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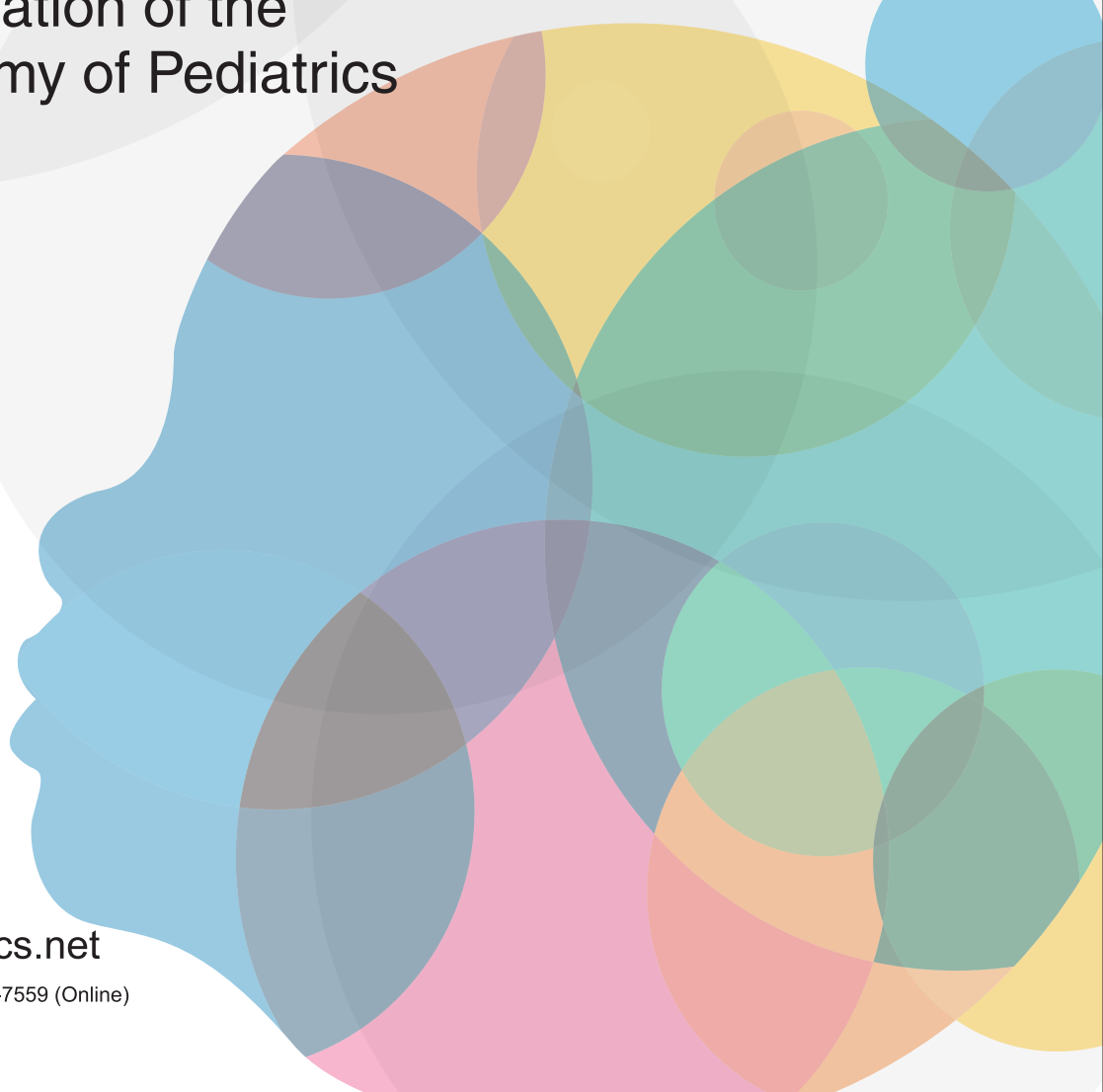

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IAP Guidelines for pediatric skincare

state that the newborn skin undergoes various structural and functional changes[1*]

Use of appropriate evidence-based skincare practices are important[2]

Protect the infant skin barrier function with safe, appropriate, and evidence-based products

Johnson's® CHOOSE gentle™

CLINICALLY mildness PROVEN®

Evidence-based skincare regimen

Cleansing regimen

As mild as pure water to maintain the skin barrier function[3,4]

Helps maintain the skin barrier function[5]

Moisturizing regimen

Improves the skin barrier integrity[6,7]

Helps enhance the skin barrier function for moisture retention[8]

*From birth to the first five years of life

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EDITORIAL COMMENTARY

What Is the Best for Colon Preparation: Single-Dose, Split-Dose or Add-ons to Polyethylene Glycol?

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Colonoscopy is an important diagnostic and therapeutic modality used in pediatric practice. Chronic bloody diarrhea, bleeding per rectum, surveillance for polyposis syndromes, obtaining tissue biopsies for pathologies such as intestinal tuberculosis and Crohn's disease, and documenting endoscopic mucosal healing in the above conditions are common indications for colonoscopy. Adequate bowel preparation is the essential step in colonoscopy for proper visualization of the mucosa to identify these lesions. It has been estimated that one-third of the colonoscopies are done in suboptimal preparation [1]. In adults, cleanliness of bowel preparation is assessed by Ottawa Bowel Preparation Scale (OBPS). Other commonly used scales in pediatric practice include Aronchik Scale and Boston Bowel Preparation Scale (BBPS) [2]. While Aronchik scale is a global assessment scale, OBPS and BBPS rate the bowel preparation per each colonic segment (such as right colon, transverse colon and left colon). With technological advancement, use of artificial intelligence systems has begun. A software "ENDOANGEL" assesses bowel preparation using BBPS while withdrawing the colonoscope; accuracy was found to be greater than experienced endoscopists [3]. However, it needs further validation studies. In the absence of a universal protocol of bowel preparation, North American Society of Pediatric Gastroenterology, Hepatology and Nutrition (NASPGHAN) endoscopy and procedures committee provided a clinical report in 2014 to guide pediatric bowel preparations for colonoscopy [1].

Polyethylene glycol (PEG), an osmotic laxative, is one of the most commonly used agents for bowel preparation. Various centers use PEG alone or in combination with other agents. The drawback of using PEG is that larger volumes are required. To overcome the issue of using larger volumes in a single go, split-dose regimen has been preferred in adults. NASPGHAN endoscopy and procedures committee had advocated the use of PEG plus electrolytes alone, or in combination with bisacodyl, as a one- or two-day regimen. PEG plus electrolytes when used alone, can be administered as a single or split-dose regimen. Tripathi, et al. [4] have shown better efficacy and tolerability (lesser side effects and improved sleep pattern) of using PEG in a split-dose regimen even in children. The study had a good inter-observer agreement of the findings. Longer regimens of up to four days have also been used [5]. Many practitioners add other osmotic laxatives like magnesium oxide, glycerol or stimulant laxatives such as sodium picosulphate or bisacodyl to enhance bowel preparation [1]. European Society of Gastrointestinal Endoscopy (ESGE) 2019 Guidelines advise the use of low residue diet prior to colonoscopy [6]. Federal Drug Administration (FDA) has recently approved the use of Pure-Vu system, which enhances the bowel preparation quality [7]. It uses a mixture of water and air to break up the fecal matter similar to that of a flush pump. HyGleaCare is another novel system which helps in bowel preparation pre-procedure. It contains a nozzle which infuses a steady stream of warm water that breaks the stool [2]. It is unlikely that small children will cooperate with the use of this device.

In this issue of *Indian Pediatrics*, a randomized trial by Hein, et al. [8] evaluated 129 children assigned to one of the two bowel preparation strategies by block randomization technique. One group received PEG plus bisacodyl and the other group received PEG plus glycerol enema. Researchers and endoscopists were blinded to the regimens followed. Primary outcome measure was the efficacy of bowel preparation that was assessed by BBPS and the secondary outcome measures were tolerability, acceptability and compliance to the regimens used. No difference was found in the rates of successful bowel preparation using the two regimens. However, side effects such as nausea and bloating were found to be significantly higher in patients receiving PEG plus glycerol. Compliance to therapy was found to be better in patients receiving PEG plus bisacodyl. There was better tolerance to PEG plus bisacodyl in the

form of willingness to repeat the process. Overall, the use of PEG plus bisacodyl was found to be more acceptable. Strengths of the study include the fact that it was a randomized trial, though open label. The main drawback of the study is that the volume of PEG used in the two regimens was different; 50 mL/kg in the PEG plus bisacodyl group and 70 mL/kg in PEG plus glycerol enema group. As has been discussed above, the side effects such as nausea, bloating and poor compliance are related to the use of higher volume of PEG in PEG plus glycerol enema group. The second issue was use of glycerol enema twice in the latter group. Obviously, acceptability of a regimen will be poor if an invasive method (such as rectal enema) is used as compared to oral tablet. Whether all the colonoscopies were performed by a single pediatric endoscopist or multiple persons was not highlighted, and if there was any inter-observer bias was not clearly mentioned. Despite these drawbacks, the article has shown that the dose of PEG can be lowered by adding a stimulant laxative to achieve a good bowel preparation with fewer side effects. Ideally, comparisons should have been made between low dose PEG alone and low dose PEG plus bisacodyl to obviate the above-mentioned drawbacks.

To conclude, in children, appropriate bowel preparation is especially needed due to issues arising from sedation and technical difficulties, which makes repeating the procedure a difficult experience for the patients and attendants. There are various regimens of bowel preparation. PEG alone used as a split-dose regimen is a safe and efficacious regimen validated by a randomized controlled trial in children [4]. There is an unmet need to have a larger randomized controlled trial to compare low dose PEG with low dose PEG plus and stimulant laxative.

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EDITORIAL COMMENTARY

Intermittent Mixed Venous Oxygen Saturation in Pediatric Septic Shock

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Across the globe, sepsis is the leading cause of morbidity and mortality in the pediatric population. An estimated 1.2 million cases of childhood sepsis are reported worldwide [1]. Given the global burden of disease in 2001, Surviving Sepsis Campaign (SSC) was initiated to collaborate and improve research and education for sepsis survival [2,3]. Shortly after the SSC campaign was initiated, the American College of Critical Care Medicine (ACCM) and Pediatric Advanced Life Support (PALS) guidelines recommended the use of targeted mixed venous oxygen saturation (ScvO2) therapy for Early Goal Directed Therapy (EGDT) in pediatric septic shock. Targeted ScvO2 monitoring is a critical tool for guiding therapy in children with septic shock both in the developed and developing countries [4-6]. Despite extensive use of ScvO2 monitoring in clinical practice, there are not many studies supporting its use. Clinical practice guidelines for management of pediatric septic shock by ACCM (2014) strongly recommended the use of targeted ScvO2 levels, a surrogate for cardiac index, as a marker of therapeutic end point for resuscitation, based on case control and cohort studies [7]. However, these recommendations are based mostly on the adult studies from developed world. Feasibility of continuous ScvO2 monitoring is question-able in resource-constrained settings of low- and middle-income countries (LMICs).

De Oliveira, et al. [8] with their randomized controlled design provided a head on comparison of continuous targeted ScvO2 >70% in children with fluid refractory shock guided monitoring (n=51 in each group). The authors reported a significant reduction in mortality in children with targeted ScvO2 therapy with numbers needed to treat (NNT) of 3.6. The study provided a premise for use of ScvO2 monitoring in clinical practice in advanced pediatric intensive care units (PICU) in the developing world. Subsequently, Sankar, et al. [9] published a prospective cohort study of children with fluid refractory shock admitted to a PICU in India. They used intermittent ScvO2 values obtained at 1, 3 and 6 hours after initiation of therapy to guide treatment against clinical variables and lactate among controls. The catheter was placed in subclavian/internal jugular vein, and the patients in whom catheter could not be placed served as controls. The authors observed a lower mortality in ScvO2 group as against controls (33% vs 54%; NNT=5). The limitation of the study was its design with a possible selection bias but it provided a balanced approach in resource-limited set ups, where continuous monitoring was not feasible.

The 2020 SSC International guidelines support the use of advanced hemodynamic monitoring such as ScvO2, but categorize it under weak low quality evidence [2,10]. Jain, et al. [11] report on a randomized controlled trial targeting intermittent superior vena caval saturation (ScvO2) above 70% for EGDT in patients with pediatric septic shock, in the current issue of *Indian Pediatrics*. The study population included children from 1 month to 12 years of age with fluid refractory shock admitted to the PICU. The authors found a significantly lower 28-day mortality and lower new organ dysfunction in the group with ScvO2 targeted therapy group. There was no difference noted in the time to achieve therapeutic end points, need for organ support and length of PICU or hospital stay. The study protocol is similar to the previous study from India except that fluid refractory shock was defined as shock not responding to 40 mL/kg of fluids as compared to 60 mL/kg used by Sankar, et al. [9]. All these studies support the utility of continuous and intermittent ScvO2 monitoring as a cost-effective tool for improving the survival of children with sepsis.

Dr. Joseph Carcillo, whose work has been instrumental in the field of pediatric septic shock, refers ScvO2 as a poor man's mixed venous oxygen saturation [12]. The author warns us of the limitations of the use of ScvO2 measurements. True ScvO2 requires oxygen saturation measurement of the pulmonary artery and this value can be 2% to 8% lower than catheters placed at SVC-RA and IVC-RA junction. High ScvO2 can indicate improved oxygen delivery but may also be reflective of poor oxygen consumption due to sepsis induced dysfunctional tissue perfusion and mitochondrial dysfunction [12,13]. In 2018,

Goonasekera, et al. [14] studied global oxygen extraction ratio (gO2ER) as a marker of oxygen consumption in fluid refractory pediatric septic shock on a cohort of 62 children admitted to the PICU with fluid refractory shock. They concluded that gO2ER of >0.48 with a blood lactate >4.0 mmol/L and metabolic acidosis are better predictors of death as compared to ScvO2.

Children are not young adults. With the limited evidence available in pediatric population, the study by Jain, et al. [11] continues to support the use of intermittent targeted ScvO2 monitoring for improving survival in pediatric septic shock refractory to 40 mL/kg of fluid resuscitation. However, the results mostly apply to sick children with septic shock having low SvcO2. With the clinical world constantly searching for more non-invasive methods, implementing the principles of EGDT using echocardiography and point of care ultrasound (POCUS) in children with septic shock may give newer insights in future [6,14].

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RESEARCH PAPER

Efficacy of Two Regimens for Colon Cleansing Using Polyethylene Glycol 4000: *A Randomized Open Label Trial*

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Aim: To compare effectiveness, safety and tolerance of two colon cleansing regimens using polyethylene glycol 4000 (PEG) in children.

Methods: Prospective, randomized, open clinical trial carried out in 129 children, 3 to 18 years old undergoing colonoscopy. Patients were randomized into two groups, 64 children received PEG with electrolyte (50 mL/kg) and oral bisacodyl (PEG+B group) or 65 other children received PEG with electrolyte (70 mL/kg) and glycerol enema (PEG+G group).

Results: Both regimens showed a good colon cleansing effectiveness with the percentage of successful cleansing being 93.8% for PEG+B regimen and 89.1% for PEG+G regimen (*P*=0.51). There was no statistically significant difference between the pre-regimen and post-regimen laboratory values. The rates of nausea (65.6% vs 31.3%; *P*<0.001) and bloating (50% vs 17.2%; *P*<0.001) of PEG+G group were significantly higher than that of PEG+B group.

Conclusion: Both regimens had good efficacy and safety for colon cleansing in children. The tolerance of PEG+B regimen was better.

Keywords: *Bisacodyl, Bowel preparation, Colonoscopy, Glycerol enema.*

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Appropriate bowel cleansing before colonoscopy for children requires consideration of the efficacy, safety, and tolerance of the regimen. The North American Society for Pediatric Gastroenterology, Hepatology, and Nutrition (NASPGHAN) and American Society for Gastrointestinal Endoscopy (ASGE) recommend polyethylene glycol (PEG) with or without stimulant laxative (Bisacodyl) as the most common clean-out regimen for colon preparation in children [1,2]. Recent studies have shown that PEG alone (single or split dose) or difference in length of preparation (1-4 days) is good enough [3-7]. Hence, PEG plus bisacodyl (low-volume solution) may be considered as one of the many effective methods of colon cleansing. Glycerol suppositories, recommended for the treatment of constipation without any side-effects [8], can be added in the colon cleansing regimen in children. However, PEG 3350 and glycerol suppositories are not available in Vietnam, whereas glycerol enema and PEG 4000 are available in Vietnam. Besides, there have been studies showing the effectiveness and safety of regimen using PEG 4000 with electrolytes for colon cleansing in children [9].

In our hospital, we used PEG 4000 with electrolytes in combination with glycerol enema before colonoscopy, which showed a dramatic improvement of cleansing effectiveness. However, in children this regimen requires drinking a large amount of fluid, limiting the compliance level and the efficacy. Therefore, this study was conducted in order to compare the effectiveness and safety of two colon cleansing regimens using PEG 4000 with electrolytes in combination with either oral bisacodyl or glycerol enema.

METHODS

This study was a prospective, randomized, open-label clinical trial, conducted in our hospital from October 1, 2016 to June 31, 2017. We enrolled consecutive children aged 3–18 years undergoing colonoscopy in our hospital. A written parental consent was obtained for all the enrolled patients in this study.

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We excluded children with; severe systemic disease that require parenteral nutrition, known chronic cardiovascular, liver, kidney, neurological or systemic diseases, known coagulopathy and/or thrombocytopenia with a decreased platelet function, known chronic granulocytopenia and/or immune deficiency, electrolyte

imbalance, and finally children with acute intestinal obstruction.

Patients' colonoscopy eligibility for enrollment selection was done at the outpatient department. A complete clinical physical examination was performed with the blood tests: hemoglobin, hematocrit, sodium, potassium, chloride, phosphorus, calcium, glucose, urea, creatinine, and alanine transaminase and aspartate transaminase. If all results were normal, the patient moved to next step.

Eight paper notes were prepared in a box, which contained four options for PEG+B regimen and another four options for PEG+G regimen. Then, selected patients were openly randomized to receive an option regimen in a box. When all paper notes in a box were selected, other 8 paper notes were prepared and the random selection process was repeatedly performed. The researchers recorded clinical symptoms and laboratory parameters before the beginning of the trial medication. The researchers, then prescribed, distributed a guide explaining to the patients' parents how to implement the regimen for CC, including diet, how to prepare drugs and PEG solution according to the selected regimen. Researchers also explained the possible side effects of drugs, instructed how to monitor and manage them initially when encountering these problems. The patient was then discharged home for the beginning of the selected regimen with a patient parental questionnaire reporting all clinical manifestations, side effects, therapeutic compliance and tolerance during the selected study regimen.

After applying the selected regimen, patients were monitored by researchers with a complete clinical physical examination, and patients or patients' parents were interviewed with the questionnaire during the selected regimen. The researchers recorded clinical manifestations, side effects, therapeutic compliance and tolerance of the used regimen, then the second blood tests were performed, as those before colonoscopy, and results recorded.

Colonoscopy was performed by an experienced pediatric endoscopist and evaluated with the Boston scale. Researchers recorded all endoscopic information, completed clinical research records, and requested a second physical examination, if any abnormalities occurred during colonoscopy.

All researchers and endoscopists were blind to the used regimens during monitoring visit before colonoscopy, during colonoscopy and in the post-colonoscopy period.

Colonic cleansing process: Both groups received a diet including snacks until 4 PM on the day before the colonoscopy. PEG+B regimen; after 4 PM on the day before the colonoscopy, children received an oral bisacodyl tablet of 5 mg according to their bodyweight (Bisacodyl, fabrication: 1 tablet ≤20 kg, 2 tablets 20-30 kg, and 3 tablets > 30 kg) [2,3,9]. From 6 PM to 9 PM on the day before the colonoscopy, children were given PEG 4000 solution with electrolytes (Fortrans, fabrication), with a dose of 50 mL/kg of body weight with a maximum amount of two liters [9]. One Fortrans package contains 64g macrogol and is dissolved in one liter of water before drinking.

For PEG+G regimen, children were given PEG 4000 with a dose of 70 mL/kg of body weight with a maximum amount of 4 liters. Children had to drink half the dose of solution from 4 PM to 6 PM, take 2 hours off, and then drink the remaining half dose from 8 PM to 10 PM on the day before the colonoscopy [10]. Children received two glycerol 9g (Microlismi fabrication) by rectal enema. The first enema was done at 4 PM on the day before colonoscopy and the second one was done in the morning at 8 AM on the colonoscopy day.

Colon cleansing efficacy was evaluated by endoscopists according to the Boston Bowel Preparation Scale (BBPS) [11], consisting of a 4-point scoring system applied to each of the three broad regions of the colon: right colon, transverse colon, and left colon. Overall colon cleansing was scored by summing up the scores of each segment. The total score ranging from 0 to 9 was divided into 4 grades: excellent cleansing (total score, 8-9), good cleansing (total score, 6-7), poor cleansing (total score, 4-5) and inadequate cleansing (total score, 0-5). Successful colon cleansing was defined with a total score of at least 6.

Vital signs, physical examination, and blood tests were performed at the time of patient enrollment and after a colonoscopy that included hematological parameters, liver and kidney function test, sodium, potassium, chloride, calcium, phosphorus, glucose. Immediately before the procedure, each patient was asked about his or her experience by using a standardized questionnaire and answered about tolerability, acceptability and compliance. Tolerability assessment was based on the recording of the occurrence and severity of gastrointestinal symptoms such as nausea, bloating, abdominal pain and anal discomfort. We evaluated the acceptability of colon cleansing regimens by willingness to repeat with three grades: willingness to repeat, difficulty to repeat and no acceptance to repeat.

All participants were recorded completely using oral Bisacodyl 5 mg tablets as well as glycerol rectal enema 9g. Hence, treatment compliance was based on the volume of PEG; it was considered as excellent when the patient intake was >90% of prescribed volume of PEG, moderate between 50 to 90%, and poor <50%.

Statistical analysis: The statistical analyses were performed by using absolute and relative frequency tables and contingency tables. For categorical variables we used Chi-square test and Fisher exact test, and Student *t*-test for continuous variables. Differences in pre-post laboratory variables for each group were assessed using the Wilcoxon signed-ranks test. The statistical significance was set at $P<0.05$. The analyses were conducted using SPSS version 20.0.

RESULTS

Our study had 136 patients who had an indication for colonoscopy, of which 129 were finally randomized to two groups: 65 patients received PEG+B regimen and 64 other patients received PEG+G regimen (**Fig.1**). However, one patient in PEG+B group did not complete intervention because this patient drank under 30% of PEG solution and passed solid stools before the procedure. This patient had delayed colonoscopy 1 day later with another colon cleansing regimen. There were no differences in gender and age between the two groups **(Table I)**.

The total score was observed by 4 grades without statistically significant difference in two groups. The rates of PEG+G group and PEG+B group were 9.4% vs 15.6% in excellent cleansing; and 4.7% vs 1.6% in inadequate cleansing (**Table II**).

CC was also evaluated by each colonic segment and there was no significant difference in the efficacy of the two protocols in the different colonic segments. Mean BBPS score of PEG+G group and PEG+B group were similar (**Table II**).

Both regimens were equally efficient with a high rate of successful colon cleansing (95.3% of PEG+B regimen and 89.1% of PEG+G regimen) by per-protocol analysis or intention-to- treat analysis (93.8% vs. 89.1%; $P=0.51$). 128 patients underwent colonoscopy up to the cecum.

Table I Baseline Characteristics of Children Receiving Two Colon Cleansing Regimens (*N*=128)

Variable	*PEG+B, (n=64)*	*PEG+G, (n=64)*
Male sex	39 (60.9)	44 (68.8)
Age (y)[a]	5.80 (2.67)	5.67 (2.53)
Body mass index (kg/m^2)[a]	15.1 (2.18)	16.26 (2.94)
Reason for colonoscopy[b]		
Bloody stools	62 (96.8)	59 (92.1)
Persistent diarrhea	1 (1.6)	3 (4.7)
Persistent abdominal pain	1 (1.6)	1 (1.6)
Colonoscopy findings		
Normal	10 (15.6)	9 (14.1)
Polyp	38 (59.4)	37 (57.8)
Anal fissure	15 (23.4)	13 (20.3)
Other	1 (1.6)	5 (7.8)

Values in no. (%) or [a]mean (SD). Cleansing regimen – polyethylene glycol with electrolytes and oral bisacodyl (PEG+B) or glycerol enema (PEG+G). [b]One child in PEG+G group underwent colonoscopy for anal mass.

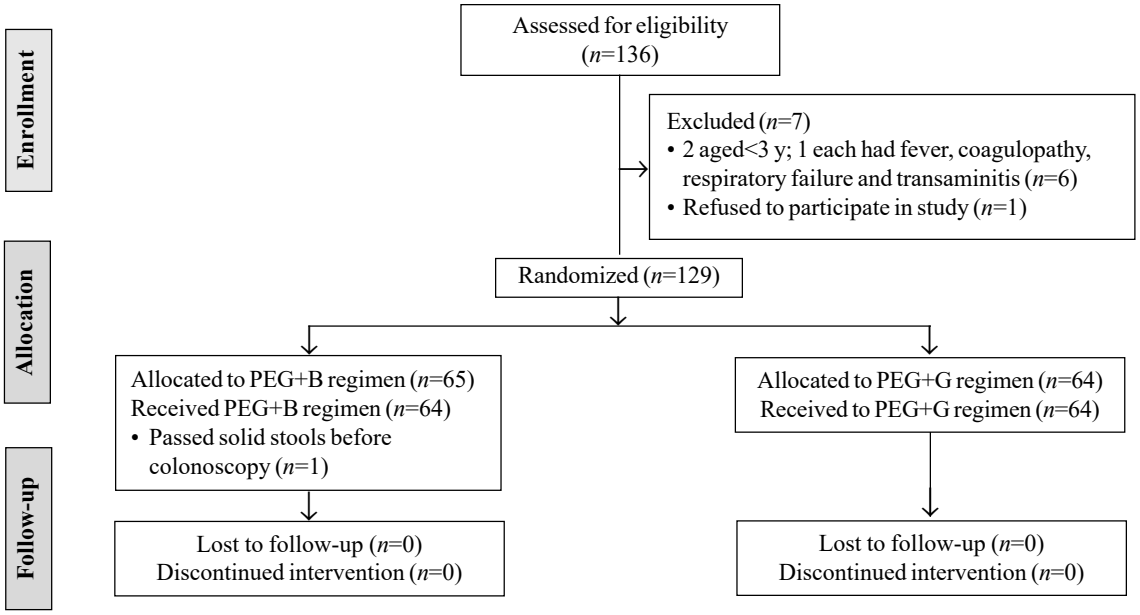

Fig. 1 Study flow chart.

Table II Efficacy of Two Colon Cleansing Regimens Among Children Undergoing Colonoscopy (*N*=128)

Variable	*PEG+B (n=64)*	*PEG+G (n=64)*
Qualitative cleansing rating		
Excellent (BBPS score: 8-9)	6 (9.4)	10 (15.6)
Good (BBPS score: 6-7)	51 (79.7)	51 (79.7)
Poor (BBPS score: 4-5)	4 (6.2)	2 (3.1)
Inadequate (BBPS score: 0-3)	3 (4.7)	1 (1.6)
Successful colon cleansing	57 (89.1)	61 (95.3)
BBPS score per segment[a]		
Overall	6.1 (1.2)	6.4 (1.3)
Right colon	2.02 (0.4)	2.13 (0.4)
Tranversal colon	2.05 (0.4)	2.16 (0.4)
Left colon	2.03 (0.4)	2.13 (0.45)

Values in no. (%) or [a]mean (SD). All patients in both groups had cecal intubation. Polyethylene glycol with electrolytes and oral bisacodyl (PEG+B) or glycerol enema (PEG+G). BBPS-Boston bowel preparation scale. All P>0.05.

In this study, none of the enrolled children from both used regimens developed any severe side effects. The hemoglobin and hematocrit tended to decline in both the groups. Glucose level tended to decrease slightly in both groups; however, there were no children having hypoglycemia after implementing the regimen. There were 2 children with high blood glucose at 199.8 mg/dL and 176.4 mg/dL but without any clinical features. The rest of the laboratory indicators were normal.

The rates of nausea (65.6% vs 31.3%; *P*<0.001) and bloating (50% vs 17.2%; *P*<0.001) of PEG+G group were significantly higher than that of PEG+B group. However, the rate of vomiting (18.8% vs 10.9%) and abdominal pain (32.8 vs 27.9%) were not different between the both groups.

We found that 79.7% (*n*=51) of families and children had willingness to repeat the same colon cleansing regimen if needed in the PEG+B group. This rate was significantly higher compared to that of 35.9% (*n*=23) in the PEG+G group *(P*<0.001). The rate of excellent compliance (children with ≥90% of fluid intake) in PEG+B group (62.5%), significantly higher than that in the PEG+G group (42.2%), (*P*=0.03).

DISCUSSION

Our study found that the two regimens were equally effective for colonic cleansing, with success rate of PEG+G group of 89.1% and PEG+B of 93.8%. Different reports in children report a successful rate of 72-95% [4-7,9,12,13]. The wide range of these results are related to the regimen used in research. Our study; thus, demonstrated similar efficacy of low-volume PEG 4000 solution with oral bisacodyl in colon preparation as the other protocol (high-volume PEG 4000 or 3350, split-dose, length preparation) in children [4-7].

None of the children in this study developed any severe side effects with use of either regimen, compared to the study by Di Nardo, et al. [9] who reported a 10-year-old girl developing severe dehydration, and orthostatic hypotension, with use of PEG 4000 requiring intravenous fluid for 6 hours. There were no biochemical abnormalities due to these regimes, except the blood glucose levels slightly decreased in both groups but without any documented hypoglycemia. There were two children with hyperglycemia that could be resulted from the fact that we had instructed patients to take sugar 3 hours before endoscopy. These results were similar to other studies using PEG 3350 and PEG 4000 [9,13]. In contrast, another study showed the rate of hypokalemia was 24%, but without clinical manifestations, in electrolyte-free PEG-3350 regimen [4].

Among our patients, none presented with extra-intestinal symptoms such as seizures. However, digestive symptoms such as nausea and bloating after bowel preparation occurred significantly more commonly in the PEG+G group vs the PEG+B group. This could be related to the higher ingested fluid volume in the PEG+G regimen vs the PEG+B one. Likely, these symptoms are more common when patients need to drink more fluid. Such symptoms also affect the patient's ability to comply with the regimen [9]. However, there were no differences between the groups for vomiting and abdominal pain.

Our study showed the rate of children complying with ≥90% of the fluid in PEG+G group (42.2%) was lower than the other group PEG+B (62.5%). Some recent studies presented the percentage of compliance in split-dose or low-volume solution or length of preparation was higher than full single dose [4-7,9]. The PEG+B group in our study had significantly higher acceptability in the willingness to repeat than PEG+G group, which was similar to a previous report. This result was also similar to another study on split-dose versus full single-dose regimen of PEG [6].

Limitation of our study was that the dose of PEG 4000 volume in two groups was different and made it difficult to compare the compliance. Other limitations of this study are the small sample size and done in only one centre. As a matter of fact, our results of Boston scores in both regimens were not as high as our expectation, around 6 points. Therefore, we hope to conduct a meta-analysis in future to find the optimal protocol for bowel preparation in Vietnamese children.

Both regimens used had a good colon cleansing efficacy in children, with a high safety by both clinical and

WHAT IS ALREADY KNOWN?

- Using PEG 4000 with electrolyte is effective and safe in CC in children.

WHAT THIS STUDY ADDS?

- Using either of PEG+B or PEG+G regimen was effective and safe for colon cleansing in children; however, the tolerance of PEG+B regimen was better.

biochemical indicators. The tolerance of PEG+B regimen was better.

Ethics clearance: Research Institute of Child Health of Vietnam National Children's Hospital; No. 113/QĐ-BVNTU, dated: September 16, 2016.
Contributors: HPT: concept and designed the study, analysed data and drafted the manuscript; TVH: collected the data and helped in data analysis; Ha NT: helped in data analysis; KN: helped in drafting the manuscript and methodological comments. All authors approved the final version of manuscript, and are accountable for all aspects related to the study.
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RESEARCH PAPER

Early Goal-Directed Therapy With and Without Intermittent Superior Vena Cava Oxygen Saturation Monitoring in Pediatric Septic Shock: *A Randomized Controlled Trial*

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Objective: To compare early goal-directed therapy (EGDT) 'with' and 'without' intermittent superior vena cava oxygen saturation ($ScvO_2$) monitoring in pediatric septic shock.

Design: Open label randomized controlled trial.

Setting: Pediatric intensive care unit in a tertiary care center.

Participants: Children aged 1 month to 12 year with septic shock.

Intervention: Patients not responding to fluid resuscitation (up to 40 mL/kg) were randomized to EGDT 'with' (*n*=59) and 'without' (*n*=61) $ScvO_2$ groups. Resuscitation was guided by $ScvO_2$ monitoring at 1-hour, 3-hour, and later on six-hourly in the 'with' $ScvO_2$ group, and by clinical variables in the 'without' $ScvO_2$ group.

Outcome: Primary outcome was all-cause 28-day mortality. Secondary outcomes were time to and proportion of patients achieving therapeutic endpoints (at 6 hours and PICU stay), need for organ supports, new organ dysfunction (at 24 hours and PICU stay), and length of PICU and hospital stay.

Results: The study was stopped after interim analysis due to lower mortality in the intervention group. There was significantly lower all-cause 28-day mortality in EDGT with $ScvO_2$ than without $ScvO_2$ group [37.3% vs. 57.5%, adjusted hazard ratio 0.57, 95%CI 0.33 to 0.97, *P*=0.04]. Therapeutic endpoints were achieved early in 'with' $ScvO_2$ group [mean (SD) 3.6 (1.6) vs. 4.2 (1.6) h, *P*=0.03]. Organ dysfunction by sequential organ assessment score during PICU stay was lower in 'with' $ScvO_2$ group [median (IQR) 5 (2,11) vs. 8 (3,13); *P*=0.03]. There was no significant difference in other secondary outcomes.

Conclusion: EGDT with intermittent $ScvO_2$ monitoring was associated with reduced mortality and improved organ dysfunction in pediatric septic shock.

Keywords: *Mortality, Organ dysfunction, Septic shock.*

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Worldwide, sepsis in children is a significant cause of mortality [1,2]. Despite the understanding of septic shock and novel therapeutic strategies, the mortality rate is reported up to 20% in high-income and 57% in low-middle income countries (LMICs) [3-5]. The pediatric septic shock guideline has been extrapolated from adult studies, but significant pathophysiological differences exist bet-ween adults and children [6]. Early goal-directed therapy (EGDT) has been reported to be associated with reduced mortality in adult septic shock [7]. EGDT approach involves adjustments in cardiac preload, after-load, and contractility to balance oxygen supply with oxygen demand [6,7]. Hemodynamic assessment based on clinical findings, central venous pressure (CVP), and urine output may fail to detect persistent global tissue hypoxia [7].

Superior vena cava oxygen saturation ($ScvO_2$) is a surrogate marker of cardiac index, and it is one of the targets to be achieved during hemodynamic stabilization [7]. Few studies examined the role of $ScvO_2$ monitoring in pediatric septic shock [4,5]. However, there are constraints in adopting EGDT in LMICs, despite the recommendation of surviving sepsis campaign [4]. There are significant differences in the organizational structure of critical care and demographic characteristics between high and LMIC.

Editorial Commentary: Pages1117-18

Limited studies in pediatric septic shock reported a favorable outcome in EDGT [5,8]. The crucial need to reduce septic shock mortality, and the paucity of high-quality controlled studies in children warrants examining the role of EGDT in LMICs [9]. Hence, this study was conducted to compare EGDT with and without intermittent $ScvO_2$ monitoring in pediatric septic shock and its effect on all-cause 28-day mortality.

METHODS

This open-label randomized controlled trial was conducted

in the pediatric intensive care unit (PICU) of a tertiary care center from September, 2015 to June, 2019. The study was approved by the institutional ethics committee and written informed consent was obtained from parents/caregivers. Children aged 1 month to 12 years diagnosed with septic shock and who continued to have impaired perfusion despite fluid bolus (up to 40 mL/kg) within the first hour of resuscitation were included in the study. Septic shock, sepsis, and organ dysfunction were defined as per the international pediatric sepsis consensus conference [10]. Fluid bolus was discontinued, if clinical signs of fluid overload (hepatomegaly, basal lung crepitation) developed. Children with contraindication to insertion of a central venous catheter (CVC), cardiac disease, severe malnutrition, and referred with a CVC in-situ and/or already received more than 6 hours of care were excluded.

Computer-generated block randomization with a variable size of blocks was generated by a person not directly involved in the study. Individual assignments were kept in serially numbered, opaque sealed envelopes (SNOSE). The investigator opened the envelopes, and eligible patients were enrolled sequentially. The study intervention was not blinded because of the nature of the interventions. However, the person handling the data and the statistician were blinded for treatment assignment during the analysis. The assignment was disclosed after finalizing the first draft of the results.

Before starting the trial, we conducted multiple discussion sessions among investigators, resident doctors, and nursing staff about the nature of the trial, its components, and how EGDT had to be delivered. The basic concept of EGDT was adopted from Rivers, et al. [7], and the concept of without $ScvO_2$ monitoring was adopted from Sankar, et al. [5]. Supplemental oxygen and mechanical ventilation were administered based on clinical need. CVC was inserted in all patients in the internal jugular vein by study investigators. A 5Fr CVC was used for infants and younger children and 7Fr for older children. The CVC tip position was confirmed at the junction of the superior vena cava and right atrium by ultrasound and *X*-ray. $ScvO_2$ values were analyzed using a blood gas analyzer with a co-oximetry module (Cobas b221 blood gas system, Roche Diagnostics). Values of $ScvO_2$ were recorded in both groups, but they were not used to guide treatment in EGDT the without $ScvO_2$ group.

In EGDT with $ScvO_2$ group, resuscitation was carried out as per the protocol (**Web Fig. 1a**). After achieving target CVP and mean arterial blood pressure (MABP) by fluid and vasoactive drugs, the $ScvO_2$ ≥70% was targeted and estimated at enrollment, 1, 3, and every 6-hourly. Epinephrine was the initial choice for cold shock and nor-epinephrine for a warm shock. Packed red blood cells (PRBC) was transfused when ScvO2 was <70%, and hematocrit was ≤30% during the first hour of resuscitation. If $ScvO_2$ remained <70% even after PRBC transfusion, dobutamine was started and titrated according to hemodynamic parameters. In EGDT without $ScvO_2$ group, resuscitation was carried out as per the protocol (**Web Fig. 1b***)*. After achieving target CVP and MABP by fluids and vasoactive drugs, the arterial lactate level was targeted to <1.6 mmol/L. If the arterial lactate was ≥1.6 mmol/L, PRBCs and inotropic support were provided. Lactate values were obtained at enrollment, 1 hour, 3-hour, and then every 6-hourly. In both groups, the additional choice of vasopressor, inotropic agents, and fluid bolus was decided according to the hemodynamic parameters, CVP, $ScvO_2$/lactate values, and type of shock. The therapeutic endpoints of septic shock were defined as capillary refill of ≤2 second, normal blood pressure for age, normal pulses with no differential between peripheral and central pulses, warm extremities, urine output ≥1 mL/kg/hour, normal mental status (without sedation), arterial lactate <1.6 mmol/L or decreasing trend and $ScvO_2$ saturation ≥70% [5,11]. The only difference between the two groups was that the $ScvO_2$ ≥70% was the final therapeutic endpoint in EGDT with $ScvO_2$ group, whereas $ScvO_2$ was not used as a therapeutic endpoint in the without $ScvO_2$ group. The standby extracorporeal membrane oxygenation (ECMO) service was not available in our unit. After achieving the therapeutic endpoints, patients were monitored and if any worsening occurred, the protocol was repeated as per the assigned group. Details of hourly hemodynamic parameters, investigations and interventions were noted in a predesigned proforma.

Our primary outcome was all-cause 28-day mortality. Secondary outcomes were time to and proportion of patients who achieved therapeutic endpoints (at 6-hour and PICU stay), need for organ supports (mechanical ventilation, vasoactive support, and renal replacement therapy-RRT), new organ dysfunctions (at 24-hour and PICU stay by sequential organ failure assessment score-SOFA, Pediatric Logistic Organ Dysfunction-(PeLOD) score, and length of PICU and hospital stay.

Unpublished data from our center from January – December 2014 and Sankar, et al. [5] reported a 55% mortality rate in the EDGT without $ScvO_2$ group and 30% mortality 'with' $ScvO_2$ group [5]. With the assumption of 25% absolute risk reduction in the intervention group and 95% power, alpha error of 5%, we calculated the sample size of 110 in each group (total of 220), including a 10% attrition rate (nQuery Advisor+nTerim 3.0). These results assumed that two sequential tests at equally spaced intervals were made. The study progression was monitored by the

independent data monitoring and ethics committee. An independent statistician who was also a physician analyzed the data in a blinded manner. The trial had to be stopped after an interim analysis of 120 patients contended lower mortality in the intervention group. The power of the study decreased from 95% to 80% with a two-sided alpha error of 5% after the planned interim analysis.

Statistical analysis: Data was analyzed according to the assigned group (intention to treat analysis). Kolmogorov-Smirnov *Z*-test was used to check the distribution of data. Normally distributed continuous data were compared by Student *t*-test and by Mann-Whitney U test if skewed data. Chi-square test or Fisher exact test were used for analyzing qualitative data. Kaplan-Meier survival estimates with log-rank test was used for time to event analysis. Cox proportional hazard analysis was done to adjust the predefined variables (age, sex, shock type-compensated/hypotensive). Relative risk and hazard ratio with 95%CI were calculated as necessary. The general linear model-repeated measures analysis of variance (RM-ANOVA) was performed to compare the trends of the first 72-hour of hemodynamic and laboratory variables in the study groups. The missing values in the data (amounting to 9.8%) during the RM-ANOVA analysis were handled using the last observation carried forward (LOCF) method. All the tests were two-tailed, and a P value <0.05 was considered statistically significant. IBM SPSS version 20.0 (SPSS Inc.) and Epi Info 7 (7.0.9.7, CDC) were used for data analysis.

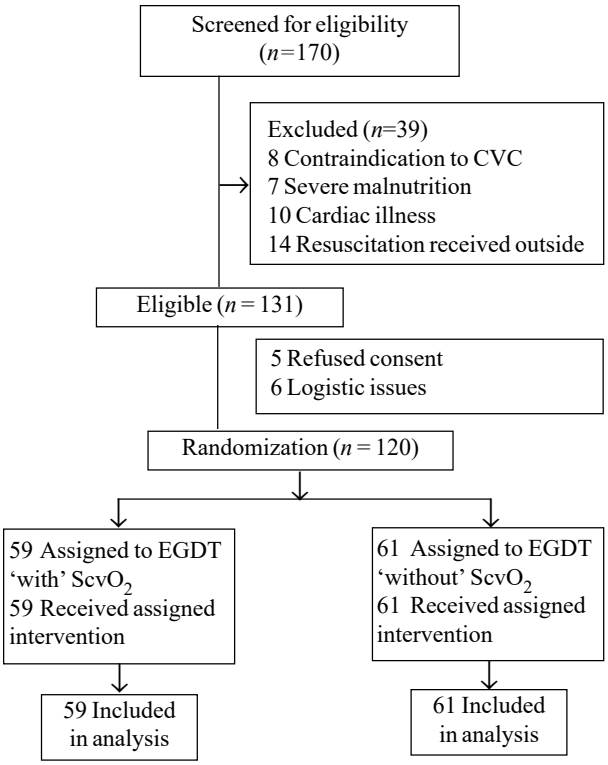
RESULTS

Of the 131 eligible children screened, 120 were randomized and analyzed (59 in 'with' $ScvO_2$ group and 61 in 'without' $ScvO_2$) (**Fig. 1**). No protocol violation was noted. Baseline characteristics of both the groups are shown in **Table I**. The most common focus of infection was pulmonary (n=67, 55.8%), followed by bloodstream infections (n=20, 16.7%). Culture were positive in 34 (28.3%) patients, and the most common gram-negative and gram-positive organisms isolated were *Pseudomonas aeruginosa* (31.4%) and *Staphylococcus aureus* (14.3%), respectively. A significantly higher number of children received dobutamine in the EGDT with $ScvO_2$ group (61% vs. 33%, P=0.002); no significant difference was seen in the need for other interventions (**Table II**).

The overall all-cause 28-day mortality rate was 47.5% (n=57). EGDT with $ScvO_2$ group had 20.1% absolute reduction in all-cause 28-day mortality which was significant than EGDT without $ScvO_2$ group [37.3%, n=22 vs. 57.4%, n=35; RR (95%CI) 0.66 (0.45-0.97); P=0.028] and adjusted hazard ratio 0.57 (0.33-0.97), P=0.038] (**Table III** and **Fig. 2**). The number needed to treat was 5 (2.7-38.6). In EGDT with $ScvO_2$ group, the mean time to achieve all therapeutic endpoints at 6-hour (P=0.035) and the SOFA score calculated daily (24 hrs) (P=0.039) was significantly lower than the without $ScvO_2$ group. No significant difference was noted in other secondary outcomes (**Table III**). Seven patients had minor bleeding at the site of CVC placement (3 with $ScVO_2$ and 4 without $ScvO_2$ groups). No serious complications occurred like significant bleeding, air leak, or other CVC-related complications.

DISCUSSION

In our study, EGDT with intermittent $ScvO_2$ guided management in pediatric septic shock was associated with 20.1% absolute reduction of all-cause 28-day mortality than without $ScvO_2$ monitoring. Both the mean time to achieve all therapeutic endpoints at 6 hours, and the SOFA score were significantly lower in EGDT with $ScvO_2$ group. $ScvO_2$ is a surrogate marker of oxygen utilization at the tissue level. Hence, targeting $ScvO_2$ ≥70% ensures adequate microcirculation and organ perfusion [5-8]. American College of Critical Medicine (ACCM) guidelines emphasized using $ScvO_2$ ≥70% as a therapeutic endpoint in pediatric and neonatal septic shock [6].



CVC-central venous catheter, EGDT-early goal directed therapy, $ScvO_2$-superior vena cava oxygen saturation.

Fig. 1 Study flow chart.

Table I Baseline Characteristics of the Study Participants

Characteristics	*EGDT (with ScvO2) group (n=59)*	*EGDT (without ScvO2) group (n=61)*
Age, mo[a]	12 (7-39)	12 (4-54)
Males	31 (52.5)	29 (47.5)
PRISM III[a]	17 (13 - 21)	16 (13 - 23)
PeLOD[a]	12 (8 - 24)	16 (11 - 23)
SOFA[a]	8 (5 - 13)	9 (6 - 13)
Transferred from		
Pediatric emergency	42 (71)	43 (71)
Pediatric ward	10 (17)	13 (21.3)
Others	7 (12)	5 (8.2)
Prior antibiotic therapy	27 (46)	33 (54)
Focus of infection		
Lung (pneumonia)	34 (57.6)	33 (54.1)
Bloodstream infection	8 (13.6)	12 (19.7)
Abdominal infection	6 (10.2)	6 (9.8)
Skin and musculoskeletal infection	6 (10.2)	5 (8.2)
Central nervous system infection	2 (3.4)	1 (1.6)
Renal system infection	1 (1.7)	1 (1.6)
Without focus	2 (3.4)	3 (4.9)
Cold shock	54 (91.5)	53 (87)
Warm shock	5 (8.5)	8 (13)
Hypotensive shock	38 (64.4)	41 (67.2)
Compensated shock	21 (35.6)	20 (32.8)
Clinical and laboratory parameters		
Mean arterial blood pressure, mmHg[b]	52 (13)	49 (13)
Central venous pressure, cmH2O[b]	8.3 (2)	8.3 (1.9)
Hemoglobin, gm/dL[b]	8.7 (2.1)	9 (2.1)
Hematocrit, %[b]	26.2 (6.4)	26.8 (6.5)
Lactate, mmol/L[b]	4.6 (3.1)	66.3 (10.4)
ScvO2, %[b]	4.6 (2.9)	64.7 (10.8)
ScvO2 <70%	43 (73)	46 (75.4)
ScvO2 among patient with <70%[b]	61.4 (6.5)	60.2 (7.4)
Culture positive	18 (31)	16 (26.2)

Values presented as no. (%) except [a]median (IQR) or [b]mean (SD). EGDT-early goal-directed therapy, $ScvO_2$-superior vena cava oxygen saturation, PRISM-pediatric risk of mortality, PeLOD-pediatric logistic organ dysfunction, SOFA-sequential organ failure assessment.

Table II Treatment and Hemodynamic Variables in the Two Study Groups During the First 72 Hours

Parameter	*EGDT (with ScvO2) group (n=59)*	*EGDT (without ScvO2) group (n=61)*
Time to first antimicrobial dose, min[a]	24 (12)	22 (11)
Bolus received, mL/kg[b]	64.8 (27.1)	65.4 (29.9)
Need for colloids	5 (8.3)	9 (14.8)
Need for any vasopressor	55 (93.2)	57 (93.4)
Need for dobutamine[c]	36 (61)	20 (33)
Need for milrinone	10 (17)	5 (8.2)
Inotropic score[b]	23.3 (1.8)	22.7 (1.8)
Vasoactive-Inotropic score[b]	35.6 (3.7)	37.4 (3.6)
Need for PRBC	33 (56)	24 (39.3)
PRBC transfused, mL/kg[a]	19.2 (7.7)	18.4 (10.7)
Need for FFP	7 (11.9)	9 (14.8)
Need for platelet concentration	10 (17)	11 (18)
Received steroid	26 (44.1)	29 (47.5)
Fluid balance (%FO)[a]	0.70 (0.50)	0.74 (0.39)
Mean arterial blood pressure[a]	57.3 (1.6)	57.3 (1.6)
Central venous pressure, cmH_2O[b]	8.7 (0.2)	8.9 (0.2)
$ScvO_2$ (%)[b]	70.4 (1.5)	65.1 (1.5)
Lactate, mmol/L[b]	4.1 (0.6)	4.6 (0.6)
Delta-lactate at 6h,[a,e] mmol/L	- 0.43 (1.24)	0.65 (2.72)

Data presented as no. (%) except [a]mean (SD) or [b]mean (SE). EDGT- early goal-directed therapy, $ScvO_2$-superior vena cava oxygen saturation, PRBC-packed red blood cell, FFP-fresh frozen plasma, %FO-percentage fluid overload. [c]P=0.002, [d]P=0.012, [e]P=0.007.

Previous reports [5,8] also reported mortality reduction similar to ours in the $ScvO_2$ group. However, the patient population in our study was sicker than those in the previous study [8]. The timely administration of bundled care is the cornerstone for improved outcomes in septic shock. In EGDT with $ScvO_2$ group, the mean time taken to achieve therapeutic endpoints at the first 6-hour was significantly lower, and the $ScvO_2$ values (0 to 6-hour and 0 to 72-hour) were significantly higher. Thus, our study showed that $ScvO_2$ monitoring helps early recognition of septic shock and guides interventions to correct this pathophysiology, thereby improving outcomes, similar to previous work [5,8]. The mortality rate in our study was almost the same as previously reported [7]. However, in three major controlled trials in adults [12-14] conducted in high-income countries, no difference in mortality was found by the EGDT compared with usual care and protocol-based standard therapy, respectively. Their low baseline mortality could be one of the reasons for this difference.

Timely administration of resuscitation bundle and targeting tissue perfusion variable, namely $ScvO_2$, in septic shock has been associated with improved organ

Table III Outcome Comparison of Primary and Secondary Between the Two Study Groups

Outcome	*EGDT (with ScvO2) group (n=59)*	*EGDT (without ScvO2) group (n=61)*	*Relative risk (95% CI)*	*P value*
Primary outcome				
All cause 28-d mortality	22 (37.3)	35 (57.4)	0.66 (0.45-0.97)	0.028
Secondary outcomes				
Achieved therapeutic endpoint at 6h	37 (62.7)	33 (54.1)	1.16 (0.86-1.57)	0.34
Time to achieve therapeutic endpoints during 6h, h[a]	3.6 (1.6)	4.2 (1.6)	–	0.03
Achieved therapeutic endpoints during PICU stay	45 (76.3)	38 (62.3)	1.22 (0.96-1.56)	0.097
Time to achieve therapeutic endpoints during PICU stay, h[a]	6.3 (8.1)	7 (11.1)	–	0.77
Organ support				
Need for invasive ventilation	45 (76.3)	45 (73.8)	1.03 (0.84-1.27)	0.75
Duration of ventilation, d[b]	4 (3-7)	4 (2-7)	–	0.72
Average Vasoactive-inotropic score (first 7d)[a]	30 (25.6)	31.5 (22.9)	–	0.73
Need for renal replacement therapy	11 (18.6)	10 (16.4)	1.14 (0.52-2.48)	0.75
New organ dysfunction				
Patients with new-onset organ dysfunction	25 (42.4)	30 (49.2)	0.86 (0.58-1.28)	0.45
No. of new organ dysfunction[b]	2 (1-3)	2 (1-2)	–	0.68
PeLOD score				
At 24h[b]	12 (8-24)	16 (11-23)	–	0.21
During PICU stay[b]	8 (3-18)	13 (4-20)	–	0.17
SOFA Score				
At 24h	8 (5-13)	10 (6-14)	–	0.08
During PICU stay[b]	5 (2-11)	8 (3-13)	–	0.04
Duration				
PICU stay, d[b]	5 (3-9)	5 (2-10)	–	0.72
Hospital stay, d[b]	8 (6-13)	7 (4-13)	–	0.40

All data presented as no. (%) except [a]mean (SD) or [b]median (IQR). EGDT-early Goal-Directed Therapy, $ScvO_2$ - superior vena cava oxygen saturation, CI-confidence Interval, PeLOD-pediatric Logistic Organ Dysfunction score, SOFA-sequential Organ Failure Assessment, PICU -pediatric intensive care unit.

dysfunction and reduced mortality [5,15]. Otherwise, inadequate resuscitation leads to progressive organ dysfunction and death [16]. We found that new-onset organ dysfunction was similar in study groups; however, during PICU stay, the SOFA score was significantly lower in EGDT with $ScvO_2$ group. This contrasts to previous pediatric studies that have reported a lower new-onset organ dysfunction in EGDT with $ScvO_2$ group [5,8].

In our study, the need for vasoactive therapy and blood transfusion were similar in both groups; however, the need for dobutamine was higher in EGDT with $ScvO_2$ group. Previous adult and pediatric studies reported a higher proportion of patients receiving both inotropic and blood transfusion in the $ScvO_2$ targeted group [7,8]. This could be due to sepsis-associated myocardial dysfunction in septic shock [5-8]. We used the same blood transfusion threshold (10 g/dL) in both study groups. A similar observation was reported by Sankar, et al. [5]. The need for other interventions was similar in both groups including fluid bolus, ventilation, and additional vasoactive therapy.

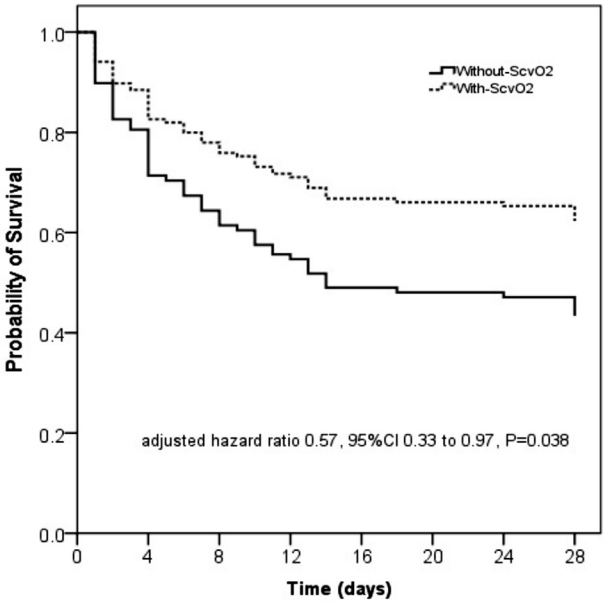


Fig. 2 Kaplan-Meier survival curve showing mortality up to 28-day in the study groups.

WHAT IS ALREADY KNOWN?

- Limited studies on early goal-directed therapy in pediatric septic shock reported reduced mortality and improved organ dysfunction.

WHAT THIS STUDY ADDS?

- Early goal-directed therapy with intermittent superior vena cava oxygen saturation monitoring reduces mortality and improves organ dysfunction in pediatric septic shock.

In our study, the baseline lactate was higher and the declining trend was significantly more in EGDT with $ScvO_2$ group, which contrasts with previous studies [5,8]. Hence, targeting the $ScvO_2$ with an inotropic agent and bundle care might improve the myocardial contraction and optimize tissue perfusion.

The limitations of the study were that $ScvO_2$ was not monitored continuously. However, recent studies have showed non-inferiority of intermittent $ScvO_2$ monitoring in pediatric septic shock [17]. It is a single center, and our baseline mortality was high, which limits the generali-zation of our results to other settings. The strengths of our study are that it enrolled children with various types of infections presenting as septic shock, measurement of $ScvO_2$ was done using blood gas analyzer with a co-oximetry module, which contrasts with the previous study [5]. Future studies in different settings are required to validate the generalizability of our results.

Our study concludes that EGDT with intermittent $ScvO_2$ monitoring (and targeting ScvO2 ≥70%) was associated with reduced mortality and improved organ dysfunction in pediatric septic shock.

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Ethical clearance: Institutional Ethics committee, JIPMER; No.JIP/IEC/2015/16/598, dated June 25, 2015.
Contributors: RR: had full access to all the data in the study and took responsibility for the integrity of the data and the accuracy of the data analysis; RR, SM: study concept and design; PJ, PS: acquisition, analysis, or interpretation of data; PJ: drafting of the first manuscript; RR: critical revision of the manuscript for important intellectual content; RR, SM: study supervision. All authors approved the final version of the manuscript.
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Conflict of interests: None stated.
Note: Additional material related to this study is available with the online version at *www.indianpediatrics.net*

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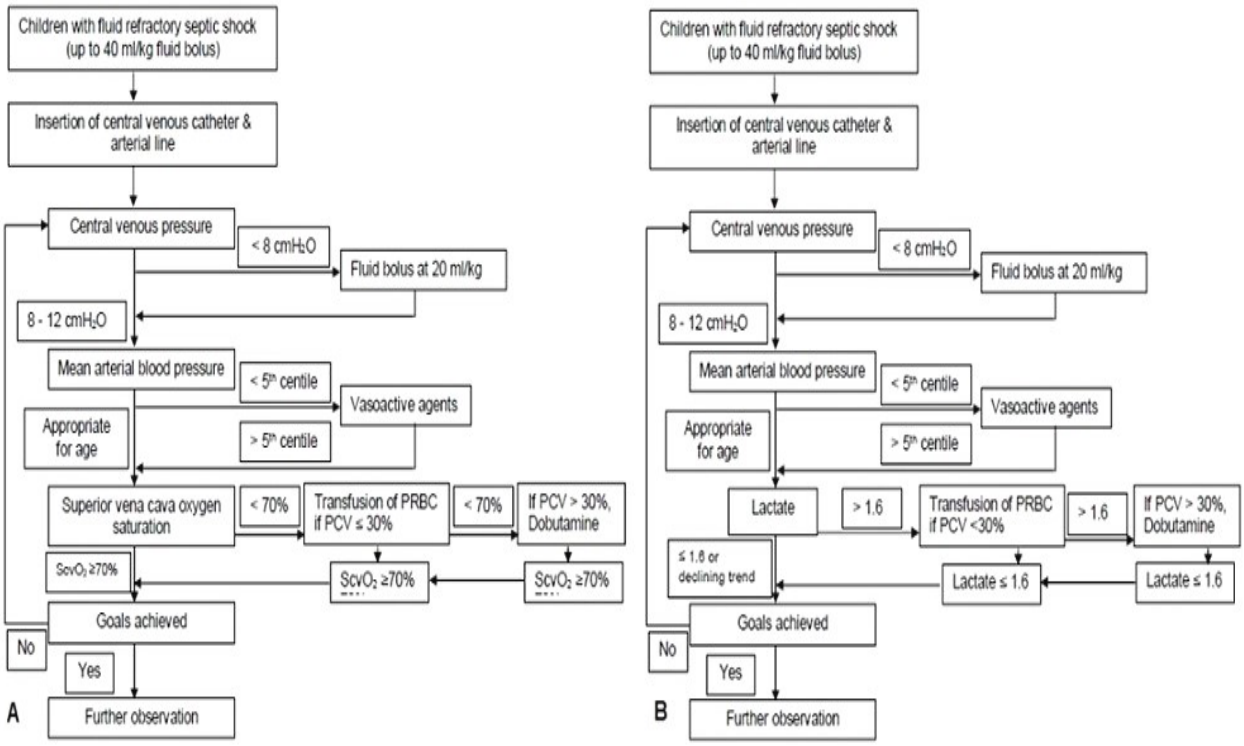

Web Fig. 1 (a) Early goal-directed therapy with superior vena cava oxygen saturation protocol; **(b)** Early goal-directed therapy without superior vena cava oxygen saturation protocol.

RESEARCH PAPER

Immunogenicity and Safety of Three WHO Prequalified (DTwP-HB-Hib) Pentavalent Combination Vaccines Administered As Per Iranian National Immunization Plan in Iranian Infants: *A Randomized, Phase III Study*

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Background: The pentavalent vaccine Pentavac was officially introduced in the Iranian National Immunization Plan in November, 2014.

Objective: To compare the immunogenicity and safety of Pentavac vaccine (Serum Institute of India Ltd.) with two other pentavalent vaccines available in Iran, i.e., Pentabio (PT Bio Farma (Persero)) and Shan 5 (Shantha Biotechnics Ltd.).

Design: Randomized, phase III study.

Participants: 900 infants attending the study sites to receive the vaccine at 2, 4, and 6 months of age.

Intervention: Infants were randomly assigned to one of the Pentavac, Pentabio, and Shan 5 vaccine groups.

Outcomes: The antibody titers were measured against five antigens, diphtheria, tetanus, pertussis, *Haemophilus influenzae B*, and hepatitis B before receiving the first dose and one month after the last dose. The adverse events following vaccination after each dose were recorded in the adverse events diary.

Results: All vaccines showed similar immunogenicity against four of the five antigens except pertussis. While vaccination with Shan 5 resulted in the highest immunogenicity against pertussis, Pentabio was significantly lower than the other two vaccines (P<0.001). The incidence of local adverse events significantly differed among the three vaccine brands (P<0.001), but the incidence of most of the evaluated systemic adverse events was similar (P>0.05).

Conclusion: Pentavac and Shan 5 had similar immunogenicity, the former having better immunogenicity against pertussis than Pentabio. Pentavac and Pentabio had a comparable safety profile.

Keywords: *Adverse effect, National immunization program, Vaccination.*

Clinical Trial Registration: IRCT2015051222234N1

The use of combination vaccines to immunize children against several diseases simultaneously is a well-known strategy to increase vaccine coverage in the pediatric vaccination program [1]. These vaccines have benefits such as reduced injection number, low cost, and better patient compliance. It is also essential to ensure that adding different components does not change the efficacy, safety, or immunogenicity of each component [2].

Diphtheria (D), tetanus (T), and pertussis (P) antigens are critical components of the World Health Organization's Expanded Program on Immunization (EPI) [2]. In 1992, the World Health Organization (WHO) recommended that the hepatitis B (HB) vaccine be added to the EPI and later recommended the *Haemophilus influenzae B* (Hib) vaccine be included in the pediatric vaccination program in all countries after six weeks of age. The administration of Hib conjugate vaccine led to a decrease of over 90% in the prevalence of severe Hib diseases in countries with universal coverage of the vaccine.

The pentavalent vaccine officially entered the Iranian National Immunization Plan (NIP) in November, 2014 as a DTwP-HB-Hib vaccine administered at the age of 2, 4, and 6 months [3]. The adverse events and immunogenicity of Pentavac (Serum Institute of India Ltd.) were evaluated in two studies in 2017 [3] and 2018 [4], respectively, indicating

that Pentavac was safe, although it did not induce proper immunogenicity against pertussis. Arjamand, et al. [5] indicated acceptable seroprotection against HB by Pentavac six months after three doses of vaccination.

Pentavalent vaccines such as Pentabio [PT Bio Farma (Persero)] and Shan 5 (Shantha Biotechnics Ltd.) have been produced by various countries [6,7]. A study conducted in Indonesia showed that Pentabio is as immunogenic and safe as the Hib monovalent vaccine given simultaneously with DTwP-HB [8]. However, due to lack of research on comparing Pentavac vaccine in Iranian children with other pentavalent vaccines [9-15], this study was designed to evaluate and compare the immunogenicity and safety of Pentavac vaccine with two other pentavalent WHO-prequalified vaccines, Pentabio and Shan 5 [13].

METHODS

In this prospective, randomized, double blind, multicenter, phase III study, we enrolled healthy infants 50 to 70 days of age, born after full-term pregnancy with birth weight ≥2.5 kg, who had not received previous doses of Hib, HB, or DTP vaccines. The protocol and informed consent form were approved by the institutional review board of the study center, and the protocol of the study was registered in the Iranian Registry of Clinical Trials. Written informed consent was taken from one of the parents.

Eligibility of participants was assessed using the infant's health documents archived in the health centers or by interviewing the parents. The exclusion criteria were axillary temperature >37.1°C on the day of inclusion, current or planned involvement in another clinical trial during the clinical trial period, mother with known history of human immunodeficiency virus infection, known immunodeficiency or immunosuppressive conditions, history of blood transfusion or use of blood products or immunoglobulin use since birth, acute symptoms or severe chronic illness that could interfere with conduct or completion of the trial, hypersensitivity to any of the vaccine components, any contraindication to intramuscular injection, and use of any vaccine or research drug other than that of the study during the study period or 30 days before inclusion in the study except for oral polio vaccine, which was allowed at 2, 4 and 6 months of age, along with the study vaccines.

Six health centers in different districts of Tehran city were selected for participant enrolment. The brand of pentavalent vaccine was selected randomly for each health center using block randomization method. The same brand was used for immunization of the study participant in all the three immunization visits. The study was performed between September 2019 and October 2020.The investigated vaccines were Pentavac, Pentabio, and Shan 5.

The participants received intramuscular injection of one of the pentavalent vaccines into the anterolateral aspect of their right thigh at 2, 4, and 6 months of age. All the vaccine vials were covered with the same coating to ensure similar appearance to enable blinding. The parents and laboratory technicians were blinded to the type of vaccine each participant received.

A researcher-made diary was used to record the adverse events of the vaccine [3,7,9]. The parents were taught how to complete the side effect diary. Adverse events were categorized as local adverse events (redness, pain, stiffness, warmth, injection-site lesion/abscesses), and systemic adverse events (fever, drowsiness, skin allergies, lymphadenitis, paralysis, loss of appetite, diarrhea, vomiting, rhinorrhea, cough, asthma, encephalitis, toxic shock syndrome, hospitalization, and death).

Blood samples were collected prior to the first dose of study vaccine and 28 days after the third dose to assess antibody responses. Blood specimens were maintained in a sterile capped test tube and transferred to the main laboratory within 4 hours for serum separation. Serum samples were maintained at -70°C until enzyme-linked immunosorbent assay (ELISA) was done to determine antibody titers.

IgG titers for the DTP and Hib components were determined by ELISA kits (Demeditec). HBV antibodies were measured using ELISA kits (Antisurase, General Biologicals). The cut-off value for seroprotection against diphtheria and tetanus was ³0.1 IU/mL. AntiHBs ³10 mIU/mL was considered protective for Hepatitis B. For HiB, antibody titer ³0.15 g/mL was considered to provide short-term protection, and ³1.0 g/mL was considered to provide long-term protection.

Pertussis specific IgG antibodies (anti-pertussis toxin and anti-filamentous hemagglutinin antibodies) were measured using Bordetella pertussis IgG ELISA- based kit (IBL international kit) according to the manufacturer's instructions, with the lowest detectable level of 1 IU/mL. The cut-off value of >25 U/mL was regarded as a protective value for pertussis [14].

Statistical analysis: Statistical analysis was performed using SPSS (Version 25) (IBM SPSS Statistics for Windows, IBM Corporation). For categorical variables, counts with percentages were presented. Pre- and post-vaccination antibody titers (immune or non-immune; dichotomous variable) were compared using Chi-square test (or Fisher exact test if appropriate) at a significance level of $P<0.05$. Immunogenicity and safety analyses were based on the eligible immunized subjects who completed the study and provided pre- and post-vaccination blood samples.

RESULTS

A total of 900 participants were enrolled, 300 participants in each group. Of these, 292 children (150 males) in the Shan 5 group, 285 (130 males) in the Pentabio group, and 298 (156 males) in the Pentavac group received all the three vaccine doses and were evaluated for immunogenicity and safety of vaccines (**Fig. 1**). The mean (SD) age of infants in the three groups at enrollment was 1.76 (0.36) months in Pentavac group, 1.77 (0.30) months in Pentabio group, and 1.74 (0.32) in Shan 5 group.

The vaccines had the same immunogenicity against all the five antigenic components except for pertussis. In the case of pertussis, the Pentabio vaccine exhibited significantly less immunogenicity than the other two types. **Table II** presents the percentage of immunized subjects in each group in terms of antigen.

After receiving the first to the third doses of each vaccine, the local adverse events (except for the injection-site abscess/ local reactions) were significantly different between the three vaccines. There was no significant difference among various vaccines in most systemic adverse events. Encephalitis, toxic shock syndrome, hospitalization, and death after vaccination were not observed in any of the infants. There was a significant difference in the incidence of anorexia and drowsiness between the vaccines.

DISCUSSION

This study evaluated the immunogenicity and safety of three pentavalent vaccines. Arjmand, et al. [4] showed that only 17.3% of infants receiving the Pentavac vaccine had an acceptable level of antibodies against pertussis six months after the last dose of the injection. A study on the DTP vaccine's immunogenicity against pertussis in Iran showed protective immunity in 76.8% of the participants [14]. In the present study, the most significant change, from 3.3% to 71%, in the pertussis antibody levels was related to the Shan 5 vaccine.

Consistent with the results of Gandhi et al. [1], the results showed that the Shan 5 and Pentavac vaccines were similarly efficient against all the five antigens.

Rusmil, et al. [6] reported protection rates of 99.7%, 100%, 99.3%, and 84.9% against diphtheria, tetanus, hepatitis B and pertussis, respectively. The Pentabio vaccine was reported as a suitable alternative for other certified pentavalent vaccines. [6]. In the present study, pertussis antibodies were measured by the IgG ELISA kit based on sandwich principle, and cut-off values of less than 25 U/mL were considered negative. An important caveat is that antibody testing does not reliably predict protective immunity against pertussis. Although there are no agreements upon the correlates of protection for pertussis, the data of antibody levels have been a basis on which

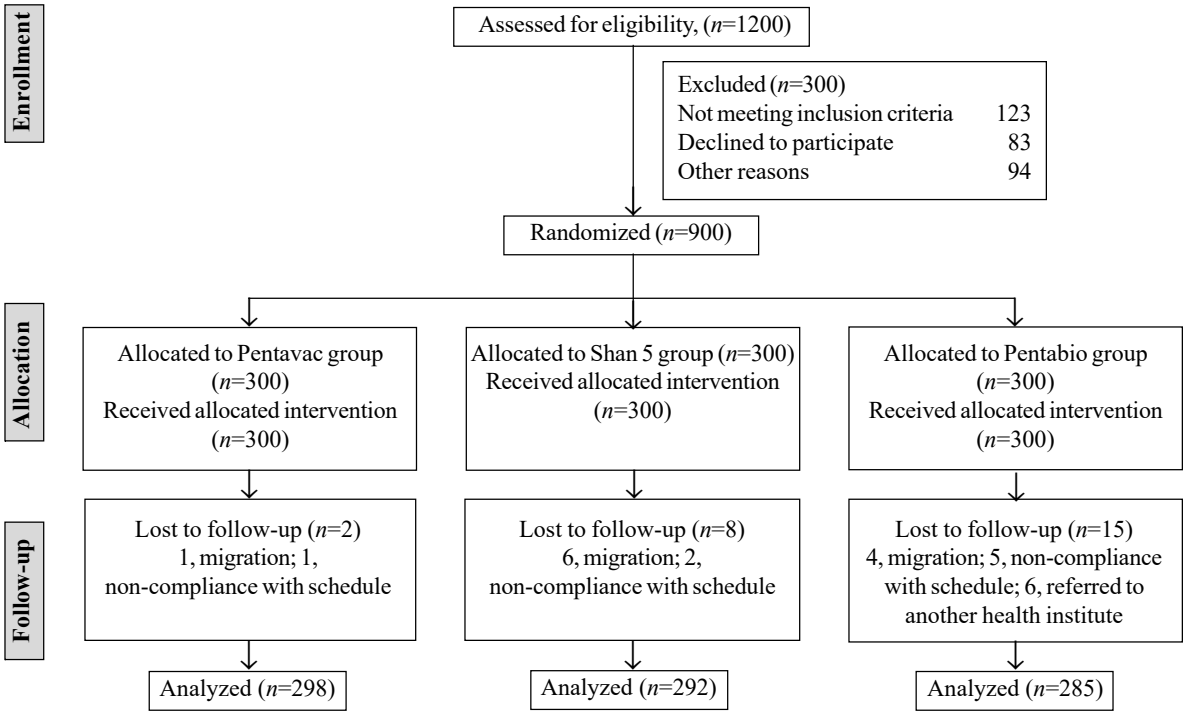

Fig.1 Study flow chart.

Table I Infants Having Post-immunization Antibody Titers Above Minimum Protective Levels After Third Dose of Injection

Immunogenicity	*Before vaccination (at the age of 2 mo)*				*After vaccination (at the age of 7 mo)*			
	A n= 298	*B n=285*	*C n =292*	*P Values*	*A n =298*	*B n=285*	*C n =292*	*P Values*
Tetanus (≥0.1 IU/mL)	245 (82.2)	251 (88)	256 (87.6)	0.15	292 (98)	282 (98.9)	287 (98.2)	0.69
Pertusis (>25 U/mL)	58 (19.5)	45 (15.7)	10 (3.4)	1 (<0.00)	199 (66.8)	131 (43.9)	207 (71)	01 (<0.0)
Diphtheria (≥0.1 IU/mL)	165 (55.4)	149 (52.2)	51 (17.5)	1 (<0.00)	271 (90.9)	255 (89.4)	268 (91.8)	0.79
Hib (≥1.0 g/mL)	262 (87.9)	240 (84.2)	271 (92.8)	0.03	296 (99.3)	280 (98.2)	290 (99.3)	0.39
HB (≥10 mIU/mL)	117 (39.3)	91 (31.9)	110 (37.7)	0.19	272 (91.3)	244 (85.6)	263 (90.0)	0.13

Data presented as no. (%). A, B, C indicate Pentavac, Pentabio, and Shan 5 vaccines, respectively. P values indicate difference differences between the babies in the three groups.

other whole cell pertussis-containing vaccines have been licensed and in routine use [17].

A recent review [18] comparing combined DTP-HepB-Hib vaccine with separately administered DTP-HepB and Hib vaccines showed that minor adverse events such as pain and redness were more common in children given the combined vaccine. Consistent with the results of Sharma, et al. [16], our results showed that stiffness, pain, and redness of the injection site were the most common local adverse events. Dalvi, et al. [19] reported tenderness as the commonest local reaction in infants receiving Pentavac vaccine, followed by swelling, redness and induration. Gandhi, et al. [1] showed a similar rate of redness, pain, and abscess prevalence in infants vaccinated with Pentavac and Shan 5 vaccines. These differences could be due to the differences in how the symptoms were recorded in the the studies.

Rao, et al. [7] showed that one-third of Shan 5 vaccine injections were associated with injection adverse events; the most common local complication was pain and the most common systemic complication was fever. Sharma, et al. [16] reported that Pentavac vaccine recipients had less injection-site pain and limb movement restrictions than those receiving the EasyFive vaccine. In the present study, limb movement restriction was observed only in one Pentavac vaccine recipient and only after the first injection.

A limitation of the present study was that participant's demographics other than age and sex were not recorded. In addition, the vaccine adverse events were not recorded by professionals. Center-to-center reporting bias and failure to assess the geometric mean concentration or titer of antibodies were the other, limitations of this study.

In conclusion, Pentavac vaccine has immunogenicity similar to the Shan 5 vaccine and better immunogenicity against pertussis than the Pentabio vaccine. All the three pentavalent vaccines have a good safety profile.

Ethics clearance: National Institutes for Medical Research Development; No. IR.NIMAD.REC.1395.002 dated: 4 April 2016.

Contributors: All authors approved the final version of manuscript, and are accountable for all aspects related to the study.

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WHAT IS ALREADY KNOWN?

- The comparative evaluation of safety and immunogenicity of licensed pentavalent vaccines in use in Iran is not available.

WHAT THIS STUDY ADDS?

- Pentavac vaccine had immunogenicity similar to that of Shan 5 vaccine and better immunogenicity against pertussis than the Pentabio vaccine.

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CLIPPINGS

Serum sodium concentration and mental status in children with diabetic ketoacidosis (Pediatrics. 2021;148: e2021050243)

This prospective study conducted across 13 centres in USA, used data from the Paediatric Emergency Care Applied Research Network (PECARN) Fluid Therapies Under Investigation in Diabetic Ketoacidosis Trial (FLUID) and compared children who had declines in glucose corrected sodium concentrations with those who has stable or rising concentrations. Children (2-18 years) with DKA, were randomly assigned to 1 of 4 intravenous fluid protocol which differed in infusion rates and sodium concentrations. Data from the first 4, 8, and 12 hours of treatment were analyzed for 1251, 1086, and 877 episodes, respectively. On multivariate analysis, children who had higher sodium and chloride concentrations at presentation and who were previously diagnosed with diabetes had significant declines in glucose corrected sodium concentrations. rates of fluid infusion and 0.45% normal saline was also associated with declines in glucose corrected sodium levels however, higher rates of fluid infusion were associated with declines in sodium concentration only at 12 hours. The risk of cerebral injury was similar in patients with and without declines in glucose corrected sodium concentrations. Therefore, this study highlights that patient who have high sodium concentrations at presentation should be carefully monitored for sodium levels so that timely intervention can be initiated.

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RESEARCH PAPER

Profile of Children With Tuberculosis in a Pediatric Intensive Care Unit in Mumbai

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Objective: This study was done to analyze the profile of confirmed pediatric TB patients seen in an intensive care setting.

Methods: Data of all children admitted in our PICU with bacteriologically proven tuberculosis (smear, culture, poly-merase chain reaction, genotypic testing or Pyrosequencing) between January, 2007 and December, 2019 were retrieved. Drug resistance was classified as per World Health Organization definitions.

Results: 59 children (28 boys) met the inclusion criteria (median (IQR) age 8 (4,13) years). About a third (22/59) had past history of treatment with antituberculosis drugs. The indications for admission to PICU were monitoring and management of neurological status in 31 children, post procedure monitoring in 20 children and respiratory failure in 8 children. Severe ARDS was seen in 2 children. Out of 37 children with neuro-tuberculosis, 19 children had TB in additional sites, and 9 children died. Sample positivity rate for CSF culture was 66%. Drug sensitivity testing (DST) of positive culture was done in 35 cases and showed multidrug resistance in 4 children, pre-XDR (extreme drug resistance) in 10 and XDR in 5 children.

Conclusion: Neurotuberculosis was the commonest reason for admission to PICU. Concerted efforts should be made to obtain samples for culture and drug sensitivity testing in critically ill children with tuberculosis.

Keywords: *Drug-resistance, Management, Mortality, Neurotuberculosis, Outcome.*

Tuberculosis continues to be an important cause of morbidity and mortality [1]. The commonest reasons for intensive care unit admission in adults with tuberculosis are acute respiratory failure, multi-organ dysfunction and shock [2]. There is limited data on critically ill children with tuberculosis [3,4].

METHODS

All children admitted to the pediatric intensive care unit (PICU) with suspected tuberculosis between January, 2007 and December, 2019 were identified from the PICU database and hospital medical charts. Those with bacteriologically proven tuberculosis (smear, culture, or molecular testing) were then studied. A chart review was carried out to extract clinical details including age, sex, weight, history of anti-tubercular treatment (ATT), history of contact, time from symptom onset to diagnosis, tuberculosis location, investigations and outcome. Ethics committee approval was obtained prior to start of the study.

Bacterial TB culture was obtained by Mycobacterial Growth Indicator Tube (MGIT) 960 system (Becton Dickinson). The following molecular tests were used: polymerase chain reaction (PCR), CBNAAT using Xpert MTB/RIF assay (Cepheid), Line probe assay (Genotype MTBDR plus, Hain life science) and pyrosequencing reactions were conducted using a repurposed Qiagen PyroMark Q96 ID system (Qiagen, Valencia). Genotyping testing and drug sensitivity testing were performed at the discretion of the treating physician. Clinical and laboratory standards were used for drug sensitivity testing (DST) by phenotypic testing. Drug resistance was classified as per WHO definitions [5].

Statistical analysis: Data recording was done in MS Excel format. Descriptive statistics are used to present the data.

RESULTS

The total number of PICU admissions during the study period was 4628. There were 75 patients suspected to have tuberculosis and 108 samples were sent from these children. Fifty nine children were bacteriologically proven tuberculosis; mycobacterial culture or smear was positive in 48 patients, molecular testing or pyrosequencing were

positive in an additional 11. The cultutre positivity from 108 samples were as follows: 28/42 for cerebrospinal fluid, 13/22 for bronchoalevolar lavage, 16/23 for tissue, 4/7 for ascitic/ pleural/pericardial fluid and 1/8 for gastric lavage.

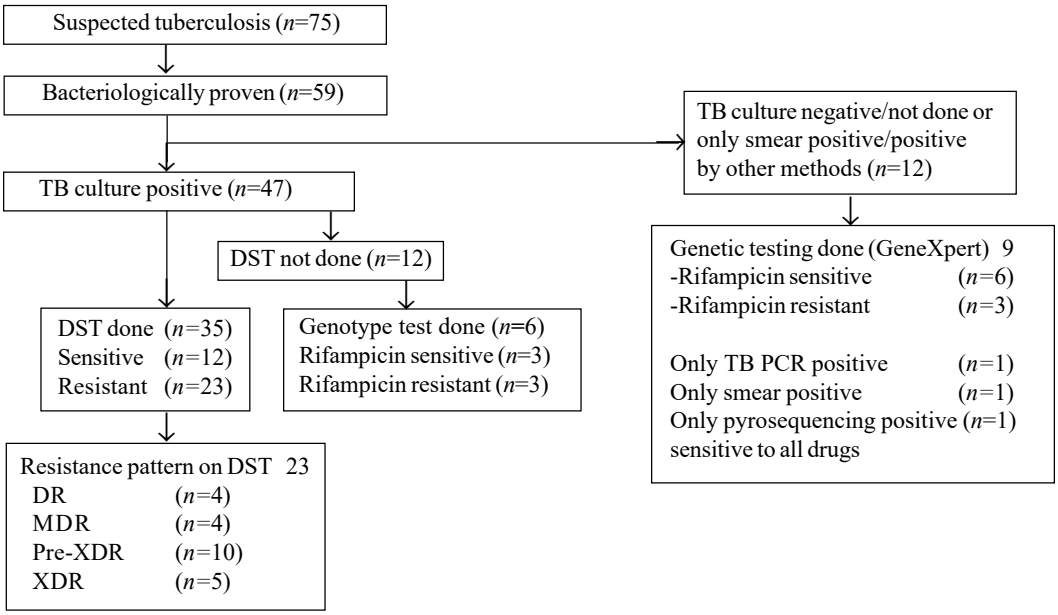
Data of only these 59 children (28 boys) were further analyzed. The median (IQR) age was 8 (4,13) years. Indication for the initial admission to PICU were as follows: 31 children were admitted for monitoring of neurological status (10 for management of raised intracranial pressure, 17 for altered sensorium, and 4 for status epilepticus), 20 for procedure or post- operative monitoring (post-operative 12, BAL 7), and eight with respiratory failure of whom two children had severe acute respiratory distress syndrome (ARDS) and three had underlying neuromuscular disease with pneumonia, and 3 had inability to maintain airway. Operative procedures included spinal cord decompression, pneumonectomy, pericardiectomy, hemicolectomy, and temporal lobe granuloma biopsy.

The commonest symptom was fever seen in 45 (76%) patients. Neurotuberculosis was present in 37 children (62%) of whom 19 also had tuberculosis in additional sites (lung, spine, peritoneum, abdomen, eyes). Tuberculosis of the spine was seen in 8 children. There were five children on immune-suppressive therapy (chemotherapy for malignancy or long term steroids), 26 children (44%) were malnourished, one child was HIV-positive, and one was on home ventilator therapy for a congenital myopathy.

History of treatment with ATT for more than 1 month was found in 22 children (37%) with a median (IQR) duration of 5 (1,9) months. History of contact with a case of tuberculosis in the previous two years was seen in 9 (15%) children ; 2 children had family contacts with poor compliance for ATT, 1 child had lost both parents due to tuberculosis and HIV, and for 1 child DST of the contact (Extremely drug resistant TB, XDR TB) was available. The median (IQR) time to appropriate ATT was 2 (2,6) months. Chest *X*-Ray was abnormal in 30 children; miliary TB was seen in 9, cavitation in 4 (2 were younger than 6 months), parenchymal lesions in 9, nodal involvement in 3 and mixed lesions in 5 children.

Mycobacterial culture was positive in 62 samples from 47 patients (47/75, 62%). Tuberculosis was confirmed by smear or molecular testing in an additional 12 patients (**Fig. 1**). Drug sensitivity testing (DST) was done in 35 (74%) cases out of 47 with positive MTB cultures; multi-drug resistance (MDR) was seen in isolates from 4, pre-extensively drug resistant (pre-XDR) in 10 and XDRTB in 5 children. An additional 4 children had isolates showing sensitivity to rifampicin but drug resistance to INH and/or streptomycin and/or ethambutol and/or ethionamide. All isolates were sensitive to clofazimine (**Web Fig. 1**).

GeneXpert testing was positive in an additional 15 patients who were culture negative or culture positive but DST was not done, and showed rifampicin resistance in 6.



DST: drug sensitivity testing; Drug resistance: resistance to streptomycin and/or INH and/or ethambutol and/or ethionamide; multidrug resistance: resistance to INH, rifampicin; pre-XDR: resistance to INH, rifampicin, any fluoroquinolone; XDR: resistance to INH, rifampicin, any fluoroquinolone (oofloxacin, moxifloxacin) and any injectable second line aminoglycoside (amikacin, capreomycin, kamamycin).

Fig. 1 Mycobacterial testing in patients with suspected tuberculosis.

These children were treated as MDR-TB. Pyrosequen-cing was done in 1 child who was GeneXpert and culture negative and was positive for sensitive mycobacteria. Drug sensitivity or GeneXpert testing was not available in 8 children who were treated with first lineATT.

Mechanical ventilation was needed in 12 children, of whom 2 had acute respiratory distress syndrome (ARDS), 3 had pneumonia with underlying neuromuscular disorder and 7 had raised intracranial pressure or inability to maintain airway. Inotropic support was needed in 3 children; 2 for peritonitis with septic shock and 1 for severe ARDS with shock and multi organ dysfunction,

On discharge after their first ICU admission, 36 children had improved, 19 had sequelae and 4 children died. A further 4 children died during subsequent admissions to PICU; 3 children within 6 months of presentation and 1 child after 2 years of treatment failure. An additional child with severe sequelae post-TB meningitis died at home 8 months after presentation. All 9 children who died had TB meningitis, and apart from 1 child, the rest had involvement at other sites as well (lung =7, spine=5, abdomen=3, eye=1). The causes of death included extensive infarcts from vasculitis, ARDS, peritonitis with septic shock, aspiration pneumonia and ventriculitis. The sequelae included hydrocephalus, stroke, paraparesis, cognitive deficit, seizures, optic atrophy, visual deficit and hearing loss. Six children had multiple ICU admissions for ventriculo-peritoneal shunt related complications.

DISCUSSION

Children with tuberculosis may need intensive care for tuberculous meningitis, ARDS or septic shock [6]. Majority of children with tuberculosis requiring ICU admission in our study had neuro-tuberculosis, similar to a study from Pune [3]. However, a study from South Africa reported that 72% of 57 children were admitted with respiratory failure and only 8 children had tuberculous meningitis [7]. Although chest *X*-ray findings were abnormal in 30 children in our study, severe ARDS was seen in only two children. A case series from Europe of children with miliary tuberculosis involving lung and brain showed that vasculitis due to TB meningitis leads to poor outcome rather than the lung involvement; in fact the extent of lung involvement on chest *X*-ray did not correlate with respiratory insufficiency [6]. Children with TB meningitis had a high mortality (24%), similar to previous reports [3,7].

An important finding in the study is the high rates of culture positivity in our patients (66%) – many of them having neurotuberculosis, a particularly pauci-bacillary disease thought to have low rates of culture positivity. Earlier rates of positive cultures in clinically diagnosed tuberculous meningitis were often as low as 10-20%, especially from developing countries [8]. However, studies from Vietnam have shown a high rate of bacteriologic confirmation similar to ours (positive smear in 58% and culture in 71%), especially with use of large CSF volume and increased time spent while doing microscopy [9]. It is also possible that the high rates of MDR-TB may have contributed to a higher bacteriological load with lower rates of culture-conversion in treated patients, as has been demonstrated in sputum samples of pulmonary TB [10]. In addition, diagnostic yield was increased by sampling from multiple sites such as broncho-alveolar lavage, and pleural and ascitic fluid [11].

Similar to our findings, increasing drug resistance is being reported in children [12]. Out of 21 TB patients who were resistant to rifampicin on DST, 13 were resistant to quinolones, which would be missed by Gene Xpert testing. It is therefore important to make all efforts to perform drug sensitivity and avoid treating only on the basis of GeneXpert [13]. A meta-analysis of 8955 patients with MDR-TB and XDR-TB found that drug sensitivity testing provides useful information to guide treatment, and that in vitro susceptibility to a drug was significantly associated with treatment success. In our study, one patient was detected only by pyrosequencing with all her other tests being negative [14]. It should be used as an adjunct to culture-based DST methods due to its rapid turnaround time, especially if drug-resistant tuberculosis is suspected [14].

The limitation of our study is that besides the retrospective nature, it suffers from significant referral bias as our cohort consists mostly of severely ill patients admitted in an intensive care setting of a tertiary care center. More than a third of patients had a poor response to first line ATT. DST was also available in only 35/47 of all culture positive patients.

In a South African study by Schaaf, et al. [15], the correlation between the drug susceptibility results of the child's and adult source case's isolates was 68%. Whenever available, the source's DST should be sought and followed, and if necessary, second line drugs started early in the treatment if MDR-TB has been found in the source. It is important to do screening and follow up of all children who are household contacts of adult cases with MDR-TB; this would need collaboration between adult physicians and pediatricians.

In conclusion, neurotuberculosis was the commonest presentation of children with tuberculosis needing PICU admission in our cohort. In children with suspected tuberculosis, all efforts should be made to get samples for culture and drug sensitivity testing.

WHAT THIS STUDY ADDS?

- Critically ill children with tuberculosis most commonly present with neurotuberculosis.
- Drug sensitivity testing /other available tests should be done early to determine appropriate treatment at the first presentation.

Ethics clearance: Institutional ethics committee: PD Hinduja Hospital and Medical Research Center; No.1426- 20 - SR dated Dec 17, 2020.
Contributors: SR, RS and DW did the data collection and initial preparation of work. SU and VU contributed to the conception and design of the work, finalization of the draft and critical revision of the work. All authors approved the final version of manuscript and are accountable for all aspects related to the study.
Funding: None; *Competing interests*: None stated.
Note: Additional material related to this study is available with the online version at *www.indianpediatrics.net*

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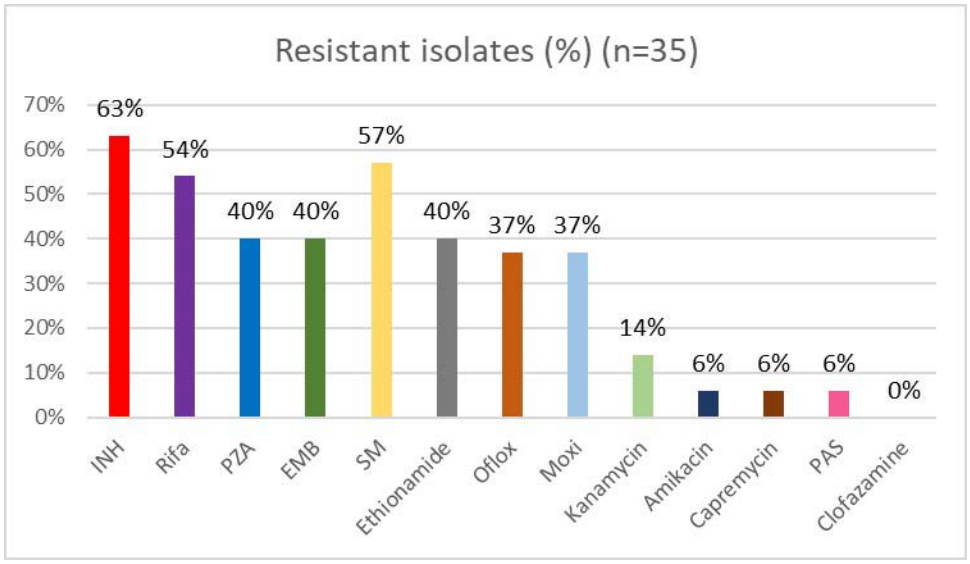

Web Fig. I. *Resistance to anti-tuberculosis drugs on drug sensitivity testing of mycobacterium tuberculosis isolates from 35 patients.*

RESEARCH PAPER

Efficacy of Growth Hormone Treatment in Children With Chronic Kidney Disease: Tunisian Experience

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Objective: To study the effect of using recombinant human growth hormone (rhGH) in growth retarded children with chronic kidney disease (CKD) **Methods**: This was a non-randomized controlled study over 2 years including children in CKD stages 4-5 suffering from growth retardation. Children were divided into rhGH-treated or non-rhGH treated groups. **Results**: A total of 70 children (35 in each group) were enrolled. While the mean (SD) height of 35 children with CKD had increased from 109.5 (26) cm to 116 (26) cm (mean growth velocity 6.5 cm/year; *P*=0.09) prior to rhGH therapy, the same was found to increase from 116 (26) cm at the start of therapy to 125 (25) cm after one year of therapy (*P*=0.02). **Conclusions**: Therapy with rhGH was helpful in catch-up growth in Tunisian children with CKD.

Keywords: *Renal failure, Growth failure, Height velocity.*

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Chronic kidney disease (CKD) is defined as the presence of kidney damage or an estimated glomerular filtration rate (eGFR) less than 60mL/min/1.73 m^2, persisting for three months or more [1]. Growth retardation, which is a major problem for many children with CKD, can result from several causes like cachexia, chronic vomiting, metabolic acidosis, fluid and electrolyte loss, decreased levels of Insulin-like-growth factor 1 (IGF1) and its receptor, puberty delay, renal osteodystrophy and the use of certain growth-inhibiting treatments. However, random fasting serum levels of GH are normal or increased in these children with CKD [2]. The primary aim of this study was to assess whether recombinant human growth hormone (rhGH) therapy was associated with improvement of height and weight in children with CKD. Secondary aims were to assess whether serum protein, hemoglobin level and parathyroid hormone (PTH) had an impact on the growth in children with CKD receiving rhGH therapy.

METHODS

This observational study was conducted in the Pediatric Nephrology Unit of Charles Nicolle Hospital from January, 2016 to December, 2018. Children in CKD stages 4-5 with growth retardation were included and divided into rhGH-treated (Group A) or non-rhGH treated (Group B). Staging was based on eGFR, calculated by the Schwartz formula [3]. After evaluation of the history, anthropometric parameters, clinical examination and routine laboratory investigations, the children were enrolled in the study. Those with growth retardation due to a pathology other than CKD, those with severe osteodystrophy or with contraindications to rhGH were excluded.

As per recommendations for the use of rhGH for children with CKD by the National Kidney Foundation (NKF) 2005 [6], hip *X*-ray and a wrist *X*-ray for bone age estimation were performed, prior to initiation of GH therapy, in children of Group A . Also, GH therapy was not initiated until the PTH and phosphorus levels were greater than 1.5-times the upper limit for age. All rhGH-treated cases and non-rhGH-treated controls were pre-pubertal at study entry. Children with CKD stage 4-5 in regular follow-up, who did not receive rhGH treatment because of socioeconomic issues, or because parents refusal, were enrolled as controls (Group B).

Both cases and controls were regularly followed up by an experienced dietician, ensuring adequate nutritional intake of 100% of the recommended energy intake and 100-120% of the recommended protein intake [4]. Metabolic acidosis and anemia, were treated as per the recommendations of the KDOQI Guidelines [4,5]. Thyroid function was evaluated at the beginning as well as during the course of the study for both groups.

Statistical analysis: Analysis was done on SPSS software, version 23. Unpaired independent *t* test was used to compare the two groups, taking a *P*-value <0.05 as statistically significant.

Parental consent was taken and approval from hospital's ethics committee was obtained before starting the study.

RESULTS

Thirty-five children in each of group A and group B were included in our study. Baseline characteristics of both groups are represented in **Table I**. While the rate of increase in mean height, over the one year prior to initiation of GH therapy was 7.2 cm/year, the rate increased significantly (9 cm/year) after therapy (P=0.02) (**Table II**). Infants had the highest growth rate (13.5 cm/y), followed by children aged over the age of 12 years (7.35 cm/y). The statural gain was 7.27 cm/y for children aged 4 to 12 years, and 6.5 cm/y for children aged 2 to 4 years. This difference in growth rate across age groups was statistically significant (P=0.036). We also noted an increase of 3.9 kg in weight after one year of treatment (P=0.39). The increase in height and weight was significantly greater in treated children compared to the untreated group (**Table II**). Two children from group A, and 5 children from group B entered puberty, the average delay was 10 months for treated children and 16.25 months for the control group. The mean statural gain was 6.25 cm/y for group A and 5.5 cm/y for group B (P=0.7).

We found that none of the parameters like serum protein, hemoglobin, or PTH levels had an influence on growth (P=0.367, P=0.203, and P=0.841, respectively).

Table I Baseline Characteristics of Children With CKD

	rhGH-treated cases (n=35)	*Non-rhGH-treated controls (n=35)*
Age at baseline (y)	9.7(1.1)	9.4 (1.8)
Males[a]	20 (57.1)	26 (74.3)
Age groups[a]		
< 2 y	3 (8)	2 (5)
2-4 y	2 (7)	3 (10)
4-12 y	18 (50)	23 (65)
> 12 y	12 (35)	7 (20)
Dialysis[a]	HD: 11 (33)	HD: 14 (40)
Height SDS at baseline	-2.5 (1.2)	-2.3 (1.02)
Height <-2 SDS (%)[a]	30 (85)	29 (83)
BMI SDS	-0.7 (1.25)	-0.65 (1.13)
Etiologies of CKD[a]		
CAKUT	26 (74)	31 (85.7)
Glomerular nephropathies	2 (6)	1 (2.8)
Hereditary nephritis	4 (11)	1 (2.8)
Urea (mg/dL)	172.3 (3.0)	163 (2.4)
Creatinine (mg/dL)	5.27 (1.9)	4.68 (1.6)
Hemoglobin (g/dL)	9.9 (1.3)	9.58 (1.0)
Serum total protein (g/L)	69.2 (5.2)	67 (4.3)
Serum albumin (g/L)	37.2 (2.4)	36 (1.9)
PTH (pmol/L)	456 (23.9)	389 (22.0)

Values in mean (SD) or [a]no. (%). All P values>0.05. Unknown etiology in 3 and 2 children in the two groups, respectively. CAKUT: congenital anomalies of kidney and urinary tract, CKD: chronic kidney disease, BMI: body mass index, PTH: parathyroid hormone. HD:hemodialysis.

DISCUSSION

Impairment of linear growth in children with CKD reflects both the severity of renal disease and the quality of health care. Failure to grow is most pronounced if renal diseases arise during those vulnerable phases of life when growth velocity in healthy children is at its maximum: during the first year of life and during puberty [7-8]. We found that, without use of rhGH, our patients gained a mean of 7.2 cm/year in height and mean of 2.2 Kg/year in weight, which is the result of comprehensive management including nutritional and conservative treatment [9]. After receiving rhGH, patients gained height at a mean velocity of 9 cm/year, higher than the normal velocity of 5 cm/year. Our results are comparable to the report published by the Food and Drug Administration in 1987, where the annual growth velocity increased from 4.94 (1.4) cm/year for the year before treatment to 10.08 (1.97) cm/year after treatment (P<0.01). A subsequent report published in 1989 noted that the actual velocity after one year of treatment in these five children was 9.8 (1.2) cm/year (P=0.006).

Our study shows significant increase in mean weight after rhGH therapy, of 3.9 kg/year, indicating that GH might help in improving weight, though nutritional care remains the cornerstone for optimal weight gain. Moreover, by comparing the rate of increase in weight in the year before

Table II Comparison of Changes in Height and Weight in Treated and Control Groups

Time point	*Height (cm)*		*Weight (kg)*	
	Group A n=35	*Group B n=35*	*Group A n=35*	*Group B n=35*
1 y before initiation of therapy	109.5 (26)	110 (15)	18.9 (9)	18.1 (7)
At initiation of therapy	116 (26)	115 (9)	21.1 (9)	20.7 (7.05)
After 6 mo of therapy	122.5 (25)	120 (20)	23.6 (10)	22.2 (8)
After 1 y of therapy[a]	125 (25)	120.7 (21)	25 (11)	22.9 (8.1)

Values in mean (SD). [a]P=0.03 both for height and weight comparison between the groups.

WHAT THIS STUDY ADDS?

- This study documents the favorable response of recombinant growth hormone therapy in Tunisian children with chronic kidney disease.

and after therapy, it appears that the nutritional care and routine treatment given to those patients are the main determinants of the weight gain and growth hormone therapy only affects it to a minor degree, in comparison to its effect on height velocity effect [10-13]. As GH therapy raises serum level of PTH, it is advisable to have a close follow-up of PTH levels and readjustment according to the NKF recommendations [14,15].

The limitation of our study is the small number of patients and a non-randomized recruitment design of the study.

To conclude, growth retardation is a major complication of children with CKD. In our study, we found that giving rhGH to children with CKD in Tunisia helps them to catch up their growth due to an increase in the growth velocity. rhGH therapy may be considered in the treatment regimen of these children, provided other factors exacerbating growth retardation are corrected prior to initiation of therapy.

Ethics clearance: Ethic committee of Charles Nicolle Hospital; No. Tun23032016, dated March 23, 2016.
Contributors: AB: concept and designed the study, analyzed data and drafted the manuscript; RL: collected the data, helped in data analysis; MJ: helped in collecting data, and manuscript drafting; ON: supervised cognitive and behavioral assessments; TG: supervised cognitive and behavioral assessments;
Funding: None; *Competing interest*: None stated.

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RESEARCH PAPER

Serum Ferritin as a Diagnostic Biomarker for Severity of Childhood Sepsis

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Objective: To explore association between serum ferritin and severity of sepsis among children, and relate levels to the final outcome. **Methods**: This observational study was conducted in a tertiary care hospital between I February and 30 July, 2019. Serum ferritin level was estimated in children (age 6 months to 12 years) suffering from sepsis, irrespective of the probable etiology. Children with hemoglobinopathies, autoimmune diseases, previous blood transfusion, severe acute malnutrition, hemophagocytic lymphohistiocytosis and chronic hepatitis were excluded. The ferritin level was measured sequentially at pre-defined stages of illness viz., sepsis, severe sepsis, septic shock and multiorgan dysfunction syndrome (MODS). Association between serum ferritin and severity of sepsis was analyzed, and ferritin level was related to the final outcome of death or recovery by receiver operating characteristic (ROC) curve analysis. **Results**: The study group included 47 children with sepsis who progressed to a state of MODS; 32 recovered from MODS. Significant differences in serum ferritin level were observed with severity of sepsis. There was clear demarcation of ferritin levels between sepsis severity stages. The proportion of death among the 47 MODS cases was 31.9% (95% CI 18.6 – 45.2%). ROC analysis in the MODS group indicated that serum ferritin >1994.3 ng/mL predicts mortality (AUC 0.73 [95% CI 0.58-0.85]) with sensitivity 66.7% [95% CI 38.4-88%] and specificity 100.0% [95% CI 89.1-100%]. **Conclusions**: There is clear demarcation of serum ferritin levels that can help differentiation of sepsis severity stages in children with sepsis. There is no such demarcation between survivors and non-survivors in MODS cases.

Keywords: *Mortality*, *Multiorgan dysfunction syndrome, Outcome, Septic shock*.

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Sepsis in pediatric age group contributes approximately 10-15% to childhood mortality worldwide, with the risk being higher among those admitted to pediatric emergency in state of septic shock [1]. In the natural course of sepsis, development of septic shock and multiorgan dysfunction syndrome (MODS) have several determinants [2,3]. Categorizing the patients into sepsis stages has a role in administering appropriate management and it is done largely on the basis of clinical features and laboratory investigations [4,5]. Hence, attempts have been made to identify biomarkers in blood samples that can reliably indicate severity stage transitions in the absence of florid signs and symptoms [6,7]. However, serum ferritin has not been adequately studied in sepsis in children as a marker of severity of sepsis [8]. We have tried to address this lacuna through a prospective observational study in a tertiary care setting.

METHODS

The study was conducted between February and July, 2019 after approval from the institutional ethics committee. Children of either sex, between 6 months to 12 years of age, admitted with proven sepsis, were screened. These children were included through purposive sampling. Children with significant comorbidities like hemoglobinopathies, autoimmune diseases, previous blood transfusion, severe acute malnutrition, hemophagocytic lymphohistiocytosis and chronic hepatic illness were excluded. While the subjects were managed conservatively in intensive care, few amongst them deteriorated further to MODS and some eventually succumbed to multiorgan failure. Those who recovered were followed-up for their clinical condition till one week after being discharged or shifted from intensive care. Definitions adopted by International pediatric sepsis consensus were followed to define the different stages of sepsis [9].

The predetermined phases evaluated for serum ferritin level were the stages of sepsis, severe sepsis or septic shock and MODS. The stage of severe sepsis and septic shock were clubbed together into one stage to avoid bias in categorizing them as small margin of discrimination allowed in their differentiation criteria. Another blood sample for ferritin level assay was obtained at 7th day post-recovery in subjects who recovered from MODS. The ferritin level was measured by electrochemilumine scence immunoassay (ECLIA) using COBAS E411 analyzer (Roche Diagnostics). The lower and upper limits of quantification were 0.1 ng/mL and 2000 ng/mL, respectively.

Statistical analysis: Comparison between subgroups was done using Mann-Whiney U test, with $P< 0.05$ as the cut-off for statistical significance in such comparisons. Receiver operating characteristics (ROC) analysis was done to identify cut-offs of serum ferritin for predicting poor outcome.

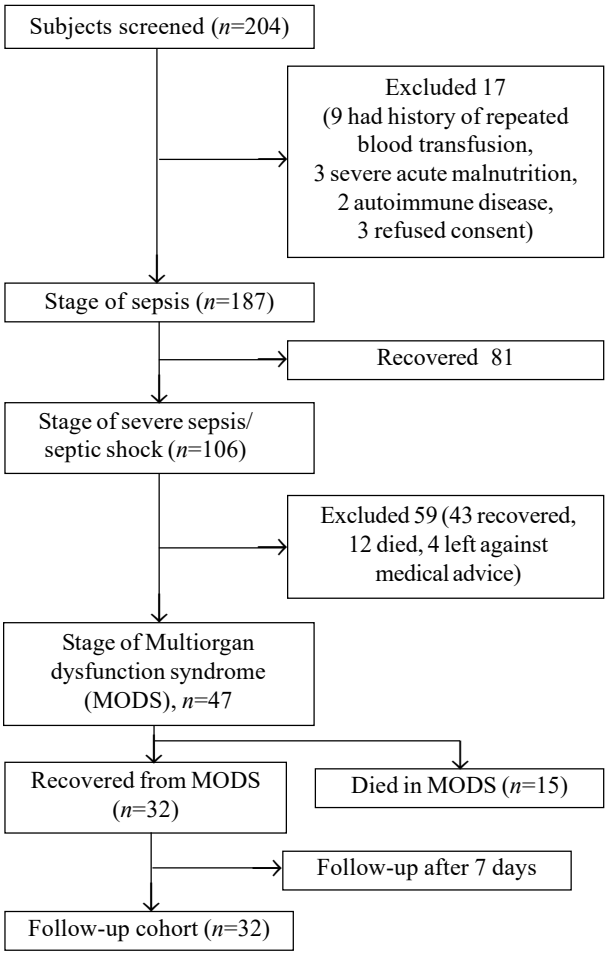

Fig. 1 Study flow chart.

RESULTS

We screened 204 children and finally 187 children with sepsis were recruited; of these 47 (25.13%) advanced from the stage of severe sepsis or septic shock to the stage of MODS (**Fig. 1)**. Within the MODS group, 15 children died (31.9%, 95% CI 18.6-45.2%) and 32 recovered, who were followed-up for one-week post-discharge or transfer-out from the intensive care unit.

The median (IQR) serum ferritin amongst MODS subjects were 371.5 ng/mL (265.1-442.33), 892.2 ng/mL (764.5-1029.4), and 1784.9 ng/mL (1657.5-1916.4) in the stage of sepsis, stage of severe sepsis or shock, and stage of MODS, respectively. The change of serum ferritin level during transition from one phase to another was found to be significant ($P<0.001$). There was statistically significant difference between the ferritin level in severe sepsis versus MODS ($P<0.001$). The ferritin level median (IQR) observed in the recovery cohort was 808.85 ng/mL (672.5-929.8) at one-week post-discharge or transfer-out from ICU. The trend of ferritin levels found at different stages along the course of illness among the children who recovered from sepsis induced MODS are depicted graphically in **Fig. 2**.

Correlation analysis in the MODS cohort indicated weak to moderate correlation between the ferritin levels across the spectrum of illness (Spearman's rank correla-tion coefficient 0.454 between sepsis and severe sepsis; 0.367 between severe sepsis and MODS). This indicates that although there is elevation of ferritin as a whole, the pattern in individual subjects is variable. **Table I** presents the descriptive summary of serum ferritin levels in survivors and non-survivors in the MODS cohort, along their spectrum of illness. The data indicate that there is

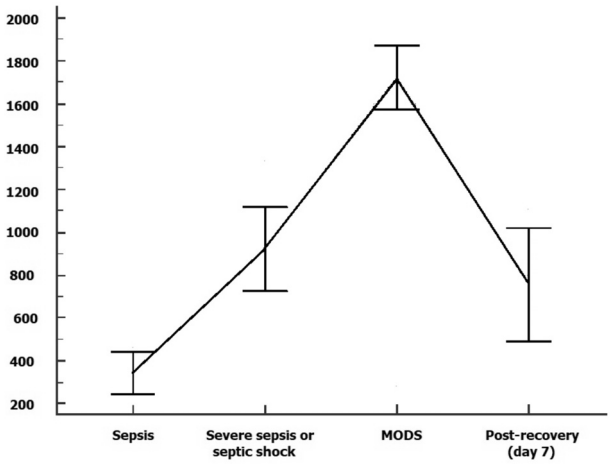

Fig. 2 Ferritin level (ng/mL) at successive stages of illness in children with sepsis who recovered. Error bars indicate median (IQR).

WHAT THIS STUDY ADDS?

- Serum ferritin levels can help in demarcation of sepsis severity stages in children.

Table I Serum Ferritin in Children With Sepsis-Induced Multiorgan Dysfunction Syndrome (MODS) (*N* = 47)

Severity of illness	*Death cohort (n=15)*	*Recovered cohort (n=32)*	*P value*
Stage of sepsis	378.6 (249.93-451.73)	358.1 (280.55-433.35)	0.715
Stage of severe sepsis / shock	879.2 (649.83-1032.23)	895.2 (788.40-1012.75)	0.615
Stage of MODS	2001.0 (1700.60-2001.00)	1750.2 (1615.60-1837.30)	0.011
Stage of recovery	–	808.8 (672.50-929.80)	–

Ferritin values are in median (IQR) ng/mL.

statistically significant difference only in the MODS stage, the median value being higher in the non-survivors compared to survivors (*P*=0.011). Accordingly, ROC curve analysis of serum ferritin in these subjects to identify mortality cut-offs was successful only in the MODS stage – serum ferritin >1994.3 ng/mL predicted mortality (area under ROC curve 0.73 [95% CI 0.58-0.85]) with sensitivity 66.7% [95% CI 38.4-88%] and specificity 100% [95% CI 89.1-100%].

DISCUSSION

Classically, ferritin biology focuses on its role in iron storage and homeostasis, with low ferritin levels indicative of deficiency and high levels suggesting primary or secondary hemochromatosis. However, iron, redox biology and inflammation are linked and serum ferritin has been established as an acute phase reactant [10]. Its rise can be observed with inflammatory response mediated through various pro-inflammatory cytokine stimulants like tumor necrosis factor, interleukin-1 and interleukin-6 [10,11].

In children, sepsis staging can be difficult because differentiating signs are often subtle [12-14]. Therefore, a single biomarker that can reliably differentiate stages in the spectrum of sepsis illness can be quite helpful in guiding management step-up and prognostication. In the present study, a significant rise of serum ferritin value could be observed among children who suffered from sepsis induced MODS. The study indicates that ferritin can fulfill the differentiating biomarker role in childhood sepsis and, although there is some overlap at extremes of range.

Earlier studies have reported that high serum ferritin value is associated with unfavorable outcomes in pediatric sepsis [15]. In our study the highest values occurred in the MODS stage and a cut-off value of serum ferritin of nearly 2000 ng/mL could be identified to predict mortality. However, no cut-off could be identified for the earlier stages. Garcia, et al. [8] studied ferritin levels in children with severe sepsis and septic shock and found that level >500 ng/mL was associated with 58% mortality. Our study had mortality of around 32% in the MODS stage.

Consistent with the fact that ferritin is an acute phase reactant, the levels declined with resolution of the sepsis and the 7-day post-recovery values were considerably lower than the peak levels attained in the MODS state. However, we did not follow-up these children till normalization of ferritin levels. Despite the limitations of our study, we can conclude that there is clear demarcation of serum ferritin levels which can help differentiation of sepsis severity stages in children with sepsis. There is no such demarcation between survivors and non-survivors in MODS cases, but serum ferritin >1994.3 ng/mL is associated with poor outcome in MODS cases.

Ethics clearance: Institutional ethics committee of NB Medical College; No. IEC/NBMC/2018-19/91, dated: February 01, 2019.
Contributors: AN: primary investigator, data collection, making draft; TM: making draft, literature search, interpretation, statistical help; DD: draft, patient management, literature search; SR: study review, Review draft and interpretation; NK: technical inputs, data Collection, data interpretation; IM: reviewing draft, study interpretation, literature search: AH: literature search, review draft, statistical analysis; RM: conception of study, reviewing draft, study design, and literature search.
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CLIPPINGS

Breaking bad news: what parents would like you to know (Arch Dis Child 2021;106:276-281)

This is a qualitative study conducted in Netherlands consisting of a constant comparative analysis of in-depth interviews conducted with parents to analyse parents' experiences (barriers and facilitators) of communication of bad news. The study was conducted on bereaved and nonbereaved parents of children (1-12 years) with life threatening conditions. The parents were interviewed face to face at their place of choice (residence). The interviews were recorded on audio media and the transcribed verbatim. Sixty-four parents of 44 children with life threatening condition were interviewed, 24 of them were bereaved. Both facilitators and barriers of communication of bad news were part of interview. Parents were very explicit about barriers to good communication of good news. Ten barriers to good communication like lack of (timely) communi-cation, physicians' failure to ask parents for input, parents feel unprepared during and after the conversation, a lack of clarity about future treatment, physicians' failure to voice uncer-tainties, physicians failure to schedule follow-up conversations, presence of too many or unknown healthcare professionals, parental concerns in breaking bad news to children, managing indications of bad news in non-conversational contexts, and parents' misunderstanding of medical terminology were identified and analysed. The authors concluded that health care professionals should timely communicate the bad news in a language that is understandable to parents. An appropriate place should be chosen for communication and accompanying professionals should be introduced to parents. The results provide practical pointers on how the communication of bad news can be improved to better suit the needs of parent.

Anti-cardiolipin antibody/D dimer/C-reactive protein and coronary artery lesions/multiple-organ damage in children with Kawasaki disease (Front Pediatr. 2021; 9:704929)

This retrospective study was conducted with the objective to study the corelation of anti-cardiolipin antibody (ACA), D dimer, C reactive protein (CRP), coronary artery lesions (CAL) and multiple-organ lesions in children with Kawasaki disease (KD). Two hundred and eighty-four children with KD and incomplete KD (iKD) were analysed from May 2015 to April 2016. Patients were divided into six groups namely ACA+ group and ACA- group, elevated D dimer group (DDE) and normal D dimer group (DDN), coronary artery injury (CAL) group and non-coronary artery injury (NCAL) group. ACA was most likely tested positive in younger KD children (P<0.05). ACA+ and hypoproteinaemia were correlated with CAL, thrombocytosis, and granulocytopenia (p < 0.05–0.01). There were no significant differences in myocardial and liver damages between CAL and No-CAL groups (P>0.05). CAL occurred frequently in patients who were younger, with prolonged fever, later IVIG treatment, CRP elevated over 100 mg/L. However, three were no significant difference (P>0.05). In the KD with DDE group, the incidence of granulopenia, thrombocytosis, myocardial damage, cholestasis, hypoproteinaemia, and aseptic urethritis was significantly higher than that in the KD with DDN group (P<0.05–0.01). However, elevated D dimer was not associated with CAL, whereas CRP elevation was highly correlated with D dimer, but not with CAL. Therefore, this study concluded a higher incidence of CAL and myocardial damage occurred in KD patients with positive ACA and hypoproteinaemia. An elevated D dimer was associated with increased multi organ dysfunction. CRP was closely correlated with D dimer, but were not associated with CAL and ACA.

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RESEARCH PAPER

Comparison of a Voiding Diary With Clinical Management Tool As an Outpatient Screening Tool for Childhood Functional Voiding Disorders

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Objective: To study the agreement of questionnaire-based assessment with voiding diary for differentiating primary mono-symptomatic nocturnal enuresis from voiding disorder in children. **Method**: Children 5-12 years old with bedwetting after exclusion of secondary enuresis were enrolled and parents filled a clinical management tool (CMT) questionnaire and a 48-hours voiding diary. Point prevalence and agreement of classification as primary mono-symptomatic nocturnal enuresis or voiding disorder were compared. **Results**: Of 1276 children screened, 143 (11.2%) reported enuresis. Of 100 (82 males) children finally analyzed, constipation and positive family history occurred in 14% and 37%, respectively. Questionnaire-based assessment and voiding diary identified 65% and 71%, respectively as voiding disorder [Cohen's kappa 0.542 (95%CI: 0.367-0.717)]. Discordance of classification was noted in 20%. Voiding diary identified additional 7% cases of voiding disorder. **Conclusion**: While CMT and voiding diary have moderate agreement, voiding diary should be used for cases screened negative by a questionnaire-based tool.

Keywords: *Bladder, CMT questionnaire, Enuresis, Lower urinary tract symptoms.*

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Bedwetting or enuresis, due to immaturity of bladder control, occurs in ~15% children, and may persist in 2-3% into adulthood [1]. Isolated nighttime bedwetting is labeled as primary mono-symptomatic nocturnal enuresis (PMNE). Associated daytime and lower urinary tract symptoms (LUTS) may be due to functional disturbance of micturition either in filling and/or voiding phases, in the presence of an intact neuronal pathway [2,3]. Undetected voiding disorders may result in long term adverse consequences of the urinary tract, and sexual and psychosocial functions [4].

In an outpatient setting, voiding disorder can be assessed by detailed history and clinical examination [4,5] along with questionnaire-based screening tools or voiding diary. Voiding diary enquires compliance, motivation as well as additional visits [6,7]. A questionnaire-based assessment appears more practical in the outpatient setting. Clinical management tool (CMT) is a validated one-time recall based questionnaire, developed by International Children's Continence Society (ICCS), for evaluation of enuresis in children [2]. Concerns remain about missing markers of bladder dysfunction and mislabelling a bedwetting child as PMNE based on questionnaire filled by patient/parent from recall. There is sparse data comparing CMT and voiding diary [2,8]. We conducted this prospective cohort study to assess the agreement of clinical management tool and voiding diary for classifying children presenting with enuresis as having PMNE or voiding disorder.

METHODS

Between January, 2014 and March, 2015, twice weekly, a systematic random sampling was carried out in the general pediatric outpatient department of a referral armed forces hospital. Twice weekly a random number between 1 and 10 was generated using a web-based random number generator. Every 8th child with general pediatric OPD registration number starting from this generated number, aged 5-12 years with parents with high school education and comprehension of Hindi/English language was enrolled in the study. Children with structural anomalies or pre-existing neurological/psychiatric/renal disorders were excluded. Institutional ethics clearance was obtained for the study. After obtaining informed consent, parents were administered a CMT questionnaire [2] and also taught to complete a voiding diary at home.

The children were classified as PMNE or voiding disorder, as per ICCS criteria [2], after assessing CMT and voiding diary. A single lower urinary tract symptom was sufficient to classify as voiding disorder as per CMT. For

interpreting voiding diary, standard ICCS parameters were used. Children were subsequently managed and followed up in a weekly pediatric nephrology clinic over the next 18 months. The proportion of children with bedwetting who were diagnosed to have PMNE or voiding disorder was calculated.

Statistical analysis: Data were analyzed using Microsoft Excel. Difference between proportions was analyzed by Chi square test. Cohen's Kappa was calculated to assess the agreement of classification as primary mono-symptomatic nocturnal enuresis versus voiding disorder using either clinical management tool or voiding diary as the assessment tools. Sample size was calculated with an assumption of Cohen Kappa 0.9 with 15% precision at 95% confidence level as per a previous study [9]. At an assumed prevalence of 10% of voiding dysfunction and 5% drop out rate, a minimum of 98 children with enuresis had to be enrolled for the agreement study.

RESULTS

A total of 100 children (64 males) were analyzed (**Fig. 1**). Most children (53%) were <7 years of age, family history being positive in 37%. Symptom distribution is shown in **Table I**. No significant male predilection was noted for children diagnosed as voiding disorder by CMT or voiding diary (53% vs 55%, *P*=0.89). Frequency and urgency on voiding diary were not significantly correlated with constipation. Reduced maximum voided volume (MVV) was seen in 66% children on voiding diary.

Table I Symptom Distribution From Clinical Management Tool (*N*=100)

Symptoms[a]	*Male*	*Age distribution*		
		5-7 y	*8-10*	*>10 y*
Increased frequency, *n*=30	19	23 (76.7)	6 (20)	1 (3.3)
Decreased frequency, *n*=14	9	3 (21.4)	6 (42.8)	5 (35.7)
Dribbling, *n*=8	6	3 (37.5)	6 (75)	5 (62.5)
Leakage, *n*=17	10	10 (58.8)	5 (29.4)	2 (11.7)
Poor stream, *n*=4	4	1 (25)	1 (25)	2 (50)
Dysuria, *n*=5	2	4 (80)	1 (20)	0
Urgency, *n*=30	13	17 (56.6)	13 (43.3)	0
Abdominal pain, *n*=3	2	2 (66.6)	1 (33.3)	0
Constipation, *n*=14	11	10 (71.4)	4 (28.5)	0
Family history, *n*=37	27	19 (51.3)	13 (35.1)	5 (13.5)

Data in no. (%). One child aged 8-10 y each had fever and lethargy.

Using CMT, 65% and 35% of children got classified as voiding disorder and PMNE, respectively; whereas, voiding diary reclassified them as 71% voiding disorder and 29% PMNE. The pattern was independent of the age group. Increased frequency of micturition was recorded significantly more in voiding diary compared to CMT (51% vs 30%, *P*=0.004). Overall agreement for classification between the two tools was 80% [κ (95% CI) 0.54 (0.37-0.72)]. Diagnostic classification discordance was noted in

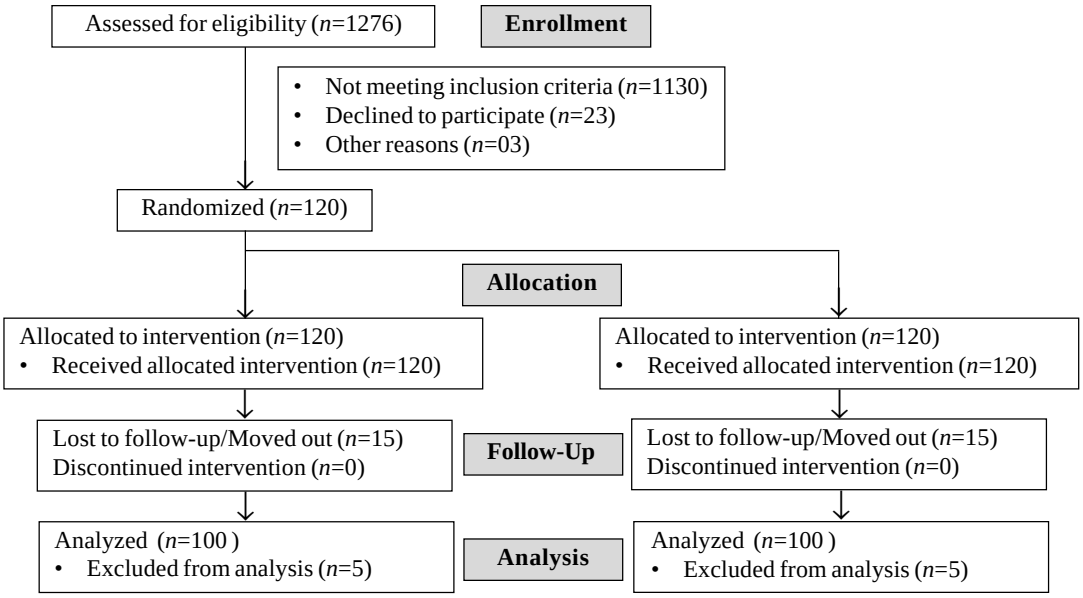

Fig. 1 Flow of subjects in the study.

20% cases with 7 additional cases diagnosed by voiding diary (**Fig. 2**).

The CMT has a sensitivity of 81.7% (95% CI 70.7-89.9) and specificity of 75.9% (95% CI 56.5-89.7) to diagnose voiding dysfunction in comparison to voiding diary (AUC 0.79; 95% CI 0.70 to 0.88). The positive and negative predictive values of CMT is 89.2% (95% CI 79.1-95.6) and 62.9% (95% CI 44.9-78.5), respectively, to diagnose voiding dysfunction.

DISCUSSION

The frequency of enuresis and its characteristics like male preponderance, family history, higher prevalence between 5-7 years and progressive decline thereafter, noted in our study, is concordant with published literature [10]. We noted a moderate agreement of 80% between CMT and voiding diary for classification as voiding disorder versus PMNE, this aspect not having been studied so far. Significant discrepancies in LUTS were noted between questionnaire-based screening and bladder diary in few previous studies [11,12]. A recall bias with CMT may have led to a lower detection of decreased urinary frequency with CMT than the voiding diary. Using CMT, incontinence was reported in 17% cases.

Akin to published literature, we noted urgency and dysuria more commonly amongst females while leakage and poor stream was commoner in males [2,4]. Also, similar to these studies, urgency was found to be equally distributed across genders between 5-8 years age, progressively declining thereafter.

We chose to simplify the existing tools of CMT and voiding diary for a quick administration in the OPD, maintaining the core components whose reliability has been proven [7]. Also, since ICCS guidelines suggest that a 2-day voiding diary suffices to evaluate bladder capacity and fluid intake, we used a simplified 2-day diary, omitting additional parameters [2].

Using either of the two tools, 78% cases could be classified as voiding disorder. Voiding diary led to a label of voiding disorder in additional 7% cases while discounting any abnormality in 13% cases whose initial diagnosis was PMNE based on CMT, implying advantage of voiding diary over CMT. The cut-offs to define frequency on a voiding diary and utility of calculating MVV have been questioned [13,14].

Limitations of our study include a possibility of referral bias, resulting in improper estimation of true community prevalence of enuresis, PMNE or voiding disorder. Being a questionnaire, a recall bias exists in CMT and having been administered to patients presenting with enuresis rather than all cases, a selection bias might be there. A parallel control group would have been preferable. Further, the questionnaire is not adapted to the Indian context. Bladder-bowel habits of our population are vastly different from Western populations and constipation is a common accompaniment of enuresis as well as daytime wetting reported in Western literature [15]. Agreement of the findings of CMT and voiding diary with results of invasive urodynamics, ultrasound and uroflowmetry would have been scientifically more valid.

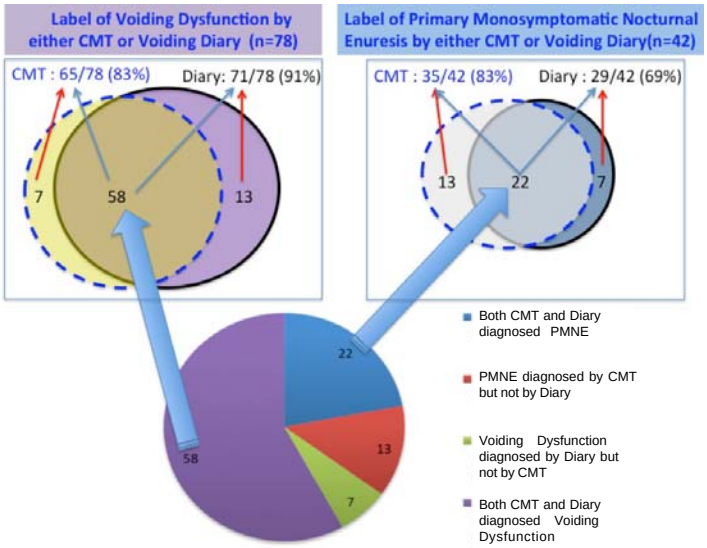

Fig. 2 Schematic depiction of the concordance of diagnosis of primary monosymptomatic nocturnal enuresis (PMNE) and voiding disorder using the Clinical management tool (CMT) questionnaire and the 48-hour voiding diary.

WHAT THIS STUDY ADDS?

· Voiding diary has additional utility over questionnaire-based tool in pediatric outpatient settings for identification of a voiding disorder and reconfirmation of primary mono-symptomatic nocturnal enuresis identified by clinical management tool questionnaire.

To conclude, we found a moderate agreement between a modified CMT and a simplified 48-hour voiding diary for classification of children with enuresis into primary mono-symptomatic enuresis and voiding disorder. More prospective studies and adaptations are required to ascertain the utility of CMT questionnaire before recommending its universal application as a screening tool in preference to a voiding diary.

Disclaimer: The work reported is those of the individual authors and in no way reflect the official position of the Directorate General Armed Forces Medical Services of India or Ministry of Defence.

Ethics clearance: Institutional ethics committee, Base Hospital Delhi Cantt; No. 139/2013 dated August 17, 2013.

Contributors: MK: conceptualization of study, drafting, finalization and approval of manuscript; SS: case recruitment, initial evaluation, literature review, manuscript drafting and approval: SKP: clinical assessment, analysis, drafting and approval of manuscript. All authors approved the final version of manuscript, and are accountable for all aspects related to the study.

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RESEARCH PAPER

Therapeutic Plasma Exchange in Children – Experience From a Tertiary Care Center

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Objective: To assess the safety, efficacy and outcomes of therapeutic plasma exchange (TPE) in children. **Methods**: Data were retrieved from hospital records for all children ≤18 years who underwent TPE between August, 2011 and July, 2018. **Results**: 46 children [median (range) age 96 (8-204) months] underwent 293 sessions of TPE by membrane plasma separation technique. Renal disease was the commonest indication (24, 52.2%) followed by neurological illnesses (17; 36.9%). 36 (78.2%) patients belonged to American Society for Apheresis category I. Overall, the most common indication was atypical hemolytic uremic syndrome (aHUS) (16; 34.8%). Fresh frozen plasma plus albumin was used as replacement fluid in aHUS, while albumin was used in others. 40 (86.9%) patients had complete/partial recovery while six did not show any sign of recovery. Complications were seen in 21 (7.1%) sessions; majority of which were minor in the form of blood pressure fluctuations. **Conclusion**: TPE can be performed safely and effectively for renal and non-renal indications, even in small children.

Keywords: *Atypical hemolytic uremic syndrome, Neurological indications, Plasmapheresis*

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Therapeutic plasma exchange (TPE) has become increasingly popular and effective therapy for many renal and immunological diseases and has proved to be life-saving in certain conditions [1-3]. TPE is a procedure where a part of the plasma of an individual is removed by an extracorporeal procedure and replaced with either fresh frozen plasma (FFP) or albumin, retaining the cellular component of the blood while removing pathogenic circulating antibodies, immune complexes, cytokines and toxins [4]. In addition, it can replace a deficient molecule such as complement factor H (CFH) in atypical hemolytic uremic syndrome (aHUS) [5]. Although the principles of TPE are the same in adults and children, there are technical differences unique to children such as poor vascular access and high volume of distribution [4,6].

The American Society for Apheresis (ASFA) has assigned disease conditions to one of four categories based on the quality of published evidence and strength of recommendation for TPE. The recommendations are mainly based on adult studies and do not distinguish between childhood and adult-onset diseases [7]. The literature on TPE for children is mostly limited to single-center, retrospective studies, hence, the recommendations for TPE are usually extrapolated from adult studies [1-2,4].

The primary objective of this study was to review the indications and technical details of cohort of children treated with TPE at our center. The efficacy of the treatment was also studied for individual diseases and different ASFA categories along with the complications related to the procedure.

METHODS

We conducted a review of hospital records of children ≤18 years, who underwent TPE at our institution between August, 2011 and July, 2018. The study was approved by the institutional ethics committee and informed consent was waived. Data were collected from the hospital medical records, which included indications, technical details of procedure and complications. Indications were catego-rized into renal, neurological and others, and also as per ASFA guidelines [8].

Decision for TPE was taken by the pediatric nephrologist based on the indications. All procedures were performed according to the hospital protocol by pediatric renal nurses and technicians, along with pediatric nephrologist in the pediatric intensive care unit (PICU). Appropriate site for venous access was selected as per the age of the patient. Size of the membrane filter was selected and exchange volumes were calculated. Procedure was performed by membrane filtration technique using Fresenius 4008S dialysis machine (Fresenius Kabi). Anticoagulation was done with heparin.

The outcome was measured at the time of discharge as complete response, partial response and absent response. Efficacy of the treatment was defined according to the

underlying pathology and assessed using the criteria published by Paglialonga, et al. [9]. Complications encountered related to the treatment were evaluated and categorized as access-related complications and proce-dure-related complications.

RESULTS

During the study period, 293 procedures were performed in 46 patients [30 males; median (range) 96 (8-204) months]. The demographic characteristics and technical details of the procedure are presented in **Table I**. Three children were younger than two years and weighed <10 kg. Most common access used was femoral vein (25; 54.3%). The median (range) TPE sessions per patient was 5 (1-21).

Renal disease was the commonest indication for TPE (24; 52.2%) followed by neurological illness (17; 36.9%). Also, majority of the sessions were performed for renal indications (197; 67.2%). The most common diagnosis was aHUS (16; 34.8%) accounting for 153 sessions. The indications for TPE and ASFA categories are shown in **Table II**. Maximum indications belonged to ASFA category I (36; 78.2%), while none to category IV. Median (range) duration of initiation of TPE from onset of symptoms was 12 (1-60) days.

Amongst 36 patients in ASFA category I, 22 (61.1%) had complete recovery, 12 (33.3%) had partial recovery and 2 (5.6%) showed no sign of recovery (**Table II**). ASFA category I was found to have significantly better recovery than category III (P=0.004). No significant difference was found between other groups.

Complications were seen in 21 (7.1%) sessions. Two cases of catheter-related bloodstream infection along with access thrombosis were seen. They recovered following relocation of the venous access and intravenous antibiotics. Among the procedure-related complications, hypertension (n=3) was self-resolving and required no additional treatment. For hypotension (n=4), transient stopping of diuretic and fluid resuscitation was required in one case. Among serious complications, one patient developed pulmonary edema, which resolved with diuretics but required discontinuation of the procedure. The second patient had seizures, likely due to clearance of anti-epileptic drugs, requiring an extra dose. There were no deaths or chronic sequelae directly related to TPE; however, two patients died due to the underlying disease.

DISCUSSION

In the current study, the commonest indication for TPE were renal (52%) and neurological (37%), which is consistent with previous reports [5,9]. However, the most common indications in the World apheresis registry were neurological disorders [10]. The difference in indications of TPE is likely due to difference in each centers' specific subspecialties, center-specific patient selection criteria and classifications [5].

The number of patients classified in ASFA category I or II was higher than other reported studies. Two recent analyses from developed countries reported 56.7% and 61% of the patients in category I or II [9,5]. A large analysis performed in US reported under-utilization of TPE with only 13.4% patients with ASFA category I receiving TPE [3]. This difference could be due to better adherence to the ASFA guidelines at our center and early referral for TPE. Moreover, due to non-availability of eculizumab in India, aHUS patients are primarily being managed with TPE.

Majority of adult centers in India prefer centrifugal methods [11], while pediatric centers use membrane filtration methods [12]. In contrast, centrifugal method is the most common apheretic procedure both in pediatrics and adults in the USA [3]. For the substitution fluid used, similar findings were reported by Paglialonga, et al. [9] with indication being the deciding factor for type of replacement fluid. However, Sinha, et al. [12] reported FFP alone to be the most common replacement fluid. For anticoagulation, heparin was used solely by us, while citrate was the most common documented anticoagulant in the World Apheresis Registry [10].

Overall, 86.9% patients showed either complete/partial recovery. The highest recovery rates were seen for renal (91.6%) disorders in our cohort. On the contrary, only 64% patients with renal disorders recovered in a previous study [5]. Our response rate in neurological disorders is also better than in the European survey, where only 55.5% had a full/partial recovery [9]. Higher overall response in our study may be due to the larger proportion of aHUS patients, majority

Table I Demographic Profile of Patients and Details of Therapeutic Plasma Exchange

Characteristics	*Value*
Weight (kg)[a]	23.2 (17.8-35)
Duration of hospital stay (d)[a]	33.5 (18-51)
Vascular access	
Femoral vein	25 (54.3)
Internal jugular vein	16 (34.8)
Both	5 (10.9)
Sessions per patient	5 (4- 6)
Exchange volume 60 mL/kg[b]	25 (54.3)
Filter membrane surface area 0.6 sq.m[c]	35 (76.1)
Replacement fluid	
Albumin + normal saline	30 (65.2)
Albumin + fresh frozen plasma	16 (34.8)

Data presented as no. (%) or [a]median (IQR). [b]40 mL/kg exchange volume was used in the rest; [c]0.3 sq.m membrane used in the rest.

Table II Indications and Outcomes of Therapeutic Plasma Exchange in Children as per ASFA category (*N*=46)

Clinical diagnosis	*N (%)*	*No. of sessions, n=293*	*ASFA category*	*Recovery*		
				CR n=25	*PR n=15*	*NR n=6*
Renal	24 (52.2)	197 (67.2)		16 (66.7)	6 (25)	2 (8.3)
Atypical HUS						
Anti CFH +ve	11	117	1	10 (90.9)	1 (9.1)	0
Anti CFH –ve[a]	5	36	1	3 (60)	2 (40)	0
FSGS						
Steroid resistant (native kidney)	1 (2.2)	5	III	0	0	1 (33.3)
Post renal transplant recurrence	1 (2.2)	4	I	0	1 (33.3)	0
Pre renal transplant FSGS	1 (2.2)	5	NC	1 (33.3)	0	0
Antibody mediated rejection	1 (2.2)	6	I	0	1 (100)	0
ANCA associated vasculitis	2 (4.4)	11	I	1 (50)	0	1 (50)
ABOi renal pre-transplant desensitization	1 (2.2)	2	I	1 (100)	0	0
Anti GBM antibody nephritis	1 (2.2)	11	III	0	1 (100)	0
Neurological	17 (36.9)	85 (29)		7 (41.2)	8 (47)	2 (11.8)
Autoimmune encephalitis	13 (28.3)	65	I	7 (53.8)	6 (46.2)	0
Guillain-Barre syndrome	2 (4.4)	10	I	0	1 (50)	1 (50)
Fulminant SSPE	1 (2.2)	3	NC	0	0	1 (100)
ADEM	1 (2.2)	7	II	0	1 (100)	0
Others	5 (10.9)	11 (3.8)		2 (40)	1 (20)	2 (40)
Methemoglobinemia	2 (4.4)	7	III	1 (50)	0	1 (50)
Hepatic encephalopathy	1 (2.2)	1	III	0	1 (100)	0
Autoimmune hemolytic anemia	1 (2.2)	2	III	1 (100)	0	0
Sepsis MODS	1 (2.2)	1	III	0	0	1 (100)

Data in no. (%). [a]Considered in category I as mutation analysis not done. CR, complete recovery; PR, partial recovery; NR, no recovery; HUS, hemolytic uremic syndrome; CFH, complement factor H; FSGS, focal segmental glomerulosclerosis; ANCA, anti-neutrophil cytoplasmic antibody; GBM, glomerular basement membrane; SSPE, subacute sclerosing pan encephalitis; ADEM, Acute disseminated encephalomyelitis; MODS, multi organ dysfunction syndrome; NC, not classified; TPE, therapeutic plasma exchange; ABOi, ABO incompatible

having anti-CFH antibody, who showed good response to TPE. Other pediatric studies from India have shown a variable response in aHUS ranging from 27-87.5% [12-15]. We found significantly better response of ASFA category I patients to TPE than category III patients. But others have reported no such association [9]. However, the recovery in these patients could not be attributed solely to TPE as 63% of our patients received concomitant immunosuppression also. We observed complications in 7.1% of TPE sessions, which is comparable to previously published reports from India and abroad [5,9,12,15]. Previously reported adverse event rate is 4-10% [10,16]. Despite the presence of many young children in our cohort, no increase in complication was noted in this group. This finding further confirms the safety of TPE in small children.

The major limitations of the study are its retrospective design, relatively small number of patients per indication, and the fact that it is a single-center analysis. Moreover, there was a lack of genetic testing in children with aHUS without anti-CFH antibodies. Nevertheless, this work adds to the limited data available on TPE use in Indian children.

In conclusion, TPE is an effective therapeutic modality with minimal complications in pediatric renal and non-renal disorders. It is safe, even in small children, in well-equipped settings.

Ethical clearance: Ethics committee, Sir Ganga Ram Hospital; No. EC/08/18/1415 dated August 30, 2018.
Contributors: RM: designing of study, data collection, analysis, drafting the manuscript, approval of the final version; KA, PKP: conception and designing of study, analysis, revision of manuscript, approval of the final version. All authors approved the final version of manuscript, and are accountable for all aspects related to the study.
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REVIEW ARTICLE

Macrophage Activation Syndrome in Children: Diagnosis and Management

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Macrophage activation syndrome is a severe yet under-recognized complication encountered in pediatric rheumatology. It manifests as secondary hemophagocytic lymphohistiocytosis leading to a hyper-inflammatory state resulting from an underlying cytokine storm. If unchecked, it may lead to multiorgan failure and mortality. Early diagnosis and timely initiation of specific therapy is pivotal for a successful outcome. This review outlines the key clinical and laboratory features and management of macrophage activation syndrome.

Keywords: *Hyper-inflammation, Secondary hemophagocytic lymphohistiocytosis*, Juvenile idiopathic arthritis.

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Macrophage activation syndrome (MAS) is a form of secondary hemophagocytic lymphohistiocytosis (sHLH) and one of the most commonly encountered pediatric rheumatic emergencies [1]. While these terms are often used interchangeably, MAS is typically used for sHLH with an underlying rheumatic disorder. MAS is most often reported with systemic juvenile idiopathic arthritis (sJIA) [2]. Additionally, it is seen with Kawasaki disease (KD), juvenile systemic lupus erythematosus (SLE), and juvenile dermatomyositis (JDM) [3,4]. A single centre analysis of 31 children with MAS identified the under-lying disease as sJIA in 84%, SLE in 13% and KD in 3% with the overall mortality being 32% [5]. MAS can compli-cate any chronic inflammatory condition at onset or during the course of the disease [6-8]. sHLH can also be triggered by a variety of other inflammatory conditions viz., underlying infection, malignancy or primary immuno-deficiencies [9].

MAS leads to fulminant manifestations in nearly 10% of children with sJIA, though up to 30% of children with sJIA may have subclinical MAS [2,7,10]. A retrospective study from India reported MAS affecting 1.3% of 950 children diagnosed with KD and coronary artery abnormalities were more frequent, affecting 41.7% of the subset with MAS [3]. Although, MAS is increasingly recognized since its early reports in the 1980s, it seems that a significant proportion of children are either not diagnosed or diagnosed late. This review highlights the pathogenic mechanisms, salient clinical features and the management strategies for MAS.

PATHOGENESIS

The cytolytic dysfunction of natural killer (NK) cells and cytotoxic T-lymphocytes (CTL) is the key inciting event in MAS/primary HLH [9]. While recessive null mutations in *PRF1*, *UNC13D*, *STXBP2*, *STX11*, *RAB27A*, and *LYST*, which adversely affect granule processing in the CTL and NK cells' function, are known to be associated with primary HLH, there is increasing evidence to suggest that the susceptibility to cytokine storms in sHLH could be along a continuum of genetic deficiency, with monoallelic, biallelic and polygenic defects [11-13]. Of the various implicated genetic defects in fHLH, *LYST*, *MUNC13-4*, and *STXBP2*, nearly one-third were detected in subjects with sJIA, MAS on whole-exome sequencing. The described heterozygous mutations may constitute the first hit in the genetically-susceptible individual resulting in a subtle underlying impairment in the cytolytic pathways, that turns into an exaggerated immune response subsequent to a second trigger by either an unknown/self-antigen (as in juvenile SLE) or an infectious agent (viral, bacterial, or fungal). This would potentially culminate in an unrestrained activation of immune cells, and subsequent frank hyper-inflammatory state. Antigen-induced T cell hyper-stimulation in MAS/sHLH is not matched with degranulation due to the underlying cytolytic dysfunction. Such persistent activation of CTL and macrophages results in a cytokine storm; most notably involving the massive release of TNF and interleukins: IL-6, IL-1b, and IL-18 [9]. The resultant cytokine storm usually exhibits good

correlation with serum ferritin levels. Ferritin is the storage form of iron, and is an acute phase reactant. Cytokine-mediated macrophage activation in MAS results in hepatic injury and release of ferritin [14]. One of the most consistent laboratory markers of MAS/sHLH is hyper-ferritinemia. Amongst other effects, ferritin has been shown to inhibit lymphocyte division and granulocyte proliferation.

Activation of macrophages leads to hemo-phagocytosis, a cardinal feature of MAS that can be demonstrated histopathologically in the bone marrow, liver, and spleen. However, bone marrow examination shows HLH with positive CD163 macrophages in only up to 60% of cases, and this finding may not be evident during the initial stages [15]. CD163 and CD25 are markers of activated macrophages which mediate endocytosis of haptoglobin-hemoglobin complexes, and the elevated levels of these surface markers, i.e., soluble interleukin-2 receptor alpha chain (sCD25) and soluble CD163 (sCD163) may be sensitive indicators of detecting MAS. **Fig. 1** demonstrates the pathogenic mechanism for MAS.

The prototype animal model of sJIA MAS is the IL-6 transgenic mouse, wherein LPS induces a hyper-inflammatory state akin to MAS, thus recapitulating the occurrence of infection-triggered sJIA MAS. While IL-6-induced stimulation of macrophages accentuates further cytokine production (excess IL-1, IL-6 or IL-18), in vitro experiments have suggested its association with a transient NK cell dysfunction resulting in decreased degranulation i.e., low perforin and granzyme expression on stimulated NK cells in the inflamed microenvironment [16]. The other crucial arm of inflammation is macrophage hyperfunction, with various defects translating into a systemic hyperin-flammatory state. The macrophage phenotype also changes from M1 (pro-inflammatory) to M2 (anti-inflammatory) in response to external stimuli amid the changing inflammatory milieu [9]. The underlying hyper-cytokinemia and inflammatory cascade form the basis for anti-cytokine therapies in sJIA.

Apart from sJIA, MAS can complicate other rheumatic disorders like juvenile SLE, KD and JDM. In juvenile SLE, defective clearance of self-antigens (due to defects in the dendritic cells and the complement cascade) may contribute to MAS. There is some evidence that the different cytokines may have a major role in the pathogenesis of MAS depending on the underlying rheumatic disorder: soluble TNF receptor (sTNFR)-I levels for SLE, serum IL-18 levels for JDM, and serum sTNFR-II levels for KD and sJIA [17]. A study from Japan, which measured cytokine levels in 36 patients with MAS complicating sJIA, showed a positive correlation of serum sTNFR-II/I ratio with disease activity. This correlation still appeared to exist in the patients treated with the anti-IL-6 drug tocilizumab [18]. The authors suggest that the serum sTNFR-II/I ratio may be a helpful biomarker to assist diagnosis of MAS in sJIA patients receiving tocilizumab.

CLINICAL AND LABORATORY FEATURES

Timely recognition of MAS is of utmost importance for suitable management and successful outcome. A high degree of suspicion is warranted, and it should be considered in any febrile child with underlying rheumatic disease. The

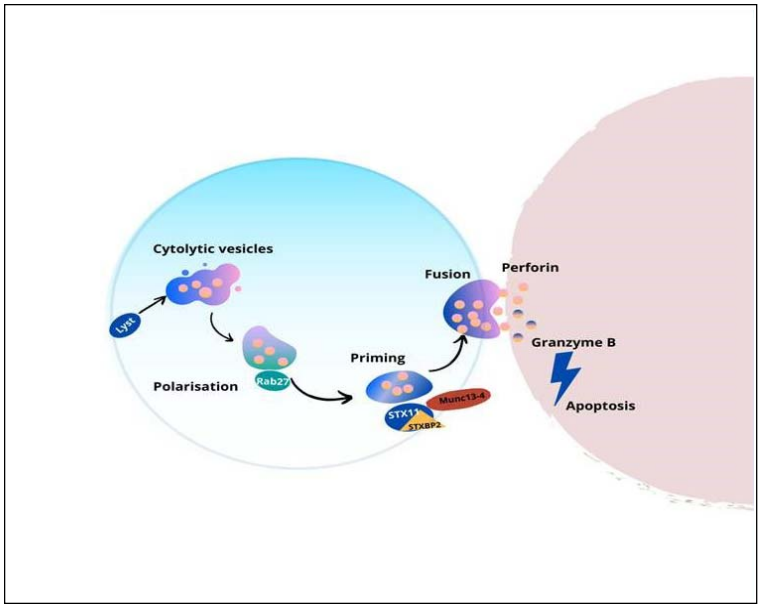


Fig. 1 Immune pathways implicated in macrophage activation syndrome.

persistent high-grade fever, especially in those who are not on any long term immunosuppression, is one of the most important features of MAS. The typical fever in sJIA changes from an intermittent pattern to a non-remitting fever in MAS, and this change in the pattern of fever may be an early clue to differentiate MAS from the underlying disease flare. However, sometimes children with ongoing immunosuppression (steroids) may not mount the typical high persistent fever but have other clinical and laboratory features suggestive of MAS. Absence of fever has also been observed in children on biologicals [15], and it seems that fever may not be an absolute finding in all cases of MAS.

The underlying cytokine storm in MAS results in multiorgan involvement and can have varied clinical features. Many patients will have hepatosplenomegaly and lymphadenopathy. Hemorrhagic manifestations secondary to thrombocytopenia, coagulopathy and liver dysfunction include petechial spots, mucosal bleeding, severe gastrointestinal bleeding, and disseminated intravascular coagulation. Central nervous system (CNS) involvement in the form of drowsiness, headache, seizures and altered behavior; from mild confusional state to frank coma, has been reported in 30-35% patients; and often heralds a worse prognosis. Unless promptly recognized, MAS can lead to multiorgan involvement, including cardiac, pulmonary, and renal failure. The reported mortality of MAS varies from 10-35%; however, early recognition and prompt treatment can improve survival rates. Differentiating the underlying disease flare and MAS is often challenging **Table I** summarizes the major differences.

MAS in SLE: MAS is most commonly described with sJIA; however, it is increasingly being recognized with other rheumatic disorders, particularly SLE. Based on international cohort data, patients with oral/nasal ulcers, arthritis, serositis, renal, CNS or hematologic involvement at diagnosis of SLE are more likely to develop MAS [19]. Borgia, et al. [8] have reported that 9% of children with SLE had MAS, and the majority (68%) had features of MAS at the disease onset. The diagnosis of MAS in SLE is often more challenging because of the overlapping features like fever, organomegaly and cytopenias in both disease flare and MAS. However, lymphadenopathy and cytopenias are more frequently observed in MAS as compared to active SLE. While comparing the clinico-laboratory differences between MAS and active SLE without MAS, all clinical features of MAS except fever have better specificity than sensitivity. In contrast, fever is the most sensitive with low specificity. Hyperferritinemia, hypertriglyceridemia, elevated lactate dehydrogenase and hypofibrinogenemia are known to have the best sensitivity and specificity in differentiating MAS from active lupus [15]. Akin to sJIA, MAS in SLE can be life-threatening, with mortality ranging from 5-11% [8,15] and therefore needs a high index of suspicion for timely diagnosis of MAS in this population.

CLASSIFICATION CRITERIA AND DIAGNOSIS

The diagnosis of MAS is often challenging, especially

Table I Differentiating Features Between Macrophage Activation Syndrome and Disease Flare

Clinical and laboratory parameter	*Macrophage activation syndrome*	*Disease flare*	
		SLE	*sJIA*
Fever	+++	++	++
Hepato-splenomegaly	+	±	±
Hemorrhages	++	-	-
CNS dysfunction	++	±	-
Hemoglobin	Low	Normal to low	Normal
Platelet count	Low	Normal to low	Highly elevated
Erythrocyte sedimentation rate	Normal to low	Normal to elevated	Normal to elevated
C-reactive protein	Elevated	Normal to elevated	Normal to elevated
Aspartate aminotransferase	Elevated	Elevated	Normal
Alanine aminotransferase	Elevated	Normal	Normal
Lactate dehydrogenase	Elevated	Normal	Normal
Ferritin[a]	Elevated	Normal	Normal
Fibrinogen	Low	Normal	Normal
Triglycerides	Elevated	Normal	Normal

[a]*Hyperferritinemia has the best sensitivity and specificity, followed by increased lactate dehydrogenase level, hypertriglyceridemia, and hypofibrinogenemia for diagnosis of MAS. SLE: systemic lupus erythematosus, SJIA: systemic onset juvenile idiopathic arthritis.*

Box I Pediatric Rheumatology International Trials Organisation Macrophage Activation Syndrome Classification Criteria (2016) [2]

A febrile child with known or suspected sJIA is classified as having MAS if the serum ferritin >684 ng/mL and ≥2 of the following:

- Platelets ≤181×10^9/L
- Aspartate aminotransferase (AST) >48 U/L
- Triglycerides >156 mg/dL
- Fibrinogen ≤360 mg/dL

during the early phase of the disease. The HLH-2004 criteria, originally designed for enrolment in clinical treatment trial, are often used for classification of MAS but lack sensitivity, especially for early detection of MAS. In addition, some of the features described in the HLH-2004 criteria overlap with features observed in active rheumatic diseases in the absence of MAS. Some of these challenges have been addressed in the proposed 2016 MAS in sJIA classification criteria by the Pediatric Rheumatology International Trials Organisation (PRINTO) (**Box I**), which have a sensitivity of 73% and specificity of 99% [2]. Although originally described for children with sJIA, these criteria have also been found to be useful for other rheumatic conditions like SLE and KD. Recently, these criteria have also been used to classify MAS associated with pediatric multisystem inflammatory syndrome temporally related with COVID-19 (PIMS-TS) [20].

The various laboratory parameters including falling total white cell count, low platelet counts, low fibrinogen and low ESR, suggest the diagnosis of MAS. It is difficult to clinch the diagnosis of MAS using a single clinical or laboratory parameter. Kostik, et al. [21] proposed a combination of more than three of the following laboratory parameters to predict an early diagnosis of MAS in sJIA reliably: declining platelet (≤211×10^9/L) and white blood cell counts (≤9.9×10^9/L); decreased albumin (≤2.9 g/dL) and fibrinogen (≤1.8 g/L); elevated ferritin (>400 µg/L), aspartate aminotransferase (>59.7 U/L) and lactate dehydrogenase (>882 U/L); and proteinuria.

More recently in 2019, the novel MAS/sJIA (MS) score was developed in an attempt to improve discrimination between active sJIA and MAS [21]. An international cohort of several hundred patients with sJIA, with and without MAS, were analyzed to identify clinical and laboratory parameters which distinguished the conditions. The MS score includes seven variables: central nervous system dysfunction, hemorrhagic manifestations, active arthritis, platelet count, fibrinogen, lactate dehydrogenase and ferritin. The total score ranges between –8.4 to 41.8 with a value ≥–2.1 showing the best performance. Although the authors reported a good performance in a validation cohort, a separate study compared the MS score with the H-score for identification of MAS in 71 patients with sJIA and found that the H-score performed slightly better [23].

An additional measure to help recognition of MAS in the context of sJIA, which is simpler than the MS score or H-score, is the ferritin to ESR ratio. A study using data from a large international cohort found that a ferritin/ESR ratio >21.5 has a sensitivity and specificity of 82% and 78%, respectively for diagnosing sJIA-MAS versus active sJIA without MAS [24]. In light of the challenges faced while applying the available criteria in clinical practice, it is crucial to meticulously observe for evolving clinical features and trends of laboratory variables in order not to miss a diagnosis of MAS.

Hyperferritinemia forms part of the HLH-2004 and the 2016 MAS in sJIA criteria with thresholds of 500 ng/mL and 684 ng/mL, respectively. In clinical practice, in patients diagnosed with MAS, ferritin is usually >1000 ng/mL and frequently >10,000 ng/mL. In a systematic review by Sarangi, et al. [15], the median ferritin in children with MAS, sepsis and familial HLH was 37 680 ng/mL, 8775 ng/mL and 3234 ng/mL, respectively. One study, which analyzed all children admitted to Texas Children's Hospital over two years with ferritin > 500 ng/mL, found that a ferritin >10 000 ng/mL was 90% sensitive and 96% specific for HLH [26]. A retrospective analysis over three years of children with ferritin >10 000 ng/mL at 11 UK centres identified 153 patients, but HLH/MAS was not entertained as a diagnostic possibility in 23.2% of these children [27]. This

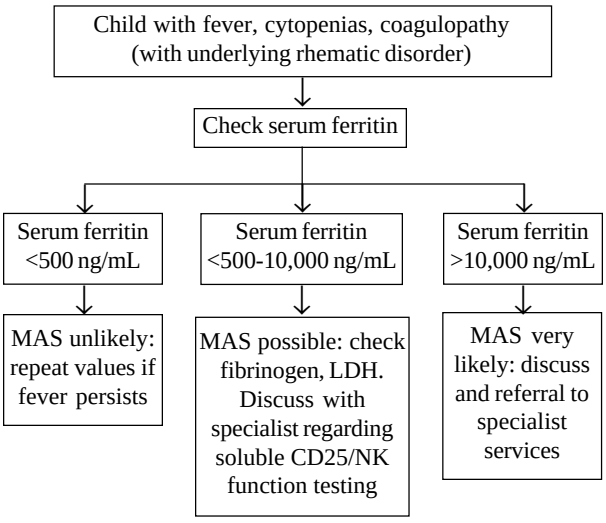


Fig. 2 Guide to early diagnosis and referral for macrophage activation syndrome (*adapted from: Sen ES, Steward CG, Ramanan AV. Diagnosing haemophagocytic syndrome. Arch Dis Child. 2017;102:279-84 [28]*).

highlights the importance of improving education and awareness of HLH/MAS amongst all clinicians caring for children. We recommend that a child with fever, cytopenias and/or coagulopathy with ferritin >10 000 ng/mL be referred to specialist services for evaluation and management of probable MAS/sHLH (**Fig. 2**) [28].

TREATMENT

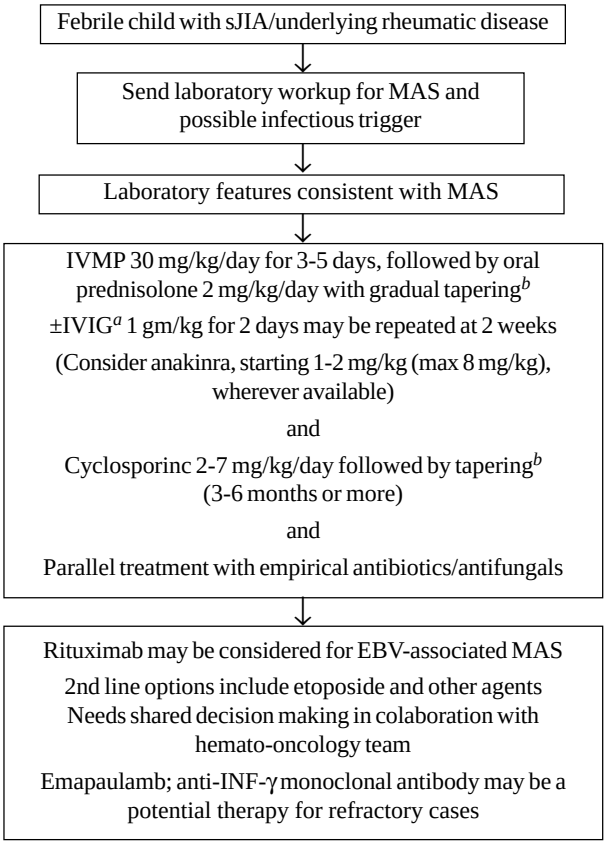
Timely diagnosis and intervention are pivotal for dampening the underlying cytokine storm and curtailing the morbidity and mortality associated with MAS. The treatment is aimed at mitigating the underlying cytokine storm using immunosuppression and controlling the inciting trigger. In clinical practice, ascertaining the underlying infectious trigger is often not possible. Empirical antibiotics and/or antifungals are instituted in parallel with the immunosuppressive therapy to cover the probable infectious trigger. The treatment of MAS secondary to sJIA is based on the evidence generated from case series, and the same protocol is usually extrapolated for management of MAS secondary to other rheumatic disorders [29-31].

Intravenous methylprednisolone (IVMP) pulse therapy, 30 mg/kg/day (maximum 1g) for 3 to 5 days, followed by oral prednisolone is the most widely used first-line therapy [31,32]. The oral steroids are initiated at a dose of 2 mg/kg/day and gradually tapered while monitoring the clinical and laboratory parameters. They are usually continued for a few weeks until normalisation of hematological abnormalities. Subsequent doses of steroids are titrated as per the need of the underlying disease. There are a few reports of replacing IVMP with dexamethasone palmitate (dose varying from 7.5 to 10 mg/day) in refractory cases or CNS involvement in MAS [33].

The calcineurin inhibitor cyclosporine A has shown efficacy in other histiocytic disorders, and this has led to its use in MAS. Addition of cyclosporine with steroids in the management of MAS results in rapid control of the underlying cytokine storm, in addition to limiting the excessive use of corticosteroids. However, in the acute setting of patients with multiorgan dysfunction, cyclosporine should be used with caution due to its neurotoxic and nephrotoxic side effects. The optimal duration of cyclosporine in MAS is unclear, and it has been used for a variable duration from 3 months to 2 years [34]. It should be continued until the normalization of all laboratory parameters, followed by tapering to prevent relapses. We usually consider oral cyclosporine in a dose of 2-7 mg/kg/day for a period of 3-6 months.

Intravenous immunoglobulin (IVIG) has also been used in the treatment of MAS, and it may be considered as adjunctive therapy to IVMP [35,36]. Given the relative safety and favorable adverse effect profile, it seems prudent to use IVIG as an adjunct to IVMP, especially in Indian settings where underlying infection always remains a possibility. We usually consider using IVIG as initial therapy when the clinical suspicion of concomitant infection is high.

Cytokine-specific therapy using biological agents like anti-IL-1 (anakinra) is increasingly used in the treatment of MAS in sJIA; with improved benefit with an early institution [37]. The use of anakinra is usually considered at an early stage if there is no response to IVMP [30]. Although a common starting dose of anakinra is 2 mg/kg/day, it may be escalated up to 8 mg/kg/day in refractory cases. In cases of shock and poor peripheral absorption, intravenous anakinra can be recommended [38]. Other anti-cytokine therapeutic options for use in MAS include IL-1α and IL-18 blockers [39]. However, non-availability of these agents precludes their use in India. The utility of other biological agents like anti-IL-6 receptor monoclonal antibody tocilizumab and anti-TNF agents (etanercept) in treating MAS secondary to various



[a]Initiating IVIG where underlying infection cannot be ruled out seems a reasonable practical approach. In such scenarios IVMP can be given after 48 hr of IVIG and antibiotic coverage; [b]gradual tapering based on clinical and hematological parameters.

Fig. 3 Management strategy for macrophage activation syndrome (MAS).

rheumatic disorders remains unclear. The children with sJIA on tocilizumab may remain afebrile with lower cell counts and ferritin levels while they develop MAS, compared to those developing MAS without tocilizumab therapy [40]. As discussed previously, the serum sTNFR-II/I ratio may be a helpful biomarker to identify the onset of MAS in these patients on tocilizumab treatment [18]. Rituximab, a monoclonal antibody to CD20, may be considered in EBV-triggered MAS [41]. A dose of 375 mg/m^2 weekly for four weeks has shown successful results in this setting.

MAS refractory to initial IVMP and cyclosporine is often challenging to manage. In refractory cases, additional use of etoposide may be considered [42]. However, this may be complicated with fatal myelosuppression and opportunistic infections. There are anecdotal reports of successful outcomes with the use of cyclophosphamide in refractory MAS [42,43]. Recently, emapalumab, a fully human IgG1 anti–INF-γ monoclonal antibody has shown its efficacy in children with primary HLH [44]. There is preliminary data suggesting effectiveness of emapalumab in MAS associated with sJIA, although this, and its potential role in MAS secondary to other underlying rheumatic disorders, needs to be further studied [45]. Treatment of refractory MAS is often challenging and should be tailored judiciously in consultation with the hemato-oncology team. **Fig. 3** summarizes the treatment approach for MAS. Hematopoietic stem cell transplantation (HSCT) is a curative option for primary/familial HLH. ten Cate, et al. [46] shared their experience of allogenic stem cell transplant carried out in a child with refractory sJIA who succumbed to MAS following this modality [46]. With the availability of more specific cytokine-targeted therapy with favorable outcome, HSCT is not advocated for treatment of refractory MAS.

CONCLUSION

Macrophage activation syndrome is one of the commonest pediatric rheumatic emergencies. It is most frequently reported with sJIA; although, it can be a complication of any underlying rheumatic disorder. Impaired cytolytic function and the resultant cytokine storm lead to laboratory and clinical manifestations, including, in some cases, fulminant multiorgan dysfunction. Timely diagnosis and judicious use of immunosuppressive agents is pivotal for a successful outcome.

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Diagnosis and Management of Gastroesophageal Reflux Disease in Children: Recommendations of Pediatric Gastroenterology Chapter of Indian Academy of Pediatrics, Indian Society of Pediatric Gastroenterology, Hepatology and Nutrition (ISPGHAN)

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Justification: Gastroesophageal reflux (GER) related symptoms are a major cause of parental concern and referrals at all ages. These guidelines have been framed to inform pediatricians regarding current diagnosis and management of gastro-esophageal reflux disease (GERD). **Process**: A group of experts from the pediatric gastroenterology sub-specialty chapter of Indian Academy of Pediatrics (ISPGHAN) discussed various issues relating to the subject online on 25 October, 2020. A consensus was reached on most aspects and a writing committee was constituted. This committee had three meetings for a detailed discussion. The statement was sent to the entire group and their approval obtained. **Objective**: To formulate a consensus statement to enable proper diagnosis and management of GERD in children. **Recommendations**: GER is physiological in most infants and it improves as age advances. The pathological form, called GERD causes distressing symptoms that affect daily activities and may result in complications. The presentation would vary from regurgitation to severe symptoms due to esophageal or respiratory tract disease. In older children, esophagitis is the commonest manifestation of GERD. A careful history and clinical examination are adequate to make a diagnosis in most patients, but judicious investigations are necessary in a few. Upper gastro intestinal tract endoscopy may be required in those with esophageal manifestations, dysphagia and hematemesis. In children with extra-esophageal symptoms, MII-pH monitoring and scintigraphy are necessary. Empirical treatment with a Proton pump inhibitor (PPI) has not been proven useful in infants, but a four-week trial is recommended in older children without complications. While positioning and feed thickening have limited benefit in infants, life-style modifications are important in older children.

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Symptoms attributed to gastroesophageal reflux (GER) are a major reason for parental concern irrespective of the age of the child. GER is a physiological event in infants that improves with increasing age. While 50% infants reflux below 6 months, the prevalence reduces to 10 % by 2 years of age [1,2]. However, in 22 to 25% of infants, it is pathological termed Gastroesophageal reflux disease (GERD), and requires medical intervention [3]. In older children and adolescents, the overall prevalence of GERD is 5-8% and most of them will need some intervention [4]. A comprehensive history and clinical examination are adequate in most infants and children, but judicious investigations are necessary in some. In India, due to lack of clarity amongst pediatricians; there is a flawed approach, unnecessary investigations and inappropriate medications. These guidelines aim to improve awareness amongst pediatricians regarding management of gastroesophageal reflux disease.

PROCESS

A group of experts from the pediatric gastroenterology sub-specialty chapter of Indian Academy of Pediatrics (ISPGHAN) discussed various issues relating to the subject in an online meeting of select members on 25 October, 2020. A broad consensus was reached and a writing committee was formed. This committee had two meetings online on 21 November, 2020 and 1 December 2020 for a detailed discussion. The statement was sent to the entire group by e-mail and their approvals were obtained. A consensus statement was formulated after incorporating suggestions on 24 December, 2020.

GUIDELINES

Clinical Manifestations

In infants and children, GER has various manifestations with a range of severity. Symptoms may be mild, like random regurgitation, or severe symptoms due to esophageal or

respiratory tract disease (**Table I**). In some infants the symptoms can be non-specific and difficult to recognize. Amongst older children, about 60% of children present with epigastric pain, regurgitation and extra-esophageal symptoms [5,6]. Nausea and vomiting are seen in 21% of children [6]. The classic "reflux syndrome" or "reflux chest syndrome" consists of substernal, burning chest pain, with or without regurgitation, and cannot be expressed by most children below 8 years of age [4]. Only 6-8 % of children below eight years are able to give a classical description. Definitions used in this guideline are provided in **Box I**. Risk factors for GERD in **Web Box I** and features which suggest an alternate diagnosis than GERD are in **Web Box II**.

Diagnosis

GER is physiological in most infants and needs no tests for documentation. In GERD, investigations depend on the clinical manifestations. A thorough history and clinical examination has a reliability of 60-70% in adolescents and older children [7]. However, it is less reliable in infants and in those with extra-esophageal symptoms. Hence investigations are required in a small subset of patients. The choice of investigation is based on the clinical symptomatology of a given case. When typical symptoms (**Table I**) are present, upper gastro-intestinal endoscopy must be the first investigation. Tests like pH/MII (multichannel intraluminal impedance) may be the first choice in those with atypical symptoms or after failure of empirical therapy.

Infant GER Questionnaire

I-GERQ-R is an infant reflux symptom based assessment scale containing 12 items, completed by the caregiver. It has been validated in western settings as a screening tool to identify those with GERD and also to monitor outcome of therapy [8]. Data is not sufficient to recommend it in developing countries, where artificial feeding is much less prevalent than in the west.

Upper GI Endoscopy

Endoscopy helps direct visualization of esophageal mucosa, assess the function of the lower esophageal sphincter and obtain biopsies to rule out conditions like eosinophilic esophagitis. Endoscopically visible damage to the distal esophageal mucosa is the most reliable sign of reflux esophagitis. The typical features of GERD on esophageal histology are basal zone hyperplasia (>20% of total thickness) and elongation of papillae or rete pegs (>50% of total thickness). These may be useful in non-erosive reflux disease (NERD), but they lack sufficient sensitivity and specificity [9]. Endoscopy can also diagnose conditions like achalasia and hiatus hernia, as well complications like stricture and Barrett metaplasia. Indications for endoscopy are summarized in **Box II.**

Box I Definitions

Gastroesophageal reflux (GER): Physiological, involuntary passage of gastric contents into the esophagus with or without visible regurgitation.

Regurgitation: Bringing up of gastric contents into the oral cavity and sometimes out of the mouth. While regurgitation is the visible form of GER, absence of regurgitation, does not rule out reflux related disease.

Vomiting: Forceful expulsion of gastric contents through the mouth, which involves intense muscular activity of the respiratory and abdominal muscles and is a centrally mediated somatic reflex response, sometimes triggered by reflux.

Gastroesophageal reflux disease (GERD): Pathological form of GER resulting in symptoms that affect daily activities or complications that cause systemic disease. It occurs either from loss of calories or the consequence of the esophagus/respiratory tract being exposed to the gastric contents.

Table I Clinical Features of Gastroesophageal Reflux Disease

Typical features	*Atypical features*
Infants	
Regurgitation/vomiting	Apnea
Irritable during and after feedings	Chronic stridor
Refusal to feed /failure to thrive	Wheezing/recurrent pneumonia
Inconsolable crying	Hematemesis/Anemia
	Neurobehavioural changes
	Sandifer's syndrome
Children	
Nausea with or without vomiting	Chronic cough /recurrent pneumonia
Pain abdomen mostly epigastric	Recurrent stridor/ bronchospasm
Heartburn/chest pain	Laryngitis/hoarseness of voice
Drooling/spitting/water brash	Sinusitis
Choking during feeding or night	Otitis media
Poor appetite/refusal to feed	Gastrointestinal bleeding/ anemia
Nausea with or without vomiting	Dental erosion/halitosis
Pain abdomen mostly epigastric	Sleep disturbance
Heartburn/ chest pain	Chronic cough/recurrent pneumonia
Drooling/spitting/water brash	Recurrent stridor/ bronchospasm
Choking during feeding or night	Laryngitis/hoarseness of voice
Poor appetite/refusal to feed	Sinusitis
	Otitis media
	Gastrointestinal bleeding / anemia
	Dental erosion /halitosis
	Sleep disturbance

Box II Indications for Endoscopy

- Hematemesis
- Dysphagia
- Feeding aversion and a history of regurgitation
- Persistent faltering growth associated with overt regurgitation
- Back arching or features of Sandifer's syndrome.
- Persistent retrosternal or epigastric pain refractory to PPI trial
- Unexplained iron deficiency anemia
- A referral for fundoplication

24-hour Esophageal pH Monitoring

Esophageal pH monitoring is used to measure the magnitude of acidic reflux over 24 hours using a pH probe placed at the lower esophagus. It helps in establishing a temporal association between the patient's symptom episodes and acid reflux. Reflux index (percentage of time esophageal pH <4) of >10% in infants and >7% in children is considered significant. However sensitivity varies from 41% to 81% for diagnosis of GERD and currently it is not considered ideal for routine use after the advent of impedance pHmetry [7]. It may be used to correlate episodic symptoms like heartburn with reflux, confirm if acidic reflux is the cause of eosinophilic esophagitis and also assess if drug therapy is effective.

Combined esophageal multichannel intraluminal impedance and pH monitoring (MII-pH): At present, this is considered the best investigation for diagnosis of GERD. Multi-channel intra-luminal impedance measures electrical impedance changes as a bolus of solid, liquid or air passes between the sensors along the catheter (air>liquid). Here a pH electrode is incorporated into the impedance catheter. It monitors not only acid (pH<4) but also weakly acidic (pH 4-7) reflux episodes as well as non-acid/ alkaline (pH>7). This is an important improvement, since 50% of children with GERD will be missed, if only acidic reflux is measured. MII-pH also provides details about the duration and degree of the reflux as well as the contents of the refluxate, whether liquid, gas or both together. It may also be of help to correlate extra-esophageal symptoms with reflux [10]. MII-pH study in a symptomatic patient with a normal endoscopy will reveal three patterns: *a*) Functional heart burn (normal acid exposure with no symptom correlation) *b*) Non-erosive reflux disease (abnormal acid exposure irrespective of symptom correlation) and *c*) Esophageal hypersensitivity (normal acid exposure but symptoms correspond to acidic/nonacidic reflux episodes).These findings provide important clues in deciding whether a given patient requires acid suppression, neuromodulators or anti-reflux surgery.

MII-pH study improves management decisions in children when compared to conventional 24-hour pH study [11]. However, it is still unclear if the treatment based on MII-pH study leads to long term reduction of symptoms. High cost, non-availability of expertise and equipment, as well as paucity of reference values in children are constraints for recommending their routine use in India. Restricted availability of esophageal manometry equipment for the precise placement of MII-pH catheter is also a limiting factor. Indications for MII-pH study are given in **Box III**.

Trial of Proton Pump Inhibitor

Many randomized control trials (RCT) have supported the use of a 2-4 week trial of PPI administration in children with typical symptoms of GERD [12,13]. Though there is no data from India, a trial of PPI may be judiciously used in older children when there are no alarm signs. However in infants, no study has shown any benefit irrespective of the duration of the therapy [14]. Hence empirical therapy with PPIs cannot be recommended in infants.

Esophageal Manometry

Manometry has poor specificity and sensitivity in the diagnosis of GERD. It is useful to rule out esophageal motility disorders during pre- operative evaluation of children undergoing fundoplication. It is also useful in the diagnosis of achalasia, wherein symptoms may sometimes mimic that of GERD [15].

Scintigraphy

Scintigraphy or milk-scan is a radionuclide based study for the diagnosis of pulmonary aspiration in GERD. It has low sensitivity, lacks standardized technique and there are no accepted normal values. In addition, it assesses only the immediate postprandial reflux. Hence it is not useful for documentation of esophageal reflux. It may be done to confirm pulmonary aspiration in patients with refractory respiratory symptoms or those with recurrent aspiration pneumonia, but normal esophageal pH monitoring study [16]. However a normal scan does not always rule out aspiration.

Upper GI Contrast Study

It is not useful for confirming GERD in infants and children. It has a role in evaluation for anatomic abnormalities like

Box III Indications for MII-pH Monitoring

- Infants with extra-esophageal symptoms (non-epileptic seizure-like events or apneas)
- Feed refusal in infant with failure to thrive in the absence of other diagnosis
- Infants with unexplained crying, before considering acid-suppressive therapy
- Follow-up of esophageal surgical conditions (atresia, diaphragmatic hernia)
- Assess efficacy of antireflux therapy
- Evaluation of preadolescent children with unreliable GERD clinical picture

hiatal hernia, duodenal web, achalasia and malrotation of the gut, all of which may present with symptoms similar to GERD. In children with recurrent aspiration, video-fluroscopic swallow studies are useful to differentiate oropharyngeal dysphagia from GERD, especially in neurologically impaired children [17].

Management

Treatment depends upon specific symptoms, disease severity, age of presentation, and associated comorbidity. The aim is to reduce the troublesome symptoms, avoid complications and maintain normal growth without any side effects of treatment. Management can be categorized as non-pharmacological, pharmacological and surgical

Non-pharmacological Measures

Feed thickeners: This is an option in non-breast fed infants. They increase the stickiness and weight of the feed so that it remains in the stomach for a longer time and decreases the risk of regurgitation [18]. Esophageal intra-luminal impedance and pH studies in term healthy infants have demonstrated that feed thickeners decreased visible regurgitation by decreasing maximum height reached by the refluxate [19]. Various studies with use of different types of feed thickeners such as locust bean gum, cabor bean gum, xanthum gum, alginate with trials ranging from 1 to 8 weeks did not show superiority of any one over the other. Use of thickeners decreases reflux by 2 episodes/day and reduces anxiety of parents probably due to a placebo effect. It may be advised to term infants with mild disease [18]. However, thickeners can sometimes cause diarrhea due to increase in osmolality, and increased weight gain from high calorie density. In some babies, it may paradoxically increase symptoms of reflux by slowing down gastric emptying. Thickeners have not been found to be useful in preterm babies with some studies reporting an increased incidence of necrotizing enterocolitis [20,21]. In India, commercial infant feed thickeners are currently not available, but many use rice cereal as a cheap and acceptable alternative, even though there are no studies to support it.

Feed volume and position: It is necessary to assess for over-feeding when evaluating infants with GERD. There are no RCTs comparing volume of feed and its effect on GER [22]. Despite various intra-luminal impedance and pH studies showing that prone and left lateral positions decrease GER; only the supine position is still recommended due to increased risk of sudden infant death syndrome. Elevation of the head end of the crib and use of chairs can compromise respiration of infants by increasing pressure over the abdomen, and hence these are not advised [23]. Adolescents and older children with GERD can use left lateral position and head end elevation to decrease reflux-related symptoms [4].

Life style modification: Avoiding modifiable external factors like chocolates, tobacco, passive smoking, high fat meals, alcohol, late dinner and weight loss help to decrease reflux in adults. It can be recommended in adolescents and older children, wherever applicable [4].

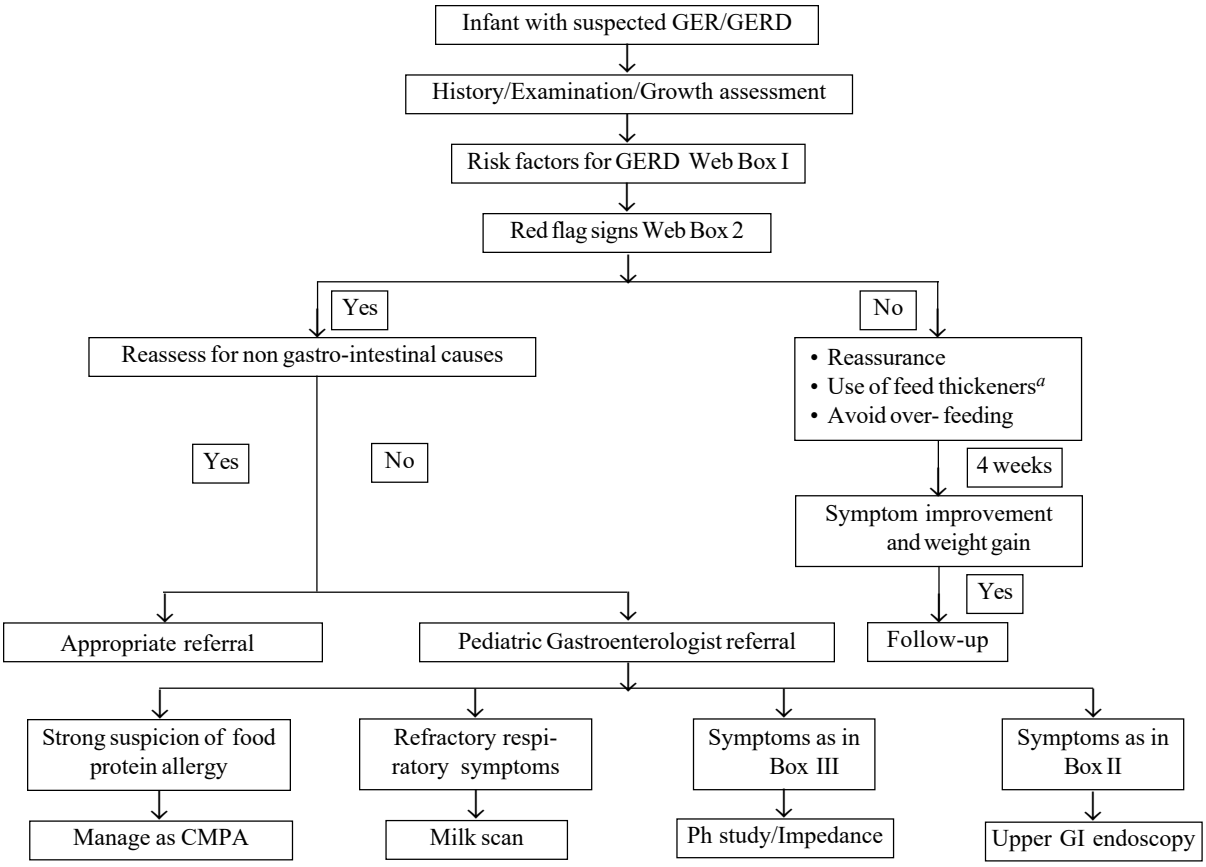
Hypo-allergenic formula: Symptoms of GERD and milk allergy in infants are non-specific and mimic each other. Since both the conditions are common in the West, some guidelines recommend a short trial of hypo-allergenic formula in babies who are not breast-fed. Since the prevalence of milk allergy in India is not known it is not scientific to support such a recommendation. It may be considered if there is a strong suspicion of milk allergy, after evaluation by a specialist.

Pharmacological Management

Proton pump inhibitors: Proton pump inhibitors (PPIs) are the anchor of medical treatment of GERD. They inhibit gastric acid secretion by noncompetitively inhibiting the active H^+-K^+-ATPase proton pumps in the gastric parietal cells. For optimal efficacy, PPI should be administered 30–60 minute before a meal so that it is absorbed before the proton pump is activated. They are the first-line therapy of erosive esophagitis in all age-groups and in the empirical treatment of typical symptoms of GERD i.e., epigastric or retrosternal pain in adolescents and older children. [11,24,25].

Evidence available so far does not support the use of PPIs for infants with unexplained crying or distress. In a recent study, where esophageal MII-pH monitoring tracings of 62 infants with unexplained distress were examined, it was found that the episodes of distress did not significantly correlate with GER [26]. Similarly, the role of PPIs in extra-esophageal symptoms like cough, wheezing or asthma is questionable [25]. A large randomized placebo controlled study of children with poorly controlled asthma showed no improvement in asthma control scores with the addition of a PPI to their asthma management plan [27].

The recommended duration of empirical PPI therapy for uninvestigated GERD symptoms is 2-4 weeks. In those in whom there is a complete resolution of symptoms, the PPI is tapered and stopped over 4-8 weeks. In partial responders, one may consider administering the same PPI twice daily rather than the conventional once daily dosing [28]. In those who do not show any response within 4 weeks, investigations (including an endoscopy) to rule out alternate causes for the symptoms should be performed (**Fig. 2**). Those with documented erosive esophagitis should receive 8 weeks of treatment [29]. Long term PPI at



[a]For babies on top feeding. CMPA-cow's milk protein allergy, GER-gastroesophageal reflux, GERD-gastroesophageal reflux disease.

Fig. 1 Algorithm for management of an infant with suspected gastroesophageal reflux disease.

the lowest effective dose should be advised for those who have a recurrence of symptoms after stoppage of the drug. It is important that such patients are periodically evaluated for its ongoing need with a specialist referral, when appropriate [30].

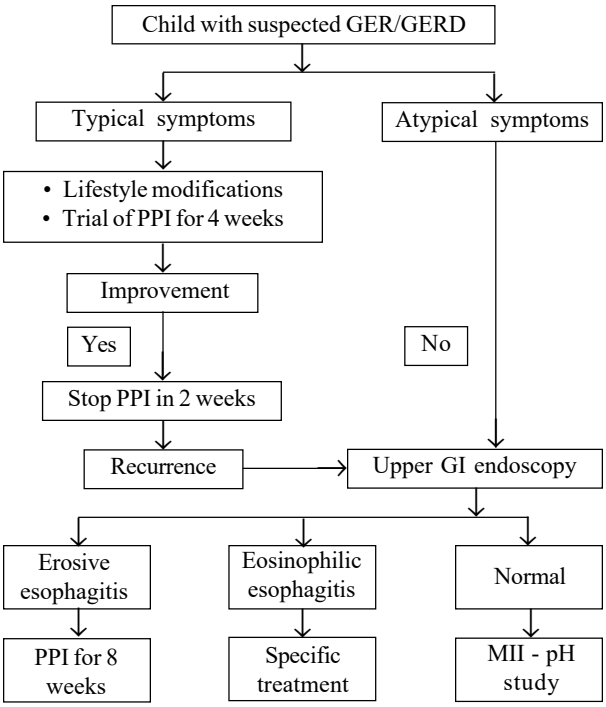
Available data does not support the use of PPIs in infants unless there is evidence of erosive esophagitis. In cases where a referral to a specialized center for an endoscopy is not possible, a trial of a PPI for up to 4 weeks may be acceptable, although not scientific.

There is no difference in efficacy between the various available PPIs (omeprazole, lansoprazole, esomeprazole, rabeprazole, pantoprazole) [24]. As a result of differences in pharmacokinetics and pharmacodynamics children require higher doses on a per kilogram basis as compared to adults (**Table II**).

Long term PPI use may have potential adverse effects which may include gastrointestinal and respiratory infections, acute and chronic kidney disease and adverse bone health [30,31]. However, the overall risk of these complications is low, with an absolute excess risk of 0.03-0.4% per patient/year, and it should not deter prescribers from using appropriate doses of PPIs for appropriate indications. It is important that PPIs are used appropriately and judiciously.

H2-receptor antagonist: H2-receptor antagonists (H2RAs) act by inhibiting the histamine H2 receptors of the gastric parietal cells. In a systematic review comprising 8 studies (276 children), H2RAs were better than a placebo in GERD symptom relief and tissue healing [32]. However, they are less efficacious than PPIs in both rendering a patient symptom-free and the healing of esophagitis and are indicated only if PPIs are unavailable or contra-indicated [4, 33].

H2RAs have a rapid onset of action and is therefore useful to provide immediate relief of symptoms. This is in contrast to PPIs, which are less efficacious for on-demand treatment. This is because a single dose of PPI does not inhibit all proton pumps immediately and takes almost 3



CMPA-cow's milk protein allergy, GER-gastroesophageal reflux, GERD-gastroesophageal reflux disease, P P I -proton pump inhibitors, MII-Multi channel intra luminal impedance.

Fig. 2 Algorithm for management of an older child with suspected gastroesophageal reflux disease.

days to reach a steady state. H2RAs are not suitable for long-term acid suppression, since they develop tachyphylaxis within a few weeks of use.

Ranitidine (dose: 5-10 mg/kg/day divided into two to three doses; Children > 30 Kg : 150 mg twice a day) is the most widely used. In view of the presence of N-nitrosodimethylamine (NDMA), a probable carcinogen, the Food and Drug Administration (FDA) and European Medicines Agency (EMA) have presently withdrawn the license of all ranitidine preparations.

Prokinetics: Prokinetics like domperidone and metoclopramide (cisapride, which is not available now) are used in treating GERD assuming that they would help in improving symptoms of GERD by increasing gastric emptying and consequently reducing the risk of reflux into the esophagus. However, there is little evidence supporting their use and individual meta-analyses for each of these prokinetics have found no benefit in the management of GERD [33,34]. Moreover, these drugs also have a potential for side-effects with metoclopramide associated with extrapyramidal symptoms and domperi-done and cisapride having been implicated in cardiac arrhythmias. Prokinetics are therefore not recommended for routine use in GERD.

Baclofen: Baclofen is a GABAβ receptors agonist that reduces the number of transient lower esophageal sphincter relaxations and accelerates gastric emptying. A meta-analysis of 9 RCTs in adults found baclofen to be useful in GERD. There are very few controlled trials in children. In addition, significant side effects such as lower seizure threshold, drowsiness and fatigue, have been reported and therefore it cannot be recommended for routine use in children [35].

Conventional antacids: Conventional antacids have no role for routine use in children because of low efficacy and potential side-effects such as aluminum toxicity with aluminum containing preparations and milk-alkali syndrome with calcium preparations.

Surface agents: Alginates are polysaccharides derived from brown seaweed and act as surface agents by forming a 'foam raft' on the top of gastric contents. A Cochrane review which included 5 RCTs in children concluded there is moderate evidence that alginates improve symptoms [24]. Currently it is available in India only in combination with conventional antacids (sodium bicarbonate 133.5 mg, sodium alginate 250 mg and calcium carbonate 80 mg per 5 mL) and the dose recommended is 10-20 mL/dose (after meals) in children ≥12 years old.

Table II Proton Pump Inhibitors Available in India

Drug	*Pediatric dose (mg/kg/day)*	*Adult dose (mg)*	*Approved age*	*Available formulations*
Omeprazole	1-4	20	1-17 y	Tablet, capsule, intravenous granules for oral suspension
Lansoprazole	0.7-1.44	30	1-17 y	Tablet, capsule, oral disintegrating tablet
Pantoprazole	1-2	40	5-17 y	Tablet, capsule, intravenous
Esomeprazole	3-5 kg-2.5 mg; >5-7.5 kg-5 mg; >7.5-<20 kg-10 mg; ≥20 kg-20 mg	40	1 mo to 17 y	Tablet, capsule, intravenous, granules for oral suspension
Rabeprazole	0.5-1	20	1-17 y	Tablet, capsule, intravenous granules for oral suspension

All drugs to be given once-a-day.

Sucralfate is a sucrose–sulfate–aluminum surface agent. It reacts with hydrochloric acid to form a cross-linking viscous material that attaches to the mucosal surface. There is limited data regarding its usefulness in children [33]. It is not indicated in the routine management of GERD in children.

Refractory GERD

It is defined as GERD unresponsive to eight weeks of optimal treatment. Refractory GERD is common in children who are neurologically impaired or have had esophageal atresia repair surgery. They usually have associated esophageal dysmotility, delayed gastric emptying and swallowing dysfunction. Such patients should be evaluated at a specialty center for anti-reflux surgery.

Since children with neurological impairment and recurrent aspiration often have swallowing dysfunction, the benefit of transpyloric feeding is difficult to establish. Presence of a nasogastric tube per se can sometimes increase reflux. However, a short trial of transpyloric feeding is justified prior to fundoplication [4].

Surgical Treatment

Surgical interventions are necessary only when medical management is unsuccessful. Surgery can be considered with GERD-related Acute life threatening events (ALTE) in infants, persisting troublesome symptoms despite adequate medical therapy, inability to withdraw medications and complications like recurrent peptic strictures and Barrett's esophagus [36].

Fundoplication: Nissen fundoplication (complete wrap), Thal fundoplication (anterior wrap) and Toupet fundoplication (posterior wrap) are done for management of GERD. Laparoscopic or open surgery can be considered based on the available surgical expertise, as both have similar short-term clinical outcome [37]. Before surgery, it is necessary to rule out associated comorbidities like esophageal dysmotility, and hiatal hernia. A meta-analysis of anti-reflux surgeries, which included 1280 children, demonstrated 86% median success rate for improvement of esophagitis related symptoms [38]. Neurologically impaired children had more postoperative complications (47%), higher mortality (13%) and failure rate (18%).

CONCLUSIONS

The recommendations are summarized in **Box IV**. Gastroesophageal reflux is an age-related phenomena in infancy, while in older children it may be related to life style. It is a clinical diagnosis and most need no investigations. When pathological, symptoms may be esophageal or extra-esophageal. Investigations like upper GI endoscopy, MII-pH are required in a select few, depending on the nature and severity of symptoms. PPIs are the mainstay in treatment. Empirical therapy has no benefit in infants, while a 4-week trial can be given in older children with symptoms suggestive of esophagitis.

Box IV Recommendations

- In most infants, GER is physiological and will improve with age. Investigations are required only if the reflux is pathological (GERD).
- In older children, esophagitis is the commonest manifestation of GERD.
- Upper GI endoscopy may be required in those with esophageal manifestations, dysphagia and hemetemesis. MII-pH monitoring and scintigraphy may be recommended in those with extra-esophageal manifestations.
- Barium contrast studies should be done only if an underlying anatomical abnormality is suspected.
- Empirical PPI therapy in infants is not justified, but a four week trial may be undertaken in older children. While positioning and feed thickening have limited benefit in infants, life-style modifications are important in older children.
- Refractory GERD is most commonly seen after esophageal surgery and in neurologically impaired children, and hence needs specialist care.

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ANNEXURE

Participants in the Online Expert Group Meeting to Develop the Guidelines

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Web Box I Risk Factors for Development of GERD
Infants
Prematurity
Medications as theophylline/caffeine for apnoea
Congenital anomalies of respiratory tract
Post-operative esophageal atresia/diaphragmatic hernia
Neurological impairment
Abdominal wall defects as gastroschisis and omphalocele
Nasogastric tube placement
Children
Obesity
Chronic respiratory disorders/wheezing/ asthma
Neurological impairment
Hiatus hernia
Post-achalasia treatment
Diet rich in drinking soda, spicy and fatty food

Web Box II Red Flags in Gastro-esophageal Reflux Disease (GERD) and Differential Diagnosis

Infants

Symptoms < 1 week of age: Necrotising enterocolitis, Congenital G I tract anomalies
Vomiting: CMPA, Pyloric stenosis, Intestinal obstruction
Seizure / Hepatosplenomegaly: Metabolic disorders
Microcephaly/dysmorphic features: Genetic syndromes
Macrocephaly/bulging fontanelle: Raised intracranial pressure
Choking, coughing with feeds: Palato-pharyngeal incompetence,Tracheo-esophageal fistula
Fever/ lethargy: CNS infections, Urosepsis
Constipation/abdominal distension: Hirschsprung's disease

Children

Abdominal pain/ GI bleeding: Acid peptic disease
Stereotypical/ cyclical episodes: Cyclical vomiting syndrome
Behavioural changes: Rumination; unchausen syndrome

Interventional Study Designs

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Interventional studies are specifically designed to evaluate direct impact of therapeutic or preventive measures on outcomes by assigning participants into treatment/intervention or control group. Main types of interventional study designs are: single-arm interventional studies, non-randomized controlled trials, cross-over trials, randomized controlled trials, and cluster randomized trials. Each of these study designs has its own set of advantages and disadvantages, which need to be assessed and reviewed in the design phase of the study to choose the most appropriate design. Purpose of this article is to provide concept and processes of various interventional study designs along with their utility and limitations.

Keywords: *Randomized controlled trial, Randomization, Research methods, Statistics*.

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Interventional studies intend to evaluate the efficacy or safety of specific therapeutic, preventive educational measures by assigning individual participants or a group (cluster) of participants to receive an experimental intervention, and often another group receiving a comparator or no intervention [1]. In observational study design, an investigator records presence of exposure and outcome without trying to change the course of natural events. In contrast, interventional study designs evaluate the direct impact of treatment or preventive measures on diseases, and have the potential to change the practice and policy. Thus, they are ranked towards the top in evidence-based medicine pyramid [2]. Interventional study designs can be broadly categorized into the following types: Single-arm interventional studies; Crossover trials; Non-randomized controlled trials; and Randomized controlled trials (RCTs). **Table I** provides a brief overview of the different types of interventional studies [3-7].

SINGLE-ARM INTERVENTIONAL STUDIES

Single-arm interventional study is the simplest trial design without a comparison group. The study participants are administered a new therapeutic or preventive (e.g. vaccine) intervention, and then followed up to evaluate its response. However, clinical equipoise exists when we are uncertain about the benefit or harm offered by the treatment to a patient. It is unethical to conduct a trial of a drug whose efficacy has not been established, and thus availability of preliminary data in form of animal experiments, case reports or case series is essential before conduct of such studies. The ethical decision-making process requires a comprehensive plan which incorporates consent, assent and full disclosure of information.

Few examples of published single-arm interventional studies are: study on safety and efficacy of antiretroviral drug darunavir with low-dose ritonavir in treatment-experienced patients with HIV [4]; a single arm pilot trial of brief cognitive behavioural therapy for insomnia in adolescents with physical and psychiatric comorbidities [5]; and studying the outcomes of flash glucose monitoring in children with type 1 diabetes [6]. Single-arm trials have a unique role when controlled design is not feasible, desirable or ethical. These studies pave the way for providing important preliminary efficacy and safety data.

CROSSOVER TRIALS

In a crossover trial, participants are randomly allocated to study arms where each arm comprises of two or more treatments given sequentially. In this type of interventional study, the study participants are intentionally crossed over to the other treatment arm after they have received one treatment for a specified duration [8]. It begins as a usual RCT but at the end of first phase of treatment, the participants are crossed over to the other arm (**Fig. 1**). There is usually a washout period between the two intervention periods. Washout period is defined as "a period of time during a clinical study when a participant is taken off a study drug or procedure in order to eliminate the effects of the treatment" [8].

Table I Comparison of Different Types of Interventional Studies

	Single-arm	*Randomized controlled trial*	*Cluster randomized trial*	*Non-randomized*	*Cross-over*
Number of study groups	One	Minimum two	Minimum two clusters of participants	Minimum two (control group may be concurrent or historical)	Minimum two (each arm receives two or more treatments given sequentially)
Prerequisites	Aware of natural history of disease or intervention through animal models or pharmacokinetic studies	Sequence generation and allocation concealment (with or without blinding) to decide allocation to intervention arm and control arm with pre-decided endpoint to evaluate outcomes (decided *a priori*)	Fix the number of clusters and cluster size depending on available resources and time	Precise inclusion and exclusion criteria, and outcome variables need to be defined *a priori* even in absence of proper randomization process	Washout period between two
Application	Phase-2 of clinical trials	Testing new intervention in comparison to standard management	Evaluation of effectiveness of interventions and public health strategies on a wider scale	Best when act of randomization is unethical or impractical	Study of short-term outcomes in chronic diseases
Advantages	•Feasible with limited study participants •Desirable when placebo is unethical e.g., Cancer trials [3]	•Robust design with ability to determine cause-effect relationship between intervention and outcome • Minimal bias	More feasible and practical when evaluating families and large population groups.	•Easy to carry out • Low cost • Design facilitates recruitment of larger population	•Risk of confounding is minimal •Requires lesser number of study participants
Limitations	• Limited validity • Lacks quantification of magnitude of effect	• Stringent ethical and regulatory guidelines	• Study design, analysis and conduct are more complex compared to RCT • Larger sample size required owing to design effect	• Susceptible to selection, attrition, detection and performance bias • May show association and trends but cannot test cause and effect	• Effect of one treatment may carry over and alter the response to next treatment
Examples	• Study on safety and efficacy of antiretroviral drug darunavir with low-dose ritonavir in treatment-experienced patients with HIV [4] • Evaluation of drugs for the treatment of chronic hepatitis C infection [5] • Studying the outcomes of flash glucose monitoring in children with type 1 diabetes [6]	• Randomized controlled trial of zinc as an adjuvant therapy for severe pneumonia in young children [7]	• Medical practitioners in trials evaluating the efficacy of disease screening programs • Communities in trials evaluating the effectiveness of new vaccines • Hospitals in trials evaluating educational guidelines directed at physicians and/or administrators	• Comparison of children receiving insulin via infusion pumps (treatment group) with children who had received standard therapy in the past from the same hospital (control group)	• A cross over trial to compare the effects of butter diet or margarine diet on lipoprotein levels [7]

To understand this type of study design, a simple XY/YX study model can be used (**Fig. 1**). In first phase of treatment, participants enrolled in the XY study arm receive treatment X whereas those in YX arm receive treatment Y. After a washout period, participants are intentionally crossed over such that participants who had received treatment X will receive treatment Y, and those who had received treatment Y will receive treatment X. The washout period is determined to ensure that during this period the effects of treatment received first wanes off. For this, investigators must know the likely maximum duration of effects of both the interventions. In this type of study design, risk of confounding is minimized as all interventions are measured on the same participant, which means participants serve as their own control. In a crossover trial, lesser number of study participants are required than in an RCT. The biggest disadvantage of crossover trial is that the effect of one treatment may carry over and alter the response to next treatment, even after the washout period.

This type of study design is best suited for study of short-term outcomes in chronic diseases. It cannot be used for acute conditions as the illness has to last for long enough to allow the crossover, and allow the investigator to measure the response to intervention. Commonly, crossover designs are used for drugs, but they can also be used for dietary interventions [7,9]. For example, a crossover trial conducted to compare the effects of butter diet or margarine diet on lipoprotein levels of 49 volunteers with polygenic hypercholesterolemia [7]. One group received butter diet, and the other received margarine diet for six weeks. After the first phase, there was a washout period of five weeks when all the participants were asked to revert to their usual diet. In the second phase, the participants who had received margarine diet were crossed over to butter diet and vice versa for next six weeks. Blood samples for various lipids were collected at the start of the study, after the end of first phase of treatment, and at the end of second phase of treatment. In this study, authors had assumed six weeks of experimental period as adequate to affect the lipoprotein level and five weeks of washout period to dissipate the affects. Crossover trials cannot be done in educational interventions or where illness is self-limiting or does not require continuous medications where washout cannot be validly done.

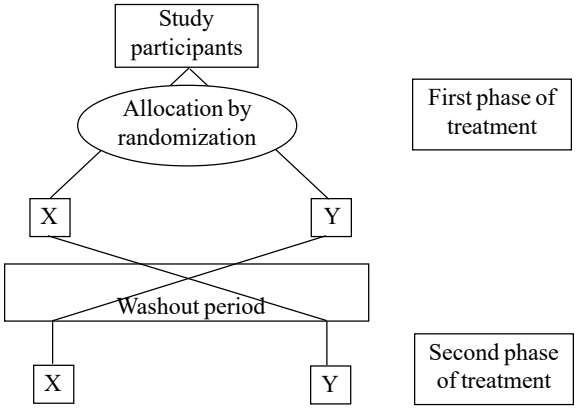

Fig. 1 Crossover randomized controlled trial.

NON-RANDOMIZED TRIALS

Non-randomized trial is a type of study design where investigator controls the allocation that is not at random. Non-randomized trials are also referred to as quasi-experimental designs as they do not meet the criteria of true experimental design such as random assignment of participants to intervention or control group. This type of study design differs from observational study in a way that allocation of intervention to patients is still in control of researchers as per research protocol. Similar to observational study, in non-randomized trials, variables need to be identified and measured to get two comparable groups. Precise inclusion and exclusion criteria need to be documented for the study population. These trials can show associations and trends but cannot validly test cause and effect hypothesis. They can be done in community settings, can involve more people from the community thus making the results more generalizable, and hence helps to increase the external validity of the study.

Non-randomized trials are best used study designs where randomization will reduce the effectiveness of intervention. For example, studies where effectiveness of any intervention largely depends on participants' active participation, which in turn is influenced by their beliefs and cultural or social preferences. They are also preferred when randomization is unethical or impractical (cost factors). These study designs have advantage of having a control group, which takes care of threats to internal validity from the unaccounted changes in clinical care, nature of disease or confounding effect of other co-interventions.

The biggest disadvantage of this type of study design is bias and confounding. As the study is non-randomized, investigators can select study participants to get the best results of the trial. Other disadvantages are susceptibility to attrition, detection and performance bias. Attrition bias would result from dropouts, detection bias if assessment of outcomes is not standardized and blinded, and performance bias if there are errors in allocation, application and recording of interventions. The selection of study sites and the allocation of participants to treatment groups are among the most challenging issues in nonrandomly assigned control group studies. There are two different types of controls viz., concurrent controls and historical controls.

Concurrent controls: Here, treatment and control group participants are matched at group level based on demographic and other characteristics. They are given different treatment conditions at the same time but in different settings. For example, in a non-randomized trial of a new oral hypoglycemic drug in adults with type 2 diabetes mellitus, we can assign the participants to control or treatment groups based on where they would receive the treatment (setting); like hospital A, where standard treatment is available and hospital B, which will give the new drug to be tested.

Historical controls: Here, investigators will compare outcomes among group of participants who are receiving new treatment (experimental group) with outcomes among participants who received standard treatment in a previous period (control group). Thus, in historical controls we are comparing the two groups in similar settings but different periods of time. We can understand this by the following example. In order to test different mode of administration of insulin in children (insulin pumps versus standard), we apply a set of inclusion criteria to get similar baseline characteristics of study population. Thereafter, we compare children receiving insulin via infusion pumps (treatment group) with children who had received standard therapy in the past from the same hospital (control group). Here we are comparing two groups in similar setting which in this case is same hospital but in different period of time.

To summarize, in non-randomized controlled trials, participants are assigned to groups using a non-random procedure. They are easy to carry out and lower in cost in comparison to RCTs, and lack of randomization may facilitate recruitment of larger population.

RANDOMIZED CONTROLLED TRIAL

The randomized controlled trial (RCT) is a study design in which participants are randomly allocated to either the experimental group, where they receive the intervention or drug that is to be tested, or other group (comparison group or control group) which receives placebo, no treatment or alternative/conventional treatment (**Fig. 2**). Both groups are then followed-up till a pre-decided endpoint to evaluate outcomes, which have been decided a priori. For example, a randomized controlled trial [10] of zinc as an adjuvant therapy for severe pneumonia in young children, where participants in the experimental group received oral zinc in addition to standard management whereas the control group participants received placebo in addition to standard management.

Randomization is the principal technique that makes an RCT effective by minimizing various biases. **Table II** enlists types of biases encountered in clinical research, with the processes which address these biases. Randomization means that each participant has an equal chance of being allocated to the experimental or control group, and the researchers have no control in deciding who is assigned to which group. The aim of randomization is to have two groups that are similar in all respects, both for measured and unmeasured factors. After recruitment, baseline characteristics of the recruited study participants such as age, gender, clinical condition, comorbidities, and all-important prognostic factors are measured before the intervention to ensure that they were equally distributed between the two groups. As per the Consolidated Standards of Reporting Trials (CONSORT) guidelines on reporting an RCT, it is important to show comparison of baseline variables in an RCT [11].

Elements of Randomization

Randomization consists of two key and essential steps: *i*) sequence generation – generating a random sequence to ensure that each participant has equal (or in a predetermined ratio) chance of being allocated to either group; and *ii*) allocation concealment – to ensure that nobody knows to which group the participant will be allocated till the intervention is administered. In addition, blinding or masking may be employed to further ensure that study participants and researchers continue to be unaware of the nature of intervention (experimental or control) till the outcomes are finally measured or sometimes even till statistical analysis.

Sequence Generation

Sequence generation for randomization is presently mostly done through computer programs. However, manual randomization is possible by use of random number table.

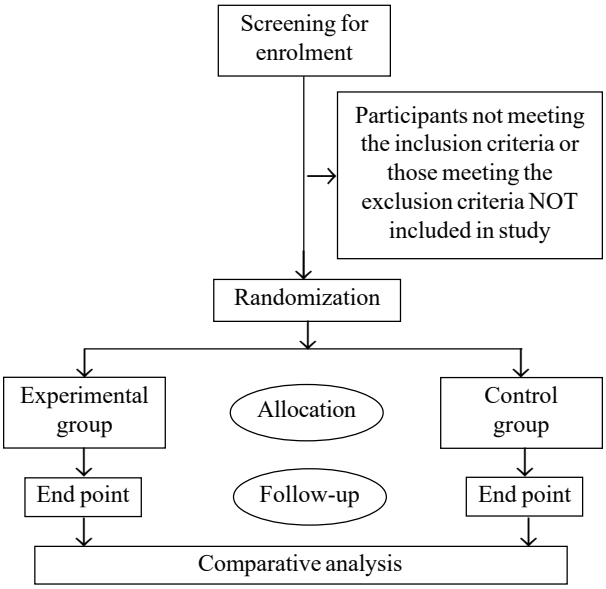

Fig. 2 Flow diagram of randomized controlled trial.

Table II Different Types of Biases Minimized by Randomized Controlled Trials

Types of bias	*Potential effects of bias*	*Measures to resolve the bias*
Selection bias	Differences in baseline characteristics of the groups that are being compared	• Sequence Generation • Allocation Concealment
Performance bias	Difference in level of care or exposure to factors other than interventions of interest between the comparison groups	• Blinding of participants and investigators
Differential compliance bias	Participants in the two arms may have different levels of compliance to assigned therapy	• Blinding of participants
Detection bias	Differences in determining outcomes between comparison groups	• Blinding of outcome assessors
Follow-up bias	Large loss to follow-up or difference in number of participants lost to follow-up	• Intention-to-treat analysis
Reporting bias	Difference in reporting of outcomes	• Trial registration

Various types of randomizations for generating sequence are described as follows:

Simple randomization: Randomization based on a single sequence of random assignments is known as simple randomization. This is one of the simplest forms of sequence generation where participants are randomly assigned into treatment/intervention group or control groups. Various methods that can be used for simple randomization are tossing of coin (e.g., heads-treatment; tail-control), shuffling of cards (e.g., hearts and diamonds-treatment; clubs and spades-control), drawing of lots, or throwing a dice (e.g., 1,2,3- treatment; 4,5,6-control) or by using random number table. A random number table found in statistics books or that generated by computer can be used. For example, in a study with two groups, A and B, we may decide that odd digits will designate assignment to treatment A and even digits (and zero) will designate treatment B. The treatment allocation that is described by the random number is written on a master list to match the sequence in which the patients are enrolled in the study. So, if the first random number is 2, treatment B will be written in the master list against patient 1; if the next random number is 7, treatment A is written against patient 2, and so on, as determined by the random numbers. Thereafter, this sequence must be concealed by appropriate methods (described later). Assignment in simple randomization can also be done unequally in the groups by assigning more random numbers to one arm. For example, if the desired case to control ratio is 1:2, the random numbers ending with 1, 2 and 3 can be assigned to intervention group whereas random numbers ending with any digit between 4 to 9 can be assigned to control group. Any number ending with zero will have to be ignored in that case, and the immediate next number is considered for generating sequence.

Simple randomization is the most unrestricted form of randomization where every participant has equal chance of being allocated to either group, and is the preferred form of randomization in large RCTs. However, it has limited applicability in studies with small sample size as it can result in unequal number of participants among two groups.

Block randomization: In block randomization, study participants are divided into blocks of size 2n so that each arm gets 'n' number of participants in each block. The sequence within the blocks is determined in a randomized manner so that it is not easy to be guessed. For example, if there are two groups A and B, blocks of size 4 will have possibilities of following sequences: AABB, ABAB, ABBA, BABA, BAAB and BBAA. These blocks are arranged in a randomized manner so that it is not known, if first patient is allocated to group A, what group (A or B) the next patient will belong to. After the enrolment of every 4th participant, it will be ensured that equal number of participants are allocated to each group. The possibility of varying sequences within the block will increase with the increasing block size.

The main advantage of block randomization is to ensure equal number of participants at the end of the study, and also earlier if the study may have to be stopped because of any reason. It also takes care of ensuring equal numbers in each group during different time periods of the study, such as different seasons of the year or different research conditions. Block randomization is especially handy in cases of studies with small sample size where a simple randomization may not result in equal sample size in both groups, sometimes compromising statistical sample size needs. The disadvantage of block randomization is that if someone knows the block size, the group of last participant can be guessed (fixed block design). Even if the block size is not known to the investigators, it is possible to guess the block size by examining the pattern of sequence of patient enrolment after few patients are enrolled,

particularly in unblinded studies. This problem can be taken care of by making the block size variable within the study e.g., some blocks having size of 4, others with size of 6 or 8 (variable block design). Block randomization is one of the most common methods of randomization used in published RCTs.

Stratified randomization: In stratified randomization, study population is initially classified into homogenous subgroups called strata, and then samples are drawn randomly from each strata. Finally, results from all strata are combined. It ensures representation (equal or in a particular ratio) of participants with baseline covariates such as age, gender, race, disease severity. It also allows analysis of applicability (or otherwise) of results to some special strata, and helps in assessing confounding effect of factors included in stratification (like age) and need of any statistical adjustments in analysis. In the RCT on efficacy of feeding regimens for home-based management of children with uncomplicated severe acute malnutrition [12], age-based stratified randomization was done for age categories 6-17 months and 18-59 months so that young children are equally represented, and the results of study are applicable to them. Disadvantages of stratified randomization is loss of precision if small numbers are being sampled in each stratum. Sample size requirements increase according to the number of strata, particularly if applicability of results to each stratum is desired to be analyzed.

Other methods of randomization: Urn randomization, Covariate adaptive randomization and minimization are also sometimes used in clinical trials. In urn randomization, number of balls in urn equals to number of treatments, which remain unchanged in the study. For example, investigator starts off with an urn that contains a red ball to represent treatment A and a green ball to represent treatment B. If the first draw pulls green ball, the green is replaced with red ball increasing the odds that red will be drawn next. This procedure works best for small sample size and helps to prevent imbalance in the two study arms.

In some clinical trials, covariate adaptive randomization (CAR) is used in place of pure randomization so as to reduce the covariate imbalance between treatment groups. CAR is preferably used in small- to moderate-sized clinical research where simple randomization can lead to inequality of important covariates among treatment groups. In CAR, first randomization is according to baseline covariates and then assignment of treatment is done based on these covariates. It helps to maintain balance between the two groups with equal distribution of covariates.

Minimization is a type of adaptive stratified sampling used in clinical trials with the aim to minimize the imbalance between the two arms. It addresses the imbalance by calculating and adding all the imbalance in the study. Minimization often maintains a better balance than traditional block randomization, and its advantage increases with the number of stratification factors.

Nowadays, computer softwares and online calculators are used for all above types of randomization. Various programmes are available for generating allocation sequence [13]. The random numbers generated by the software generators are pseudo-random. By using the same seed, we can get the same random number sequence. This provides us the possibility of reproducing a randomization schedule. These number generators are stored in the core of computer. Each study participant is provided a unique identification number which is maintained till the end of the study. Some online randomization resources are: *www.sealedenvelope.com* and *www.graphpad.com*

Allocation Concealment

The generated sequence must be implemented in such a way that the study participants and researchers are unaware of which group a participant is going to be assigned till the assignment is actually done. This is different from blinding in the manner that in 'blinding', the participants and researchers remain unaware of the type of intervention even after it is administered, and outcomes are measured without knowing whether the group is treatment arm or the control arm; whereas in 'allocation concealment' the lack of awareness is only till the group is assigned. Thus, blinding is an optional component of RCT and may not be even possible in some designs; whereas, allocation concealment is the essential ingredient, and is possible in all settings. In absence of allocation concealment, we can get a biased effect of treatment to the extent of 40% or even more [14].

For example, a new injectable vaccine is to be tested in a clinical trial, and the other group has to receive no intervention. If investigators have access to the complete list of sequence of participants and their allocation (e.g., vaccine for first participant, no vaccine for second participant, no vaccine for third participant, vaccine for 4th participant and so on), the allocation is not concealed and investigators will have the choice to assign a preferred participant to the vaccination group by altering the sequence in which that 'preferred participant' enters the study. Thus, this is a breach of randomization process. As this is a trial where one group receives an injection and the other does not receive it, blinding is not possible, but allocation concealment is still necessary so that investigators have no control in deciding who receives the vaccine and who does not. Following approaches are commonly used for allocation concealment:

Central randomization: In this process, the investigator contacts a central agency (such as a helpline or independent statistician not involved with study) as soon as an eligible participant consents to be enrolled in the study, and the centre informs the randomization code/ group to the researcher. This technique is particularly useful in multicentric studies where there is a common randomization sequence for all the sites. Alternatively, each site can have their randomization sequence as per the number of patients to be enrolled by that site.

Serially numbered opaque sealed envelope (SNOSE) technique: A pre-set sequentially numbered sealed opaque envelopes with randomization code are prepared by an independent person after referring to the generated sequence and are handed over to the investigators. The investigators preferably write the name/identifier of participant over the envelope after the participants consents to be enrolled in the study, open the envelope as per the sequence of enrolment, and allocates him/her to the group/code mentioned in the slip inside envelope. The allocated sequence of enrolment may be audited periodically by the independent person who has generated the sequence by matching with his/her own list. This is the most common and most convenient allocation concealment technique used in published research. However, there is still a scope of manipulation by researchers who can make a 'preferred participant' wait till their desired envelope is opened, and allowing another participant enter the sequence in between. If envelopes are not totally opaque or sealed, researchers may try to see the hidden code and manipulate the sequence of entry of participants.

Pharmacy-coding: For a clinical trial, allocation concealment can also be coordinated by the hospital pharmacy at a trial center. Pharmacists can dispense the trial drug to a patient based on the unique randomization code for that patient. A code list which links up with central randomization code can be provided to the pharmacist. On the other hand, the trial drugs can be labelled by the manufacturer or drug packager. The list with the labels can be provided to the pharmacist.

Blinding (Masking)

Blinding (or masking) refers to withholding information about treatment assignment from participants and investigators to prevent bias in assessment of outcomes, particularly subjective outcomes such as patient comfort, adverse events and perception scores [15]. Though, it is an important element of minimizing bias in an RCT, blinding may not be always possible or feasible. For example, in a clinical trial of medical versus surgical management of appendicectomy in acute appendicitis in children, blinding will not be possible as researchers and patients will know whether they have undergone surgery or not. Following terms are commonly referred in reference to blinding in RCTs (**Fig. 3**) [15]:

Single blind: The participants receiving the experimental or control intervention are not aware in which arm they belong, but the researchers might be knowing the same. However, this is not a true blinding as there is always a possibility of researchers disclosing the nature of intervention to the patients. Ideally, blinding should not be dependent on honesty of researchers, but it has to be inbuilt into the study design so that there is no possibility of breaching it by being dishonest or sometimes even considerate or sympathetic.

Double blind: In this process, participants as well as the investigators assigning the intervention, and those recording the outcomes are unaware of the treatment assignment until the end of the study. Sometimes, some investigators use the term triple blind when a person carrying out the analysis is also unaware of the assigned treatment. However, this is not a universally accepted terminology.

Modalities of blinding: Blinding is not just keeping the names of treatment hidden from the participants and the investigators. It is a robust procedure, particularly when the response criteria are subjective like relief of pain. Sometimes, the color or the smell of the drug to be tested becomes a clue for the study participants and researchers to decipher which group they belong to. In order to ensure effective blinding, the placebo or comparator drug must be similar to the experimental drug in appearance, odour, packaging and mode of delivery as much as possible. Placebo is a substance or a procedure (sham), which is

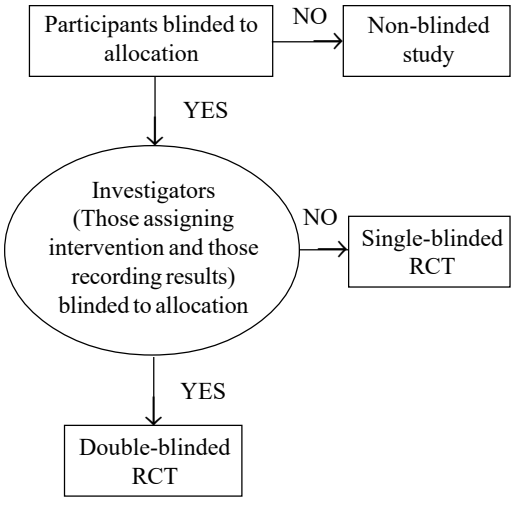

Fig. 3 Blinding (masking) in an interventional study design.

administered to the control group but it has no biological or therapeutic value. It not only achieves blinding if it is made similar in appearance, taste and smell to the experimental drug, but also takes care of the differences in psychological effect (placebo effect) the participants might perceive just because they are receiving an intervention (oral drug or injection). To ensure that blinding has been achieved, it is important to periodically ask participants which intervention (experimental or control) they think they are receiving, and recording and comparing them between the groups. Studies involving educational interventions, surgical inter-ventions, or alternative treatment strategies (e.g., yoga, physical activity) will be difficult to be blinded effectively. Whenever possible, investigators must attempt blinding, and sometimes it involves innovation and critical thinking. Blinding is not easily applicable in surgical RCT's, as there is a physical component involved. However, there are trials where patients or patients and assessors were blinded [16,17]. Randomized controlled trials which have not ensured effective blinding are known to show erroneously larger treatment effects [18]. Thus blinding should be incorporated into an RCT, wherever feasible.

Cluster Randomized Trial

Cluster randomized trial is a comparative study design in which clusters of individuals rather than independent individuals are randomly allocated to intervention groups (**Fig. 4**). Clusters are defined as groups of people who have common identifiable feature and the outcome measured in the representative sample of the individual member of the cluster will equate for the rest of the members [19]. Components or members of clusters are more likely to have comparable results than an arbitrarily nominated sample of individuals from the same population. The groups used in cluster can vary in size from families to entire communities. Examples of randomization unit in a cluster RCT can be communities – in trials evaluating the effectiveness of new vaccines, or hospitals – in trials evaluating educational guidelines directed at physicians and/or administrators.

A cluster RCT to increase childhood influenza vaccination was done in 20 primary care practices treating children between 2011-2012. Here the unit of randomization was primary care practices. These clusters (primary care practices) were randomly allocated to intervention and control arms [20].

Cluster RCT is preferred methodology when we need to evaluate public health policy and national programs. Cluster RCT in vaccine trials can be done by randomization of geographic areas to capture indirect (herd) effects of vaccination. Incidence of disease among non-vaccinated persons in the study group is compared to incidence of disease in the control group. In comparison to RCT, cluster RCT is cost-effective with decreased adminis-trative convenience and lower implementation costs. Study design, analysis and conduct of cluster RCT are more complex as in comparison to individual RCT. Total sample size in cluster RCT is function of number of clusters and cluster size. We can fix one of them and determine the other using fixed formulas; for example, we can fix the number of clusters and calculate the cluster size. To understand this, let us take the following example of a case study where number of clusters is fixed. A study is planned to test the effectiveness of newly designed kit for diagnosis of Group B streptococcus infection in pregnant patients at the time of labor. Hospitals are now randomized into kit based or standard methods to diagnose streptococcus infection. Here the limiting factor is the number of kits. Thus, we have to minimize the number of clusters. In this case, number of clusters becomes fixed. Considering the same example if we assume the trial will run for 8 months and cluster size is set as number of women meeting specific set of eligibility criteria, a fixed cluster size of 300 is set as maximum for the

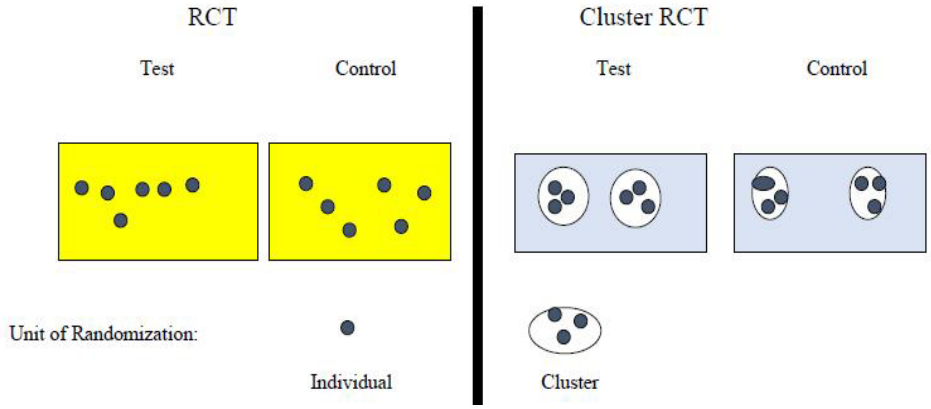

Fig. 4 Diagrammatic representation of a cluster randomized trial in comparison to randomized controlled trial at individual level.

given funding and trial duration. Decision on number of clusters and cluster size should be made simultaneously and not independently. For a cluster trial to be called a cluster RCT, it is must that a proper process of randomization of clusters to one or other intervention group is followed.

Another issue while analyzing cluster RCT is intra-class correlation. Measure used to assess degree of correlation within the clusters is called intra-class correlation coefficient (ñ). Larger the coefficient more the number of clusters required to have an adequately powered study. In order to keep power of the study, the sample size should be multiplied by 1+(*m* -1)ñ, called the design effect, where *m* is the average cluster size [19]. Nowadays statistical software take care of adjustments for the intra-class correlation coefficient. If we fail to analyze or take into account the intra-class coefficient, a falsely inflated statistical significance is obtained. Double jeopardy is seen when loss of statistical power is further exaggerated with effects of clustering seen on the treatment [20]. While analyzing cluster RCT, it must be ensured that adequate number of clusters are recruited to ensure adequate statistical power and intra-class correlation of outcome and measurement is minimized. Stepped wedge cluster RCT is an alternative to parallel cluster trials where researcher wants to evaluate service delivery or policy intervention at the level of cluster. There is an initial period where none of the clusters are exposed to intervention. Subsequently at regular intervals/steps one or a group of clusters are randomized to cross from control to intervention arm. This process would continue until all clusters have received the intervention. Finally at the end all clusters would have been exposed to the intervention. Thus each cluster would contribute to control arm and intervention arm giving a more generalizable result.

To conclude, there should be a rationale for adopting cluster design. Clustering must be incorporated into sample size estimation and analysis. There should be a chart showing flow of clusters through the trial from assignment to analysis.

Compliance and Attrition in RCT

Non-compliance is failure to adhere to treatment protocol. It tends to minimize any difference between the groups resulting in reducing the statistical power to detect a true difference and hence the true effect will be biased toward the null. RCTs are also marred with the problem of loss to follow up. This can occur in both study arm and the control arm. Loss to follow-up could be due to a number of reasons like study participants losing interest, adverse effects of the treatment or intervention, difficult to follow or complex treatment protocol, or if the protocol is socially unacceptable. Loss to follow-up is crucial factor to affect the validity of study. It needs to be calculated and proper calculation can be done by determining the correct denominator like including all the study participants enrolled in that arm. If percentage of loss to follow-up is less (say <5%), it is less likely to affect the validity of the study. But if it is high (say >20%), it may affect the validity of the study. Nobody can be excluded from an RCT once the randomization is done. We should follow the rule of once randomized always analyzed, irrespective of non-compliance, loss to follow-up, protocol deviations and withdrawal from the study. In order to deal with missing data, last observation carry forward method or last available measurement of the individual just prior to withdrawal or loss to follow-up from the study may be retained in the analysis. This methodology of including all participants as originally allocated in the final analysis has been termed as Intention-to-treat analysis (ITT) [21]. However, questions do arise about the efficacy of the treatment or intervention if we are including those subjects in final analysis who never received the treatment/ intervention or received it for inadequate duration. Thus, in RCTs, per-protocol (PP) analysis is usually also performed that includes only those patients who have adhered to the treatment protocol and completed the study period with complete availability of outcome. However, it has the disadvantage of showing exaggerated treatment effect. Both ITT analysis and PP analysis should be reported in the reporting of parallel group randomized controlled trials as per the CONSORT guidelines.

Outcome Measures of RCT

In order to assess the effect of intervention in an RCT, outcome measures or measure of effect is used. Outcome of an intervention can be assessed either through clinical examination of patient, laboratory work-up or can be patient reported. Outcome measures should be relevant to the target population of the interventional study. The primary outcome of the study should be decided according to the main study objectives which determines the sample size in each group. If there is more than one primary outcome measure, the sample size should be calculated for each of these, and the highest is taken into account. Secondary outcomes may not be statistically important as trials are not designed with power for evaluating them but they could be used to generate further hypothesis. Composite measure or combined measures are used in clinical research in which multiple end points are combined into one composite outcome. For example, poor outcome in a trial on neonates with hypoxic ischemic encephalopathy may be defined as occurrence of death or cerebral palsy or intellectual disability. They are frequently used as primary outcome measures in randomized trials and are often associated with increased statistical efficiency.

Hypothesis Testing

Hypothesis is considered as an assertion which has to be approved or rejected. Fisher, Neyman and Perason layed the foundation of hypothesis testing. Hypothesis consists of both null (H_0) and alternate hypothesis (H_1). H_1 or alternate (scientific) hypothesis is the reason for which the interventional study is conducted. Null hypothesis (H_0) is opposite to the scientific hypothesis. Null hypothesis assumes there is no effect of the intervention on outcome. A researcher would interpret the intervention or drug to be successful only if null hypothesis is rejected. For example, when a new drug is introduced by a pharmaceutical company for diabetes, in order to prove that the new drug is superior to the conventional drug, the null hypothesis, which means no difference between the two drugs, has to be proved incorrect. Interpretation of statistical tests would lead to either rejection of null hypothesis in favor of alternate hypothesis or not being able to reject it. Not being able to reject null hypothesis may not always mean that it is true, it only implies that the present study could not find a difference between intervention and control groups. Clinical trials based on their purpose can be classified into superiority trial (i.e. the drug or intervention to be tested is considered superior to control group), non-inferiority (new drug/intervention to be tested is not inferior to conventional regimen), or equivalence (i.e. there is no difference between the two regimens). Sample size calculation, data analysis, and interpretation of analysis results all depend on the type of hypothesis specified.

Interim Analysis in RCT

In clinical trials, occasionally an interim analysis is done before data collection is completed. This is done particularly when treatment in intervention arm is showing clear benefits or harm compared to the standard therapy. Based on a pre-defined evaluation of partial data set while the study is continuing, the investigators may stop the study early. It helps to save time, resource and would decrease the exposure of study participants to less useful drug or intervention.

To summarize, properly conducted RCTs are the gold standard of study designs. Every RCT should have the following components:

- Well defined scientifically relevant research question
- Randomization techniques should be explained
- Use of placebo control or blinding in order to decrease bias.
- Unbiased analysis of report mentioning all significant and nonsignificant results.

While reporting RCT, CONSORT guidelines are to be followed [11]. It is a 25-item checklist and flowchart which has been particularly designed for RCTs in order to standardize reporting of key components such as study design, analysis and interpretation of the RCT. Advantages and disadvantages of RCT are summarized in **Box 1**.

To conclude, interventional studies are useful study designs that are placed at higher pedestals in hierarchy of evidence. They determine the true efficacy and safety of interventions, and hence have the potential to influence policy decisions. However, every research question is not suitable to be answered by an interventional design, and other designs retain their unique role in different circumstances. Also, interventional study designs are prone to numerous biases, especially if not designed, conducted or interpreted properly. Thus, process of every interventional study design should be carefully scrutinized from its conception to publication, and even beyond – such as using the results for framing policy and recommendations.

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Box I Advantages and Disadvantages of a Randomized Controlled Trial

Advantages

- Allows direct comparison of the efficacy of one intervention to another which helps to establish the cause-effect relationship.
- Minimizes allocation and selection biases.
- Ensures equal distribution of unknown variables (confounders), which might have a bearing on effects of the intervention.
- Blinding of the participants helps to minimize performance bias on their part.
- Can be effectively analyzed in a systematic review.

Disadvantages

- May lack external validity.
- An intervention that works in patients recruited in trials under controlled settings may not work as well in real life situation.
- Insufficient study periods and lack of long-term follow-up leads to failure to pick up rare adverse effects, which may occur in later course.
- Require a lot of planning, and are labor- and cost-intensive.
- Marred by ethical challenges, particularly in conducting trials with new drugs and vulnerable population.
- Poorly conducted RCT may be a disaster as RCTs being ranked higher in hierarchy of evidence have the potential to influence policy, and if not conducted or reported properly, it may end up doing more harm than benefit to the society.

Key Messages

- Interventional study designs evaluate precise impact of therapeutic or preventive measures on diseases. In interventional studies, investigators, rather than circumstances, decide the nature of intervention to be assigned to study participants.
- Single-arm interventional studies, randomized controlled trials, cluster randomized trials, non-randomized controlled trials and cross-over trials are the different types of interventional studies.

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OBITUARY

Dr. (Mrs.) Shakuntla Saxena

(1929 - 5th November, 2021)

The sad demise of Dr. (Mrs.) Shakuntla Saxena is a great loss to Indian pediatric fraternityas well as the state of Rajasthan. She was the architect of pediatric services and education in Rajasthan.

She completed her DCH and MD Pediatrics under the guidance of (Late) Prof. PM Udani from Bombay. She joined Rajasthan state health service at SP Medical College, Bikaner and SMS Medical College, Jaipur and was head of the department there for 27 years.

She started Indian Academy of Pediatrics, Rajasthan at Jaipur with just six-seven members in 1970-71, and was its founder chair.The first five IAP state conferences were held under her chairpersonship at Jaipur.

She was a perfect amalgam of knowledge, simplicity, and excellent humanistic and teaching qualities. An honest and bold administrator, she had great interest in teaching clinical skills to her students. She was affectionate to all and was known for her wit, administrative skills and ability to take everyone alongside.

She will always be remembered and her memories would be cherished as pride possession in our hearts.She is survived by her only daughter Dr. Sangeeta, who is settled in USA with her family. We respectfully pay homage to the departing soul, who has left behind a legacy of masterpiece teaching and humanitarian values.

Basal Ganglia Disease Mimicking Acute Encephalitis Syndrome Among Infants of Bodo Tribe, Assam

We conducted a review of hospital records of infants with acute encephalitis syndrome with bilateral symmetrical basal ganglia infarcts, between 2011-2015, at a single center in Assam. Thiamine (as part of multivitamin injection) was used in the treatment of 23 infants and not used in 27; Only 1 (3.7%) infant died in the former group and 20 infants (86.9%) died in the latter [RR (95% CI) 0.04 (0.006,0.29); *P*<0.001). Two infants on follow-up had normal development, both in the thiamine group. The study suggests the possibility of subclinical thiamine deficiency, mitochondrial diseases, or *SLC19A3* gene mutation in this population.

Keywords: *Outcome, Thiamine, Vitamin-responsive.*

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Bilateral symmetrical basal ganglia infarcts were observed among infants, who presented with features of acute encephalitis syndrome. Authors believe that patients had good response to multivitamin containing thiamine, and share their experience.

An audit of medical records of children admitted between 2011 and 2015 with a diagnosis of acute encephalitis syndrome with bilateral basal ganglia infarct was conducted at a secondary-level hospital in Tezpur, Assam. Fifty infants had bilateral basal ganglia infarct. Depending on the exposure to multivitamins (thiamine) 27 infants were grouped in the non-exposure group (September, 2011 to April, 2014), and 23 infants in the exposure group (May, 2014 to Sepember, 2015). As thiamine was not separately available, an intravenous multivitamin injection with thiamine was given as a once-a-day infusion during the hospital stay. The constituent was vitamin B1 (thiamine) 100 mg, niacin 100 mg, vitamin B12 1000 mcg, vitamin B2 (riboflavin) 5 mg, vitamin B6 (pyridoxine) 100 mg, d-panthenone 50 mg in 3 mL.

The mean (SD) age at presentation was 6.7 (2.7) months. Common presenting symptoms included seizures (100%), lethargy (90%), fever (70%), and feeding difficulties (76%). There was a preceding illness like fever, lower respiratory tract infection, or acute diarrheal disease in 38 (76%) infants (**Table I**). Laboratory parameters and CSF analysis were unremarkable. Serum lactate was 1.95 mmol/L (normal 0.7-2.1 mmol/L) done for 4 infants in the exposure group. C-reactive protein (CRP) was found to be 3.24 mg/L (normal 0-10 mg/L) done for 5 infants in the exposure group. Japanese encephalitis virus JEV-specific IgM in CSF by IgM-capture ELISA was done for four infants, scrub typhus IgM rapid for six infants, malaria parasite antigen rapid for 20 infants, and automated blood culture and sensitivity for 10 infants, which were all negative. **Web Fig. 1** shows the CT brain of the infants showing bilateral symmetrical infarcts involving the caudate, putamen, globus pallidus, and medial thalamus.

For analysis of outcome, in addition to the infants who died, a moribund child leaving against medical advice was also labelled as death. There were 16 (*n*=27) moribund leave against medical advice infants in the non-exposure group, and 1 (*n*=23) in the exposure group. In the exposure group, 1 (3.7%) infant died, and in the non-exposure group 20 infants (86.9%) died [RR (95% CI) 0.04 (0.006-0.29); *P*<0.001)]. The infants in the exposure group had 96% less risk of death, compared with the non-exposure group. There were subsequent outpatient follow-up data for 7 patients available in the exposure group and none in the non-exposure group. Among them, two infants had normal development, and all others had neurological sequelae. Among the infants with neurological sequelae, two infants were able to walk with support.

Acute encephalitis syndrome (AES) is a public health problem in India, characterized by acute onset of fever, change in mental status with new-onset seizures [1]. Thiamine is successfully used in the treatment of many neurological conditions with basal ganglia involvement like infantile Leigh-like *SLC19A3* gene defect, THTR2 deficiency, biotin thiamine responsive basal ganglia disease [2]. Basal

Table I Characteristics of Children of Bodo Tribe With Acute Encephalitis Syndrome in Assam, 2011-2015 (*N*=50)

	Non-exposure group (n=23)	*Exposure group (n=27)*
Age (mo)[a]	6.8 (2.9)	6.6 (2.8)
Male sex	13 (56.5)	12 (44.4)
Weight for age (z-score <-2)	6 (26.1)	11 (40.7)
Bodo tribe	21 (91.3)	25 (92.6)
Other tribes	2 (8.7)	2 (7.4)
Chief complaints		
Fever	15 (65.2)	21 (77.8)
Cough/coryza/breathing difficulty	8 (34.8)	16 (59.3)
Loose stool/vomiting	3 (13.0)	3 (11.1)
Neurological complaints		
Seizures	23 (100.0)	27 (100.0)
Lethargy	20 (87.0)	25 (92.6)
Feeding difficulties	16 (69.6)	22 (81.5)
Associated Illness		
Respiratory tract Infection	8 (34.8)	14 (51.9)
Acute gastro enteritis	3 (13.0)	3 (11.1)
Management		
Antibiotics	21 (91.3)	23 (85.2)
Acyclovir	2 (8.7)	0 (0.0)
Antipyretics	15 (65.2)	19 (70.4)
Intubated	4 (17.4)	4 (14.8)
Antiseizure medication		
One (Phenytoin)	6 (26.1)	13 (48.1)
More than one	17 (73.9)	14 (51.9)

Data presented in no. (%) or [a]mean (SD). All P>0.05.

ganglia being rich in mitochondria are prone to hypoxia, toxic poisoning, and metabolic, and mitochondrial diseases [3]. Thiamine serves as a cofactor for numerous enzymes, predominantly with mitochon-drial localization. Moreover, the brain is extremely vulnerable to thiamine deficiency due to its dependence on mitochondrial ATP production [3,4]. Furthermore, *SLC19A3* gene mutation is implicated in the deterioration of thiamine transport in neurons via thiamine transporter-2. Depending on the age of the patients, this gene mutation has different clinical pictures. During neonatal period, it presents as Leigh-syndrome-like phenotype, characterized by acute encephalopathy and lactic acidosis. During the early infancy period, presents as a severe disease characterized by epileptic spasms, and bilateral thalamic and basal ganglia lesions [2]. The study suggests the possibility of subclinical thiamine deficiency, mitochondrial diseases, or *SLC19A3* gene mutation in this population.

The present study has a few limitations as it is a single institution experience and a retrospective audit, and has inadequate follow up. Moreover, those who were moribund and left against medical advice were also considered to have died, in addition to 4 deaths that occurred in the non-exposure group and none in the exposure group. Magnetic resonance imaging of brain and extensive metabolic and genetic work up were not performed in the present study. Despite these limitations, our study showed that, multivitamin (thiamine) supplementation may be associated with less risk of death in this group of infants. It raises an important question regarding the status of thiamine deficiency in the affected population. The study provides avenue for future research to explore the possible cause of thiamine responsiveness in acute encephalitis syndrome in the Bodo tribal community of Assam. We feel that multivitamin (thiamine) supplementation could be considered in the management protocol in infants with AES and symmetrical basal ganglia involvement.

Ethics approval: Approved by Emmanuel Hospital Association Institutional Ethics Committee (No. 233; 22-07-2020) dated July 22, 2020.
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CLIPPINGS

National, regional, and state-level pneumonia and severe pneumonia morbidity in children in India: modelled estimates for 2000 and 2015 (Lancet Child Adolesc Hlth. 2020; 4: 678-87)

This is a modelling study which estimates the burden of pneumonia and severe pneumonia in under five children using a risk factor-based model. Systematic literature view was done in the study to find out estimates of community acquired pneumonia. State-specific incidence rates for WHO-defined clinical pneumonia between 2000 and 2015 using Poisson regression and the prevalence of risk factors in each state was obtained from National Family Health Surveys. As per findings of study, between 2000 and 2015, the estimated number of pneumonia cases in Indian HIV-uninfected children younger than 5 years decreased from 83·8 million cases (95% uncertainty interval [UI] 14·0-300·8) to 49·8 million cases (9·1-174·2), representing a 41% reduction in pneumonia cases. The incidence of pneumonia in children younger than 5 years in India was 657 cases per 1000 children (95% CI 110-2357) in 2000 and 403 cases per 1000 children (74-1408) in 2015. The estimated number of pneumonia and severe pneumonia cases among children less than five years old decreased from 2000 to 2015. Improvement in socioeconomic status and government directed initiatives might have contributed to decline in the number of cases. However, number of cases remain high in few states like Uttar Pradesh (565 cases per 1000 children) and Madhya Pradesh (563 cases per 1000 children). India has the largest cohort of under five years children who will grow up to be economically productive population. In order to prevent and protect this population from pneumonia, interventions like exclusive breast feeding upto 6 months, adequate nutrition, immunisation with pertussis, measles, Hib, pneumococcal vaccines, and reduction of indoor air pollution should be strengthened.

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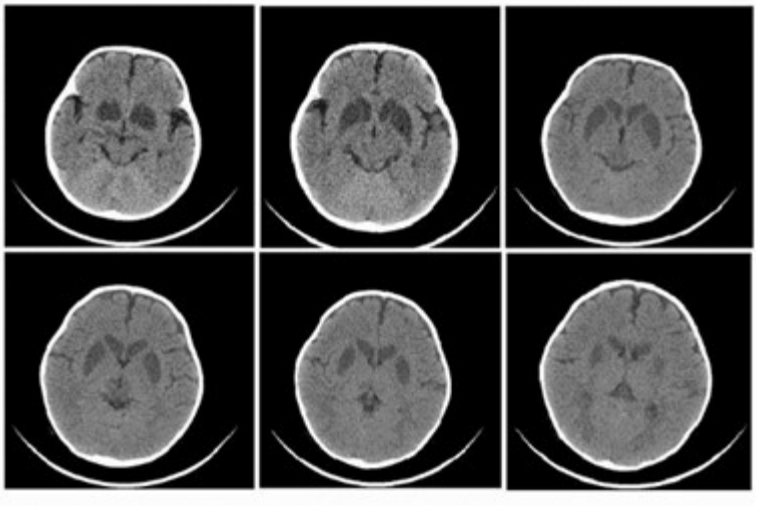

Web Fig. 1 CT Brain showing bilateral basal ganglia infarcts.

Monogenic Diabetes –A Case Series

Monogenic diabetes is uncommon, accounting for approximately 1-6% of pediatric diabetes patients [1]. They are rare forms of diabetes resulting from mutations in single gene as against type 1 and type 2 diabetes, which are polygenic. Neonatal diabetes (NDM) and maturity onset diabetes of young (MODY) are the two main forms of monogenic diabetes. NDM occurs in newborns and young infants, while MODY occurs in adolescence or early adulthood. It is important to distinguish monogenic diabetes from type 1 and type 2 diabetes.

For this case we reviewed 295 children diagnosed as diabetes as per ISPAD criteria [2] and admitted in a tertiary care center in Chennai between January, 2014 and December, 2018. Ethics clearance was obtained from institutional ethics committee. Out of these, monogenic diabetes was considered in 10 patients who were diagnosed with diabetes in first 6 months of life, two of those who were diagnosed between 6-12 months and were negative for auto-antibodies and three children diagnosed beyond 12 months who had a sibling with diabetes or clinical features consistent with known genetic syndromes [1]. The GAD-positivity among newly diagnosed children with type 1 diabetes in our center was found to be 79%. One of the patients in whom the diagnosis of monogenic diabetes was considered, died during the course of illness before genetic evaluation, and remaining 14 children underwent genetic analysis. Clinical data including age of presentation, gender, birth weight, associated clinical features and history of consanguinity were collected for all children. Three milliliters of whole blood in EDTA vial of the children and both parents was sent to Royal Devon and Exeter NHS Foundation Trust Laboratory, Exeter, UK for genetic analysis. The samples were first tested for most common causes of NDM (*ABCC8, KCNJ11* and *INS* genes) by Sanger sequencing. If a causative mutation was not identified in first test, the patient's sample was tested for mutation of all known NDM genes using targeted next generation sequencing assay [3]. Genetic diagnosis was confirmed in 11 (3.7%) children, whereas no genetic mutation was identified in three children subjected to genetic analysis. Out of 11 children, 5 were males. Seven children presented with diabetic ketoacidosis, while three children were noted to have developmental delay. Third degree consanguinity was noted in all three children with Thiamine Responsive Megaloblastic Anemia and the child with Wolcott Rallison syndrome. Three children with permanent NDM, the child with Wolfram syndrome, and the child with Rabson Mendenhall syndrome were born with low birth weight.

Genetic analysis confirmed permanent neonatal diabetes mellitus (PNDM) in five children. Two children had *ABCC8, 1 KCNJ11, 1 INS* and 1 *GCK* mutations. Three children were detected to have *SLC19A2* mutation characteristic of Thiamine responsive megaloblastic anemia (TRMA). One child had *WFS* mutation suggestive of Wolfram syndrome, one *EIF2AK3* mutation typical of Wolcott Rallison syndrome and one *INSR* mutation typical of Rabson Mendenhall syndrome. Children with *ABCC8* and *KCNJ11* were switched over to oral sulfonylurea as per standard protocol. All three children with TRMA were started on oral benfothiamine along with insulin, which resulted in better glycemic control and improvement of anemia. The child with Wolfram syndrome had buphthalmos and overlapping toes which are not described in literature. The child with Wolcott Rallison syndrome developed two episodes of acute liver failure from which she recovered. This child did not have any skeletal manifestations, which are considered essential component of the syndrome. The child diagnosed with Rabson Mendenhall syndrome had a poor glycemic control with high doses of insulin prior to genetic diagnosis, and was switched over to oral metformin after genetic diagnosis. The glycemic control continued to be poor in that child and eventually the child died. The remaining 10 children are on follow-up with good glycemic control. All of them except the child with Wolfram syndrome are thriving well.

This series describes children diabetes reporting to a tertiary care center catering exclusively to children less than 12 years of age. Hence, the spectrum described includes mainly neonatal diabetes and the genetic forms of diabetes presenting in early childhood. Prevalence of neonatal diabetes is 4% in our series as against 8% reported in a previous study done in the same center [4], and 7% reported from another center [5]. The number of children who presented with diabetic ketoacidosis and developmental delay was similar to that reported in previous studies [4].

Mutations in genes coding for K_{ATP} channels are common and the detection of these mutations has a therapeutic implication as these children can be successfully switched over from insulin to oral sulfonylurea therapy [6]. The child with Rabson Mendenhall syndrome was difficult to treat and had poor glycemic control as described in literature. Genetic diagnosis guided in switching over this child to oral metformin. Genetic diagnosis guides us to anticipate future complications and initiate swift and appropriate action, as in the child with Wolcott Rallison syndrome. Genetic counseling, and prenatal and postnatal testing were offered to mothers who conceived subsequently. Though molecular testing is expensive and not easily available, it is essential to establish a genetic diagnosis so that we can offer appropriate therapy, prognosticate and offer genetic counseling in future pregnancies.

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Excessive Weight Loss in a Neonate - Novel Mutation Causing Primary Hypoaldosteronism

Excess weight loss in a term neonate is a worrying symptom. We report a full-term male neonate who presented with jaundice and was noted to have progressive weight loss. This prompted us to evaluate further, uncovering an underlying endocrine disorder.

A 9-day-old male baby, born at 39 weeks, presented to the outpatient department with jaundice. As serum bilirubin was at treatment threshold (17.7 mg/dL), the neonate was admitted for phototherapy. The neonate had excess weight loss (admission weight- 3.45 kg, birth weight- 3.82 kg, weight loss- 9.7%). With phototherapy, the jaundice subsided in next 36 hours, but the child continued to lose weight over next two days (3.37 kg by day 11). The baby was born to a 30-year-old third gravida mother, with one previous first trimester abortion and a healthy male child. Antenatal scans showed mild left hydronephrosis. Her blood sugars were deranged at 36 weeks and late onset polyhydramnios was noted at 38 weeks (amniotic fluid index-17.5). It was a vaginal birth with smooth perinatal transition. There were no evident physical anomalies/dysmorphism. Baby had jaundice on day 2 of life, for which phototherapy was given for 24 hours and discharged on day 4 of life. At discharge, the child weighed 3.52 kg, breast feeding was well established, and there was adequate urine output. At home, the mother had adequate lactation with signs of good attachment and milk output. Since the continued weight loss was worrisome, the child was evaluated with serum electrolytes, venous blood gas, urine specific gravity, urine culture and sepsis screen. Hyponatremia (124 mEq/L) and hyperkalemia (6.9 mEq/L) were uncovered. C-reactive protein, white blood cell count, blood gas and urine examination were normal. Urine culture revealed growth of *Escherichia coli*. Genitalia, skin pigmentation, blood pressure, urine output and blood sugar were normal. The possibilities considered were salt wasting congenital adrenal hyperplasia, adrenal hypoplasia/hemorrhage, and type 4 renal tubular acidosis.

The child was initiated on liberal fluids, sodium supplements and anti-hyperkalemic measures. However, serial investigations revealed persistence of hyponatremia, worsening of hyperkalemia, new onset normal anion gap metabolic acidosis, and natriuresis (fractional excretion of sodium- 5.8%), fitting into the picture of type IV renal tubular acidosis. On further evaluation, ultrasound of adrenals, expanded newborn screening, 17-hydroxy progesterone, testosterone, dehydroepiandrosterone and cortisol levels were normal, and an appropriate response was noted with ACTH stimulation test, ruling out the possibility of congenital adrenal hyperplasia (CAH) and other structural adrenal pathologies. Trans-tubular potassium gradient was low (0.5) and urinary pH was elevated (6.5) suggesting decreased aldosterone activity. Aldosterone level was low (4.05 ng/dL; normal: 5-90 ng/dL) and plasma renin activity was high (>120 ng/mL/h; normal range: 2-35 ng/mL/h), indicating a possibility of primary hypoaldosteronism. The child was continued on sodium and bicarbonate supplements, and fludrocortisone was initiated. Following this, the child started gaining weight, with normalization of electrolytes and was discharged on day 28 of life.

Whole exome sequencing revealed a novel heterozygous contiguous deletion of 3 kb involving exons 5-9 of *CYP11B2* (ENST00000323110.2) gene at chr8: g.(142913452_142914263)_(142917844_142910557), that results in corticosterone methyl oxidase type I (Type 1) and II (Type 2) deficiency, conclusive of aldosterone synthase deficiency (ASD) (primary hypoaldosteronism). 18-hydroxycorticosterone levels would have differentiated these subtypes, but they were unavailable. Parents were counseled regarding risk of recurrence and the need for antenatal diagnosis in future. At last follow-up, at 3½ months of age, child was on fludrocortisone and sodium supplements, with good weight gain (5.4 kg), and normal serum electrolytes (Na-131 mEq/L and K-5.1 mEq/L).

Excess weight loss with abnormal electrolytes in neonatal period heralds the presence of an underlying life-threatening disorder. Diagnostic approach primarily rests on ruling out congenital adrenal hyperplasia due to 21-hydroxylase deficiency; while X-linked adrenal hypoplasia congenita, ASD and aldosterone resistance (pseudohypoaldosteronism, PHA) are the other less common causes [1,2]. A normal 17-hydroxy progesterone rules out 21-hydroxylase deficiency and normal cortisol and response to ACTH stimulation rules out adrenal hypoplasia. Normoglycemia, stable hemodynamics, and abnormality in electrolytes, acid-base balance, and weight points

to an exclusive aldosterone pathway defect. Disorders of aldosterone pathway, namely, ASD and PHA can be differentiated by aldosterone levels [3]. A decreased/ inappropriately low levels of aldosterone clinches the diagnosis of ASD [4]. The presence of low/near normal aldosterone levels favors the diagnosis of ASD type 2 [5,6]. While most common mutations are missense and nonsense, we noted a deletion in the index case. This mutation was not noted in about 1,670 variants described MGeND database and 942 variants described in gnomAD database. ASD type 2 is an autosomal recessive condition and this is the first report of a heterozygous mutation resulting in ASD type 2. The child requires lifelong mineralocorticoid replacement and continued monitoring of electrolytes and growth.

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Expanding the Neuroradiological Phenotype of 18q Deletion Syndrome

Central nervous system (CNS) abnormalities characterize several rare genetic diseases and syndromes. Case reports on rare conditions, and their uncommon findings can help to delineate their clinical phenotypes. Deletions of the long arm of chromosome 18 (18q-) (MIM 601808), occur in 1/40,000 liveborn infants [1]. The phenotype is variable, characterized by facial dysmorphisms, short stature, foot and hands deformities, congenital aural atresia (CAA), variable intellectual disability (ID), microcephaly and cerebral white matter (WM) abnormalities [1,2]. Kidney malformations, bone dysplasia, congenital heart disease, and IgA deficiency are less common [1]. Autoimmune diseases have been associated to 18q- [1].

We report a 10-years-old girl carrying an 18q22'!qter heterozygous deletion and showing dysmorphic features, juvenile idiopathic arthritis (JIA), autoimmune thyroiditis and IgA deficiency. She also developed hydrocephalus due to stenosis of aqueduct of Silvius, suggesting this might represent an expansion of the spectrum of the CNS abnormalities in 18q-syndrome.

The patient was born at term, with normal weight and length. She received percutaneous pulmonary valvuloplasty at one year for a pulmonic stenosis and has also got an interventricular septum defect.

She came to our observation at 10 years, for unsteady gait. Her height, weight and cranial circumference were normal (138.3 cm, -0.14 SD; 38 kg, +0.97 SD; 52.5 cm, +0.5 SD, respectively). Physical examination revealed: heart murmur, arthritis of the left knee, horizontal nystagmus, dysmetria at the finger-to-nose test, hyporeflexia, and unstable gait. Romberg test was negative. Psychomotor and cognitive development were normal. No data on previous cranial circumference measurements were available. Patient's parents reported unsteady gait from the very first steps. Nystagmus has always been complained as well.

Blood tests revealed increase in C-reactive protein and erythrocyte sedimentation rate, antinuclear antibody was positive with a titer of 1:640. IgA deficiency and increased thyroglobulin antibody levels, with normal thyroid hormones were also detected. JIA, autoimmune thyroiditis and IgA deficiency were diagnosed [2]. Ophthalmological evaluation revealed neither uveitis, nor coloboma. Dysmorphic features, hands and feet abnormalities were noted (**Web Fig. 1 J-O**), array CGH analysis was performed (ISCAv2 8x60K, Agilent Technologies) revealing a *de novo* heterozygous 18q22'!qter deletion of about 13.3 Mb (**Web Fig. 1P**). The brain magnetic resonance imaging (MRI) showed supra and infratentorial dismyelination of white matter, and a segmental stenosis at the third distal part of the aqueduct of Sylvius with secondary enlargement of the third and lateral ventricles (**Web Fig. 1A-H**). Follow-up brain MRI showed worsening of ventriculomegaly and subependymal transudation (**Web Fig. 1I**), so she received a successful endoscopic ventriculocisternostomy at 11 years.

Monosomy 18q is a well-recognized chromosomal abnormality comprising deletions ranging from 18q21.2, 18q21.3 to 18q22.2'!qter, that can be associated with autoimmune disease and neuroradiological findings [1,2]. We report a 18q22.2'!qter deletion in a patient presenting with rheumatological and neurosurgical issues.

Feenstra, et al. [3] defined the critical regions for

microcephaly (18q21.33), short stature (18q12.1-q12.3, 18q21.1-q21.33, and 18q22.3-q23), white matter disorders and delayed myelination (18q22.3-q23), growth hormone insufficiency (18q22.3-q23), and CAA (18q22.3). There are 28 molecularly confirmed genes inside our deleted region of which *TSHZ1, MBP, NFATC1*, *NETO1* and *CYB5A* are sensitive to haploinsufficiency.

Cody, et al. [1] proposed *GALR1* as a trigger factor for short stature. Despite *GALR1* being involved in 18q deletion, our patient did not show short stature suggesting another deleted gene in the 18q22.2 region might be involved in growth deficiency.

The incidence of cardiac defects ranges from 24% to 36%, the most common represented by atrial and ventricular septal defects, pulmonary and aortic valve defects [4]. According to literature data, our patient presented pulmonic stenosis and ventricular septum defect. *NFATC1* could be involved in their etiology [4].

Our patient showed a stenotic external auditory canal. CAA incidence in the 18q deletion syndrome is 78% [5]. CAA has been associated with *TSHZ1* haploinsufficiency.

Linnankivi, et al. [3] demonstrated that IgA deficiency, present in about 25% of cases, is associated with deletions at q22.3 and at q23, as in our case. Further studies looking for candidate genes included into the critical region, responsible in developing autoimmune diseases are desired.

From a literature review of neuroradiological features of 18q deleted patients, WM poor differentiation and dismyelination represents the most common MRI findings occurring in around 66 and 62% of patients, respectively (**Web Table I**). Particularly Linnankivi, et al. [6] published on MRI findings of the largest cohort of 18q deleted subjects, identifying dismyelination in 10 of 14 individuals with 18qdel syndrome. The authors reported that it was not related with patients' cognitive function. Dismyelination seems to be more frequent in patients with terminal deletion than those with interstitial deletion. In fact, the myelin basic protein (*MBP)* gene, localized at 18q23, plays a crucial role in the formation and maintenance of CNS [6]. According to literature data our patient presented dismyelination on brain MRI. Additional reports described other MRI abnormalities including supratentorial atrophy, Chiari I malformation, porencephalic cyst, and empty sella [6] (**Web Table I**). Aqueductal stenosis has never been reported and might represent an additional feature of the syndrome. Kato, et al. [7] reported isolated and primary ventricle dilatation. The grade of ID seems to be mild in patients with deletions distal to 18q21.33 and severe in those with deletions proximal to 18q21.31 [2]. Our patient did not present ID. Among neurological issues, more than half of patients presents a certain degree of psychomotor delay, and/or dyscoordination, and/or hypotonia and/or unstable gait (**Web Table I**). Our case well recapitulates these common neurological characteristics.

On the best of available knowledge, it seems that the "typical" 18q deletion syndrome, intended as the association of short stature, delayed myelination, congenital aural atresia, feet deformities, and characteristic facial features is due to the deletion of the critical region localized to a 70.6-74.9 Mb interval within the 18q22.3 to 18q23 chromosome region [8].

Clinicians should periodically check either cranial circumference, and signs and symptoms related to potential intracranial hypertension during follow up of these patients. In case of suspicion of intracranial hypertension, a brain MRI should be promptly performed in order to diagnose and treat it.

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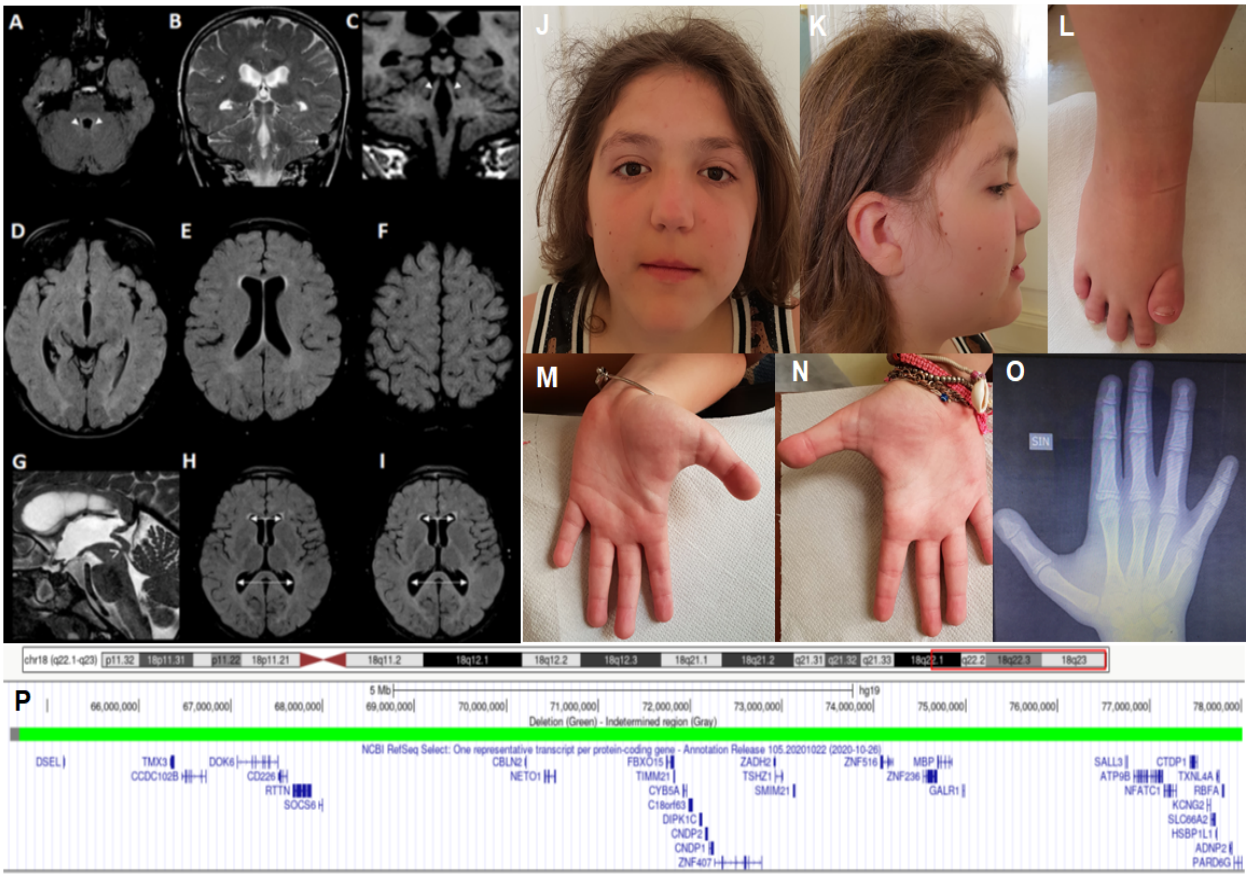


Web Fig. 1. Radiological features observed in the proband, Pictures of our proband and representation of the found deletion**.**

Brain MRIs of our patient showing bilateral high signals in the superior cerebellar peduncles (SCP) on the axial view (arrows head) and atrophy of the SCP on the coronal view (arrows head) (A-C); poor differentiation of gray and white matter due to slight hyperintensity of the periventricular and subcortical white matter (B, D-F); enlarged 3rd ventricle with expansion of both infundibular and pineal recess with normal size and configuration of the fourth ventricle (G). Aqueduct was stenotic with web (>) seen in its inferior third part. H-I: The first (H) and follow-up (I) MRI exams showed progression of the enlargement of both lateral (+) and third ventricle, secondary to aqueduct stenosis. (A, D-F, H-I: FLAIR axial image; B and C T2w and T1w coronal images at the level of the SCP; G (T2-weighted steady-state midsagittal image).

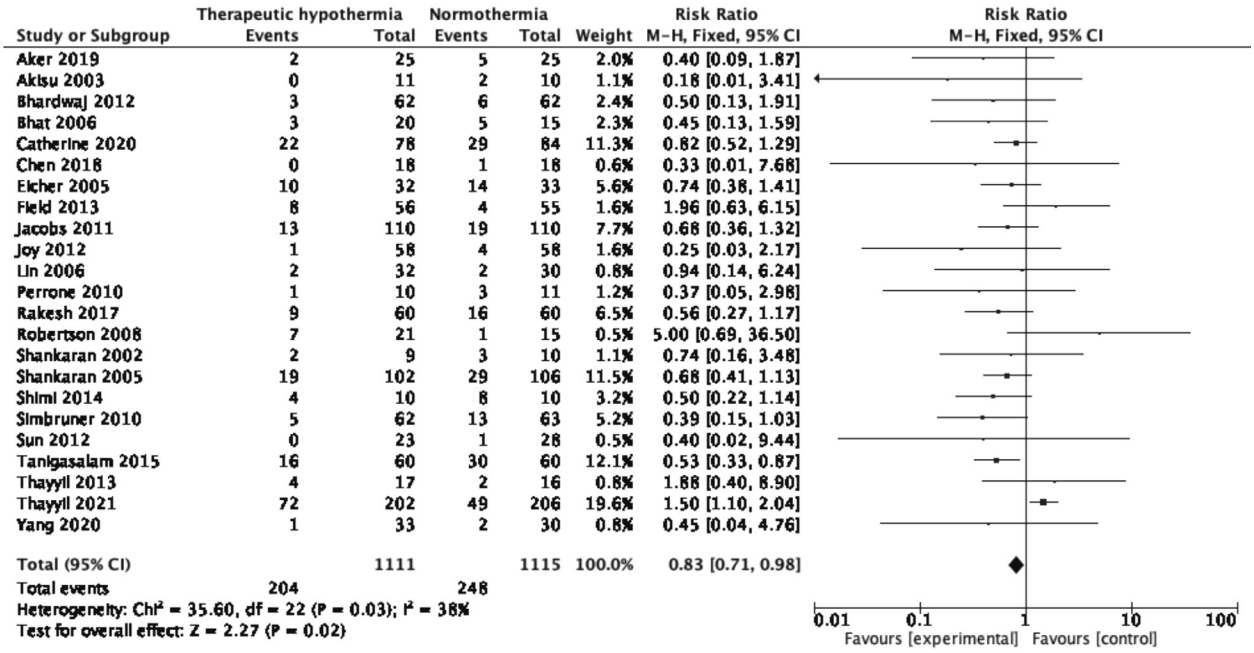
J and K pictures show squared face, mild hypertelorism, wide nasal bridge, down-slanting palpebral fissures, squared tip of the nose, smooth and long nasolabial filter, prominent chin, thin upper lip, thickened, slightly posteriorly rotated, ears with prominent antitragus. L picture shows short I metatarsus with clinodactyly. The proximal implant of the first finger of both hands with hypoplastic last phalanx and both fifth fingers clinodactyly, and shortness of fifth metacarpal are documented in pictures M and N.

X-ray of the left hand (O) shows the proximal implant of the first finger with hypoplastic last phalanx, shortness of fifth metacarpal bone, fifth finger's clinodactyly, and cone shaped last phalanges.

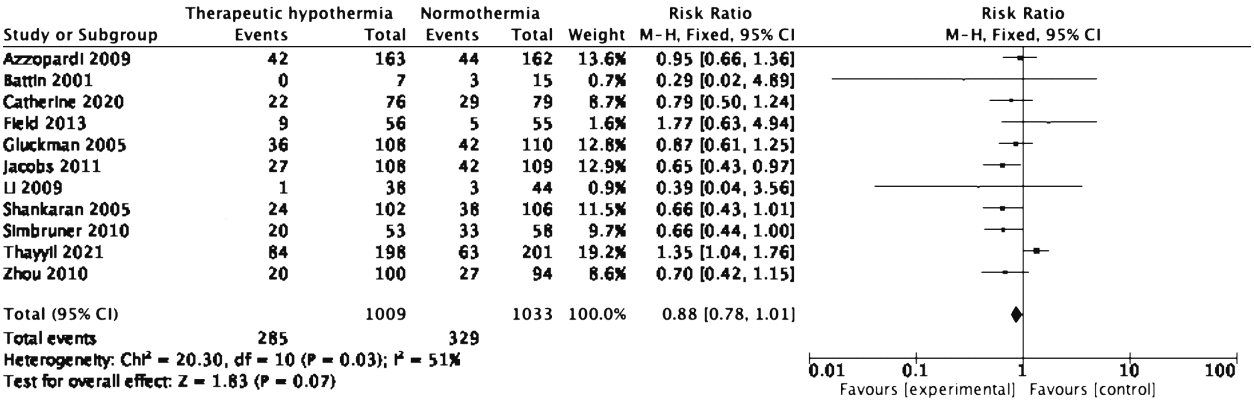
P, University of California Santa Cruz (UCSC) graphic view of the de novo 18q22.2'!qter deletion identified in the proband. The green bar corresponds to the minimal aberration length, while the flanking grey bars indicate the 5' and 3' breakpoint boundaries as determined using ISCAv2 8x60K microarray. The identified deletion of about 13.3 Mb includes 28 genes.

Up-to-date Systematic Review and Meta-analysis of Therapeutic Hypothermia for Neonatal Encephalopathy: Is the Crown Losing Its Sheen?

The extensive critical appraisal [1,2] of the recently published HELIX trial [3] prompts this brief communication. Therapeutic hypothermia (TH) holds the crown as the most effective intervention for neonatal hypoxic encephalopathy (HIE) [4]. The Cochrane review of 2013 had reported that TH reduces mortality [5], and this has been reiterated by another recent systematic review [6]. However, the latter has several methodological errors, including duplication of data from some trials, combining short-term and long-term mortality, as well as errors in data analysis [6]. In contrast to the findings of these, a systematic review, including trials exclusively from developing countries, did not find any benefit of TH on neonatal mortality [7]. More alarming, the HELIX trial [3],

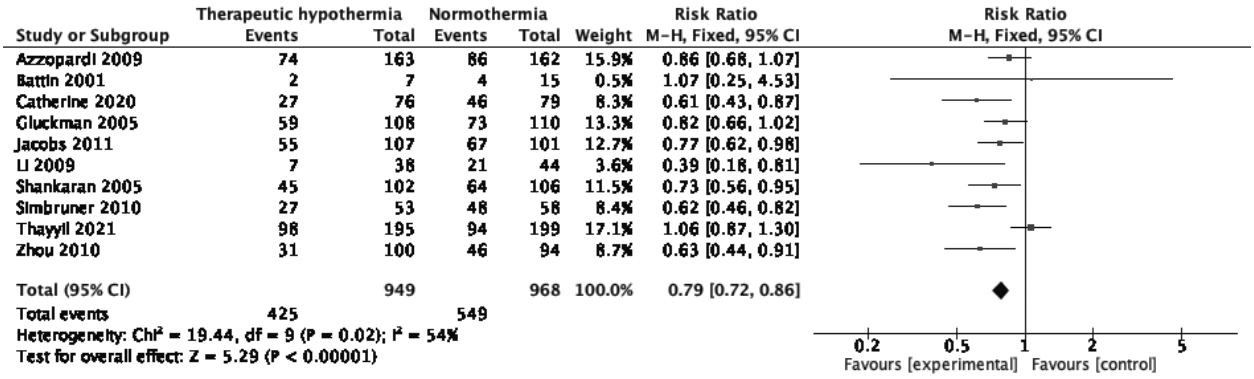


Panel A: Therapeutic hypothermia vs Normothermia for neonatal encephalopathy: Mortality before discharge

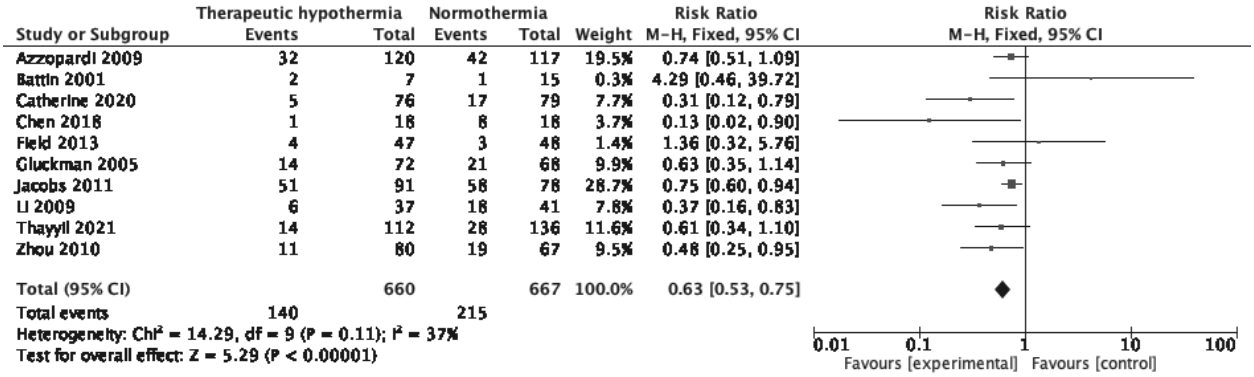


Panel B: Therapeutic hypothermia vs Normothermia for neonatal encephalopathy: Mortality at 18-24 months of age

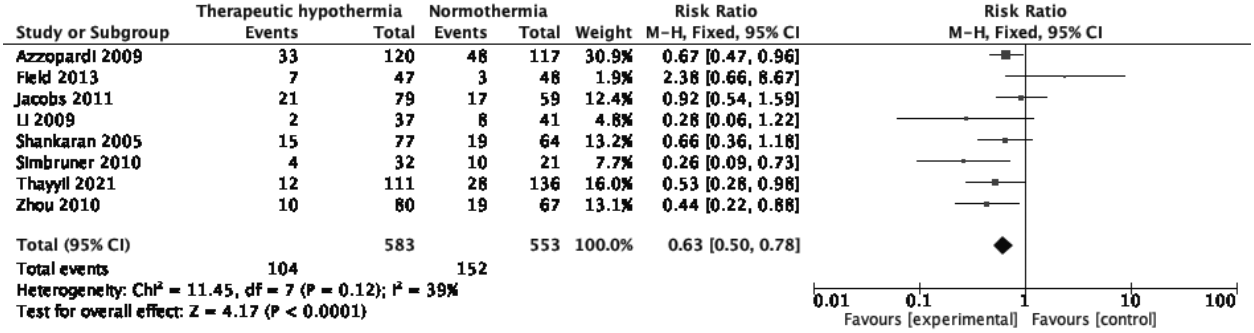
Fig. 1 Meta-analyses of therapeutic hypothermia vs normothermia for neonatal hypoxic encephalopathy, for short-term and long-term outcomes.



Panel C: Therapeutic hypothermia vs Normothermia for neonatal encephalopathy: Mortality or disability at 18-24 months of age



Panel D: Therapeutic hypothermia vs Normothermia for neonatal encephalopathy: Disability at 18-24 months of age



Panel E: Therapeutic hypothermia vs Normothermia for neonatal encephalopathy: Cerebral palsy at 18-24 months of age

reported increased short-term and long-term mortality, in low and middle income country settings. Such divergent results necessitate an up-to-date systematic review to evaluate the effect of therapeutic hypothermia (inter-vention) versus normothermia (comparison) in neonatal hypoxic encephalopathy (population), on mortality and neuro-development (outcomes).

We searched multiple databases without language or date restrictions, published up to 30 September, 2021. We included randomized controlled trials (RCT) comparing therapeutic hypothermia (defined as whole-body or selective head cooling, to temperature <34.5 °C for 48-72 hours) initiated within 6 hours of birth, versus no hypothermia, in neonates with hypoxic encephalopathy (defined by Apgar scoring and/or cord blood analysis, and supportive clinical findings), and reporting any of the following outcomes: mortality before discharge, mortality at 18-24 months, mortality or neurologic disability at 18-24 months, disability at 18-24 months, and cerebral palsy at 18-24 months.

We identified 36345 citations, of which 149 citations were

short-listed, and 32 publications (reporting 29 trials), were included. Using Cochrane Risk of Bias (RoB) 2 tool [8], two authors independently categorized, 11, 8, and 10 RCT as having high, moderate, and low RoB. Meta-analysis using Cochrane Review Manager [9] (fixed effect model) [10] revealed pooled relative risks (95% CI) as follows (**Fig. 1**): Mortality before discharge: 0.83 (0.71, 0.98), 23 trials, 2221 participants, I^2 38%; mortality at 18-24 months: 0.88 (0.78, 1.01), 11 trials, 2042 participants, I^2 51%; mortality or neurologic disability at 18-24 months: 0.79 (0.72, 0.86), 10 trials, 1914 participants, I^2 54%; neurologic disability at 18-24 months: 0.63 (0.53, 0.75), 10 trials, 1327 participants, I^2 37%; and, cerebral palsy at 18-24 months: 0.63 (0.50, 0.78), 8 trials, 1136 participants, I^2 39%. These data suggested statistically significant benefit for all outcomes except mortality at 18-24 months of age.

Subgroup analysis by study setting (developed versus developing countries) showed marked differences in mortality before discharge: RR 0.68 (95% CI 0.51, 0.92), 8 trials, 790 participants, I^2 0% versus RR 0.91 (95%CI 0.75, 1.10), 15 trials, 1431 participants, I^2 49%; and mortality at 18-24 month: RR 0.79 (0.66, 0.93), 7 trials, 1212 participants, I^2 7%, versus RR 1.05 (0.86, 1.29), 4 trials, 830 participants, I^2 65%. Other outcomes showed benefit of TH in both developed and developing countries, the magnitude of effect being greater in developing countries for disability and cerebral palsy.

The respective risk ratios (95% CI) for trials with low versus moderate/high RoB were as follows: Mortality before discharge: 1.04 (0.84, 1.29), 7 trials, 1186 partici-pants, I^2 62%, versus 0.63 (0.49, 0.80), 16 trials, 1035 participants, I^2 0%; mortality at 18-24 months: 0.97 (0.82, 1.15), 5 trials, 1011 participants, I^2 60%, versus 0.78 (0.64, 0.96), 6 trials, 1031 participants, I^2 7%; mortality or neurologic disability at 18-24 months: 0.86 (0.76, 0.97), 5 trials, 997 participants, I^2 55%, versus 0.71 (0.62, 0.81), 5 trials, 920 participants, I^2 40%; neurologic disability at 18-24 months: 0.66 (0.54, 0.82), 5 trials, 734 participants, I^2 40%, versus 0.58 (0.43, 0.78), 5 trials, 593 participants, I^2 41%; and, cerebral palsy at 18-24 months: 0.70 (0.46, 1.05), 2 trials, 385 participants, I^2 44%, versus 0.60 (0.46, 0.78), 6 trials, 751 participants, I^2 45%.

These data confirm that some of the benefits of TH reported in trials and systematic reviews are biased by studies with moderate/high RoB. TH reduces neurologic disability and cerebral palsy in later infancy in diverse settings. However, the expected benefit on short-term and long-term mortality is uncertain, especially in developing country settings. A systematic review with several additional outcomes is in progress (PROSPERO 2021 CRD42021279682). Meanwhile, these findings will help physicians, families, and policymakers, to make evidence-informed choices and decisions about therapeutic hypothermia for neonatal encephalopathy.

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NOTICE FOR ANNUAL GENERAL BODY MEETING OF IAP

Notice is hereby given that the **Annual General Body Meeting** of Indian Academy of Pediatrics for 2022 is scheduled to be held on **Tuesday, 18th January 2022, during PEDICON 2022 at Hall No: 4, India Exposition Mart Ltd. Plot No. 23 –25 & 27- 29, Knowledge Park – II, Gautam Budh Nagar, Greater Noida – 201306 from 4:30 pm** onwards to consider the following agenda.

Kindly make it convenient to attend the meeting.

With kind regards,

Dr Piyush Gupta
President, IAP 2021

Dr GV Basavaraja
Hon. Secretary General, IAP 2020 & 2021

Dr Remesh Kumar R
President, IAP 2022

Dr Vineet K Saxena
Hon. Secretary General, IAP 2022 & 2023

Place: Navi Mumbai
Date: 30th November 2021

AGENDA

1. Confirmation of the minutes of the Annual General Body Meeting held on **6th February, 2021 at Mumbai.**
2. Business arising out of the minutes.
3. Consideration and adoption of Annual Report of the Society.
4. Consideration and adoption of the audited Statement of Accounts for the year ended 31st March, 2021 and the Budget for the year 2022-2023.
5. Appointment of Auditors and fixing their remuneration for 2022-23.
6. Appointment of Honorary Legal Advisor for 2022-23.
7. Consideration of matters related to IAP Election for 2023.
8. Update on IPA Congress 2023.
9. Any other business, notice of which has been circulated with the agenda.
10. Any other business of which 30 days' notice has been given to the Secretary General in writing.
11. Any other business with the permission of the chair.

Note:

(1) If there is no quorum within half an hour of time fixed for the meeting, the meeting shall be adjourned to a later time on the same day and same place. No quorum is needed for the adjourned meeting.

(2) Kindly note that entry into the meeting hall will be permitted to only those members who give their Central IAP membership number and who bring their personal photo ID (such as Driving License with photo/PAN Card/Voter ID Card/Valid Passport/IAP Identity Card/Aadhar Card)

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AUTHOR INDEX 2021

INDIAN PEDIATRICS

Volume 58, January-December, 2021

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SUBJECT INDEX 2021

BRUE guidelines

Brief resolved unexplained events in infants (BRUE) are every pediatrician's nightmare. Earlier termed as apparently life-threatening events (ALTE), in 2016 the American Academy of Pediatrics tried to make the criteria more specific and renamed it BRUE. This was done to evaluate clinical interventions such as hospital admission or diagnostic testing. It includes all events in well looking infants associated with a change in color (cyanosis/pallor), change in tone (increased/decreased), change in sensorium or change in breathing pattern (cessation/ irregularity/ decreased) and lasting less than 1 minute.

The previous term 'apparently life-threatening event' was coined in 1987 for episodes which were "frightening to the observer and had some combination of color change, tone change usually marked limpness, choking or gagging." ALTE differs from BRUE in several ways. BRUE is a disorder of exclusion in an apparently healthy infant, while ALTE may occur with underlying illnesses and at any age not just infancy. Choking or gagging is not included in BRUE and color change is restricted to cyanosis or pallor while ALTE may include redness / erythema / plethora.

BRUE is further classified as high risk if any of the following are associated-age <60 days, born preterm with a corrected gestational age <45 weeks, CPR provided by a trained provider, duration >1 minute, more than one event or high risk concerns based on history or physical examination.

How seriously should one take these episodes? A meta-analysis suggested that the risk of death 4 months after a BRUE episode is 1:800. A retrospective cohort study including around 2000 infants found a serious diagnosis in 4%. They included seizures in 1%, airway abnormalities in <1% and abusive head trauma in <1%. Stratifying it as low risk and high risk had a negative predictive value of 90% and positive predictive value of 23%.

Clinical practice guidelines by the AAP suggest that these children may be observed for 1-4 hours with oximetry. One may obtain an ECG, evaluate for pertussis and abusive head trauma. They do not recommend routine hospital admission, neuroimaging, CSF, detailed blood biochemistry or work up for inborn errors of metabolism.

Though BRUE has received acceptance in the US, Europeans are still reluctant to separate it from ALTE. One of the chief advantages documented is a lower hospital admission rate in the US with a reduction of detailed investigations in view of the low risk of mortality. (*Eur J Pediatr28 Aug2021*)

COVID-19 vaccination in children

The CDC has approved COVID vaccination using the pediatric Pfizer mRNA vaccine in children. Vaccinations in children between 5-11 years have begun in the US. The dose is 10μg, one third of that of adults. The booster follows in 3 weeks. Vaccination of children is purported to slow down transmission, reduce risks of multisystem inflammatory syndrome and increase parental confidence in sending children to school.

The vaccine can be co-administered with other vaccines albeit at a different site. Children who have had COVID-19 infection earlier may also be vaccinated. Children with MIS-C may be vaccinated 90 days after the diagnosis, if they have recovered clinically and are at increased risk of viral exposure.

Clinical trials in about 3000 children reported a vaccine efficacy of 90.7% in preventing symptomatic infections. Adverse effects were mild to moderate and included headache, fatigue and pain. Myocarditis was not observed in these small trials though it was possibly underpowered to detect that. (*AAP News, 3 November 2021*)

Oral anti-virals for COVID-19

Two new oral drugs effective against COVID-19 have recently been announced. Molnupiravir was developed by Merk and has been approved in some countries such as the UK and Sri Lanka for mild to moderate infections with associated comorbidities. It must be given within 5 days of symptom onset in patients with comorbidities such as obesity, age > 60 years, diabetes or heart disease. It has an unusual mechanism of action. This nucleoside analog (chemical formula is N- hydroxycytidine) is mistaken for both cytidine triphosphate and uridine triphosphate during RNA replication by the SARS COV-2. This results in multiple RNA mutations resulting quickly in the death of the virus. Clinical trials have shown that it may reduce of death or hospitalization by 50%.

The second drug from Pfizer is a protease inhibitor which has shown a reduction of hospitalizations by 89% when used within 3 days of symptom onset. The drug PF-07321332 was given with ritonavir twice a day for 5 days. Ritonavir is used to reduce the drug's break down. Besides reducing severity it also has been shown to reduce transmission. The data as of 8th November, 2021 is yet to be published or peer reviewed. (*Science 8 November 2021*)

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