sup>/day. The height velocity in first year of rhGH is 8-12 cm. Fig 2

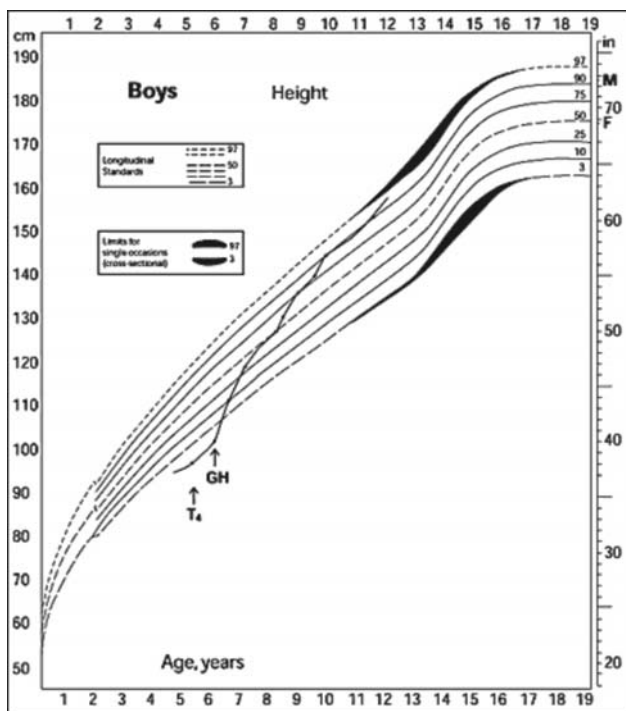


Figure 2. Growth chart of a patient with growth hormone deficiency before and during treatment with GH.

For children with GHD, whose linear growth can be within the normal range (e.g. craniopharyngioma) replacement therapy may be considered for its beneficial action on metabolism, body composition and pubertal growth.

Puberty should be induced by 12 to 13 years of age in patients who do not have spontaneous pubertal development owing to gonadotrophin deficiency. rhGH is continued until near final height ( $HV < 2\text{cm/year}$ ) when GH status should be reassessed to identify patients likely to benefit from continuing adult rhGH replacement.

### PRADER WILLI SYNDROME (PWS)

Prader Willi Syndrome (PWS) is the common genetic cause of progressive obesity.<sup>9</sup> A significant proportion of patients fulfill the biochemical criteria of GHD (low spontaneous and stimulated GH secretion taking obesity into account, with low IGF-1 and IGFBP-3 levels) and have clinical features consistent with this<sup>9</sup>. Pituitary hypoplasia and an abnormal posterior pituitary bright spot are frequently observed on MRI. These endocrine abnormalities, in addition to hypogonadotrophic hypogonadism and some typical traits (dysregulated food intake and high pain threshold) can be attributed to hypothalamic dysfunction. Declining height velocity, delayed puberty and the absence of pubertal growth spurt result in short stature with patients mean height falling below normal 5<sup>th</sup> centile by 12-14 years of age and deficit in adult height of 15-20 cm. In addition to normalizing linear growth, the aims of rhGH treatment are to improve body composition by promoting muscle mass, reducing body fat and increasing bone mineral density. Additional benefits reported include improvements in respiratory function, physical activity, physical appearance, behavior and quality of life. It is important to have calorie consumption under strict control if growth hormone treatment benefit is to be reaped. The growth response with rhGH in children with PWS is comparable to that seen in GHD. Respiratory disturbance and disordered breathing during sleep, including central sleep apnea and obstructive sleep apnea are recognized in PWS. The dosage of GH is 25-35ug/kg/day. The height velocity in first year of rhGH is 8-12 cm. Before starting rhGH, sleep studies should be done in all children who have PWS or are obese, and an ENT evaluation in those with a history of snoring and disturbed sleep.

### TURNER SYNDROME

Turner Syndrome occurs in approximately 1/1500 to 2000 female births and is a common pathological cause of short stature. Of the numerous manifestations recognized, the only consistent ones are short stature and ovarian failure. Although the stature of girls with TS varies considerably, the pattern of growth is characteristic. Mild intrauterine growth results in mean birth weight and length about 1 SD lower than the healthy newborn girls. There is a gradual decline in HV (height velocity) throughout childhood, absence of pubertal growth spurt and adult height deficit of about 20 cm. Girls with TS do not have GH or IGF-1 deficiency but levels of both are relatively low, particularly during adolescence, and can be attributed to estrogen deficiency and increased adiposity. A degree of GH and IGF-1 insensitivity is considered to contribute to growth failure and this forms the basis of treatment with supraphysiological doses of rhGH. Unlike GHD and PWS, GH provocation testing is not required in girls with TS. Much of the defect in height is caused by haploinsufficiency of the short stature homeobox-containing gene (SHOX) located on the X-chromosome. Although girls with TS are not growth hormone deficient, treatment with biosynthetic recombinant human GH accelerates height velocity and increases adult height.

In this condition a supraphysiological dose of GH is given. There are several factors which may influence effect of GH treatment, age and height at start, GH dose and injection frequency, non –

compliance, genetic factors, the addition of oxandralone, and the estrogen dose regimen and the timing of puberty induction. The dosage of rhGH used here is 45-50ug/kg/day. The height velocity in first year is 5.5-8 cm. **Fig 3**

Overall the safety profile of this treatment is good; however long term follow up of the girls using the supraphysiological doses of growth hormone is required.

Although the general experience is that most girls seem to be happy

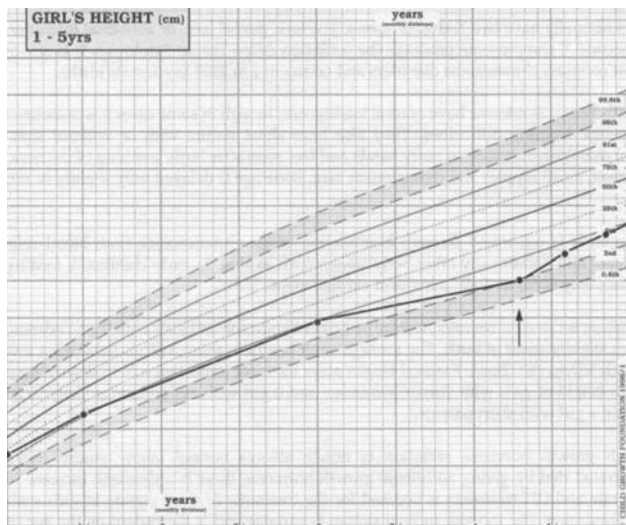


Fig 3 Typical growth chart for Turner Syndrome

with a normal height after long term treatment. At this moment there is a lack of evidence for the beneficial effect of GH treatment on the well being of patients with TS. Consequently, clinicians should not only focus on height improvement, but also consider other health related problems (including infertility) in the patients.

### SHORT CHILDREN BORN SMALL FOR GESTATIONAL AGE (SGA)

SGA is the term used to describe infants with birth weight and/or length less than -2SD for the gestational age. Up to 90% of children born SGA, experience catch up in linear growth during infancy, have height above -2SD by the first birthday and reach adult height approximately 1 SD below the normal population. Children born SGA require evaluation to identify the underlying cause and also regular growth monitoring to identify the 10% who do not have significant catch up growth and thus remain exceptionally short [10]. This is more likely in premature infants, severe IUGR and recognized syndromes (e.g., Silver Russell). Multiple factors influence growth in this heterogeneous group of children and a relative resistance to GH and IGF-1 is likely to contribute.

Assessment of the GH-IGF-1 axis is not routinely recommended but should be undertaken if an SGA child has growth failure or phenotype features of GHD. In addition, subtle changes in the GH-IGF-1 axis and a range of metabolic changes suggest an underlying reduction in insulin sensitivity. There is a growing body of evidence that GH therapy improves final height in short SGA children.

In Europe, SGA children aged 4 years or older, with failure to catch up height below -2.5 SD, HV below 0 SD and height SDS more than 1 SD below midparental height SDS are eligible for rhGH treatment.<sup>10</sup> A wide dose range is recommended and higher

doses may be considered for a limited period initially in children with marked growth retardation (height below -3 SD). The positive effects of GH therapy extend beyond linear growth and include potentially important effects on body composition, muscle mass and function, bone mass, metabolism, behavior and cognitive function. The dosage of rhGH used is 35-70 ug/kg/day and the height velocity in the first year of rhGH is 8-10 cm. **Fig 4**

GH therapy was associated with improvement in quality of life<sup>11</sup> and with a small improvement in IQ compared to historical reference data<sup>12</sup>.

GH therapy has been shown to be a safe and effective treatment for children born SGA. Remarkably few adverse events have been



Fig 4 How a SGA child grows if not treated

reported; however the effect on glucose metabolism remains a concern. Recent reports have suggested that an individual response to GH therapy might be affected by a common polymorphism of the GH receptor and the search for other polymorphisms of growth regulating genes in SGA children continues. Regarding the use of GH in Silver Russell Syndrome (SRS) little data is available. Many studies suggest a good response in younger children. Limited data suggest that GH does not exaggerate limb asymmetry.

### CHRONIC RENAL INSUFFICIENCY (CRI)

Impaired growth, short stature, delayed puberty and an attenuated pubertal growth spurt leading to reduced adult height are common in children with CRI. Growth failure is worse in younger children and in those with severe impairment in renal function. At the time of dialysis over 70% of patients have a height below 2 SD. They continue to have growth impairment despite dialysis and kidney transplantation and ultimately are short as adults.

The factors contributing to growth failure are primary renal disorder, uremia, under nutrition, metabolic acidosis and bone disease. The growth outcome, post transplantation is influenced by the dose of corticosteroid, allograft function, age of the child, pubertal status and height deficit at the time of transplantation.

Patients with CRF have relative GH insensitivity reflected by raised GH levels but also raised or normal IGF-1 and raised IGFBP-3 levels, reducing IGF-1 bioactivity. Thus high doses of rhGH are recommended to overcome this scenario. Treatment is indicated in those with significant growth impairment (height < 3<sup>rd</sup> or HV < -2SD) and CRI (GFR < 75 ml/1.73 m<sup>2</sup> BSA) before or during dialysis or following renal transplantation. To get the best result treatment should be initiated at an earlier age and earlier in the course in CRI<sup>13</sup>. Before starting growth hormone therapy nutritional and metabolic

status should be optimized and steroid treatment should be reduced to minimum. In children with CRI, initial catch-up growth followed by relatively normal growth and attainment of normal adult height can be anticipated. The dosage of rhGH is 45-50 ug/kg/day and the height velocity in the first year of rhGH is 5.5-8cm.

### IDIOPATHIC SHORT STATURE

In the last two decades, growth hormone (GH) therapy has expanded to include many children with non-GH deficient short stature such as idiopathic short stature (ISS), skeletal dysplasia, genetic syndromes and other chronic diseases associated with short stature.<sup>14</sup> The term idiopathic short stature is used to describe a child or adolescent with height more than 2 SD below corresponding mean height for given age, sex and population group, in whom with current diagnostic tools, no etiological diagnosis can be made. ISS now appears to be the most common indication for GH treatment. It is difficult to differentiate GHD from ISS with conventional growth hormone testing alone. There are subtle abnormalities of GH secretion and GH sensitivity in patients of ISS. There is no consensus on which all diseases must be excluded and how during a diagnostic evaluation a child should be labeled as ISS GH in a supra physiological dosage generally increases height velocity in children with ISS and increases the adult height up to 7 cm. The average effect on final height is modest; The dosage of rhGH used in ISS is from 0.24 to 0.37 mg/kg/week. The final gain varies between 5.4cm to 7.2 cm.

It is difficult to predict the height gain for an individual child. GH injection is well tolerated without significant side effects. However the theoretical risk of unwanted long term sequelae of elevated serum GH and IGF-1 levels have not been evaluated yet. Large scale use at present prices would consume an important part of the family resources. The psychosocial benefits and cost effectiveness need meticulous evaluation to justify GH therapy.

### SKELETAL DYSPLASIA

It is a heterogeneous group of diseases affecting the skeleton. The most prevalent is *achondroplasia* with incidence of 1 in 25,000 births. Other skeletal dysplasias include hypochondroplasia, dyschondrosteosis, congenital spondylepiphyseal dysplasia, pseudoachondroplasia and many others. Some *mucopolysaccharidosis* like Morquio Syndrome exhibit bone dysplasia. Most of the SD cause moderate to severe disproportionate short stature, most patient with SD have a normal growth hormone provocative test.

The final height differs between various disorders but often in the range of 110-130cm<sup>15</sup>. The result in skeletal dysplasia with rhGH has been less rewarding. GH therapy does not benefit patients with  $\alpha$  chondroplasia but a subgroup of patient with hypochondroplasia may benefit significantly. Other uncommon forms of skeletal dysplasia have not benefited from GH treatment.

*X-linked Hypophosphatemic rickets* is characterized by rickets, short stature, impaired renal phosphate reabsorption and vitamin D metabolism. It is treated by *oral phosphate supplementation* with an active *vitamin D analogue*; most children with XLHR demonstrate reduced height. Poorly growing children benefit from GH therapy. GH also increases phosphate tubular reabsorption and phosphate levels in blood.

### GROWTH HORMONE IN ADULTS

The action of growth hormone is not limited to physical growth. It

has an important role to play in many organ systems notably the cardiovascular system. It is also required for optimal metabolism of carbohydrates, fats, proteins, as well as for physical performance and body composition. Apart from various other functions, adequate evidence of various metabolic abnormalities and CVS dysfunction have been demonstrated in growth deficient states in adults. Adults with GH deficiency are amenable to treatment. This indicates the need for continuation of GH treatment in children with GH deficiency when they become adults provided the deficient states persist for indications other than growth. The dosage of rhGH is 0.45-0.9iu/day or 0.15-0.4 mg/day. The length of therapy to be decided in future.<sup>16</sup> rhGH is given as a daily subcutaneous injection. A variety of needle and needle free pen devices are available. Parents and older children can learn the injection technique. Although the preference is to administer rhGH in the evening to crudely mimic endogenous GH secretion the timing can be altered to accommodate family routines. The dose of rhGH should be calculated according to the body weight (in obese children according to body surface area) individualized according to the growth response and level of IGF-I levels and adjusted as the child grows. IGF-I and IGF-BP-3 levels should be maintained within age dependent normal ranges bearing in mind that the oncogenic potential is likely to be greatest with long term supra-physiological doses, high IGF-I and low IGFBP-3 levels. For patients who show a good response, rhGH is continued until significant further growth is unlikely (HV <2cm/year indicates near final adult height) or satisfactory height is attained. **Table 1**

### POOR RESPONSE TO GROWTH HORMONE TREATMENT

The response to rhGH in an individual child is variable; it can range from no discernible effect to dramatic improvement in HV and is influenced by genetic as well as non genetic factors. Factors to consider when there is a poor response (which are amenable to change) include inadequate dose, problems with an administration device, poor compliance, sub-clinical hypothyroidism or alternative hormone deficiency and other pathologies adversely affecting growth. A poor response is also likely in a GHD patient with previous irradiation damage to epiphyses and those at an advanced stage of puberty. Anti-GH antibodies and acquired GH resistance are exceptionally rare. Re-evaluation and decision to stop are critical when poor growth persists for 6 to 12 months, despite due attention to possible contributing factors.

#### *Side Effects of Growth Hormone therapy*

Recombinant Human Growth hormone (rhGH) has proved to be a safe medication and relatively free of untoward side effects. The reported side effects occur with a frequency of about 2-5% per patient year of treatment. Adverse effects are generally seen in less than 3% of the recipients. As the use of GH has expanded to include other indications such as idiopathic short stature, small for gestational age (SGA) babies and Prader Willi Syndrome, it becomes even more important to continuously monitor the safety of rhGH<sup>17</sup>. After the initiation of therapy with rhGH, transient edema due to fluid retention, transient headaches and even benign intracranial hypertension is reported. BIH is generally reversible with discontinuation of GH treatment. Severe edema and carpal tunnel syndrome are rare in pediatric patients. These effects are usually transient and reverse when treatment is stopped for a short time and generally does not recur on re-initiation of therapy. Enhanced risk of leukemia or brain neoplasia in children without specific risk factors is not proven. GH marginally increases the risk of slipped capital femoral epiphyses in children

with GHD, and return of limb edema and worsening of kypho-scoliosis in some patients with Turner syndrome. There is some concern about the effects of growth hormone on carbohydrate metabolism in (SGA) small for gestational age children but frank diabetes is very rare. Recent studies have not substantiated increased risk of transplant rejection in patients with renal failure. rhGH/IGF-1 may worsen the probability of sleep apnea in patients with Prader-Willi Syndrome, hence carefully pretreatment evaluation and monitoring is advocated. **Table 1**

Evaluation before starting and monitoring during growth hormone treatment

Before starting treatment	During rhGH treatment
Previous and baseline growth measurements and parent's heights plotted on growth chart.	Follow-up 3-6 monthly
Pubertal status	Growth response > Height, weight and head circumference at each visit. > Pubertal status at each visit. > Bone age annually.
Bone age	
GH provocation tests (if GHD suspected and in PWS)	rhGh administration technique dose and compliance at each visit
Baseline serum IGF-1 (and IGFBP-3) levels	> Dose modification based on weight, height growth and IGF-1. Serum IGF-1 annually for dose optimization and compliance . > Latent hypothyroidism unmasked. > Pituitary hormone deficiencies can evolve particularly in patients with ectopic posterior pituitary, septo-optic dysplasia and PIT-1..
TFT (GHD and TS)	
BP	
Fasting insulin ,glucose ,lipids(PWS,TS,SGA ,CRI and any obese patient)	
Sleep studies (PWS and any obese patient)	> Features of potential side effects > Benign intracranial hypertension (TS and CRI) > Peripherical edema (TS) > Arthralgia > Slipped capital femoral epiphysis > Worsening scoliosis(PWS,RSS ,TS) > Impaired insulin sensitivity(PWS,TS,SGA,CRI)

Abbreviations : chronic renal insufficiency(CRI),growth hormone deficiency(GHD),Prader Willi syndrome(PWS),Russell Silver syndrome(RSS),small for gestational age(SGA),Turner syndrome(TS).

Minor side effects such as injection site pain, numbness, redness, swelling, bleeding and sweating at the local site as well as generalized pruritus are reported. Other report effects include prepubertal gynecomastia and increased growth rate of cutaneous nevi. There are very few published studies done in India reporting the safety and efficacy of rhGH. The side effects experienced by Indian children were headaches, urticarial rash and local reaction in the form of itching and erythema. rhGH should also be used with **caution in fanconi anemia** and **Bloom syndrome** due to the inherent tendency for malignancy in these conditions.

**FUTURE PROSPECTS**

GH is recommended in catabolic wasting states such as HIV infections. In many conditions GH therapy is being tried on an investigational basis such as cystic fibrosis, steroid dependent states and chronic diseases which retard growth but where the use of GH proved to be safe. Pathophysiology of ISS is gradually being unraveled by the development of new genetic tools,

GH can be used in Thallesemia with short stature. It is still in the experimental stage.

The anabolic effects of GH have also led to its use in many catabolic states like severe burns, HIV induced cachexia, chronic high dose glucocorticoid treatment, chronic obstructive pulmonary disease, surgery, trauma, cancer, organ failure etc. In severely burnt children, GH has shown to decrease whole body catabolism, increase protein synthesis,

accelerate wound healing, and reverse growth arrest<sup>18</sup>. GH is approved by the food and drug administration for administration to adult patients with HIV associated cachexia. The GH treatment in these patients resulted in a positive nitrogen balance, increased lean body mass, decrease in body fat and improved work output.<sup>19</sup>

In the future we anticipate the following things in growth hormone development

- The availability of GH in weekly doses or monthly doses, instead of daily injections
- GH being available in dermal patches, inhaled and tablet form.
- GH combined with LHRH analogues for early puberty with short stature.

In future the use of GH is likely to include many more conditions beyond those proven at present. Although generally safe, potential side effects of GH need to be carefully noted. Children receiving GH must be monitored closely by Physicians who are experienced in the use of this pharmacological agent.

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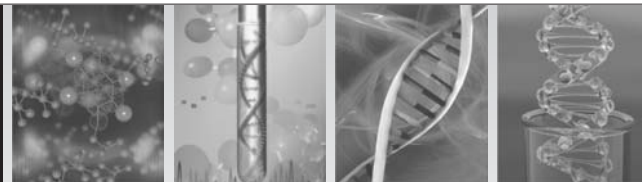
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