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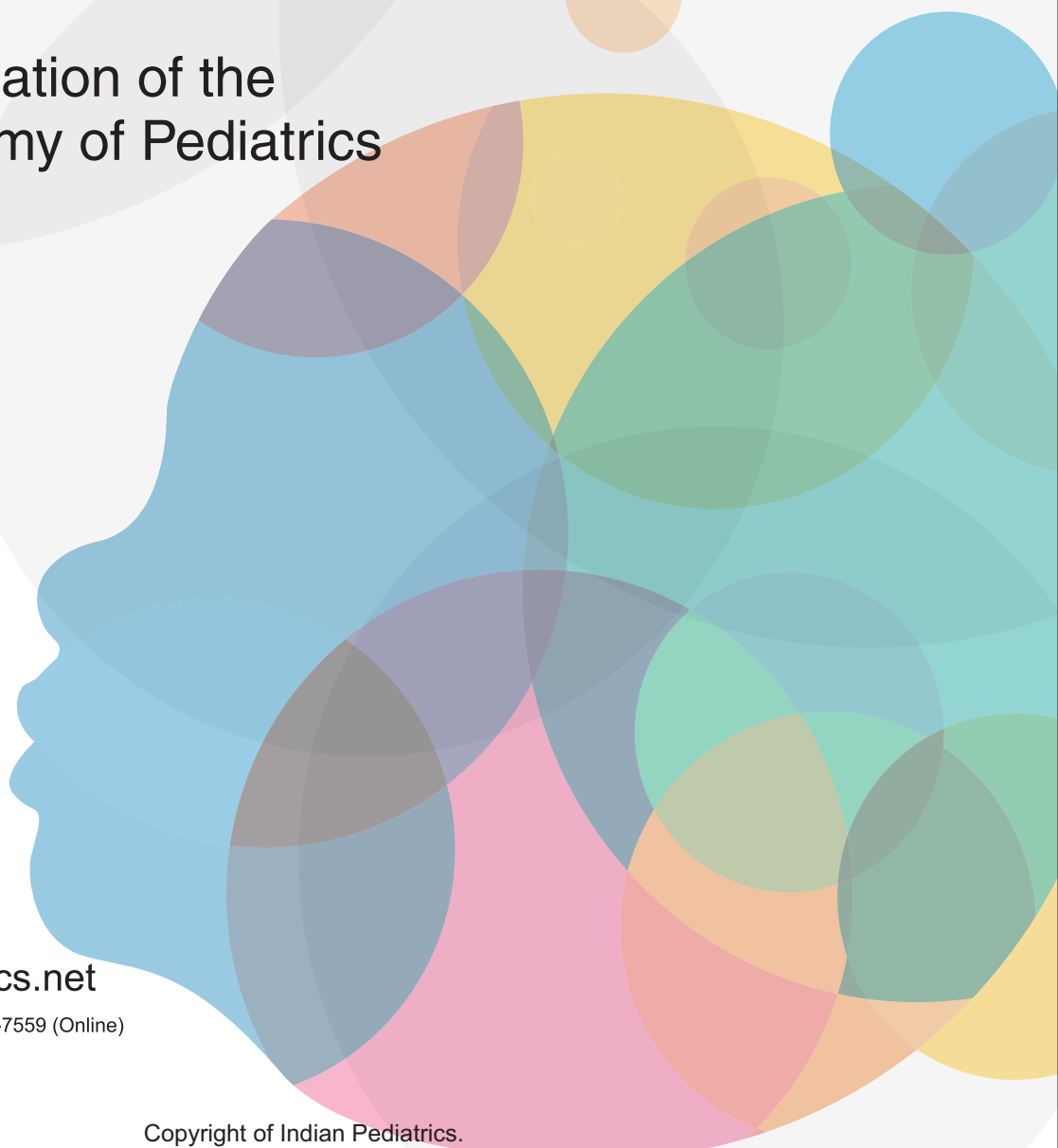

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# Mumbai 2021 Call for Action
# Addressing the Need to Incorporate 'Nurturing Care for Early Childhood Development' in Pediatric Office Practice

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We, the members of Indian Academy of Pediatrics (IAP) participating in Mumbai PEDICON 2021 in collaboration with National Neonatology Forum (NNF) and the Federation of Obstetric and Gynaecological Societies of India (FOGSI); supported by the World Health Organization (WHO) and United Nations Children's Fund (UNICEF), are committed to adopt all the components of the WHO/UNICEF Framework for Nurturing Care for Early Child Development (NC–ECD) in pediatric practice. In continuity with the IAP Consensus Statement on Early Childhood Development 2020 [1], we commit ourselves and our team members to provide an optimal healthy, safe, enabling, and nurturing environment for all children from birth till three years of age.

### *Nurturing Care for Early Childhood Development Protects the Human Capital of the Country*

Every child has the right to a standard of living that is adequate for physical, mental, spiritual, moral, and social development. As pediatricians, we must strive to ensure that all children are able to attain their maximal growth and developmental potential. This is especially important in the first three years of life, a critical period in which the foundation for good health as well as academic, behavioral, socio-and emotional competencies are laid. Providing such an environment will promote good health, alleviate non-communicable diseases in adulthood, and enhance educational, professional, and economic potential of the future citizens on our country.

### *Threats to Early Childhood Development*

We note with due concern that a large cohort of Indian children fail to attain their growth and developmental potential due to multiple risk factors like preventable health conditions, poor nutritional status, exposure to an unsafe environment, poverty, and lack of a conducive family environment.

### *Moving Ahead*

We recognize that pediatric care should include the promotive, preventive, curative and rehabilitation services to addresses the holistic health needs of children starting from birth, as a universal approach for all children as well as for children at risk of poor development or children with additional needs. We move towards making a paradigm shift in our approach to pediatric practice that is focused on improving survival and decreasing morbidity, to 'Survive, Thrive and Transform' in alignment with the Global Strategy for Women's, Children's and Adolescents' Health, 2016-30 [2].

## THE PLEDGE

We the members of the Indian Academy of Pediatrics (IAP), National Neonatology Forum (NNF), and The Federation of Obstetric and Gynaecological Societies of India (FOGSI), with support from our partners WHO, and UNICEF, do solemnly pledge to ensure that all the five components of NC–ECD (Good Health, Adequate Nutrition, Safety and Security, Responsive parenting, and Opportunities for Early Learning) are incorporated in the practice of our members, making incremental progress over the next three years (2021-2023) and sustaining the gains. To achieve this goal, we recommend the following actions for nurturing care that will be nation-wide, culturally sensitive, evidence based, and integrated with our existing practice.

### Action 1: Change Knowledge and Perception of Member Pediatricians

- Sensitise IAP members on domains of Nurturing Care Framework through various IAP platforms and journals;
- Hold consultative meetings with the Government and other major stakeholders to mobilise support; and

- Develop a Position Paper in continuity with the IAP Consensus statement on ECD 2020

### Action 2: Change Attitudes and Practices of Our Members

- Undertake nationwide capacity building by National, Zonal, State and District level training workshops to attain competency in communication, counseling and imparting knowledge and skills on nurturing care to parents and caregivers, in, practicing a uniform system of well child visits in children less than 3 years by developing appropriate resource materials for pediatricians, and supporting mother's own health and mental wellbeing.
- Strive to make our facilities ECD compliant though innovative approaches.

### Action 3: Change Perceptions and Practices of Parents

- Counsel and teach parents activities that promote good health, adequate nutrition, safety and security, responsive caregiving, and early learning, for their children and for their own wellbeing by:
- Developing resource material for parents on ECD.
- Display and dissemination of parent resource material.
- Capacity building of parents by individual and group counseling.
- Developing mobile applications and linking to the existing systems, where feasible.

### Action 4: Change Perceptions and Practices of Medical Students and Allied Professionals

- Pre-service capacity building by proposing modification in undergraduate and postgraduate training curriculum.
- In-service capacity building of medical, nursing and paramedical staff by training workshops.

### Action 5: Documentation for Knowledge Management

- Monitor progress, document, share experiences and disseminate good practices on the finalized ECD model. Evidence generation and measuring progress can further support the strategy.

We believe that this Mumbai 'Call for Action' will ensure improvement in outcomes related to child health, growth, and development, and that we will move one step forward towards attaining 2030 Sustainable Developmental Goals. Not even one child should be left behind.

(Signed on 7th February, 2021 at Mumbai)

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EDITORIAL COMMENTARY

# Immunization in Special Situations

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Protection against vaccine preventable diseases (VPDs) constitutes one of the most effective ways to impact child health worldwide. The World Health Organisation (WHO) launched the Expanded Programme on Immunisation (EPI) in 1974 [1]. India implemented the EPI in 1978. The Universal Immunisation Programme was launched in 1985 with a focus on infants below the age of one, and included tetanus toxoid for pregnant women [2]. Over the last three decades, there have been major advances in coverage against VPDs in our country. As a result, there has been a significant reduction in occurrence of VPDs in India as also in other developing countries.

With improvements in provision of health care in our country, an increasing number of children with serious medical disorders are now surviving childhood and growing up to be adults. Many amongst these medical disorders also have a component of secondary immunodeficiency, as for instance ß- thalassemia major, nephrotic syndrome, chronic renal failure and chronic liver disease, and human immunodeficiency virus (HIV) infection.

While there have been several published studies of immune responses following immunization in children with primary immune deficiencies [3], there is a paucity of literature on immune protection in patients with secondary immunodeficiencies. In this issue of the journal, there are three studies that fill up some of these lacunae in the literature [4-6].

Gomber, et al. [4] have determined anti-HBs antibody levels in 85 multi-transfused children with β-thalassemia major who had received primary hepatitis B vaccination at least 5 years ago. The authors report that seroprotection rates of hepatitis B vaccine after a mean duration of 10.8 years of completion of primary immunization were significantly higher amongst children with β-thalassemia major compared to healthy controls. Authors also found that the 23 seronegative children with β-thalassemia major were able to achieve adequate seroprotection after a single booster dose of hepatitis B vaccine. There are two important messages in this study viz., *i*) majority of multi-transfused children with ß-thalassemia major had adequate seroprotection titers even after 5 years of primary vaccination, and *ii*) following a single booster dose, children who were seronegative were able to mount an adequate protective response to the vaccine.

This study suggests that regular assessment of anti-HBs titres following primary hepatitis B vaccination needs to be incorporated in the management protocol of this condition. This is important because it is known that children with multi-transfused ß-thalassemia major are at high risk of acquiring hepatitis B infection. Though the numbers recruited in this study are admittedly small, it needs to be noted that this is a single center study and there were time constraints for completion. Nevertheless, the results are likely to impact management of children with β-thalassemia major in our country.

Jain, et al. [5] report a randomized control trial in children with HIV infection on antiretroviral therapy (ART) that compared seroprotection rate and anti-HBs titres following primary immunization with double strength (20 μg) recombinant hepatitis B vaccination. The authors compared two vaccination schedules- 3 dose (0,1 and 6 months) vs 4 dose (0,1,2 and 6 months). Data on an accelerated 3 dose schedule (0,1 and 2 months) within the 4 dose group were analyzed separately. Study sample consisted of HIV infected children aged 18 months- 12 years who had received at least 6-months ART, had not received any previous dose of hepatitis B vaccine and were anti-HBs negative. Authors found that while the median anti-HBs titres at 7 months were significantly higher in children who had received 4 doses, the difference had plateaued down by 12 months. Further, the accelerated 3 dose schedule resulted in comparable anti-HBs titers when compared to the conventional three dose schedule.

In this elegant study [5], it has been shown that the three dose double strength recombinant hepatitis B vaccination schedule offers comparable seroprotection to a four dose schedule for HIV infected children receiving ART. However, we need more data on long-term immunogenicity of the three dose accelerated schedule. This study

needs to be replicated on a much larger sample size before results can be extrapolated for formulation of immunization policies for the country at large.

Nephrotic syndrome is one of the commonest chronic renal disorders in children. Children with nephrotic syndrome have a compromised immune system both because of primary disease as well as the accompanying treatment with immunosuppressant agents. However, there are hardly any data on seroprotection for VPDs from our country. While there have been some studies suggesting that antibody titres for common VPDs (such as diphtheria, pertussis, tetanus and measles) are lower in children with steroid sensitive nephrotic syndrome (SSNS), there is paucity of similar information in children with steroid resistant nephrotic syndrome (SRNS). Ajay, et al. [6] enrolled 76 children with nephrotic syndrome to determine seroprotective titers for diphtheria, pertussis, tetanus and measles after primary immunization. Forty amongst these had SSNS while 36 had SRNS. Authors found that seroprotection rates for diphtheria, pertussis, tetanus and measles were lower in patients with SRNS compared to SSNS. These findings appear to be in consonance with a previous study from the same centre [7], wherein it was shown that seroprotection rates of hepatitis B vaccine in SRNS were lower when compared to SSNS.

Authors have clearly shown that children with nephrotic syndrome had lower overall seroprotective titers against diphtheria, pertussis, tetanus and measles even during periods of disease remission [6]. Further, the seroprotection was lower in those with SRNS. Authors recommend a booster dose of DTP or Tdap (in children above 7 years) and an additional dose of MR/MMR for all children with nephrotic syndrome once the child is in remission.

All three studies [4-6] have the apparent lacuna of a small sample size. However, this is understandable as the inclusion criteria were well-defined and rather restrictive. These studies have been well conducted and results are of direct clinical relevance to pediatricians in India. We need more data on protection against VPDs in children with secondary immunodeficiencies from our country, and such studies are a step in the right direction.

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PERSPECTIVE

# Evolution Bites - Timeworn Inefficacious Snakebite Therapy in the Era of Recombinant Vaccines

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Snakebite is a neglected tropical disease that inflicts severe socioeconomic burden on developing countries by primarily affecting their rural agrarian populations. India is a major snakebite hotspot in the world, as it accounts for more than 58,000 annual snakebite mortalities and over three times that number of morbidities. The only available treatment for snakebite is a commercially marketed polyvalent antivenom, which is manufactured exclusively against the 'big four' Indian snakes. In this review, we highlight the influence of ecology and evolution in driving inter- and intra-specific venom variations in snakes. We describe the repercussions of this molecular variation on the effectiveness of the current generation Indian antivenoms in mitigating snakebite pathologies. We highlight the disturbing deficiencies of the conventional animal-derived antivenoms, and review next-generation recombinant antivenoms and other promising therapies for the efficacious treatment of this disease.

**Keywords**: *Antivenom, Evolution, Proteomics, Venom.*

Venom is a complex biochemical concoction that is contrasted from poisons in being actively injected by the producing animal into the target prey or predator. Given their medical importance, snake venoms have fascinated humans since time immemorial, and have been extensively studied to date. Animal venoms can be chemically constituted by proteins, amino acids, carbohydrates, salts, and polyamines [1]. Snake venoms, however, are primarily proteinaceous. Historically, an anthropocentric bias has led to an erroneous understanding that only animals capable of inflicting medically significant envenomation are 'venomous'. However, venoms represent an evolutionary adaptation for self-defense and prey capture. Therefore, venom may attain remarkable specificity towards target animals and become ineffective against non-target species. For instance, certain venom toxins in arboreal 'tree snakes' (e.g., genus *Boiga*) exhibit extreme potency towards their avian and reptilian prey, while exhibiting reduced effectiveness against mammals, including humans. The potency and composition of snake venom cocktails are driven by diverse factors, such as varying diet (e.g., ontogenetic dietary shifts), geographical distribution and environmental conditions [2,3].

## A MILLION DEATHS

Despite being the non-target species, accidental snake envenoming in humans has resulted in hundreds of thousands of deaths and disabilities worldwide. Snake envenoming affects between 4.5 to 5.4 million people globally, inflicting over 100,000 deaths and 400,000 disabilities, annually [4]. Tragically, India accounts for 58,000 snakebite deaths and three-times as many immutable morbidities, making it a major snakebite hotspot [5]. Snakebite disproportionately affects the impoverished rural populations that often lack essential health infrastructure. As most bite victims are the primary breadwinners of their families, snakebite devastates far greater numbers of lives and livelihoods than currently recognized. Although snakebites kill nearly as many people in India as HIV infections, they only receive a fraction of the research attention and medical investment devoted to HIV. Since snakebite primarily affects the poor, and young males are at the highest risk of getting bitten, it results in severe socioeconomic consequences in developing countries. These considerations have led to the enlisting of snake envenoming as a high priority 'neglected tropical disease' (NTD) by the World Health Organization (WHO) [4].

## VENOMS TO DRUGS

On the flip side, snake venoms have saved many more lives than they have taken. Millions of years of evolution has resulted in diverse snake venom toxins with remarkable target specificities, and this property is being extensively exploited for drug discovery. Many snake venom proteins have been engineered into highly specific and efficient lifesaving drugs. For instance, Captopril, an angiotensin-converting enzyme inhibitor used for the

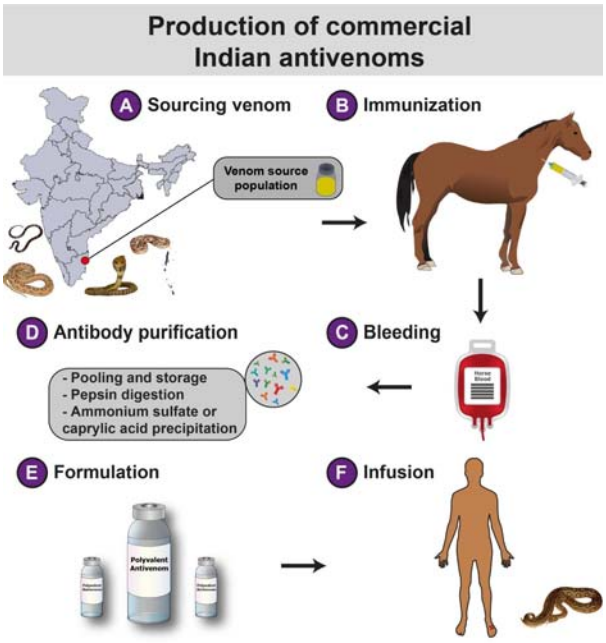
treatment of hypertension, is derived from the venom of the Brazilian pit viper, *Bothrops jararaca*, and has become the poster child for drug discovery from snake venoms. This exceptional drug has saved millions of lives globally since its introduction in the early 1980s. Many other snake venom-derived therapeutics for the treatment of various diseases, including multiple sclerosis, thrombosis, and cardiovascular diseases, are under various phases of clinical trials [6].

## CLINICAL CONSEQUENCES OF VENOM VARIATION

Antivenom is the mainstay treatment of snakebite, whose manufacturing protocols have remained essentially unchanged since their inception in the late 1800s: purification of immunoglobulins (IgG) from horses hyperimmunized with sublethal and subtoxic doses of snake venom (**Fig. 1**). The efficacy of conventional antivenom is restricted to the immunogenic potential of venoms used in its manufacture. Since venom is an adaptive trait that underpins various quotidian functions, it often exhibits dramatic inter-(between) and intra-specific (within species) variability. This variation may result in very distinct clinical outcomes and, thus, severely limits the cross-population/species antivenom efficacy – i.e., treatment of snakebites of one population/species using antivenom raised for another. However, for the commercial production of Indian antivenoms, venoms are exclusively sourced in Tamil Nadu from the so-called 'big four' snakes: the spectacled cobra (*Naja naja*), common krait (*Bungarus caeruleus*), Russell's viper (*Daboia russelii*), and saw-scaled viper (*Echis carinatus*). Moreover, India is abode to many other medically important snake species capable of inflicting fatalities and morbidities in their accidental human bite victims. Northeast India, for example, is devoid of the 'big four' snakes, but is dominated by other medically important snake species. Unfortunately, however, a single polyvalent antivenom manufactured for treating bites from the 'big four' snakes is marketed throughout the country, including in regions that lack these species. As Indian antivenoms fail to account for the inter- and intra-specific variability in venoms, they are preclinically shown to be less effective in mitigating bites from the pan-Indian populations of both 'big four' snakes and the 'neglected many', medically important snakes for which antivenoms are not manufactured [7,8].

## DISTURBING DEFICIENCIES OF ANTIVENOM

Hyperimmunization of animals with crude 'whole' venoms, which often contain antigens and other impurities, is the major shortcoming of the conventional antiserum therapy, as it increases the amount of contaminant antibodies in the finished product. In fact, the proportion of therapeutically relevant antibodies in an antivenom vial may be lower than 10-15% of the content [9], necessitating the requirement of a considerably large number of vials for efficacious treatment. This, in turn, increases the cost of treatment to the point that it becomes unaffordable to many in low- and middle-income countries. Fortunately, Indian antivenoms are heavily subsidized by the government and are freely administered without charges in government hospitals. Infusion of substantial amounts of therapeutically redundant antivenom; however, leads to complications, including serum sickness and the fatal anaphylactic shock. It is, therefore, not surprising that nearly 80% of snakebite victims who were treated with the Indian antivenoms were found to exhibit multiple adverse effects [10]. This highlights the pressing need to increase the dose effectiveness of currently available commercial antivenoms in the country.




*The manufacturing process of Indian antivenoms involves a) sourcing of venoms from the 'big four' snakes in a couple of districts in Tamil Nadu, followed by b) the immunization of healthy equines with these venoms in sublethal and subtoxic doses; c) immunized equines are then bled and the plasma is separated from the blood. The processed blood without plasma is mixed with saline and often reintroduced into the immunized animal; d) The serum is first digested with pepsin to cleave off immunoglobulin heavy chains, resulting in divalent F(ab')$_2$ fragments, followed by the treatment with ammonium sulfate or caprylic acid to precipitate antibodies, and ultracentrifugation of the precipitate to obtain purified antibodies; e) The purified antivenom is formulated either in liquid or lyophilized form before being marketed for f) the treatment of snakebite victims.*

**Fig. 1** The production of conventional Indian antivenoms.

In addition to the low dose efficacy, the poor cross-species/population neutralization capability are the other major deficiencies of the commercial Indian antivenoms. The marketed antivenoms, which are manufactured exclusively against the 'big four' snake venoms from a couple of districts in Tamil Nadu, have been shown to poorly mitigate the toxic effects inflicted by the geographically disparate populations of the 'big four' snakes and the 'neglected many' [7,8]. Unfortunately, the effectiveness of commercial Indian antivenoms has been largely evaluated by in vitro methods [e.g., enzyme-linked immunosorbent assay (ELISA), western blotting and immunochromatography (**Web Table I**)]. In contrast to in vivo experiments in the mouse model (e.g., Effective Dose 50 or $ED_{50}$), in vitro methods do not reveal the underlying neutralization potencies of antivenoms, but are only useful for understanding their venom recognition potential. Furthermore, low-molecular-weight toxins, such as three-finger toxins (3FTx), which dominate the venoms of many elapid snakes (e.g., Naja and Bungarus spp.) and are responsible for the morbid and fatal symptoms, exhibit poor immunogenicity, likely leading to a reduced proportion of neutralizing antibodies against them [22].

## UPCOMING THERAPIES FOR SNAKEBITES

To date, hyperimmunized animal-derived antivenom remains the only available treatment for snakebites. The inefficacy of such antivenoms in neutralizing the toxic effects of distinct medically important species and their geographically disparate populations have been well-documented. In recent times, several innovative strategies are being explored to develop next-generation antivenoms with increased potency, paraspecificity, and cost-effectiveness. Some of these strategies have been briefly described below.

*Phage display*: It facilitates the identification of antibodies specific to toxins of interest. Phage display essentially involves biopanning of antibody phage display libraries against a particular antigen, in this case, venom proteins, followed by the amplification and enrichment of the antigen-specific library. Selected phages are used for infecting bacteria, which are then allowed to express toxin-specific antibody fragments. Specific antibodies against various toxin types can also be combined to form a biosynthetic oligoclonal antibody (BOA) cocktail, which exhibits less batch-to-batch variation and increased efficacy and safety profiles than the conventional antivenoms [23]. Phage display technology has been shown to be effective in characterizing antibodies against medically important snake venom toxins, including crotoxin, cobratoxin, and dendrotoxin [24].

*Synthetic epitope strategy*: Next-generation antivenoms containing toxin-specific antibodies could also be produced through novel immunization strategies, such as immunization with synthetic epitope-strings. Herein, strings of nucleotide sequences coding for specific regions of various toxins are cloned into expression vectors and injected into animals for eliciting toxin-specific antibody response [25].

*Aptamers*: The use of aptamers, oligonucleotides or oligopeptides that bind to target molecules with high specificity, have also been considered for the development of novel antivenom therapies [26]. This strategy can be advantageous over animal-derived antibodies in terms of production, affordability, and ethical considerations.

*Mimotopes*: Structurally mimicking regions of clinically important toxins known as 'mimotopes' can elicit immune responses and generate toxin-specific antibodies. Examples include mutalysin-II mimotopes that have been shown to neutralize hemorrhagic activity induced by *Lachesis muta* venom [27]. These mimotopes are usually identified from a phage display library and have high specificity and stability.

*Nanoparticle engineering*: Another alternative to the current intravenous antivenom administration is the subcutaneous use of nanoparticle drug delivery systems that can facilitate the controlled release of highly stable toxin neutralizing nanoparticles. Synthetic hydrogel nanoparticles, for example, have been shown to inhibit phospholipase $A_2$ ($PLA_2$) and 3FTx pathologies [28,29]. Similarly, nanoparticles, such as $C_{60}$ fullerene, have been shown to exhibit significant neutralization against rattlesnake envenomation [30].

In addition, several small molecular inhibitors, such as varespladib are currently being tested for their ability to neutralize snakebite pathologies [28]. Unfortunatel, very few products originating from these next-generation technologies are under various phases of clinical trials, while most others are being preclinically evaluated. Thus, while the aforementioned technologies are promising and are likely to result in highly efficacious and affordable snakebite treatment therapies, they are far from fruition. It is therefore imperative, in the interim, to address the deficiencies of the current generation Indian antivenoms. Procurement of venoms from the pan-Indian populations of 'big four' and other medically important snakes by region for the production of region-specific antivenoms, while also accounting for the ecological specializations and molecular evolutionary dynamics of venoms of clinically relevant species, could be effective in countering the geographic and phyletic variations in venom

compositions and potencies. Furthermore, adoption of novel immunization strategies (e.g., the use of medically important toxin fractions and/or poor immunogenic toxin proteins for animal immunization) and purification technologies (e.g., chromatographic purification of antivenoms during manufacture) are highly likely to increase the proportion of therapeutically significant antibodies in the marketed product. Thus, in the absence of next-generation antivenoms, these measures are anticipated to save the lives, limbs and livelihoods of India's hundred thousand annual snakebite victims.

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**Web Table I Efficacy of Marketed Indian Antivenoms**

| *Snake Species* | *Venom source* | *Antivenom tested* | *Conclusion* |
|---|---|---|---|
| *N. naja, N. kaouthia* | West Bengal[e] [11] | S | *In vitro*: NA<br>*In vivo*: Antivenom is more effective against *N. naja* than *N. kaouthia*. The former venom was more potent than that of *N. kaouthia.* |
| *N. naja* | West Bengal,[e] Maharashtra,[c] Tamil Nadu[d] [12] | M (prepared against venom from Eastern India) | *In vitro*: Poor recognition of venoms from Maharashtra and Tamil Nadu.<br>*In vivo*: NA |
| *N. naja* | West Bengal,[e] Tamil Nadu[d] Maharashtra[c] [8] | H, M | *In vitro*: Specific monovalent antivenoms neutralized many enzymatic activities<br>*In vivo*: Specific monovalent antivenoms outperformed the commercial antivenom |
| *N. naja* | Tamil Nadu[d] [13] | VI, C-V1 | *In vitro*: NA<br>*In vivo*: Soy protein nanoparticle conjugated antivenom was more effective than the commercia antivenom. |
| *N. naja* | Maharashtra[c] [14] | B, P, V | *In vitro*: Poor immunorecognition of low molecular weight toxins<br>*In vivo*: NA |
| *D. russelii* | Delhi,[a] West Bengal,[e] Maharashtra,[c] Tamil Nadu [15] | M (prepared against venom from Southern India) | *In vitro*: Poor recognition of venoms from Delhi, West Bengal and Maharashtra<br>*In vivo*: NA |
| *D. russelii* | Tamil Nadu,[d] Kerala,[b] Karnataka,West Bengal[e] [16] | B | *In vitro*: Poor recognition of venoms of West Bengal and Kerala populations<br>*In vivo*: NA |
| *D. russelii* | Tamil Nadu[d] [17] | B, P, V,BE | *In vitro*: Poor recognition of low molecular weight toxins<br>*In vivo*: NA |
| *D. russelii* | Tamil Nadu,[d] Bangladesh, Pakistan, Sri Lanka [18] | I, H, VI, BE, P, ICP | *In vitro*: Efficient immunorecognition of venoms of Tamil Nadu, Sri Lanka, Pakistan and Bangladesh<br>*In vivo*: NA for venom from Tamil Nadu |
| *E. carinatus* | Tamil Nadu[d] [19] | B, P, V | *In vitro*: Poor recognition of low molecular weight toxins<br>*In vivo*: NA |
| *B. caeruleus* | Tamil Nadu[d] [20] | B, P, BE | *In vitro*: NA Poor recognition of low molecular weight toxins<br>*In vivo*: NA |
| *B. caeruleus, B. sindanus, B. romulusi* | Maharashtra and Karnataka [21] | H, P | *In vitro*: Poor recognition of low molecular weight toxins<br>*In vivo*: Antivenom ineffective in neutralizing the venoms of *B. sindanus* and *B. romulusi* |
| *B. caeruleus*, *B. sindanus*, *B. fasciatus*, *N. naja*, *N. kaouthia*, *E. carniatus*, *E. c. sochureki* | Punjab, West Bengal, Rajasthan, Arunachal Pradesh, Maharashtra and Madhya Pradesh [7] | B, P, H, VI | *In vitro*: Poor recognition of venoms of the 'neglected many' species, as well as one of the 'big four' snake venoms<br>*In vivo*: Antivenom ineffective in neutralizing the venoms of *B. caeruleus* and the 'neglected many' species except one, while neutralizing *E. carniatus*, *E. c. sochureki* and *N. naja* venoms |

*B: BSVL (Bharat Serums and Vaccines Ltd.); BE: Biological E. Limited; H: Haffkine Biopharmaceuticals Corporation Ltd.; M: monovalent antivenom raised in the study; C-VI: nanoparticle conjugated with VINS ASV; S: SIIPL (Serum Institute of India Pvt. Ltd.); P: PSVPL (Premium Serums and Vaccines Pvt. Ltd.); VI: VINS Bioproducts Ltd.; V: Virchow Biotech; I: Incepta Vaccine Ltd. (Dhaka, Bangladesh); ICP: Instituto Clodomiro Picado (San Jose, Costa Rica); NA: data not available/not performed. [a]Chest Institute; [b]Agadathantra Snake Park; [c]Haffkine Institute; [d]Irula Snake Catchers' Industrial Cooperative Society; [e]Calcutta Snake Park.*

RESEARCH PAPER

# Three vs Four Dose Schedule of Double Strength Recombinant Hepatitis-B Vaccine in HIV-infected Children: *A Randomized Controlled Trial*

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**Objectives:** To compare seroprotection rates and the anti-HBs titers following primary immunization with double strength (20 µg) recombinant hepatitis B virus (rHBV) vaccine administered intramuscularly (IM) in a 3-dose (0, 1 and 6 months) vs 4-dose (0, 1, 2 and 6 months) schedule in HIV-infected children receiving antiretroviral therapy (ART). An accelerated 3-dose schedule (0, 1, 2 months) within the 4-dose group was also compared.

**Design:** Randomized controlled trial.

**Setting**: Pediatric ART clinic of a tertiary hospital in Delhi from November, 2017 to April, 2019.

**Participants**: Fifty (25 per group) HIV-infected children aged 18 months - 12 years receiving ART for at least 6 months who had not received any prior dose of HBV vaccine, and were anti-HBs negative.

**Intervention:** Group 1 received 20 µg of rHBV vaccine IM (in deltoid muscle) at 0, 1, and 6 months, and group 2 received 20 µg the same vaccine at 0, 1, 2 and 6 months.

**Outcome variables:** Anti-HBs titers and proportion of responders in 3-dose vs 4-dose group at seventh and twelfth month and at third month after an accelerated 3-dose schedule.

**Result:** Median (IQR) anti-HBs titers at the seventh month were significantly higher in group 2 [225.7 (151-300) IU/L] compared to group 1 [138.2 (35.2-250) IU/L], but were comparable at the 12th month. Seroprotection rates were comparable between group 2 and group 1 at 7th month (96% vs 80%; *P*=0.19) and 12th month (96% vs 88%; *P*=0.61). The proportion of good responders were also comparable between the groups at 7th month and 12th month (both *P*=0.29). Accelerated 3-dose schedule achieved comparable anti-HBs titers [179.9 (130.6-250) IU/L] and seroprotection rate (92%) one month after completion of schedule to the standard 3-dose schedule.

**Conclusion:** A 3-dose double strength recombinant HBV vaccine schedule offers comparable seroprotection to 4-dose schedule for HIV-infected children receiving ART.

**Keywords:** *Accelerated schedule, anti-HBs titer, Seroprotection.*

**Trial Registration:** CTRI/2017/12/010816

Human immunodeficiency virus (HIV) and hepatitis B virus (HBV) have a high prevalence of co-infection as they share similar risk factors. HIV infection is also associated with a greater chance of chronic HBV carrier state, a higher level of viral replication, increasing its potential for transmission [1]. HIV and HBV co-infection can accelerate chronic hepatitis and liver cancer [1] contributing to morbidity and mortality in HIV-infected individuals. Immunization is one of the most important public health measures to prevent HBV infection. However, a suboptimal seroconversion between 18 to 72% is reported with HBV vaccine in HIV-infected adults and children [2-4].

In order to improve seroconversion rates following immunization, several strategies like the use of double doses [5-9], additional doses [8-11], combination vaccines [12], intradermal route for vaccination [8-10], and adjuvants [13] have been suggested.

The Infectious Diseases Society of America recommends a 3-dose schedule of double dose (20 µg) HBV vaccine in children infected with HIV [14]. National Institutes of Health [15] and Advisory Committee on Immunization Practices, under the purview of the Centre for Disease Control, USA [16] recommends a 3-dose schedule of standard dose (10 µg) recombinant HBV vaccine for HIV-infected children. The Indian Academy of Pediatrics recommends double dose of recombinant HBV vaccine in a four dose schedule at 0, 1, 2 and 6 months in symptomatic HIV-infected children, and a three-dose schedule in asymptomatic HIV-infected children at 0, 1 and 6 months [17]. There is no clear consensus yet regarding the most appropriate schedule of vaccination for primary HBV vaccination in HIV-infected children [18]. Highly active

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ART (HAART) may foster better immune reconstitution in HIV-infected children, suggesting that three doses may suffice to attain adequate seroprotection.

This study was conducted to compare seroprotection rates, anti-HBs titers and proportion of good responders following primary immunization with double strength (20 µg) recombinant HBV vaccine administered in a 3-dose schedule (0, 1 and 6 months) vs 4-dose schedule (0, 1, 2 and 6 months) in HIV-infected children who were receiving anti-retroviral therapy (ART) for at least 6 months. We also compared seroprotection rates between the two 3-dose schedules (0, 1 and 2 month *vs* 0, 1 and 6 months).

## METHODS

The study was conducted in Pediatric ART Clinic of a tertiary hospital in Delhi between November, 2017 and April, 2019. Approval was obtained from the institutional ethics committee and the trial was registered with the Clinical Trials Registry of India. Permission was also obtained from Delhi State AIDS Control Society.

HIV-infected children aged 18 months to 12 years who had been receiving ART for at least 6 months, were previously unimmunized for hepatitis B and were seronegative for Hepatitis B virus (HBs antigen negative) were included. The immunization status was ascertained on the basis of previous immunization records and a negative anti-HBs status. Any child with immunological failure, as defined by National AIDS Control Organization (NACO), was excluded [19].

A written informed consent was taken from the parent or guardian. Participants were randomized by computer generated software using block randomization technique with variable block sizes. Allocation to 3-dose and 4-dose groups of the study was done using concealed envelope technique. Recombinant HBV (rHBV) vaccine (Biological E. Limited) was administered to the participants in the immunization clinic while observing all universal precautions. Children in group 1 received 20 µg of rHBV vaccine IM (in deltoid muscle) at 0, 1, and 6 months, and those in group 2 received 20 µg of rHBV vaccine IM at 0, 1, 2 and 6 months. Any adverse event following immunization was reported to the appropriate authorities.

Two mL venous samples were drawn for estimation of anti-HBs titers at beginning of the seventh and twelfth month after receiving the first dose. Additionally, in the group receiving 4-dose vaccination, a sample was also drawn one month after the third dose to be assessed as an accelerated 3-dose schedule (0,1 and 2 months). The sera were separated and stored at -20 ºC.

Anti-HBs titers were estimated after thawing the stored sera using ELISA-based kits Diapro Diagnostic Bioprobes Srl). Seroprotection was defined using an antibody to hepatitis B surface antigen (anti-HBs) threshold of ≥10 IU/L at series completion [20]. Responders and good responders were defined as participants who had anti-HBs titers ≥10 IU/L and ≥100 IU/L at series completion, respectively [20].

The primary outcome variables were anti-HBs titers and proportion of responders in both groups after one month (seventh month) of completion of primary immunization schedule. Secondary outcome variables were anti-HBs titers and proportion of responders and good responders at twelfth month in both groups, and proportion of responders in the accelerated 3-dose schedule.

Sample size was calculated based on the study by Potsch, et al. [5], where the seroconversion rates after 4-dose and 3-dose HBV vaccine were 91% and 83%, respectively. At a non-inferiority margin of 8% with one-sided type I error rate of 5% and power 80% and assuming the true difference between seroconversion rates of the two groups as zero, a sample size of 190 children per group was calculated. However, with the universal immunization practices, we did not expect a large cohort of unimmunized children so we committed to recruit at least 25 children per group in this study.

*Statistical analyses:* We used SPSS software version 22 for analyses. Mann Whitney U test was used to compare anti-HBs titers between two groups at different time points. Proportions of non-responders and good

**Table I Baseline Characteristics of Participants in the Study**

| *Variables* | *Three-dose group (n=25)* | *Four-dose group (n=25)* |
|---|---|---|
| Age (y) | 7 (4-10) | 11 (9-12) |
| Male sex[a] | 18 (72) | 15 (60) |
| Weight for age *z*-score | -0.64 (-1.35 to 0.18) | -1.80 (-2.35 to-1.05) |
| Height for age *z*-score | -0.8 (-2.05 to 0.75) | -2.50 (-4.3 to-1.6) |
| BMI *z*-score | 0.06 (-0.92 to 0.75) | -0.20 (-0.8 to 0.95) |
| On 1st line ART[a] | 24 (96) | 23 (92) |
| ART ≥24 mo[a] | 14 (56) | 18 (72) |
| *CD4 count* | | |
| Start of ART | 623 (326-959) | 542 (362-893) |
| At enrolment | 1046 (742-1434) | 882 (644-1255) |
| CD4% at enrolment | 34.7 (26.5-37.04) | 31.5 (25.9-37.6) |

*Three-dose group: Recombinant HBV at 0, 1, 6 month; Four dose group: Recombinant HBV at 0, 1, 2, 6 month; Data expressed as median (IQR) except [a]no. (%). ART: anti-retroviral therapy; BMI: body mass index; P>0.05 for all variables.*

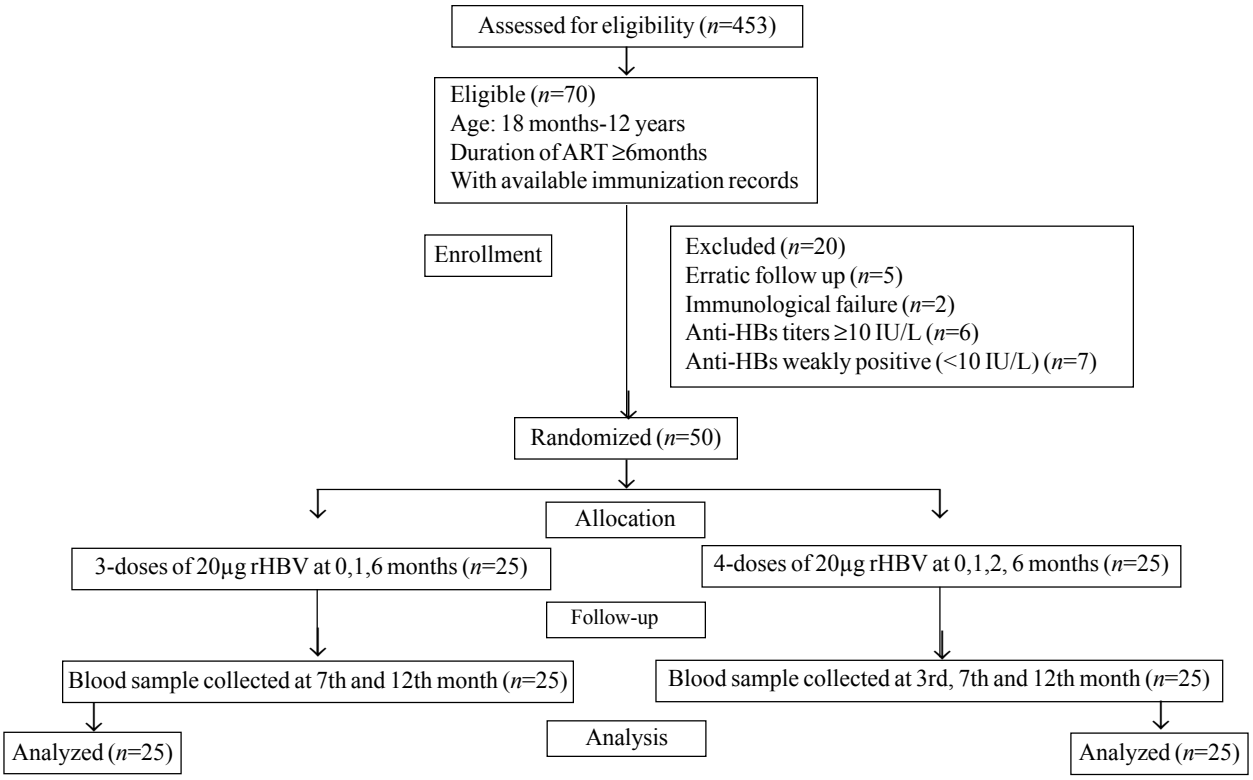


**Fig. 1** Flowchart showing the recruitment of participants in the study.

responders were compared by Fisher exact test. Odds ratio with 95% confidence interval was estimated. P-value <0.05 was considered significant.

## RESULTS

Fifty participants were recruited in the study between November, 2017 and April, 2018. **Fig. 1** depicts the flow of participants in the study. The baseline characteristics of participants in 3-dose and 4-dose groups were comparable (**Table I**).

The median anti-HBs titers and seroprotection rates achieved in the seventh and twelfth month in both groups are shown in **Table II**. No serious adverse event following immunization (AEFI) was reported in any child in either group.

The proportion of responders one month after completion in accelerated schedule were 92% which was statistically comparable to the corresponding figures in the 3-dose schedule (80%; *P*=0.42) and 4-dose schedule (96%; *P*=0.08). The median anti-HBs titers in the accelerated schedule were 179.9 (130.6-250) IU/L, which were comparable to the 3-dose schedule (*P*=0.26), but significantly lower than the 4-dose group (*P*=0.03) at the seventh month.

## DISCUSSION

The study concluded significantly higher median anti-HBs titers in the 4-dose group as compared to 3-dose group at seventh month, but not at twelfth month. The proportion of responders and good responders in both groups were comparable at both time points. This emphasizes that offering a fourth dose of recombinant

**Table II Anti-HBs Titers and Response With Four-Dose and Three-Dose Schedules of Hepatitis B Vaccination**

| | *3-dose schedule (n=25)* | *4-dose schedule (n=25)* | *P value* |
|---|---|---|---|
| *Anti-HBs titre (IU/L)*[a] | | | |
| 7th month | 138.2 (35.3-250) | 225.7 (151-300) | 0.02 |
| 12th month | 166.8 (69.7-250) | 200 (127.5-253) | 0.57 |
| *Responders* | | | |
| 7th month | 20 (80) | 24 (96) | 0.19 |
| 12th month | 22 (88) | 24 (96) | 0.61 |
| *Good responders* | | | |
| 7th month | 18 (72) | 22 (88) | 0.29 |
| 12th month | 18 (72) | 22 (88) | 0.29 |

*Data shown in no. (%) except [a]median (IQR); Responders: Anti-HBs titers ≥10 IU/L; Good responders: Anti-HBs titers ≥100 IU/L).*

**WHAT IS ALREADY KNOWN?**

- No consensus regarding the optimal number of doses of hepatitis B vaccine for primary immunization in HIV-infected children.

**WHAT THIS STUDY ADDS?**

- Three-dose vaccination offers comparable seroprotection to four-dose vaccination schedule for hepatitis B vaccination in unimmunized HIV-infected children receiving ART.

hepatitis B vaccine in HIV-positive children on ART may be unnecessary.

We found seroprotection rates of 96% and 80% one month after HBV vaccination in the 4-dose and 3-dose schedule, respectively, similar to an adult study [9]. Seroconversion rate of 94% was likewise seen after a 4-dose (double dose) schedule in unimmunized HIV-infected Indian children [21]. Similarly, a higher seroconversion rate of 95.4% was seen in seventh, than 88.6% in the twelfth month after double dose 4-dose vaccination in adults [22]. Seroconversion rate after 3-dose double dose vaccination in HIV infected children and adults vary from 60-74% [12,23,24]. The seroconversion rates at both time points in our study were either comparable or higher than the aforementioned studies. This may be accounted for by regular ART intake for atleast six months in the present study group. Universal ART in children leading to immune reconstitution and improved vaccine response appears to be a possible explanation for the robust seroconversion rates of participants in our study.

The results of our study indicate that a 3-dose schedule may be adequate for primary immunization of these children with the added advantage of having better compliance and better use of resources, while ensuring effective protection against hepatitis B. Antenatal care and prevention of parent to child transmission (PPTCT) services have improved nationwide, which have led to more timely diagnosis in mothers and children. Further, all HIV-infected children are now routinely receiving ART irrespective of clinical and immunological staging. Well-equipped ART clinics with trained doctors and paramedical staff help in better follow-up, medical care and awareness among these patients. This has led to the improved immunological status of HIV positive children and subsequently more effective response to immunization.

The 4-dose schedule had higher median value of anti-HBs titers one month after completion of the vaccination schedule, than the 3-dose schedule. This difference was not sustained as the titers continued to rise in the 3-dose group. However, whether the greater proportion of good responders in the 4-dose group compared to the 3-dose group will offer longer duration of seroprotection, needs to be confirmed with a longer follow up.

The seroconversion rate increased between seventh to twelfth month in the 3-dose group but remained static in the 4-dose group in the present study, similar to that reported before [25]. The increase in the proportion of seroconverters over time in our study, however, implied that those who do not seroconvert initially may show gradual increment in titers over time. This may be explained by gradual immune reconstitution with continued ART in HIV-infected children leading to a delayed immune response to vaccination.

The timely immunization and sampling were ensured in the present study without any lost to follow-up. Earlier studies in children and adults were conducted when ART was not being administered universally, unlike the present study where all participants universally received HAART. Limitation of our study is the small sample size. Implementation of universal national immunization schedule and practices makes it difficult to establish a big cohort of unimmunized HIV positive children. However, the fact that nearly 11% of children were found to be unimmunized, emphasizes the need to strengthen the immunization services for this vulnerable group. Further, due to the non-availability of viral load and tests for B-cell and T-cell functions, the non-response to vaccination could not be explained in a few participants who did not qualify for severe immunosuppression based on their CD4 counts.

We suggest that three doses of double strength hepatitis B vaccine may suffice in HIV-infected children receiving ART in the absence of immunological failure. The accelerated 3-dose schedule (0, 1, 2 months) may also be studied for its long term immunogenicity before it can be considered as an alternative regimen for vaccination of these children.

*Ethics clearance*: Institutional Ethics Committee, UCMS; No. IECHR/2017/32/100 dated 17 October, 2017.
*Contributors*: PD, PJ, SG, BK, AR: conceptualized the study; PJ, PD: data collection; BK: laboratory support; PD, PJ: drafted the manuscript; SG, BK, AR: critical input. All authors approved the final manuscript and are accountable for the manuscript.

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RESEARCH PAPER

# Intravenous Acetaminophen vs Intravenous Diclofenac Sodium in Management of Skeletal Vaso-occlusive Crisis Among Children With Homozygous Sickle Cell Disease: *A Randomized Controlled Trial*

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**Objective:** To compare the efficacy of intravenous acetaminophen and intravenous diclofenac sodium in the management of skeletal vaso-occlusive crisis among children with sickle cell disease.

**Design:** Single blind randomized controlled trial.

**Setting:** Tertiary care hospital.

**Participants:** 104 children with sickle cell disease and skeletal vaso-occlusive crisis.

**Intervention:** Intravenous acetaminophen at 10mg/kg/dose 8 hourly and intravenous diclofenac sodium at 1mg/kg/dose 8 hourly in 1:1 ratio.

**Main outcome measures:** Reduction in pain score (50%), number of doses needed to relieve pain after 24 hours of drug administration and decrease in pain score at 1 hour.

**Results:** A 50% reduction in pain score was seen in 35 (77.3%) and 10 (21.7%) children among acetaminophen and diclofenac sodium groups respectively (RR, 95% CI 3.6; 2.02-6.33, *P*< 0.001). The mean (SD) fall in pain score at 1 hour was significantly higher among intervention arm as compared to control arm [1.51 (0.5) and 1.06 (0.5); *P*<0.001]. Eight (17.4%) patients developed local phlebitis at the site of infusion among diclofenac group.

**Conclusion:** Intravenous acetaminophen is a better alternative to intravenous diclofenac in children with skeletal vaso-occlusive crisis.

**Keywords**: *Analgesia, Management, Pain score, Sickle cell homozygous children (HbSS).*

**Trial Registration:** CTRI/2018/01/011100

Vaso-occlusive crisis (VOCs) is one of the principal clinical manifestations of sickle cell disease (SCD) wherein pain is the main symptom that requires analgesia [1,2]. Though opioid analgesics remain central in the management of skeletal VOCs, treatment depends upon severity of pain and different analgesic drugs available [3,4]. Opioids have associated adverse effects and their dose can be reduced by combining analgesics like acetaminophen or diclofenac [5]. Oral, rectal and intramuscular nonsteroidal anti-inflammatory drugs (NSAID) like diclofenac can be used in the management of skeletal VOCs in children with SCD [6,7]. They have opioids-sparing effects with lack of sedation, but limited efficacy [8,9] and can be used to control painful VOCs in combination with opioids in severe cases [4].

Intravenous (IV) acetaminophen was approved by the U.S. Food and Drug Administration (FDA) in children two years of age and older for the management of mild to severe pain with or without opioids [10,11]. It has a quick onset of action, good analgesic efficacy and practically no side effect in the dose of 10 mg/ kg/dose 8 hourly [12-14] with varied reports of opioid sparing effects [14,15]. IV diclofenac is the current standard of care for management of skeletal VOCs in SCD as opiates have limited availability with the need to monitor for respiratory depression severe constipation and opioid dependence which develops with frequent use. Oral NSAIDs are associated with gastric side effects and non-response with regular usage. This study aimed to compare the efficacy of IV acetaminophen and IV diclofenac sodium in the management of skeletal VOCs among children with SCD.

## METHODS

The present single blind randomized controlled trial was conducted in the department of Pediatrics, VIMSAR, from October, 2016 to November, 2017 after approval from the institutional ethics committee. A pilot study was conducted for first two months (October, 2016 to November, 2016) for calculation of sample size. The inclusion criteria were children with SCD (confirmed by HPLC) of age between 6

months to 14 years of age with onset of symptoms of skeletal VOCs with in last 24 hour not relieved by home-based care. Children who were critically ill, with other serious complications (like acute chest syndrome, splenic sequestration, stroke, overwhelming sepsis, osteomyelitis, arthritis), these requiring any add-on analgesics during the study, and with hepatic or renal impairment were excluded. All enrolled children received standard management of skeletal VOCs and hydration therapy at 1.5 times of maintenance fluid at the emergency room [5].

The pilot study was done with 40 patients who were randomized by Clinical Trial Data Analyzer v1.0 software into intervention (IV acetaminophen) arm and control (IV diclofenac sodium) arm. Undiluted IV acetaminophen 10 mg/kg/dose 8 hourly [16], and diluted IV diclofenac sodium 1mg/kg/dose (1 mg diclofenac sodium in 2 mL of normal saline) 8hrly [17], were used. IV acetaminophen (1mL/10 mg) (Fresenius Kabi India Pvt. Ltd.) and IV diclofenac sodium (1mL/75 mg) (Troikaa Pharmaceuticals Ltd.) were used.

Initial pain score using age appropriate pain scale according to WHO guidelines [18-20] was assessed at 0 hour before administration of drug. A 50% reduction in pain score at 24 hours after first dose since in 14 (70%) and 7 (35%) in the intervention and control arm, respectively. Sample size estimation by *n* master v2 (BRTC, CMC, Vellore) assuming non–inferiority margin of 10%, alpha error of 5% and power of 80% was calculated as 43 in each arm. The minimum sample size required was 48 in each arm for 10% loss to follow-up.

Computer generated randomization (mixed block randomization) in 1:1 ratio was done by a faculty members not involved in the study. Allocation concealment was done with double opaque sealed envelope by a nurse. Blinding was done for the patient and/or caregiver. The patient enrolment was started from December, 2016 after taking written and informed consent from the parents or caregivers. Drugs were administered by the nurse in the prescribed dosages [21,22] in syringe pump over 30 minutes.

Pain score was assessed at 0, 1 and 24 hours after the administration of the drugs in both arms. The baseline characteristics like age in years, gender, hemoglobin (gm/dL), HPLC for HbS (%), reticulocyte count (%), duration of hydroxyurea use (months), previous hospitalization for skeletal VOCs in last 1 year and units of blood transfusion received were recorded. The pain score at 0, 1 and 24 hour, number of children with 50% reduction in pain score at 24 hours and total number of doses needed to relieve pain after 24 hours of drug administration were recorded in a predesigned case report format. Patients were followed up till adequate pain relief and/or regimen modification. Adequate pain relief was defined as an agreement between the patient and the investigator that the pain was tolerable or completely resolved and no further IV analgesics was needed. The patient was discharged home after of a pain free interval of 12 hour. Patients without adequate pain relief, requiring add-on drug like ketorolac and/or morphine were excluded from the study as per exclusion criteria.

*Statistical analyses:* Per protocol analysis was done and analysis was performed using SPSS v25 (IBM Corp.) and Dxt v 1.0 (BRTC, CMC Vellore). Data normalcy was tested using Shapiro Wilki and Kolmogorov–Smirnov test. Continuous data were expressed in mean and standard deviation. Categorical data were expressed in proportions. Independent t-test or unpaired t-test was done to compare two continuous variables. Categorical variables were compared by Fischer exact test. Comparison of categorical outcome like 50% reduction in pain score in 24 hours between intervention and control arm were expressed in terms of relative risk (RR), absolute risk reduction (ARR) and number need to treat (NNT). For all statistical purposes, $P < 0.05$ was considered to be significant.

## RESULTS

The flow of the study is shown in **Fig. 1**. The mean (SD) age was 8.33(3.2) years, Hb S 67.4 (6.4) %, hemoglobin 7.76 (1.4) g/dL units of blood transfusion received 3.04 (1.94) units and reticulocyte count 2.47 (0.82)%. The baseline characteristics were comparable between both groups (**Table I**).

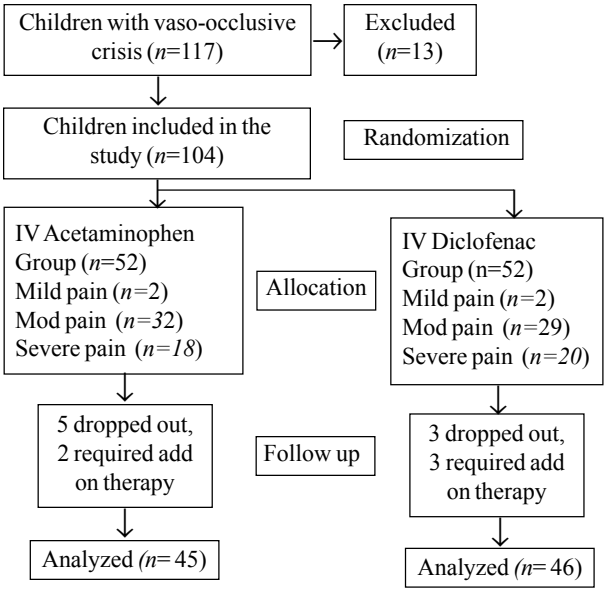


**Fig. 1** CONSORT flow chart.

**WHAT IS ALREADY KNOWN?**

- In case of unavailability of opioids, intravenous diclofenac can be used for management of skeletal vaso-occlusive crisis among children with homozygous sickle cell disease

**WHAT THIS STUDY ADDS?**

- Intravenous acetaminophen can be used as an alternative to intravenous diclofenac for management of skeletal vaso-occlusive crisis among children with homozygous sickle cell disease.

The RR (95% CI), ARR (95% CI) and NNT for 50% reduction in pain score after 24 hours of drug administration was 3.6 (2.02-6.33), -0.56 (-0.69-0.36) and -2, respectively. The mean (SD) number of drug doses needed to relieve pain after 24 hours of administration in intervention arm and control arm were [6 (4) and 8 (4); *P*= 0.011]. The mean (SD) fall in pain score at 1 hour was 1.51 (0.5) among intervention arm and in control arm it was 1.06 (0.5), *P*<0.001. Eight (17.4%) patients developed local phlebitis at the site of infusion among diclofenac group who were managed conservatively. No other major side effects were noticed in either group.

## DISCUSSION

In this study, IV acetaminophen had 3.6 times increased chance of 50% decrease in pain score after 24 hours of drug administration as compared to IV diclofenac sodium for the management of skeletal VOCs among children with SCD. The fall in pain score after 1 hour of administration of first dose was faster among acetaminophen group as compared to the IV diclofenac group.

This was a single blinded hospital-based study in which reporting bias could not be minimized. This study included only pediatric patients and the results cannot be extrapolated for all age groups. Blood level of the drugs and safety profile were not assessed.

There are different modalities of drugs used in management of skeletal VOCs in SCD ranging from acetaminophen, ketorolac, diclofenac to opioids (low and high potency) [5-7,21] which primarily depends upon severity of pain [3]. Earlier studies have shown the role of IV acetaminophen in managing postoperative analgesia [22,23], and in reducing pain of skeletal VOCs in SCD [16].

In the present study, IV acetaminophen had faster pain relief than IV diclofenac sodium as also corroborated earlier [23]. The use of IV acetaminophen for postoperative analgesia decreased the duration of hospitalization, use of opioid, opioid-related complication rates and costs [24-26].

Few cases developed mild local phlebitis at the site of diclofenac infusion even after dilution in the present study as also reported previous studies [8,11]. The incidence of local phlebitis was higher (22%) in another study [26], probably due to discrepancies in age and of drug administration.

To conclude, IV acetaminophen can be used as an effective option for management of skeletal VOCs in sickle cell disease in children as compared to IV diclofenac sodium. The findings of the current research will add to the analgesic use of IV acetaminophen.

**Table I Comparison of Baseline Characteristics of Study Groups**

| *Characteristics* | *IV Acetaminophen (n = 52)* | *IV Diclofenac (n = 52)* |
|---|---|---|
| Age (y) | 8.06 (3.1) | 8.54 (3.5) |
| Male:female | 1.02:1 | 1.06:1 |
| Hb (g/dL) | 7.66 (1.3) | 7.80 (1.5) |
| HbS (%) | 67.90 (6.7) | 67.01 (6.3) |
| Reticulocyte count (%) | 2.45 (0.7) | 2.50 (1.0) |
| Duration of pain (d) | 2.65 (1.5) | 2.32 (1.2) |
| Blood transfusion (units) | 3.16 (1.6) | 2.88 (2.2) |
| Pain score at admission | 5.75 (1.3) | 6.00 (1.3) |
| Moderate pain[a] | 32 (61) | 29 (56) |
| Severe pain[a] | 18 (35) | 20(38) |
| History of hydroxyurea use[a] | 51 (96) | 49 (94) |

*Data expressed as mean (SD) or [a]n (%).*

*Ethics approval*: Veer Surendra Sai Institutional Research and Ethics Committee (VIREC); No: 2015/P-I-RP/128, dated 15 November, 2015.
*Contributors:* PCP: conceptualization and critical inputs to manuscript writing; NRM: data collection and writing the manuscript; NRM, CP and BKN: data collection, analysis and critical inputs to manuscript writing; SKP: supervision of the work and revision of manuscript.
*Funding:* None; *Competing interests:* None stated.

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## ERRATUM

Please note the following correction in the recommendation titled "Association of Child Neurology (AOCN) – Indian Epilepsy Society (IES) consensus guidelines for the diagnosis and management of West syndrome" published in Indian Pediatr. 2020;58:54-66.

The first sentence of last paragraph, column 1, page 61 should read: "In the 2004 United Kingdom Infantile Spasms study, spasm freedom was achieved in 70% of children taking high dose oral prednisolone (**40-60 mg/day**) and 76% of children taking ACTH (40 IU/alternate day) [21]" instead of "In the 2004 United Kingdom Infantile Spasms study, spasm freedom was achieved in 70% of children taking high dose oral prednisolone (40-60 mg/kg/day) and 76% of children taking ACTH (40 IU/alternate day) [21]."

Appropriate corrections have already been done in the web version at https://www. *indianpediatrics.net/jan2021/jan-54-66.htm* on January 9, 2021.

RESEARCH PAPER

# Seroprotection for Diphtheria, Pertussis, Tetanus and Measles in Children With Nephrotic Syndrome

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**Objective**: To determine seroprotective titres for diphtheria, pertussis, tetanus and measles in children with nephrotic syndrome who had received essential immunization. **Methods**: Children (2-18 years) with steroid sensitive nephrotic syndrome (SSNS) or steroid-resistant nephrotic syndrome (SRNS) who were in disease remission and had received essential childhood immunization were included. Anti-diphtheria, anti-pertussis, anti-tetanus and anti-measles antibody titres were measured. **Results**: Seventy-six (40 with SSNS; 36 with SRNS) children with mean (SD) age 7.54 (3.96) years were enrolled. The time elapsed since last vaccination was >5 years in 68.4% patients. The seroprotection rates for diphtheria, tetanus, pertussis, and measles were 86.8%, 93.4%, 31.6% and 77.6% respectively; lower in SRNS subjects compared to SSNS. Robust seroprotection titers (1.0 IU/mL) for diphtheria were seen in 23.8% SSNS and 17.9% SRNS; *P*=0.04, and for tetanus in 69.3% SSNS and 43.8% of SRNS subjects; *P*=0.03, respectively. **Conclusions**: Children with nephrotic syndrome especially those with SRNS have lower seroprotective titers for diphtheria, tetanus, pertussis and measles, necessitating a booster dose of DPT/DT/Td and MR/MMR.

**Keywords:** *Antibody titres, DPT vaccine, Immunization, MR vaccine.*

Nephrotic syndrome is a chronic renal disorder in children requiring treatment usually with steroids and occasionally other immunosuppressant agents. Children with nephrotic syndrome have lower seroconversion to various vaccines (both live and killed) due to immune dysregulation, prolonged immunosuppressive treatment, and recurrent prolonged proteinuria [1,2]. These children are also at an increased risk of acquiring vaccine preventable diseases (both bacterial and viral) due to repeated hospital admissions, prolonged immuno-suppression and deranged immune system.

Adequate seroprotection against vaccine preventable diseases (VPDs) has been reported in only 25-56% of immunocompromised children especially those on chemotherapy, with chronic kidney disease, and with underlying congenital and acquired immunodeficiency [3,4]. The level of antibody titers for diphtheria, pertussis, tetanus (DPT) and measles were lower in children with steroid sensitive nephrotic syndrome (SSNS) during an episode of relapse compared to disease remission for all antigens [2]. There is paucity of literature on seroprotection against VPDs in children with steroid resistant nephrotic syndrome (SRNS). An earlier study from our center showed lower seroprotection rates for hepatitis B in SRNS subjects compared to SSNS [5].

The present study was aimed at looking for the seroprotective titers against diphtheria, pertussis, tetanus and measles in both SSNS and SRNS children in remission.

## METHODS

This cross-sectional study was conducted in the Departments of Pediatrics and Microbiology of a tertiary care teaching hospital during the period (January, 2016-January, 2017). The study protocol was approved by the institutional ethics committee. A written informed consent and assent was taken from the caregivers.

*Editorial Commentary: Pages 217-18*

All children (2-18 years) with nephrotic syndrome who were in remission and had previously received essential immunization were included. Essential immunization was defined as having received three primary doses of diphtheria, pertussis and tetanus (DPT) with at least one booster, and single or 2 doses of measles and/or measles, mumps, rubella (MMR) vaccines by 2 years of age. The details of prior vaccination were obtained from either the vaccination card or immunization history. Children with congenital nephrotic syndrome were excluded.

A pretested proforma consisting of clinical details,

immunization status and history regarding age of onset, type of disease (SSNS or SRNS), relapse or remission, type and duration of immunosuppressive treatment was filled. Standard definitions were used to define nephrotic syndrome, relapse, remission, SSNS and SRNS [6,7]. Five mL of venous blood was collected from each participant; 2 mL sample was used for estimation of renal function tests, serum protein, serum cholesterol, and sera was separated from the remaining 3ml sample and stored at -70ºC until further tested. Anti-tetanus, anti-diphtheria, anti-pertussis and anti-measles IgG antibodies were measured using an enzyme immunoassay (ELISA) using a commercially available kit DEDIP01 (for diphtheria), DETET01 (for tetanus), DEBPT01 (for pertussis) and DEMAS01 (for measles) (Demeditec Diagnostics GmbH). Results of the anti-diphtheria, anti-tetanus, anti-pertussis and anti-measles antibody titers using the mentioned kits were obtained in OD (optical density) units. These OD units were converted to respective antibody titers in mIU/ml using the manufacturer's instruction. The validation criteria provided by the manufacturer was fulfilled for all samples studied. The cut-offs for seroprotective titers for diphtheria and tetanus were taken as ≥0.1 IU/mL [8,9]; titers >1.0 IU/mL are associated with long-term protection according to WHO [10]. The sero-protective titers for pertussis and measles were defined as levels more than 22 IU/mL and 12 IU/mL, respectively [11,12]. The sensitivity and specificity of the mentioned kits was 94% and 94% for diphtheria, 100% and 84% for pertussis and 90% each for tetanus; the values for measles were 97% and 100%, respectively as per the manufacturer's manual.

Sero-protection for diphtheria and tetanus in immunocompromised children is reported to vary from 45-80% [2,3]; based on 75% prevalence of seroprotective titers in children with nephrotic syndrome and 95% confidence level with 10% precision, the calculated sample size was 72 and a total of 76 patients were enrolled.

*Statistical analyses*: All data was compiled in Excel spreadsheet and analyzed using descriptive statistics; mean and standard deviation (SD) were calculated for baseline characteristics and antibody titers. Chi-square test was used to compare groups with categorical data and student t-test or one-way ANOVA was used for comparing continuous data. $P<0.05$ was considered significant.

## RESULTS

Seventy-six children (60 boys) with nephrotic syndrome with a mean (SD) age of 7.54 (3.96) years were enrolled; the mean age of SSNS and SRNS was 6.9 (3.6) and 8.4 (4.2) years, respectively; $P=0.1$. Forty (53%) children had SSNS and 36 (47%) had SRNS disease. Twenty-two (55%) children with SSNS had received prednisolone alone, while remaining received drugs like levamisole, oral cyclophosphamide and mycophenolate mofetil. All children with SRNS received cyclosporine or tacrolimus along with prednisolone. At enrollment 33.3% children with SSNS and 8.3% with SRNS were off any treatment while the remaining were on minimal doses of prednisolone and other agents.

The time elapsed since last immunization for DPT was ≥5 years in 52 (68.4%) children, all of whom had received two boosters of DPT vaccine. Overall seroprotection against diphtheria, tetanus, pertussis and measles was seen in ($n$=66) 86.8%, ($n$=71) 93.4%, ($n$=24) 31.6% and ($n$=59) 77.6% children. The proportion of children with adequate seroprotection for all four antigens among SSNS and SRNS is shown in **Table I**. Good long-term antibody response > 1.0 IU/mL for diphtheria and tetanus was seen in ($n$=14) 18.4% and ($n$=41) 53.9% patients respectively; the difference being significant between SSNS and SRNS.

The mean (SD) anti-diptheria, anti-tetanus, anti-pertussis and anti-measles titres were 0.39 (0.12), 1.14 (0.42), 32.05 (10.5), 135.40 (32.89) IU/mL, respectively. The comparative antibody titres between SSNS and SRNS are shown in **Table II**. A higher proportion of children on steroids alone achieved seroprotection compared to those who received other immunosuppressants with prednisolone against diphtheria (91% vs 85.2%), tetanus (91% vs 90.7%), pertussis (36.4 % vs 29.6%) and measles (86.4% vs 74%).

**Table I Seroprotection for Diphtheria, Tetanus, Pertussis and Measles in Children With Steroid Sensitive and Steroid Resistant Nephrotic Syndrome**

| *Antibody titres* | *SSNS (n=40)* | *SRNS (n=36)* | *P value* |
|---|---|---|---|
| *Diphtheria* | | | |
| > 0.1 IU/mL | 38 (95.0) | 28 (77.8) | 0.03 |
| > 1.0 IU/mL | 9 (23.8) | 5 (17.9) | 0.04 |
| *Tetanus* | | | |
| > 0.1 IU/mL | 39 (97.5) | 32 (88.9) | 0.18 |
| > 1.0 IU/mL | 27 (69.3) | 14 (43.8) | 0.03 |
| *Pertussis* | | | |
| > 22 IU/mL | 14 (35.0) | 10 (27.8) | 0.45 |
| *Measles* | | | |
| > 12 IU/mL | 33 (82.5) | 26 (72.2) | 0.28 |

*All values in no. (%). SSNS: steroid sensitive nephrotic syndrome; SRNS: steroid resistant nephrotic syndrome.*

**Table II Antibody Titers for different Antigens in Children With Steroid Sensitive and Steroid Resistant Nephrotic Syndrome**

| *Antibody type* | *SSNS(n=40)* | *SRNS (n= 36)* |
|---|---|---|
| Anti-diphtheria titres (IU/mL) | 0.43 (0.05) | 0.35 (0.05) |
| Anti-tetanus titres (IU/mL) * | 1.48 (0.23) | 0.77 (0.16) |
| Anti-pertussis titres (IU/mL) | 32.3 (5.1) | 32 (4.8) |
| Anti-measles titres (IU/mL) | 139.52 (17.7) | 137.9 (19.6) |

*Values in mean (SD). SSNS: steroid sensitive nephrotic syndrome; SRNS: steroid resistant nephrotic syndrome; *P=0.019.*

## DISCUSSION

The present study reports protective antibody titres against diphtheria, tetanus, pertussis and measles in children with nephrotic syndrome. There has been a recent increase in incidence of diphtheria and tetanus despite the universal immunization programme in developing countries like India [13,14]. The literature on seroprotection for vaccine preventable diseases in nephrotic syndrome is scant; the seroprotection rates for diphtheria, tetanus and measles in the study population were similar to the normal comparative population [8,9,13,15].

While the tissue culture neutralization assay is regarded as the most accurate in vitro procedure for measuring anti-diphtheria antibody, ELISA and passive hemagglutination methods are more widely used due to easier availability and lower costs [10]. A higher cut-off level of >1.0 IU/mL, for both diphtheria and tetanus suggests good long-term protection [8-10]. However, a smaller proportion of children had good response against diphtheria unlike tetanus in the present study, when majority of the patients had also received the second booster of DPT vaccine at five years of age. This information clearly highlights that booster doses of diphtheria vaccine were required in this immuno-suppressed group. The SRNS group also had significantly lower titres than SSNS group against tetanus, with a poor overall seroprotection rate against pertussis (32%) in the present study. Another study [3], which looked at the seroprotective titers for diphtheria, tetanus and pertussis in 146 children who had received chemotherapy showed significantly lower protective titers for diphtheria and tetanus in patients when compared to the healthy subjects; with abysmally lower seroprotection rate for pertussis, similar to our data. The need for booster doses pertussis has been highlighted earlier [15]. The Government of India introduced Td instead of tetanus toxoid alone in the national immunization schedule in 2019 to provide for boosting of waning immunity against diphtheria during adolescence [16].

The seroprotection against diphtheria and tetanus was lower in SRNS than SSNS subjects in this study. This was possibly due to the use of more prolonged immunosuppression and recurrent proteinuria in these subjects. Lower antibody titers against diphtheria, tetanus and pertussis were likewise reported in 18 children with SSNS compared with 20 controls, which were further lower during relapse states for all 3 infections and improved during remission (irrespective of steroid therapy) indicating that proteinuria may decrease the levels of antibodies [2]. However, the difference in seroprotection between SSNS and SRNS was documented even during remission (no proteinuria) in the present study, highlighting the role of immuno-suppressant use in these patients. Lower antibody titers for diphtheria and tetanus were also seen in 400 patients with juvenile idiopathic arthritis compared to the 2176 healthy controls. Prolonged immunosuppression was cited as the reason for lower levels of protection especially for diphtheria in the study participants [17]. The present study also showed that children who received steroids alone had higher seroprotective titers for diphtheria and tetanus as compared to those receiving other immunosuppressant agents as well, similar to a previous study done in children with leukemia and hematopoietic transplant recipients [3]. Another study showed lower protection for hepatitis B in children with nephrotic syndrome compared to controls with lower antibody titres in SRNS subjects [18]. Interestingly the anti-measles antibody titers in our study were comparable in both SSNS and SRNS and could be due to boosting effect provided by subclinical infections in the community unlike diphtheria and tetanus.

The limitations of the present study are lack of control arm and the variability in the timing of antibody test from the time of immunization where role of waning immunity was not discernible. Due to the rarity of the condition, especially SRNS, too stringent criteria would substantially reduce the sample size for any meaningful interpretation.

Based on the results of our study we conclude that children with nephrotic syndrome had lower sero-protective titers for diphtheria, tetanus, pertussis and measles, even during periods of remission and the seroprotection rates were lower for those with SRNS disease. We suggest a booster dose of DPT or Tdap (if age >7 years) and MR/MMR to be administered to all children, especially those with SRNS once the child is in remission or receiving minimal doses of immuno-suppressant, preferably after measuring the antibody titers.

*Ethical clearance*: Institutional Ethics Committee of Maulana Azad Medical College; No.11/IEC/MAMC/2015/317, dated November 27, 2015.

**WHAT THIS STUDY ADDS?**

- Seroprotection against diphtheria, tetanus, pertussis and measles is lower in children with SRNS than SSNS.
- There is a need to administer dT/TdaP and MMR boosters, especially in children with SRNS disease, beyond 7 years of age.

*Contributors*: MM, SY, AC: conceptualization; AM, AA: Methodology; AM, MM, AD: software; AA, MM, SY: validation; AM, AD, MM: formal analysis; AA, AC: investigation; MM, AC: resources; AM, MM, AD: data curation, AM, MM, AD: writing - original draft; AA, MM, AD: writing - review & editing; MM: visualization; MM, SY, AC: supervision; MM, AA: project administration. All authors approved the final version of manuscript, and are accountable for all aspects related to the study.

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RESEARCH PAPER

# Requirement of a Booster Dose of Hepatitis B Vaccine in Children With Thalassemia After 5 Years of Primary Vaccination: A Prospective Study

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**Objective**: To determine anti-HBs antibody levels in multi-transfused children with beta-thalassemia major who had received primary hepatitis B vaccination ≥5 years ago, and to document their antibody response to a booster dose of hepatitis B vaccine. **Methods**: We included 85 children each of beta-thalassemia major and age-matched healthy controls, who had completed primary hepatitis B vaccination ≥5 years ago. Participants were assessed for anti-HBs titres, and those with beta-thalassemia major who were seronegative (titres<10 mIU/mL) were administered a single booster dose of hepatitis B vaccine. CD4 counts, serum levels of IL-2 and IFN-γ, and anti-HBs titres were evaluated at baseline and following booster dose of vaccine. **Results**: Seroprotection rates for hepatitis B after an average (SD) duration of 10.8 (3.8) years of completion of primary immunization were significantly higher among children with beta thalassemia major compared to healthy controls (72.9% *vs*. 52.9%, *P*=0.007). All the 23 seronegative children with beta-thalassemia major achieved seroprotection after a single booster dose of hepatitis B vaccine. **Conclusion**: A single booster dose of hepatitis B vaccine after 5 years of primary immunization is adequate to provide seroprotection to multi-transfused children with beta-thalassemia major.

**Keywords**: *Seroprotection, Infection, Immunity*.

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Children with thalassemia are particularly vulnerable to hepatitis B infection not only on account for multiple transfusions but also due to immunological derangements. The global prevalence rates for hepatitis B surface antigen (HBs Ag) positivity have been reported as 0.3% to 5.7% amongst children with thalassemia [1], with the rates being higher in Asians and South East Asians [2]. A few studies have shown these children to have immune dysfunction, especially cell mediated immunity, possibly due to iron overload [3-6]. Contrarily, a few studies have also shown that this group has a particularly active humoral immune response due to repeated antigenic stimulation [5-7]. The long term seroprotection following hepatitis B vaccination has been reported as to vary from 13-80% [8-11] in different studies and the need for booster doses remains controversial.

We conducted this study in Indian children with beta-thalassemia major who had completed primary hepatitis B vaccination five years or more back. The primary objective of study was comparing their seroprotection rates with those in age-matched healthy controls following primary hepatitis B immunisation. We also measured the responses to a single booster dose of hepatitis B vaccine, and their immune functions.

## METHODS

This study was conducted in the thalassemia day care at a tertiary care hospital over a period of one year 17 months (December, 2013 to April, 2015). Eighty-five children with beta-thalassemia major and 85 age-matched healthy controls who had documentary evidence of completion of primary hepatitis B vaccination schedule with three doses given more than five years ago, and without subsequent booster were recruited. Children having evidence of hepatitis B infection or having known immunodeficiency like HIV infection, were excluded from the study. The control group included either the healthy siblings of the children with beta-thalassemia major or the healthy participants visiting the out-patient department for minor illnesses. A written informed consent was taken from the parents/guardians of all participants prior to recruitment in our study. An informed written assent was also obtained for all participants aged more than 12 years.

*Editorial Commentary: Pages 217-18*

Ethical clearance was obtained from the institutional ethics committee.

Participants were divided into three groups on the basis of the time since completion of primary vaccination: Group 1: 60-120 months, Group 2: 121-180 months, and Group 3: 181-240 months. A 2 mL venous blood sample was drawn for baseline investigations (anti-HBc IgG, HBs Ag, anti-HBs, anti-HCV) in all participants. HBs Ag, anti-HCV, and anti-HBc were assessed using ELISA based kits (Biored, France). Anti-HBs titres were measured using ELISA based kits (Diapro). Seroprotection was defined as anti-HBs ≥10 mIU/mL.

All seronegative children with beta-thalassemia major were tested for immunological functions before being administered a single booster dose of recombinant hepatitis B vaccine intramuscularly in a dose of 10 µg to those younger than 20 years, or 20 µg for subjects aged 20 years or older. Immunological functions as ascertained by absolute CD4 count, cytokine assay (IL-2, IFN-γ), and Mantoux test (intradermal test following 5TU). IL-2, IFN-γ and anti-HBs titres were measured in children with beta-thalassemia major before and 4-6 weeks after administration of the booster dose of hepatitis B vaccine.

A 2 mL fresh whole blood was drawn in heparinized tubes for assay of CD4+ cells by flow cytometry (BD FACS Count). Cytokines (IL 2, IFN-γ) were analyzed in sera using ELISA based kits (Diaclone).

The sample size was calculated based on study by Vahidi, et al. [10] and Yazdanpanah, et al. [12]. Seroprotective rate after more than five years of primary vaccination in all patients with beta-thalassemia major was 48.7% [10] and in children without thalassemia was 84% [12]. Considering 20% difference in seroprotection rates between children with beta-thalassemia major and controls as clinically relevant, a sample size of 85 in each group was needed with 80% power and 5% alpha error.

*Statistical* analysis: Chi-square test was used for comparing the proportion of seroprotection between children with beta thalassemia major and those without thalassemia. Unpaired student *t* test was used for comparing the anti-HBs titres between groups and also the serum ferritin levels between the seroprotected and seronegative children with beta-thalassemia major. SPSS software version 20 was used for analysis.

## RESULTS

A total of 88 children with beta-thalassemia major aged 5-20 years were assessed for eligibility in the study; of which 85 (57 males) were included in the study; three subjects were excluded as two were HBsAg positive and one was anti-HBc positive. Eighty five age matched controls (56 males) were also recruited.

Participants in both groups were comparable with respect to mean (SD) age [11.4 (4.1) vs 11.2 (4.3) years; *P*=0.79] and gender [67% vs 66% males; *P*=0.87] in thalassemia group and the control group, respectively. The mean (SD) time lag between last dose of hepatitis B vaccine and inclusion in the study was 10.7 (3.7) years in the thalassemia group and 10.9 (3.9) years in the control group (*P*=0.34). The median (IQR) anti-HBs titres in the thalassemia group were higher than in control group [35.90 (9.3, 262.0) vs 14.40 (1.0, 55.8) mIU/mL; *P*=0.001]. Seroprotection rates were significantly higher in the thalassemia group (72.9% vs 27.1%; *P*=0.007).

A total of 23 children with beta-thalassemia major were found to lack seroprotective titers following primary hepatitis B vaccination. Their mean (SD) age was 11.9 (4.6) years with a mean (SD) time lag between completion of primary hepatitis B vaccination and estimation of anti-HBs titers as 11 (3.7) years. All of them were administered a booster dose of hepatitis B vaccine. Anti-HBs titers estimated after 4.7 (0.75) weeks were >10 mIU/mL for all children. The median (IQR) of anti-HBs titers before and after booster dose of hepatitis B vaccine was 5.1 (1.3-8.2) mIU/mL and 278.1 (170.1-353.0) mIU/mL, respectively; 22 children had anti-HBs titers > 100 mIU/mL.

The proportion of seroprotected children and time since primary vaccination did not show a statistically significant relationship (*P*=0.25). The proportion of seroprotected children in the beta-thalassemia major group after 60-120 months, 121-180 months and 181-240 months of primary vaccination were 77.1%, 74.3% and 60%, respectively.

Among the 23 seronegative children with beta-thalassemia major, CD4 counts were normal in all except

**Table I Response to a Single Booster Dose of Recombinant Hepatitis B Vaccine in Children With Thalassemia Having Anti-HBs Titre <10 IU/mL After Primary Hepatitis B Vaccination (*N*=23)**

| *Parameter* | *Before booster dose* | *After booster dose* |
|---|---|---|
| Anti-HBs titers (mIU/mL) | 5.1 (1.3-8.2) | 278.1 (170.1-353.0) |
| Interleukin 2 levels (pg/mL) | 0 | 0 (0-1422) |
| CD4 count (cells/mm$^3$) | 869 (682-1050) | - |

*Values in median (IQR).*

**WHAT THIS STUDY ADDS?**

- Majority of multi-transfused children with β–thalassemia major have seroprotective titers even after five years of primary vaccination.

two children, IL-2 was detectable in only two children and IFN-γ was undetectable in all children. Even following antigenic stimulus (HBV booster), only ten children had detectable IL-2 and five had detectable IFN-γ levels. There was no significant correlation of HCV infection (*P*=0.43), body mass index (*P*=0.06), serum ferritin level (*P*=0.77) and chelating agents [deferiprone (*P*=0.413), deferasirox (*P*=0.18), desferrioxamine (*P*=0.55)] with immune response to primary hepatitis B vaccination.

## DISCUSSION

In this cross-sectional study, we found that nearly three-fourths of children with beta-thalassemia major had seroprotective titres even after five years of completing the primary hepatitis B vaccination. Further, the seronegative children with β-thalassemia major mounted an anamnestic response to a booster dose of hepatitis B vaccine.

Limitations of the present study include the lack of serial annual titres of anti-HBs in the participants to find out the exact time of fall to seronegative levels. The strengths of our study include its robust sample size. We also evaluated markers of cell mediated immunity in our thalassemic cohort. However, a comparison of CD4 counts and cytokine levels in seronegative children with beta-thalassemia major and control group was not possible due to financial constraints. This information would have offered more insights into this study.

Reported seroprotection in 72.9% children with beta-thalassemia major from other studies have ranged from 13% to 80% following 3 to 6 years of primary Hepatitis B vaccination [8-11]. The waning immunity following primary hepatitis B vaccination after every passing year has been reported even in countries with high prevalence of hepatitis B infection [13]. In a follow up study from Taiwan [13], it was shown that universal hepatitis B vaccination in infancy led to adequate protection up to 14 years of age and in the absence of a booster the hepatitis B surface antibody (anti-HBs) decayed at an annual rate of 10.2%, although the new infection rates did not differ in children who received and those who did not receive booster hepatitis B vaccine.

The higher number of children with thalassemia presenting with protective titres than healthy controls could be due to higher incidence of antigenic stimuli to which children with thalassemia are exposed following repeated blood transfusions. A few studies have suggested that a possible reason for having higher protection rate is due to passive transport of anti-HBs antibodies through the donor blood [14,15]. We feel that a need for a booster dose in such individuals needs to be determined on a case-to-case basis depending upon the risk and vulnerability to hepatitis B.

Following, a single booster dose, all the previously seronegative children in this study developed protective titres showing an intact humoral response, while the cell mediated response appeared blunted as demonstrated by low rates of detection of cytokines. Previously, few studies have demonstrated dysfunctional cell mediated immunity in these children [3-6].

Based on our findings and considering the increased risk of hepatitis B in children with beta-thalassemia major, we suggest regular assessment of anti-HBs titres following primary hepatitis B vaccination and recommend administration of a booster dose whenever indicated at the earliest.

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*Competing Interest*: None stated.

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RESEARCH PAPER

# Validity and Reliability of the Turkish Version of the Pediatric Assessment Scale for Severe Feeding Problems

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**Objective:** The purpose of this study was to translate the Pediatric assessment scale for severe feeding Problems (PASSFP) into Turkish and investigate its validity and reliability. **Methods:** The study included Turkish translation of the PASSFP, and investigating its reliability and criterion validity in relation with Children's eating behavior questionnaire (CEBQ). **Results:** Cronbach Alpha reliability of T-PASSFP was 0.79, and of its subscales, i.e. Form A and B, were 0.67, and 0.73, respectively. Test-retest correlation was 0.99 for the scale and Form A, and 0.94 for Form B. There were positive correlations between total scale and Enjoyment of food and Food fussiness subscales of CEBQ. Form A had positive relationships with Food responsiveness, Enjoyment of food, and Food fussiness subscales. Form B had positive correlations with Enjoyment of food and negative correlations with Slowness in eating subscales. **Conclusion:** The Turkish PASSFP is valid and reliable in evaluating feeding in children with neurodevelopmental disorders. Form A is applied for all children, and Form B is used for partially or totally oral-fed children

**Keywords:** *Children's eating behavior questionnaire, Feeding behavior, Feeding disorders, Translation.*

Feeding difficulty, including complex biological, social, and behavioral factors, expresses all problems affecting food intake [1-5]. Gastrostomy and tube feeding are commonly used for children with neurological disorders and feeding difficulties that can stem from oral sensory-motor or behavioral problems [6-9]. To determine feeding difficulties in children with neurodevelopmental disorders, clinical and behavioral factors should be evaluated besides oropharyngeal dysphagia assessment [4,10,11]. Evaluating parent-child relationships and behaviors during feeding is essential, as these children are dependent on their parents for feeding [12]. Pediatric feeding disorders (PFD) states that diagnosis and treatment of feeding disorders require a comprehensive approach including medical, nutritional, and psychosocial aspects as well as feeding skills [5,10].

The Pediatric assessment scale for severe feeding problems (PASSFP) evaluates medical, nutritional, and psychological aspects of feeding in relation with feeding type [3,5].

The purpose of the present study was to translate the PASSFP into Turkish and investigate its validity and reliability.

## METHODS

The study was conducted at the Physical therapy and rehabilitation faculty of Hacettepe university, and was approved by the ethics committee, and written consent forms were signed by the parents. To conduct validity and reliability studies on Turkish children, written permission was obtained from the developer of the PASSFP [3].

The PASSFP consisting of 15 questions and two forms, was filled out by parents/caregivers. Form A was applied for all children and is related to feeding type and sensory responses. Form B was used for partially or totally oral-fed children and is related to oral sensory-motor responses, behavior, and quality of life. While the 1st question inquired feeding type, the 2nd question about determined the ratio of oral feeding to tube feeding. 3rd to 9th questions scored between "never=0" to "always=4" on Likert scale. Lower scores on the PASSFP indicated higher severity [3].

The study included *a*) translating PASSFP into Turkish, and *b*) investigating reliability and criterion validity in relation with Children's eating behavior questionnaire (CEBQ). Additionally, the relationship between the PASSFP and children's height-weight was also examined.

The study included children with feeding difficulties who were at least 4 months of age. A minimum of 3 months

of tube feeding was set as the inclusion criteria for tube-fed children. Have different neurological diagnoses children with feeding difficulties were divided into 3 groups: i) orally-fed, ii) partially tube-fed, and iii) tube-fed. A priori power analysis conducted on G*Power 3.1.9.2 showed that 159 participants were required to achieve 0.80 power at $P$=0.05 for a medium-sized effect (f=0.25). We could recruit only 90 participants since most parents with oral-fed and partially oral-fed children did not accept participation. For that reason, we stopped data collection after reaching the sample size reported in the original study.

Based on translation-back translation method, two physiotherapists with good English skills prepared the initial Turkish version, and translated it back into English. The original and back translated versions were compared and the final version was arranged after necessary revisions based on the feedback from the pilot study conducted on 20 parents. Original and Turkish versions are given in the supplementary material section.

The internal consistencies for whole scale, Form A and Form B were calculated separately. To investigate the scale's internal consistency, following Crist, et al. [3], we examined the relationship between the first and second questions in Form A and the total score obtained from the remaining 13 items [3]. To examine the scale's consistency in time, it was reapplied to the same sample one week later (**Table II**). The re-test was applied to a total of 87 children.

To study the criterion validity, the correlations between CEBQ and T-PASSFP were calculated [13,14]. Furthermore, partial correlation coefficients were calculated to examine the relations between T-PASSFP and its sub-scales to the weight-height of the children. The comparisons of tube-fed, partially-tube-fed and orally-fed children were also examined for T-PASSFP total score, Form A, and Form B (**Table III**).

Developed by Wardle, et al. and adapted to Turkish Culture by Yilmaz et al, CEBQ, is a 35-item questionnaire answered by parents and rated on a five-point Likert scale [13,14]. It determines the appetence of the child in eight aspects: Food responsiveness (FR), Emotional over-eating (EOE), Enjoyment of food (EF), Desire to drink (DD), Satiety responsiveness (SR), Slowness in eating (SE), Emotional under-eating (EUE), and Food fussiness (FF).

*Statistical analyses:* Windows-based Statistical Package for Social Sciences (SPSS) ver.22 was used for statistical analysis. Means and standard deviations were calculated for quantitative variables and all statistical tests were conducted at 5% significance level. Since the T-PASSFP scores of the groups violated the homogeneity of variance assumption, differences between the groups were examined using nonparametric statistics. To compare the groups, Kruskal-Wallis was performed on T-PASSFP total scores and scores of Form A, and Mann-Whitney U test was performed for the paired comparisons in Form B. The relationships between T-PASSFP and height-weight were analyzed using partial correlation coefficient.

## RESULTS

The study included 90 children with neurological disorders having feeding difficulties (48 boys, 42 girls, mean (SD) age=34.11(21.04) month), who were divided into three groups: orally-fed ($n$=45), partially-tube-fed ($n$=18), and tube-fed ($n$=27). Mean (SD) age, height and weight of children according to the groups, were 43.49 (34.13) m, 89.56 (17.48) cm, 15.24 (20.97) kg for orally-fed; 31.00(25.57) m, 82.28(13.00)cm, 17.77(21.57) kg for partially-tube-fed; 40.00 (38.94) m, 84.89 (18.88) cm, 14.41(10.89) kg for tube-fed, respectively. The distribution of the children according to their diagnoses and type of feeding are given in Table I.

The internal reliabilities of the T-PASSFP, Form A, and Form B were 0.79, 0.67, and 0.73, respectively (**Table II**). The test-retest reliabilities, of T-PASSFP and Form A were 0.99, and Form B was 0.94 (**Table II**).

The correlations between T-PASSFP total score and the 1st and 2nd questions were 0.74 and 0.78, respectively. The 1st question identifies the feeding type of the child and the 2nd question explains the percentage of the child's oral or tube feeding. Correlations regarding

**Table I Distribution of Children According To Their Diagnosis and Type of Feeding**

| *Diagnosis* | *Oral-fed n=45* | *Partially tube-fed n=18* | *Tube-fed n=27* | *Total n=90* |
|---|---|---|---|---|
| Undiagnosed | 13 (14.4) | 3 (3.3) | 7 (7.8) | 23 (25.5) |
| Cerebral Palsy | 11 (12.2) | 4 (4.4) | 12 (13.3) | 27 (30.0) |
| Different neurological disorders[b] | 8 (8.9) | 8 (8.9) | 5 (5.6) | 21 (23.3) |
| Gastrointestinal disorders[a] | 2 (2.2) | 1 (1.1) | 0 | 3 (3.3) |
| Chromosomal disorders | 3 (3.3) | 1 (1.1) | 1 (1.1) | 5 (5.5) |
| Metabolic disorders[c] | 6 (6.7) | 1 (1.1) | 2 (2.2) | 9 (10.0) |
| Muscular diseases | 2 (2.2) | 0 | 0 | 2 (2.2) |

*[a]The primary diagnoses of these three children were gastrointestinal disorders. However, they also had neurological problems that were under examination and not diagnosed yet; [b]Epilepsy, hydrocephalus, encephalitis etc.; [c]also include syndromic disorders.*

**Table II Cronbach Alpha and Test-Retest Reliabilities of the T-PASSFP and Subscales, and Their Correlations With Children's Eating Behavior Questionnaire and Its Subscales**

| | *Mean (SD)* | *Alpha* | *Test-Retest Reliability* | *Weight (kg)* | *Height (cm)* | *Children's Eating Behavior Questionnair, r* | *Food responsiveness, r* | *Emotional over-eating, r* | *Enjoyment of food, r* | *Desire to drink, r* | *Satiety responsiveness, r* | *Slowness in eating, r* | *Emotional under-eating, r* | *Food fussiness, r* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| T-PASSFP.A | 12.70 (5.00) | 0.67 | 0.99 | 0.01 | 0.07 | 0.37[a] | 0.35[a] | -0.03 | 0.47[a] | 0.28 | 0.06 | 0.01 | 0.19 | 0.44[a] |
| T-PASSFP.B | 21.01 (7.73) | 0.73 | 0.94 | 0.09 | 0.06 | 0.06 | 0.27 | 0.21 | 0.53[a] | 0.11 | -0.27 | -0.46 | -0.33[a] | (0.31)[a] |
| T-PASSFP | 27.80 (15.75) | 0.79 | 0.99 | 0.07 | 0.07 | 0.24 | 0.28 | -0.05 | 0.43[a] | 0.19 | 0.01 | -0.15 | 0.11 | 0.36[a] |

*T-PASSFP: Turkish-Pediatric Assessment Scale for Severe Feeding Problems Form (Total form), T-PASSFP.A: form A of T-PASSFP, T-PASSFP.B: form B of T-PASSFP. [a]P<0.05.*

the Criterion Validity of T-PASSFP are given in **Table II**. The T-PASSFP had significant positive correlations with EF and FF subscales of CEBQ *P*=0.002; *P*=0.012 respectively). Positive correlation between T-PASSFP and FR subscale was marginally significant (*P*=0.053).

Form A had significant positive correlations with the FR, EF and FF subscales (*P*=0.015; *P*=0.001; *P*=0.002 respectively). The relationship between Form A and DD was not significant (*P*=0.056). Form B had positive correlation with EF and negative correlation with SE (*P*=0.001; *P*=0.003, respectively).

Partial correlations of weight and height (controlling for each other) with the T-PASSFP and its subscales were not significant (*P*>0.05) (**Table II**).

To investigate criterion validity, comparing the T-PASSFP scores of the groups showed that feeding type had a significant effect on the scores of T-PASSFP and Form A (*P*<0.001, *P*<0.001). Paired comparisons revealed that the "orally-fed" group had the highest scores on both T-PASSFP and Form A, and it was followed by the "partially tube-fed" and "tube-fed" groups (*P*<0.001). Form B scores of the "partially-tube-fed" and "orally-fed" groups were not significantly different (*P*=0.11) (**Table III**).

## DISCUSSION

The PASSFP is the most appropriate test for children with neurologic disorders because of its ease of clinical implementation and its psychometric characteristics [10,15]. According to the present study, the Turkish version of the PASSFP is a reliable and valid instrument.

Cronbach Alphas of T-PASSFP at its subscales indicated sufficient internal consistencies. High test-retest correlations revealed a good consistency in time. These coefficients of reliability indicate the reproducibility of the T-PASSFP as the original study [3].

A high score in Form A indicates proper swallowing and appropriate sensory responses. The questions in Form A are related to the FR, EF, and FF, in which sensory responses examined (i.e. eating as a pleasant and desirable action, the taste and texture of food). DD, which examines to the fluid intake, is related (marginally significant) to the positive feeding skills examined in Form A. Oral-fed, partially-tube-fed and tube-fed children significantly differ on Form A reflecting their feeding-type and related sensory responses.

Form B includes questions related to oral sensory-motor responses (e.g. how resistant a child is to having their teeth/gums brushed/rubbed with a cloth), behavioral issues (e.g. how willing the child is to accept a

**TABLE III Comparisons Among Oral- Fed, Partially Tube-Fed and Tube-Fed Children on PASSFP and Subscale Scores**

| | | *Mean (SD)* | | *Mean Diff. (%95 CI of Diff.)* | *P* |
|---|---|---|---|---|---|
| T-PASSFP.A | Oral-fed | 16.64 (2.42) | Oral fed -Partially tube fed | 5.44 (3.90/6.99) | < 0.001 |
| | Partially tube fed | 11.20 (3.86) | Oral fed -Tube fed | 9.64 (8.56/10.73) | < 0.001 |
| | Tube fed | 7.00 (2.07) | Partially tube -Tube fed | 4.20 (2.46/5.94) | < 0.001 |
| T-PASSFP.B | Oral-fed | 22.33 (6.89) | Oral fed - Partially tube fed | 3.17 (-0.95/7.29) | 0.108 |
| | Partially tube fed | 19.17 (8.86) | | | |
| T-PASSFP | Oral-fed | 38.98 (8.23) | Oral fed - Partially tube fed | 10.53 (5.28/15.77) | < 0.001 |
| | Partially tube fed | 28.4 (13.07) | Oral fed - Tube fed | 31.98 (28.81/35.14) | < 0.001 |
| | Tube fed | 7.00 (2.07) | Partially tube - Tube fed | 21.45 (16.41/26.49) | < 0.001 |

*Mean Diff.: Mean Difference; %95 CI Diff.: %95 Confidence Interval for Mean Difference; *Mann-Whitney U tests were conducted as post-hoc test for probing the significant effects observed in Kruskal-Wallis Tests comparing Oral-Fed, Partially Tube-Fed and Tube-Fed groups on T-PASSFP and T-PASSFP.A. Since, T-PASSFP.B was not appropriate for Tube-Fed children, only Oral-Fed and Partially Tube-Fed groups were compared by using Mann-Whitney U test.*

spoon), and quality of life (e.g. how much the child enjoys eating). Form B is positively related to the FF, EF, and EUE (i.e. positive feeding behaviors) and negatively to SE (i.e. a negative one). Since, oral feeding let children to gain experiences in feeding, oral-fed and partially-tube-fed children were similar on Form B.

Since T-PASSFP assesses feeding responses depending on children's feeding type and analyzes various states of feeding difficulties, no correlation was found with the subscales related to the desire for eating (i.e. EOE, EUE, SR). Since tube-feeding provides children necessary nutrition, their height and weight are not correlated with T-PASSFP.

There is no golden standard test to analyze the criterion validity of the PASSFP. The CEBQ was used in the present study. However, since the CEBQ evaluates the behaviors and desire to eat, whereas PASSFP evaluates feeding difficulties, the testing parameters of these two scales do not completely coincide. This was an inevitable limitation of our study. The absence of any difference between orally-fed and partially-tube-fed children in Form B may be due to the fact that our orally-fed group also had feeding difficulties. This is another limitation of the study that healthy children without any feeding difficulties were not included. Future studies are recommended to provide comparisons with healthy orally-fed children as an additional evidence of the validity of Form B. Tube-fed and partially-tube-fed children undergo several medical interventions; causing many families reject to participate in the study, resulting in the fewer number of participants in our tube-fed and partially-tube-fed groups. Likewise, in their study, Crist et al collected data from a relatively smaller sample ($n$=74) [3]. Another limitation of the study is that the scale's construct validity could not be examined; one reason of which was the insufficient sample size for factor analysis. Furthermore, the factor structure of the scale was not examined in the original study of the PASSFP [3]. In their review study, Speyer et al. examined 12 scales evaluating pediatric feeding difficulties and reported that construct validity was not examined in any scale other than Dysphagia Disorder Survey [16,17].

The T-PASSFP has sufficient internal and test-retest reliabilities, and criterion validity, and can be used for evaluating feeding difficulties in children with neurodevelopmental disorders in Turkey.

*Ethics clearance*: Non-Invasive Clinical Research Ethics Committee of Hacettepe University; No. GO 13/433.
*Contributors:* MY: planning the study, collecting data and writing the manuscript; TA: statistical analysis and contribution to writing discussion section; SSA: contributing to data collection to data and writing discussion section; AAK: contributing to planning the study and interpretation the results.
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**WHAT THIS STUDY ADDS?**

- The Turkish version of PASSFP, which is a validated scale, is found to be a valid and reliable tool.

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## CLIPPINGS

**Reduced pancreas size and exocrine function in young children with recent-onset type 1 diabetes** *(Diabetic Med. 2020;37:1340-43)*

This study evaluated 42 children with recent onset type 1 diabetes aged 5.5 median years and 90 controls. Ultrasound imaging was used to measure transverse and longitudinal areas of pancreas. Pancreatic fecal elastase-1 was measured using ELISA to assess exocrine pancreatic function. Both pancreatic area and exocrine function were reduced in children with recent onset type 1 diabetes. The mean transverse and longitudinal pancreatic area in type 1 diabetics was 6.2 $cm^2$ and 1.28 $cm^2$, respectively, which was lower than controls (8.32 $cm^2$ and 1.55 $cm^2$). Fecal elastase -1 levels were lower (455 μg/g) in type 1 diabetics than in controls (1408 μμg/g). Pancreatic area and exocrine pancreatic function were reduced in children with recent onset type 1diabetes who presented very early in life, thus supporting role of changes in exocrine pancreas in pathophysiology of type 1 diabetes in children presenting very early in life.

**Biomarker for hypothalamic obesity in children with craniopharyngioma** *(Obesity (Silver Spring). 2021;29:402-8)*.

Craniopharyngioma (CP) is associated with multiple pituitary hormone deficiencies and/or hypothalamic obesity. The present study evaluated 31 patients (median age of 16 years) with CP to assess the levels of leptin, neurotrophic factor (BDNF), and alpha-melanocyte-stimulating hormone (α-MSH) as peripheral biomarker for hypothalamic obesity. Two control groups of children without CP with obesity (*n*=27) and without obesity (*n*=25) were also compared. Seventeen patients with CP had hypothalamic obesity. The levels of leptin and BDNF (not α-MSH) correlated with BMI in all groups. However, levels of α-MSH were higher in 17 patients with CP and hypothalamic obesity than in other groups, suggesting it to be a potential biomarker of hypothalamic obesity.

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RESEARCH PAPER

# Diagnostic Spectrum and Clinical Profile of Primary Immunodeficiency Disorders at a Tertiary Care Children Hospital in Southern India

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**Background:** Primary immunodeficiency disorders are genetically heterogeneous immune disorders with a wide range of infectious and non-infectious manifestations. **Objective:** To describe a single-center experience of primary immunodeficiency disorders. **Design:** Retrospective analysis from January 2015 to January 2020. **Setting:** Tertiary care children's hospital. **Participants:** One hundred and twelve children (<18 years) diagnosed with primary immunodeficiency disorders. **Outcome measure:** Diagnostic spectrum, clinical features, and outcome. **Results:** The median (IQR) age of the first clinical manifestation and lag time in diagnosis was 10 (27) and 11 (18) months, respectively. Twenty-seven children (24%) were diagnosed during their first presentation. Thirty-six (32%) children had phagocytic disorders, 20 (17.8%) had combined/cellular defects, 18 (16%) had predominant antibody deficiencies and 17 (15%) had disorders of immune dysregulation. Non-infectious manifestations were seen in 54 (48%). Eight children underwent hematopoietic stem cell transplantation, 44 (39%) children were on antimicrobial prophylaxis and supportive therapy, 36 (32%) were lost to follow-up and 24 (21%) children died. **Conclusion:** Congenital defects of phagocyte function, followed by combined/cellular defects are the commonest primary immune deficiencies (PIDs) identified in southern India. Long lag time in diagnosis and high mortality in our cohort emphasizes the need for early diagnosis and early referral.

**Keywords:** *Hematopoietic Stem Cell Transplantation, Phagocytic disorders, Primary Immunodeficiency, Recurrent infections, Severe Combined Immunodeficiency*.

Primary immune deficiencies (PIDs) are a group of heterogeneous immune disorders that affect distinct components of the adaptive and innate immune system. The clinical presentation is variable and includes increased susceptibility to infections, autoimmunity, auto-inflammatory diseases, allergy, and/or malignancy. It can present in the neonatal period or as late as adulthood depending upon the severity of the immune defect [1].

With the ongoing discovery of novel mutations, the numbers of genetically defined PIDs are currently estimated to be more than 400 [2]. The prevalence of PIDs may be as high as 1:1200 as reported from the United States [3]. So far, there is no nationwide data on the prevalence of PIDs in India which is expected is expected to be higher than the US due to a higher rate of consanguineous marriages. Based on statistical projections it is estimated that there could be more than one million patients with PIDs in India [4]. While awareness for PIDs is increasing, with advances in diagnosis and management, they continue to remain under-diagnosed and under-treated [3,5]. The reasons for a high drop-out rate on follow-up are probably lack of understanding of disease, denial of diagnosis, and the costs involved in the treatment and follow-up. This could be improved by establishment of patient support groups with advice, education, and support to families.

We describe the clinical spectrum and outcome of PIDs over the last 5 years in children at a tertiary care hospital from South India.

## METHODS

A retrospective analysis of case records of children (<18 years) diagnosed to have PIDs from January 2015 to January 2020 at Kanchi Kamakoti Childs Trust Hospital, Chennai was performed. PIDs were classified according to the International Union of Immunological Societies (IUIS 2017) classification [6]. Children with immune deficiencies secondary to HIV infection, chemotherapy, or chronic steroid therapy were excluded from the study. This study was approved by the institutional ethics committee.

The details collected were age at the onset of symptoms, type of clinical presentation, time of diagnosis, family history of illnesses or PID, laboratory findings, microbiological data, confirmatory diagnosis, treatment and outcome. The diagnosis of PID was confirmed based on characteristic clinical presentation, relevant laboratory data, and gene analysis. Laboratory investigations included complete blood count, peripheral smear, and relevant immunological workup. Immunoglobulin profile, nitroblue tetrazolium test, flow cytometry based analysis of peripheral blood lymphocyte subset and dihydrorhodamine assay, Bruton tyrosine kinase (BTK) expression, CD11b/18 expression, CD107a/perforin assay, Wiskott-Aldrich Syndrome Protein (WASP) expression, Dedicator Of Cytokinesis 8 (DOCK 8) expression and IFNα/IL12RB expression were done based on the clinical suspicion. Bone marrow aspiration was performed in the presence of prolonged fever, cytopenia and features of hemophagocytic lymphohistiocytosis. Flow cytometry based tests were performed at the National Institute of Immuno-Hematology, Mumbai. Next-generation sequencing based genetic test was performed at MedGenome Labs Ltd, Bangalore. Genetic testing could not be performed in all children due to cost constraints.

## RESULTS

During the study period, 112 children (65 boys, 47 girls) were diagnosed with PIDs. Most of the children (*n*=69) belonged to the state of Tamil Nadu followed by Andhra Pradesh (*n*=38, 34%).

The diagnostic spectrum and clinical profile are depicted in **Fig. 1** and **Supplementary Table I**. Molecular diagnosis was performed in 42 (37.5%) children. Sixty-five (58%) children had weight less than the 3rd centile. Positive family history was noted in 32 (28%) children, with a history of sibling death in 26 (24%) cases. Parents of 64 (58%) children had consanguineous marriage. The predominant mode of inheritance observed was autosomal recessive (*n*=28, 66%).

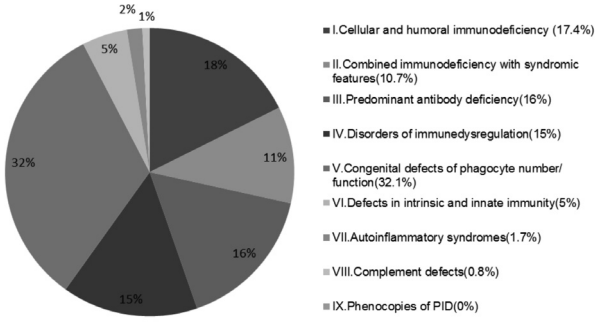


**Fig.1** Diagnostic spectrum of primary immunodeficiency disorders at a pediatric tertiary care center, 2015-2020 (*N*=112).

The median (IQR) age at onset of first clinical manifestation was 10 (27) months (range 0-12 years) and it varied depending on the underlying PID (**Supplementary Table 1)**. The median (IQR) age at diagnosis was 18 (17) months (range 0 -15 years) with median (IQR) lag time in diagnoses as 11(18) months (range 0-12 years). Twenty-seven (24%) children were diagnosed during their first presentation. The most common presentation recurrent/persistent pneumonia (*n*=39, 34%), followed by recurrent/persistent diarrhea (*n*=21, 18.5%). Non-infectious manifestations were seen in 54 (48%) children and were the main presenting symptom in 24 (21%) children, atopy being the commonest (*n*=11) presentation. Microorganisms were identified on 48 occasions in our cohort.

Forty-four (39%) children remain on supportive therapy such as immunoglobulin replacement, antimicrobial prophylaxis, and/or specific therapy. Children with autoimmune manifestations received immunosuppressive agents. One child with LRBA defect with refractory autoimmune cytopenia was started on abatacept (cytotoxic T-lymphocyte associated 4-immunoglobulin fusion protein) therapy and sirolimus after which his hemoglobin and platelet count stabilized.

Eight (7%) children underwent hematopoietic stem cell transplantation (2-CGD, 1-WAS, 1-LRBA, 1-Familial HLH, 2-DOCK8, 1-MSMD), of whom 7 children are in remission with a durable graft. One child with MSMD had a graft rejection and received anti-tuberculous therapy. Thirty-six (32%) children were lost to follow up. Twenty-four (21%) children died with the cause of mortality as severe infection (*n*=13), refractory HLH (*n*=8), refractory immune cytopenia with intracranial bleeding (*n*=2), and lymphoma (*n*=1).

## DISCUSSION

The most common PIDs observed in our study were congenital defects of phagocyte number or function followed by combined/cellular defects.

Our study had some limitations as we received children with critical acute illnesses or difficult to treat chronic conditions, which may have led to only severe forms of PIDs being recognized. The response to polypeptide vaccines was not assessed. Children were referred from southern regions of India, limiting geographical generalizability of the study findings. Detailed analysis of ethnicity, race and religion was not carried out limiting comparison to earlier Indian studies.

While, in earlier reports from India, antibody deficiencies were predominantly described from Chandigarh (42%) [4], disorder of immune dysregulation from Mumbai (33.8%) [7], and combined cellular and humoral immunodeficiency (29%) and phagocytic defect (29%) from Delhi [8]. In most parts of the world, antibody deficiency disorders are the most common PIDs reported [9,10]. The variance observed in our population could be due to genetic and racial factors with a high frequency of consanguineous marriages. Mild PIDs such as isolated immunoglobulin deficiencies may be under-diagnosed in our country [11]. The collection of pooled data from other centers in India through registries would be vital for this.

The ten warning signs of PIDs help in early recognition but do not cover the expanding spectra of non-infectious manifestations [12]. In our cohort, a high proportion of children had non-infectious manifestations, which were also the presenting symptoms similar to earlier reports [13,14].

The International Union of Immunological Society proposed a recent phenotypic and genotypic classification of PIDs [15]. A subset of patients (LRBA, DOCK8, and IL2RA defect) in our series was diagnosed based on genetic analysis. Next-generation sequencing (NGS) offers diagnostics and development of novel targeted therapies in cases where primary investigations are inconclusive [16,17].

Interventions in the form of early hematopoietic stem cell transplantation (HSCT), before the onset of serious infections, have improved 5-year survival to >90% [18,19]. Eight children underwent curative HSCT in this study. More than 100 transplants have been carried out so far in 10 centers across the country and more than half are doing well [20,21]. Barriers to cure include lack of early diagnosis and referral, lack of awareness on management of PID including HSCT and the prohibitive costs.

The high mortality of patients with SCID in our study demonstrates the need for awareness and implementation of newborn screening for SCID in the near future. The implementation of universal newborn screening (TREC – T cell receptor excision circle assay) would help in early diagnosis and timely access to treatment in these children [21].

The Indian Society for Primary Immune Deficiency (ISPID), initiated in 2011, is working towards increasing the awareness of PIDs, the establishment of diagnostic support and research centers, and also towards the development of a national PID registry. With such an initiative, there is scope for improving the outcome for children affected with PID [23].

Congenital defects of phagocyte function followed by combined/cellular defects were the commonest disorders. A consistent algorithm and a high index of suspicion will go a long way in improving early diagnosis and appropriate management of PIDs. Further modifications to include other early warning signs in the light of the growing spectra of PIDs are essential to ensure early identification.

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## CLIPPINGS

**Standard deviation scores of 17-OHP and other analytes in classical CAH** *(Horm Res Paediatr 2020;93:226-38)*

The management of congenital adrenal hyperplasia is guided by clinical assessment and measurement of biochemical parameters, chiefly 17-hydroxyprogesterone (17-OHP) and androgen metabolites. However, a single cutoff value of these analytes is difficult to define, and levels may vary with age, phenotypes and between different laboratories and assay methods. This study measured and expressed the SD scores of levels of 17-OHP, androstenedione, dehydroepiandrosterone-sulphate (DHEAS), and testosterone using liquid chromatography-tandem mass spectrometry in 38 children (aged 3-18 years) diagnosed with classical CAH. The biohemeical profile was corroborated with the clinical outcomes. The majority (86%) of the patients had elevated 17-OHP levels while consuming hydrocortisone in replacement dose of 12.6 mg/m$^2$/day. The levels of androstenedione were within ±2 SD but DHEAS levels were below -2SD. The authors reiterated the need to develop gender- and age-specific cutoffs while interpreting these hormonal levels for optimum titration of dose of replacement steroids.

**Kisspeptin levels in prepubertal obese and overweight children** *(Eur Rev Med Pharmacol Sci. 2021; 25:941-49).*

Kisspeptin is an important neuropeptide involved in regulation of the hypothalamo-gonadal axis. The concentrations of this neuropoetide were measured using radioimmunoassay in 54 prepubertal children (22 boys) who were overweight or obese and compared with 25 normal weight prepubertal children. The metabolic (glucose and insulin levels after oral glucose load, total- LDL- HDL-cholesterol, triglycerides, uric acid), hormonal (fT3, fT4, TSH, IGF-1, leptin) and total antioxidative capacity were also measured and correlated. The levels of kisspeptin were found similar in obese and normal-weight children but were lower in obese males than females. Kisspeptin did not correlate with BMI, HOMA-IR, Insulin peak levels and total antoxidative capacity; however, it significantly correlated with fT3 levels. Leptin levels were higher in obese children and positively correlated with total antioxidative capacity. The authors concluded further studies to understand this complex central regulation and interaction with oxidative stress in children.

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**Supplementary Table I Clinical and Genetic Profile of Primary Immunodeficiency Disorders, *N*=112**

| *Category* | *n (%)* | *Median age at symptom(range) (mo)* | *Infectious manifestations (n)* | *Organisms isolated (n)* | *Non-infectious manifestations (n)* | *Genetic analysis (n)* |
|---|---|---|---|---|---|---|
| Ia. SCID | 16 (14.3) | 6.5 (0.5-50) | Persistent pneumonia (4)<br>Recurrent pneumonia (12)<br>Recurrent diarrhea (9)<br>Oral thrush (5)<br>Meningitis (2)<br>Sepsis (6)<br>Orbital cellulitis (1) | MRSA (1)<br>Pseudomonas aeruginosa (4)<br>Streptococcus pneumoniae (2)<br>Candida tropicalis (2)<br>Acinetobacter baumanni (1)<br>Adenoviral pneumonia (2)<br>Pneumocystis carinii (1)<br>Cytomegalo virus (1) | Hepatospleno-megaly (5)<br>Autoimmune hemolytic anemia (2)<br>Spinal lipoma (1)<br>Developmental delay (2)<br>Seizure (2) | Homozygous ADA gene defect/AR (1),<br>Homozygous JAK3 gene defect/AR (2) |
| Ib. MHC Class II deficiency | 1 (0.8) | 12 | Recurrent diarrhea (1)<br>Sepsis (1) | Klebsiella pneumoniae (1) | | Homozygous RFXANK defect/AR (1) |
| Ib. DOCK8 defect | 3 (2.7) | 9.6 (5-18) | Deep seated abscess (1)<br>Empyema (1)<br>Recurrent diarrhea (1) | MSSA (1)<br>Pseudomonas aeruginosa (1) | Atopic dermatitis (3)<br>Food protein allergy (1)<br>Bronchiectasis (1) | Homozgous DOCK 8 defect/AR (3) |
| IIa. WAS | 4 (3.5) | 7.4 (3-10) | Recurrent diarrhea (2)<br>Recurrent pneumonia (2) | Pseudomonas aeruginosa (1) | Atopic dermatitis (4)<br>Autoimmune hemolytic Anemia (2)<br>Thrombocytope-nia (4)<br>Neutropenia (2) | Hemizygous WAS defect/XLR (3) |
| IIa. AT | 3 (2.6) | 76 (39-120) | Abdominal tuberculosis (1) | Mycobacterium Tuberculosis (1) | Ataxia (3),<br>Telengiectasia (3)<br>Myopathy (1),<br>Lymphoma (1) | Homozygous ATM gene defect/AR (1) |
| IIb. Hyper IgE syndrome | 5 (4.4) | 24 (6-60) | Empyema (1),<br>Otitis media (1)<br>Multiple pustules (1)<br>Recurrent cervical abscess (2) | MSSA (2) | Atopic dermatitis (3) | Heterozygous STAT 3 mutation/AD (1) |
| IIIa. XLA | 4 (3.5) | 67 (6-120) | Recurrent pneumonia (2)<br>Meningitis (1)<br>Septic arthritis (1)<br>Pyoderma gangrenosum (1) | Pseudomonas aeruginosa (1) | | Hemizygous BTK gene defect/XLR (3) |
| IIIa. CVID | 4 (3.5) | 49 (4-144) | Recurrent otitis media (3)<br>Recurrent diarrhoea (2)<br>Recurrent pneumonia (2)<br>Skin and soft tissue infection (1) | Cryptosporidi-um (1) | Hepatospleno-megaly (2)<br>Autoimmune hemolytic anemia (2)<br>Bronchiectasis (1) | Heterozygous NFKB1/AD (1) |
| IIIb. Selective IgA deficiency | 4 (3.5) | 11 (5-9) | Persistent diarrhoea (4) | | | |

| | | | | | | |
|---|---|---|---|---|---|---|
| IIIb. THI | 2 (1.7) | 9 (7-11)) | Persistent diarrhea (1)<br>Septic arthritis of Hip (1)<br>Cerebral Abscess (1) | MRSA (2) | | |
| IIIb. Hyper IgM syndrome | 4 (3.5) | 24.5 (5-48) | Recurrent Pneumonia (3)<br>Recurrent oral ulcers (1)<br>Meningitis (1) | Pseudomonas aeruginosa (2)<br>Candida tropicalis (1)<br>Streptococcus pneumoniae (1)<br>Eschericia coli (1)<br>Klebsiella pneumoniae (1) | Neutropenia (2) | Hemizygous CD40L defect/XLR (2) |
| IVa. Primary HLH | 6 (5.3) | 28 (1-84) | Recurrent otitis media and Impetigo (2)<br>Prolonged fever (5) | E coli (1) | Pallor with hepatosplenomeg-aly (4)<br>Partial Albinism (1)<br>Hodgkin lymphoma (1)<br>HLH (6) | Homozygous UNC13D mutation /AR (1),<br>Homozygous PRF1 mutation/AR (2) |
| IVa. Chediak Higashi Syndrome | 2 (1.7) | 38 (6-70) | Prolonged fever (2) | | Partial Albinism (1)<br>Hepatospleno-megaly (2)<br>HLH (1) | |
| IVa. Griscelli syndrome | 3 (2.7) | 79 (3-143) | Respiratory infections (2) | | Hepatospleno-megaly (3)<br>Hypopigmented skin (3)<br>Silvery hair (4))<br>HLH (2) | Homozygous RAB27A mutation /AR (1),<br>Homozygous RAB27A mutation/AR and hemi-zygous XIAP/XLR (1) |
| IVb. ALPS | 1 (0.8) | 54 | Prolonged fever (1) | | Autoimmune hemolytic anemia (1)<br>Bilateral axillary lymphadenopathy (1) | |
| IVb.XLP | 1 (0.8) | 10 | Prolonged fever (1) | EBV (1) | Hepatospleno-megaly (1)<br>HLH (1) | SH2DIA mutation/XLR (1) |
| IVb.LRBA defect | 3 (2.6) | 9 (3-14) | Recurrent Diarrhoea (2)<br>Recurrent Pneumonia (2)<br>Spondylodiscitis (1) | Cryptosporidi-um (1) | Refractory autoimmune hemolytic anemia and thrombocytopenia (1)<br>Interstitial Lung disease (1) | Homozygous LRBA gene defect/AR (3) |
| IVb ILR2A defect | 1 (0.8) | 1 | Recurrent respiratory tract infection (1)<br>Recurrent otitis media (1)<br>Febrile neutropenia (1) | | Insulin dependent diabetes Mellitus (1)<br>Neutropenia (1)<br>Hepatospleno-megaly (1)<br>Autoimmune hemolytic anemia (1) | Homozygous LRBA mutation/AR (1) |
| Va. Congenital neutropenia | 5 (4.4) | 5 (0-12) | Recurrent pneumonia (2)<br>Omphalitis (1) | | Hepatospleno-megaly (1) | Homozygous JAGI mutation/AR (1) |

| | | | | | | |
|---|---|---|---|---|---|---|
| | | | Deep seated abscesss (1) | | | |
| Vb.LAD | 5 (4.4) | 1.3 (0.5-3) | Omphalitis (2)<br>Meningitis (2)<br>Sepsis (2) | Pseudomonas aeruginosa (1)<br>Eschericia. coli (1)<br>Klebsiella pneumonia (1) | Delayed umbilical cord fall (3)<br>Non healing skin ulcers (3) | |
| Vb.CGD | 26 (23.2) | 16.8 (3-60) | Recurrent/persistent pneumonia (6)<br>Pyoderma (3)<br>Recurrent cervical adenitis (6)<br>Sepsis (4)<br>Prolonged fever (8) | Burkholderia cepacia (3)<br>Salmonella typhi (1)<br>Pulmonary aspergillosis (2)<br>Pneumocystis carinii (1) | Hepatospleno-megaly (6) | Hemizygous CYBB/XLR (2),<br>Homozygous CYBA/AR (3)<br>Homozygous NCF2/AR (1) |
| VIa. CMC | 1 (0.8) | 8 | Recurrent skin infections (1)<br>Recurrent pneumonia (1) | | Icthyosis (1) | Heterozygous STAT1/AD (1) |
| V1b. MSMD-Severe Phenotype | 4 (3.5) | 3.2 (2-5) | Disseminated BCGiosis (3)<br>BCG adenitis (3)<br>Multifocal osteomyelitis (2)<br>Submandibular abscess (1) | Mycobacterium Tuberculosis (3)<br>Mycobacterium Avium Intra cellulare (1)<br>Ecoli (1) | Lymphadenopa-thy (3)<br>Hepatospleno-megaly (3) | Homozygous IFN gamma receptor defect/AR (3) |
| V1b. MSMD Moderate phenotype | 1 (0.8) | 13 | Recurrent pneumonia (1) | Adenoviral pneumonia (1) | Seizure (1) | Homozygous TYK2 defect/AR (1) |
| VIIb. CRMO | 2 (1.7) | 59 (3-115) | Recurrent arthritis | | Recurrent multifocal culture negative osteomyeelitis (1) | Homozygous LPIN2 defect/AR (1) |
| VIII. MASP2 deficiency | 1 (0.8) | 5 | Recurrent pneumonia (1) | | Encephalopathy (1), CNS lymphoma (1) | Homozygous MASP2 defect/AR (1) |

*Severe Combined Immunodeficiency (SCID), Wiskott-Aldrich Syndrome (WAS),Ataxia Telangiectasia (AT), X-Linked Agammaglobulinemia (XLA),Common Variable Immune Deficiency (CVID),Transient Hypogammaglobulinemia of Infancy (THI), Hemophagocytic Lymphohistiocytosis (HLH),Auto immune lymphoproliferative syndrome (ALPS), Lipopolysaccharide (LPS)-responsive and beige-like anchor protein (LRBA), Leucocyte Adhesion Defect (LAD),Chronic Granulomatous Disease (CGD), Mendelian Susceptibility to Mycobacterial Disease (MSMD), Chronic Recurrent Multifocal Osteomyelitis (CRMO),Methicillin sensitive Staphylococcus aureus (MSSA),Methicillin resistant Staphylococcus aureus.*

*Autosomal Recessive (AR), Autosomal Dominant (AD), X-Linked Recessive (XLR),* Adenosine Deaminase (ADA), *Janus Kinase 3 (JAK3), Regulatory Factor X Associated Ankyrin Containing Protein (RFXANK), Dedicator Of Cytokinesis 8 (DOCK 8),* Wiskott-Aldrich syndrome *(WAS), Ataxia Telangiectasia Mutated (ATM), Signal Transducer and Activator of Transcription 3 (STAT3), Bruton's Tyrosine Kinase (BTK), Nuclear Factor Kappa B Subunit 1 (NFKB1), CD40 ligand (CD40L), Protein unc-13 Homolog D (UNC13D), Perforin 1 (PRF), Ras-related protein Rab-27A (RAB27A), X-linked Inhibitor of Apoptosis Protein (XIAP), SH2 Domain Containing 1A (SH2D1A), Lipopolysaccharide (LPS)-Responsive and Beige-Like Anchor Protein (LRBA), Interleukin 2 Receptor Subunit Alpha (IL2RA), Jagged1 (JAG1),Cytochrome b-245 Beta Chain (CYBB), Cytochrome B-245 Alpha Chain (CYBA), Neutrophil Cytosolic Factor 2 (NCF2), Signal Transducer and Activator of Transcription 1 (STAT1), Interferon gamma (IFNγ), Non-receptor Tyrosine-Protein Kinase (TYK2), Lpin2 protein coded (LPIN2), Mannan Binding Lectin Serine Peptidase 2 (MASP2)*

RESEARCH PAPER

# Measles Specific Immunoglobulin G Response in Children Aged 4-12 Year Who Received Two Doses of Measles Containing Vaccine in Infancy

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**Objectives:** To study the vaccine-induced measles specific immunoglobulin G (IgG) response in children aged 4-12 years immunized with two doses of measles containing vaccine in infancy. **Methods:** This cross-sectional study was done in a tertiary care centre for a period of 18 months from January, 2017. Measles specific IgG levels were assessed using ELISA technique in 80 children of age 4-12 years, [mean (SD) age, 7.5 (2065)] who had received two doses of measles vaccine in infancy. Measles-specific IgG levels >11 NTU were considered protective. **Results:** Protective levels of measles specific IgG were found in 86.4%. Significant association was found between negative immune response to measles vaccine and low socioeconomic status (*P*=0.03), under-nutrition (*P*=0.001), anemia (*P*=0.03), lack of exclusive breast feeding till 5 months of age (*P*=0.007) and higher parity (*P*=0.002). **Conclusion:** Less than one-seventh of children immunized with two doses of measles vaccine in infancy had measles-specific IgG below protective levels at the average age of 7.5 yr. Lack of exclusive breast feeding till 5 months of age, under-nutrition and anemia were the associated factors.

**Keywords**: *Booster dose, Efficacy, Immunization, MR vaccine.*

The global coverage with first dose of measles vaccine is 85% and that of second dose is 67%. [1]. Measles immunization has resulted in 73% worldwide reduction in measles death by year 2018 [2,3]. However, in 2010, India alone accounted for 47% of the global measles mortality [4,5].

Antibody response to measles immunization is affected by a large number of factors [6-10]. Over the last decade, there have been major advances in immunology, virology, molecular biology, bioinformatics, and related research fields and that have enhanced the under-standing of measles vaccine-induced immunity [10,11]. It is important to understand the level and determinants of measles vaccine immunogenicity to guide public health measures for measles control/eradication. Thus, we carried out this study to determine the vaccine-induced measles-specific immunoglobulin G (IgG) in children.

## METHODS

This cross-sectional study was done in the pediatrics department of a tertiary care public hospital in Kerala, India to find out the proportion of sero-protective measles specific antibody response and the factors associated in children of age 4-12 years, who had received two doses of measles containing vaccine at the ages of 9 month (measles vaccine) and 15-18 month (MMR vaccine). Children with prior natural measles infection, immune-compromised children, those who had received blood products or immunoglobulin within the past 3 months, and those on steroid therapy or cancer chemotherapy were excluded from the study. Study was initiated in January, 2017. All consecutive cases satisfying the inclusion criteria attending our center during the study period were recruited for study. Informed consent was taken for each participant from parents or primary care taker. Institutional ethics committee clearance was obtained before starting the study.

Relevant history was taken from the mother or primary care taker of the child. Semi structured proforma was used for recording the information. History of measles immunization, and dates of vaccination were confirmed from the original immunization card of individual child issued from the hospital or health care setting from where the vaccine was taken. Vital signs and anthropometric measurements were recorded. Signs of vitamin and micronutrient deficiencies were noted and systemic examination was done. Venous blood (2 mL) was taken from each study participant and their mothers (after informed consent) for measles specific IgG titres. IgG levels were assessed by ELISA technique (Novatec immunodiagnostica) at Rajiv Gandhi Institute of Biotechnology, Thiruvananthapuram, Kerala. IgG levels <9 NTU (Novatec Units) were labelled negative, 9-11 NTU equivocal, and >11 NTU as protective/positive level according to manufacturers validation criteria.

**WHAT THIS STUDY ADDS?**

- 13.6% of children aged 4-12 years immunized with 2 doses of measles containing vaccine in infancy had no protective measles specific antibody titres.

Baseline haematology investigations were done for each study participant and WHO cut-off for blood hemoglobin level was used for diagnosing anemia. Proportion of sero-protective measles specific IgG level and the associated factors were the outcome variables. The associated factors studied were age, gender, socioeconomic status, prematurity, birth weight, antibiotic use in the first month after birth, anemia, exclusive breast feeding till 5 months of age, introduction of animal milk before 1 year of age, complementary feeding practices, balanced diet, vitamin deficiencies, maternal factors like maternal age at conception, parity, weight, anemia, maternal measles-specific IgG titre, and measles infection in mother before conceiving the child.

Sample size was calculated taking the proportion of 80% sero-positivity in children who have received 2 doses of measles vaccine based on an Indian study [6]. The calculated sample size was 80.

*Statistical analyses*: This was done using the software SPSS version 24. Chi square test/ Fishers exact test was used to study the association between categorical variables. Odds ratio (95% CI) were calculated for all variables. Significance level (P value) was set as <5 %.

## RESULTS

Of the 81 children [54.3% females; mean (SD) age, 7.5 (2.65) y] recruited for the study, 38.3% were in the age group 4-6 years and 23.5% in 8-10 years. Of these, 75.3% were residing in rural areas, 61.% were from lower socioeconomic class, 25.9% were undernourished, and 51.9% were anemic. Previous history of small for gestational age (17.3%) preterm birth (4.9%), antibiotic use in the first month after birth (32.1%) were noted. Exclusive breast feeding till 5 months of age was done in 81.5% children; 59.3% had animal milk in diet before 1 year of age, and 49.4% had appropriate complementary feeding. Regarding the maternal factors, 7.4% were <20 years, 28.4% were <50 kg, 53.1% were anemic, 24.7% had prior measles infection, and 91.4% had sero-protective measles-specific IgG titre.

The proportion of sero-protective measles specific immunoglobulin G titre in children immunized with 2 doses of measles vaccine was 86.4%. after mean (SD) 6.06 (2.63) of the second dose of the vaccine [median (IQR) duration, 6.2 (3.7, 7.75) year].

On univariate analysis, significant association was found between absence of seroprotective IgG levels and lack of exclusive breast feeding till 5 months of age [OR (95% CI), 7.17 (1.45,35.71); *P*=0.007], anemia [OR (95% CI) 5.04 (95% CI), 1.86, 25.05); *P*=0.03], under-nutrition [OR (95% CI) 11.69 (2.72, 50.12)]; *P*=0.0001], low socioeconomic status [OR 7.50 (95% CI 1.42, 61.83) *P*=0.03] and higher parity [OR 9.30 (95% CI 2.30, 37.59) *P*=0.002]. No significant association was found with age group of child, gender, place of residence, prematurity, birthweight, antibiotic use in the first month after birth, intake of animal milk before 1 year of age, complementary feeding practices and maternal factors like maternal age at conception, weight, anemia, measles specific immuno-globulin G titres of mother and prior natural measles infection of mother **(Table I).**

## DISCUSSION

We found that the among children of age 4-12 years immunized with two doses of measles containing vaccine at 9 months and 15-18 months, 86.4% had protective level of measles-specific immunoglobulin G at around 7-8 year of age. Significant association was found between negative immune response to measles vaccine and under nutrition, anemia, low socioeconomic status, higher parity and lack of exclusive breast feeding till 5 months of age, on univariate analysis.

A previous study from India [5] had reported seropositivity of 21.4% after a single dose of measles

**Table I Participants Characteristics and Measles Specific Immunoglobulin G Response (*N*=81)**

| *Variable* | *Seroprotective titers*[a] | *P value* |
|---|---|---|
| Exclusive breast feeding (5 mo), *n*=45 | 43 | 0.007 |
| Anemia, *n*=42 | 33 | 0.032 |
| Undernutrition, *n*=2 | 13 | 0.001 |
| Upper socioeconomic status, *n*=31 | 30 | 0.032 |
| Primipara, *n*=35 | 33 | 0.002 |
| Protective maternal titer,[b] *n*=74 | 64 | 0.95 |
| Antibiotic use in first mo, *n*=26 | 22 | 0.74 |
| Preterm, *n*=5 | 4 | 0.46 |
| Maternal measles, *n*=20 | 17 | 0.24 |

[a]*NTU (Novatec unit);* [b]*Measles IgG.*

vaccine at 9 months. In another Indian study [6], it was noted that sero-protection rate after two doses of measles containing vaccine was 80% in children of age 4-6 years and 83.3% in children aged 9-12 years. In another study from Kenya [8], 83% had protective antibody titres. In the study by Kizito, et al. [9] regarding risk factors of negative immune response after measles vaccination, significant association was found with malnutrition and maternal retroviral infections. Genetic factors have also been reported to have significant impact on the immune response after measles vaccine, with 2-10% of individuals immunized with two doses of measles, mumps rubella (MMR) vaccine not having protective titers due to genetic polymorphism associated with response [10]. There is a growing interest in applying novel vaccinomics approaches to understand and predict vaccine-induced immune responses [11]. Limitation of this study was a single-center setting, and lack of multivariate analysis.

The number of susceptible subjects among population should be kept below 5% for control of measles (WHO). Since sub-optimal level of seropositivity in mid-childhood was seen in this study, the need for an additional dose of measles containing vaccine for Indian children may be explored in further studies. Under- nutrition, anemia, and lack of exclusive breast feeding are modifiable risk factors for poor immune response, and may be targeted through appropriate interventions.

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*Contributors*: LK: planned the study, literature search, involved in data collection and analysis, prepared the manuscript. AM: done the data collection, literature search and involved in data analysis.

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SYSTEMATIC REVIEW

# Fortification of Human Milk With Infant Formula for Very Low Birth Weight Preterm Infants: *A Systematic Review*

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**Background:** Off-label fortification of expressed human milk (HM) with infant milk formula (IMF) is common in developing countries, though its benefits and safety are unclear.

**Objective**: To study the effects of IMF fortification of HM on growth of very low birth weight (VLBW) preterm infants.

**Design**: Systematic review and meta-analysis of randomized and quasi-randomized controlled trials (RCTs).

**Data sources and selection criteria:** MEDLINE, EMBASE, CINAHL, CENTRAL and other databases were searched for articles published in English language from inception to December 2019, evaluating the effects of HM fortified with IMF as intervention, compared to unfortified HM or HM fortified with human milk fortifier (HMF).

**Participants:** Five RCTs including 423 VLBW preterm infants.

**Intervention:** Feeding with HM fortified with IMF compared to unfortified or HMF-fortified HM.

**Outcome measures:** Primary outcome measure was assessment of growth as weight, length and head circumference (HC) gain velocity. Secondary outcome measures were incidences of feed intolerance (FI), necrotizing enterocolitis (NEC), time to reach full feeds, concentration of nutritional biomarkers, duration of hospital-stay and cost of intervention.

**Results:** Of the five studies included in the review**,** pooled effects regarding weight gain velocity (SMD 0.27 g/day; 95% CI 0.08 to 0.62), length gain (MD 0.07cm/week; 95% CI 0.02 to 0.16) and HC gain (MD 0.05 cm/wk; 95% CI 0.01 to 0.11), were not statistically significant. Sensitivity analysis by pooling studies using unfortified milk as comparator yielded a statistically significant result for all growth parameters. Risk of FI or NEC was comparable. Length of hospitalstay was reduced in th intervention group.

**Conclusions:** A very-low quality evidence suggested that IMF fortification of HM is superior to unfortified milk and may be a safe alternative for HMF for short term growth of VLBW preterm infants.

**Keywords**: *Human milk, Human milk fortification, Preterm, Very low birth weight infant*

**PROSPERO Registration Number:** CRD42019138122 **Early online:** January 02, 2021**; PII:** S097475591600277

Every year, approximately14.9 million neonates, representing a birth rate of 11.1%, are born preterm, globally [1]. Though substantial advancement in medical care has led to an improved survival of preterm infants [2], significant morbidity during the hospital stay and adverse long-term neurological consequences remain major areas of concern.

Deprived of the third trimester accretion of macro and micronutrients, along with the inability to meet the increased postnatal demand due to prematurity-related illnesses and poor nutritional intake, more than half of these infants have extra-uterine growth restriction, which in turn has long-term adverse cardiovascular and metabolic consequences [3,4]. Nutritional optimization is considered vital for survival, growth, and improved neurodevelopmental outcome [5-8].

Though breastmilk is the nutrition of choice for very low birth weight (VLBW) preterm neonates [9], exclusive human milk (HM) feeding, does not meet their nutritional targets [10,11]. Moreover, after two weeks, the protein content of milk of mothers delivering preterm decreases further [12]. Multi-nutrient fortification of HM results in increased rate of gain in weight, length and head circumference of VLBW preterm infants [13-16].

Unfortunately, in low- and middle-income countries (LMICs), the concept of individualized and targeted fortification is far from implementation. Commercially available human milk fortifiers (HMF) are low in protein content (<1g/100 mL) and expensive, prohibiting routine supplementation [17]. An alternative and more economical strategy, commonly employed off-label in various neonatal units, is to enrich EBM by adding infant milk formula (IMF) to achieve the required level of protein for improved growth outcomes [18-22]. However, IMF fortification may result in increased osmolarity, non-uniform protein content and risk of contamination leading

to feeding intolerance (FI), sepsis and necrotizing enterocolitis (NEC). In addition, the quantity needed for optimum fortification and measuring technique is not validated.

This systematic review intended to evaluate the role of fortification of HM with IMF for growth in VLBW preterm infants.

## METHODS

This systematic review and meta-analysis was conducted in accordance to PRISMA guidelines [23].

*Search strategy and search criteria:* All authors independently searched the databases including PubMed, Embase, Cochrane Central Register of Controlled Trials, other clinical trial registries, Google Scholar, Scopus, Web of Science and hand searching of conference proceedings from inception to December 2019 for peer-reviewed publications in English language. The electronic search strategy included a combination of keywords along with their representative medical subjects headings (MeSH) terms. Details of search strategy are provided in **Web Appendix 1**. Reference list of all articles whose full texts were screened, was also checked to find additional articles.

We included randomized or quasi-randomized controlled trials (RCT) evaluating the effects of HM fortified with IMF as intervention, compared to unforti-fied or HMF-fortified HM on growth rate, duration of hospital-stay and other clinically relevant outcomes in VLBW preterm infants. Non-English publications were excluded.

The primary outcome was assessment of velocity of gain in weight, length, and head circumference (HC). Secondary outcomes were duration of hospital stay, incidences of FI and NEC, time to reach full feeds, concen-tration of nutritional biomarkers (calcium, phosphorous, blood urea nitrogen, prealbumin, albumin, alkaline phos-phatase) and cost of intervention.

*Data extraction and quality assessment:* Two authors independently extracted data using a pre-designed pro-forma. Disagreement, if any, was resolved by discussion with third author. Study details including location and year of study, number of infants and their characteristics, details of feeding including fortification and outcomes relevant to the study were noted. Quality of studies were assessed independently by all authors, for each study, using the risk of bias (ROB) criteria outlined in the Cochrane Handbook for Systematic Reviews of Interventions [24] in the domains of random sequence generation, allocation concealment, blinding of partici-pants and personnel, blinding of outcome assessment, incomplete outcome data, selective outcome reporting, and other bias.

*Statistical analysis*: Statistical analysis was performed using Review Manager version 5.4 (The Cochrane Collaboration, 2020). Out-come variables were calculated as risk ratio (RR) with 95% confidence interval (CI) for dichotomous data and mean differences (MD) with 95% CI for continuous data. Standardized mean differences (SMDs) were calculated where outcomes had different measurement instruments. Studies reporting dispersion of outcomes in range was converted to standard deviation using established mathematical models [25]. Results were pooled using either fixed or random effects model based on hetero-geneity which was assessed using the $I^2$ statistic. Grading of recommendations assessment, development and evaluation (GRADE) approach [26] was applied to assess the quality of evidence for predefined outcomes.

## RESULTS

Screening and inclusion of studies are summarized in **Fig.1**. Four full-text articles [18-21] and one abstract [22] were selected for this systematic review including a total of 423 VLBW preterm infants.

The characteristics of included studies are summarized in **Table 1**. The birth weight of the preterm VLBW infants included in the studies, ranged from 500g to 1499g. Fortification of HM with IMF was the intervention in all five trials. The time to start fortification varied from 100 mL/kg/d [18,19,22] to150 mL/kg/d of enteral feed [21]. Willeitner, et al. [20] introduced fortification as early as at 60 mL/kg/d, at the discretion of the treating team. In three studies [18,20,22] the comparator was HMF, while other two studies [19,21] used unfortified HM. **Web Fig. 1** depicts ROB graph summarizing each ROB item as percentage across all studies while **Web Fig. 2** summarizes ROB for each included study.

All included studies evaluated weight gain velocity as primary outcome. Four studies [19,20-22], described weight gain velocity in terms of g/kg/day, while Khorana, et al. [18] reported weight gain as g/day. Overall, pooled effects of all five studies on weight gain velocity was statistically not significant (SMD 0.27 g/kg/day; 95% CI: -0.08 to 0.62) (**Fig. 2a**). Sensitivity analysis was done due to difference in comparators. IMF fortification was found to cause a statistically significant increase in the rate of weight gain (MD 02.03 g/day; 95% CI: 1.15 to 2.92) compared to unfortified HM. Using HMF as comparator, SMD of weight gain velocity was similar (SMD -0.01 g/day; 95% CI: -0.27 to 0.25).

Four studies [18,19,21,22] with 353 participants reported data regarding rate of increase in length and HC. The pooled effect with respect to velocity of gain in length was not statistically significant (MD 0.07 cm/week; 95%

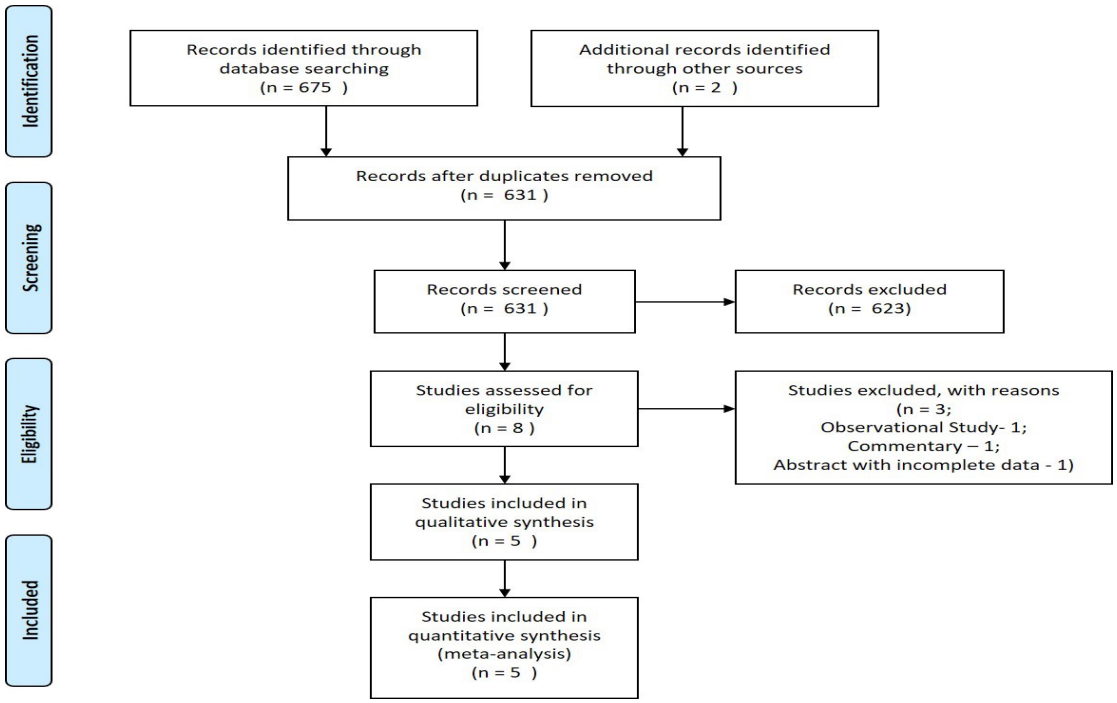



**Fig. 1** PRISMA flow diagram.

CI: -0.02 to 0.16) (**Fig. 2b**). On sensitivity analysis, when compared to unfortified HM, IMF fortification resulted in significantly higher rate of gain (MD 0.12 cm/week; 95% CI: 0.02 to 0.22), but failed to show difference when compared with HMF (MD -0.03 cm/week; 95% CI: -0.15 to 0.08). Similarly, the pooled effect with respect to velocity of gain in HC was not statistically significant (MD 0.05 cm/week; 95% CI: -0.01 to 0.11) (**Fig. 2c**). On sensitivity analysis, when compared to unfortified HM, IMF fortification resulted in significantly higher rate of gain (MD 0.08 cm/week; 95% CI: 0.03 to 0.13), but failed to show difference when compared with HMF (MD -0.04 cm/week; 95% CI: -0.14 to 0.06).

FI, reported in two studies [19,21] (*n*=208), showed no difference in risk between IMF and HMF fortification versus no fortification of HM (RR 2.29; 95% CI: 0.61to 8.59) (**Web Fig. 3a**). Though HMF fortification showed apparently higher rates of NEC [18,20], the RR was not statistically significant for either suspected NEC (RR 0.37; 95% CI: 0.07 to 1.95) (**Web Fig. 3b**) or confirmed NEC (RR 0.25; 95% CI: 0.04 to 1.39) (**Web Fig. 3c**).

Three studies [18,19,21] including 231 participants, showed that the length of hospital stay of neonates with IMF was significantly reduced (MD -4.38 days; 95% CI: -7.39 to -1.37) (**Web Fig. 3d**). Two studies [18,19] (*n*=83) found no significant difference with respect to time to achieve full enteral feeding, between those receiving formula fortified HM and those on either HMF fortified or unfortified HM. (**Web Fig. 3e**). Effect of fortification on nutritional biomarkers were reported by two studies [18,21]. No significant effect on BUN nor albumin levels was observed (**Web Fig.3f, 3g**).

Though four of the studies favored IMF intervention in terms of cost, this economical aspect was not studied as an outcome in any of them. The data presentation was not uniform and therefore, could not be pooled.

The quality of evidence pooled from included studies was assessed using GRADE approach and summary of findings table was generated on GRADE pro GDT software (Evidence Prime Inc.) (**Web Appendix 2**).

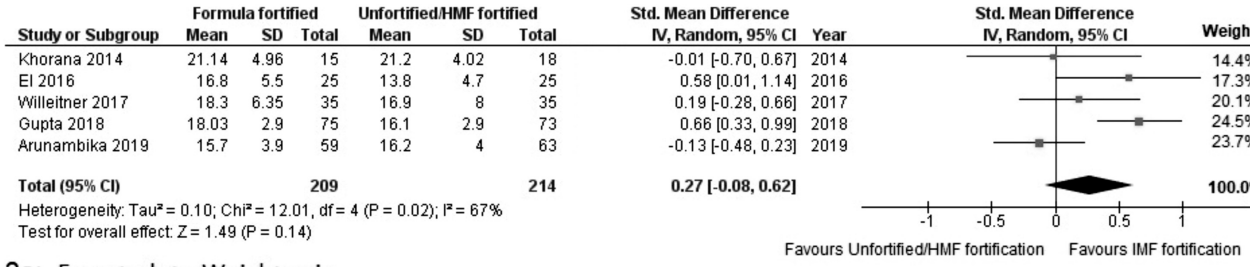
## DISCUSSION

This systematic review and meta-analysis of five RCTs, including a total of 423 VLBW preterm infants, did not show any significant benefit of IMF fortification of HM over combined HMF fortification/no fortification, on growth velocity, with respect to weight, length and HC. On sensitivity analysis for the same parameters, IMF and HMF fortifications were comparable, whereas IMF fortification was significantly better than unfortified HM, quality of evidence (QOE) being very low. No significant difference was noted in the incidences of FI/NEC and

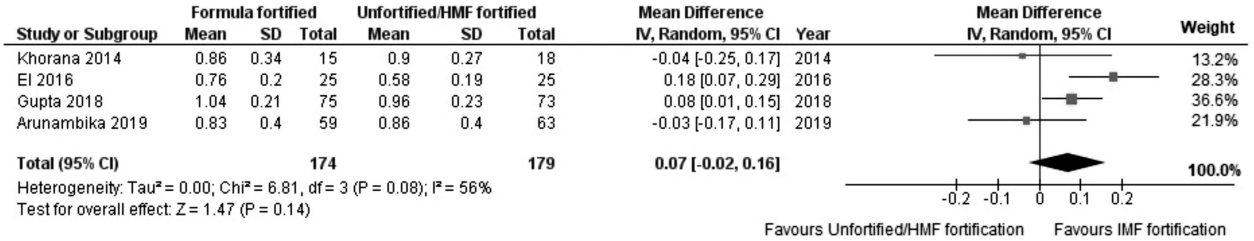
**Table I Characteristic of Studies Included**

| *Included studies/ Year/Place* | *Fortification started at enteral feeding volume* | *Outcomes assessed* | *Supplements* | *Recruitment (n)* | *Nature of fortification* | *GA (wks) Mean (SD)* | *BW (g), Mean ()* | *Cost* |
|---|---|---|---|---|---|---|---|---|
| Khorana, et al. 2014 [18], Thailand | 100 mL/kg/day | Weight gain, length gain, HC gain, BUN, creatinine, albumin, alkaline phosphatase, total calcium, phosphate, urine Ca/Cr, urine PO4/Cr, NEC, rectal bleeding, BPD, IVH, ROP, osteopenia, sepsis, length of hospital stay, cost reduction | If inadequate weight gain, MCT oil was used as additive in both arms to achieve concentration of 30 cal/oz, Iron supplementation started at 2 wk and vitamin D was started on full feeds. | Intervention (*n*=15) | Post discharge formula (Similac NeoSure Advance Powder, | 30.67 (2.32) | 1206.67 (224.99) | 605 baht per infant |
| | | | | Control | HMF fortified (*n*=18) EBM (Enfamil | 30.0 (1.88) | 1158.61 (232.94) | 11,655 baht per infant |
| | | | | Human Milk | Fortifier | | | |
| El Sakka, et al. 2016 [19], Egypt | 100 mL/kg/day | Weight gain, length gain, HC gain, Hb, hematocrit, albumin, BUN, Na, K, Ca, PO4, length of hospital stay | Vitamin D supplemented at start of enteral feeding; and iron prescribed when enteral feeding reached 150 mL/kg/day | Intervention (*n*=25) | Infant formula (Babelac premature formula) | 32.08 (2.53) | 1291.8 (105.3) | Not mentioned |
| | | | | Control (*n*=25) | Unfortified EBM | 31.37 (2.62) | 1290.3 (177.4) | Not mentioned |
| Willietner, et al. 2017 [20], USA | Started at the discretion of attending physician (not before a minimum of 60 mL/kg/day) | Weight gain, residual gastric aspirate, erythromycin treatment, NEC, blood culture, death | Not mentioned | Intervention (*n*=35) | CPF30 (Similac Special Care 30 With Iron) | 29 (24-32) | Median (Range) 1099 (530-1470) | 7 cents less than in HMF fortified group |
| | | | | Control (*n*= 35) | PHMF fortified breastmilk(Similac HMF Park, | Median (range) 29 (24-34) | Median (range) 1100 (570-1490) | |
| Gupta, et al. 2018 [21], India | 150 mL/kg/day | Weight gain, linear growth, HC, sepsis, FI, hospital stay, time in trial, apneic spells, BPD, ROP, IVH, PVL, anemia, biochemical parameters | All newborn in both groups were supplemented with extra calcium, phosphate, iron and multivitamin drops | Intervention (*n*=75) | Infant milk formula (Simyl LBW | 31.2 (1.5) | 1242.3 (170.9) | Rs.190/ infant |
| | | | | Control (*n*=73) | Unfortified EBM | 31.2 (1.6) | 1234.8 (190.8) | Rs. 2000/ infant |
| Arunambika, et al. 2019 [22], India | 100 mL/kg/day | Weight gain, length gain, HC gain, FI, NEC, sepsis, anemia, MBD, late metabolic acidosis | Not mentioned | Intervention (*n*=59) | Preterm formula made in hospital pharmacy | 30.5 (2.2) | 1161 (251) | Rs 300/ infant |
| | | | | Control (*n*=63) | PreNAN | 29.9 (2.2) | 1119 (265) | Rs 10,800/ infant |

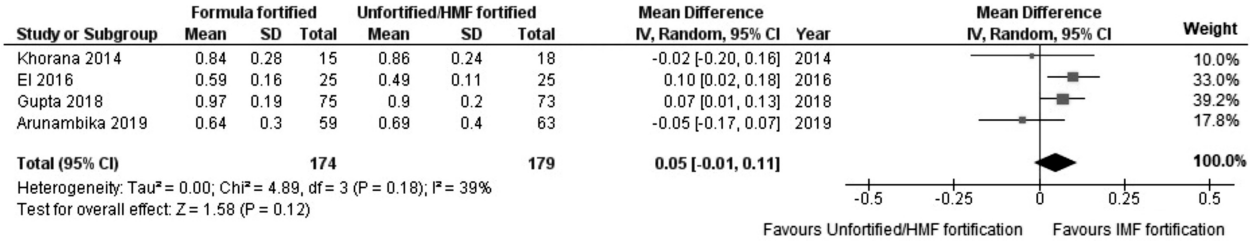
*BUN: Blood urea nitrogen; BW: Birth weight; BPD: Bronchopulmonary dysplasia; Ca: Calcium; CPF30: Concentrated preterm formula 20 Kcal/oz; Cr: Creatinine; EBM: Expressed breast milk; FI: Feeding intolerance; GA: Gestational age; Hb: Hemoglobin; HC: Head circumference; HMF: Human milk fortifier; IVH: Intraventricular hemorrhage; K: Potassium; Na: Sodium; MBD: Metabolic bone disease; NEC: Necrotizing enterocolitis; PHMF: Powdered human milk fortifier; PVL: Periventricular leukomalacia; PO4: Phosphate; RCT: Randomized controlled trial; ROP: Retinopathy of prematurity.*




2a: Forest plot - Weight gain




2b: Forest plot - Length gain




2c: Forest Plot - HC gain

**Fig. 2** Forest plot showing meta-analysis of the effect of infant milk fortification on the velocity of weight gain (2a), length gain (2b) and head circumference (HC) gain (2c).

levels of nutritional biomarkers like BUN and albumin (QOE: very low). Pooled data from three trials, showed a significant reduction in duration of hospital stay favoring IMF fortification (QOE, very low). This reduction was probably because the comparator in two of these studies was unfortified HM.

There are several limitations in the included trials. The study by El Sakka, et al. [19] was quasi-randomized with an unclear methodology. Still this study was included as its outcome measures met our inclusion criteria. The gestational age varied among the studies, with one trial [21] excluding late preterm infants. No data were available regarding long term growth and developmental outcome. Formulas and HMFs preparations used were from different manufacturers, though the protein and energy content were similar. Another area of discrepancy was non-uniform timing of initiation of fortification in included trials, which might have affected growth. The most important concern for implementation of IMF fortification in routine practice is increase in osmolarity with risk of FI and NEC. Only one trial [21] measured osmolarity of HM after IMF fortification and found it below 400 mOsm/L, the recommended upper safety limit of American Academy of Pediatrics [27]. Though no difference in the incidences of FI and NEC was noted, none of the studies was adequately powered to detect the difference. None of the trials had individualized the fortification by analysis of HM macronutrients. IMF measurement technique for fortification was described by only one study [22].

A relatively limited number of studies, with high ROB and statistical heterogeneity in this systematic review limit the generalizability of this meta-analysis. Variability in the time of initiation of feed, the maximum feeding volume and continuation of IMF as 'bridge feeding' when EBM was unavailable [20] probably limited the impact of the intervention on growth outcomes. Further, subgroup analysis based on gestation or birth weight could not be done because of unavailability of raw data. Not all biomarkers of nutrition could be evaluated due to lack of measured values. Data regarding cost could not be pooled as there was no uniformity in presentation.

To summarize, a very-low quality evidence suggests that IMF fortification of HM is superior to unfortified HM and may be a safe alternative for bovine HMFs for short term growth of VLBW preterm infants, especially in resource-limited settings. Larger well-designed studies with strict monitoring of complications including NEC with a focus on long-term outcomes are needed.

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*Contributors*: MK: conceptualized the review, literature search, data analysis and manuscript writing; JU: literature search, data analysis and manuscript writing; SB: conceptualized the review, literature search, data analysis and manuscript writing.
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**Web Appendix 1** Electronic Search Strategy

| *Database* | *Date* | *Search Strategy* | *Number of references* |
|---|---|---|---|
| PubMed | 28-11-19 | ("growth"[MeSH Terms] AND ((((("milk, human"[MeSH Terms] OR "human milk"[Title/Abstract]) OR "breast milk"[Title/Abstract]) OR "human milk fortifier"[Title/Abstract]) OR "fortification"[Title/Abstract])) AND ((("infant, very low birth weight"[MeSH Terms] OR "very low birth weight infant"[Title/Abstract]) OR "infant, premature"[MeSH Terms]) OR "preterm infant"[Title/Abstract]) | 494 |
| Embase | 28-11-19 | ('breast milk'/exp OR 'breast milk' OR 'human milk fortifier') AND ('very low birth weight' OR 'prematurity') AND 'growth rate' | 160 |
| CENTRAL | 28-11-19 | "infant, premature" in Title Abstract Keyword AND "human milk fortifier" in Title Abstract Keyword AND "growth" in Title Abstract Keyword | 21 |

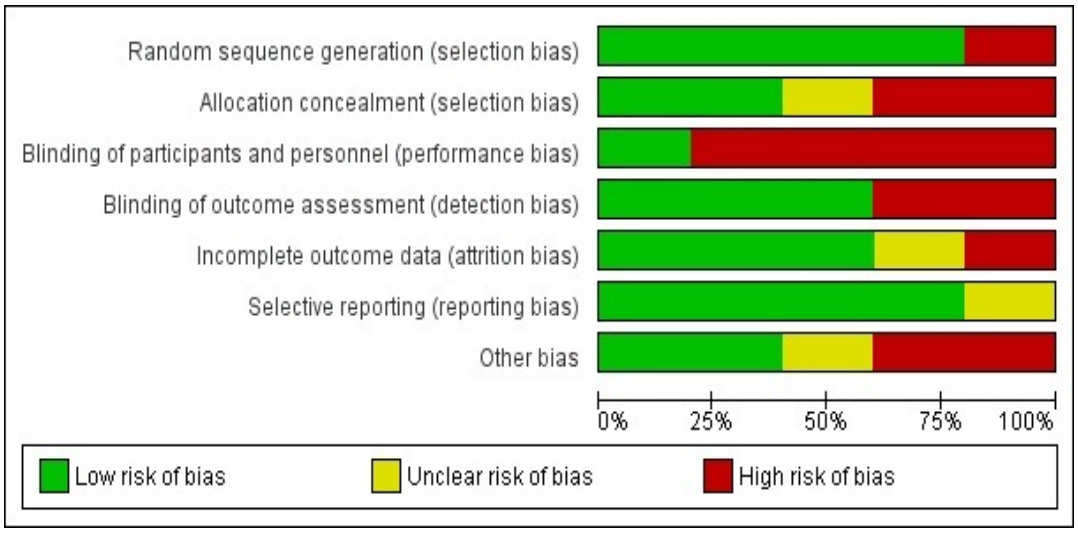
**Web Appendix 2** Grade Profile

| Certainty assessment | | | | | | | Summary of findings | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| *Nº of participants (studies) Follow-up* | *Risk of bias* | *Inconsistency* | *Indirectness* | *Imprecision* | *Publication bias* | *Overall certainty of evidence* | *Study event rates (%)* | | *Relative effect (95% CI)* | *Anticipated absolute effects* | |
| | | | | | | | *With unfortified/HMF fortified* | *With Formula fortified* | | *Risk with unfortified/HMF fortified* | *Risk difference with Formula fortified* |
| *Growth velocity* | | | | | | | | | | | |
| 423 (5 RCTs) | very serious [a] | very serious [b] | not serious | very serious [c] | all plausible residual confounding would reduce the demonstrated effect | ⨁◯◯◯ VERY LOW | 214 | 209 | - | - | SMD 0.27 higher (0.08 lower to 0.62 higher) |
| *Length gain* | | | | | | | | | | | |
| 353 (4 RCTs) | very serious [a] | very serious [b] | not serious | very serious [c] | all plausible residual confounding would reduce the demonstrated effect | ⨁◯◯◯ VERY LOW | 179 | 174 | - | - | MD 0.07 higher (0.02 lower to 0.16 higher) |
| *OFC gain* | | | | | | | | | | | |
| 353 (4 RCTs) | very serious [a] | very serious [b] | not serious | very serious [c] | all plausible residual confounding would reduce the demonstrated effect | ⨁◯◯◯ VERY LOW | 179 | 174 | - | - | MD 0.05 higher (0.01 lower to 0.11 higher) |
| *Feed Intolerance* | | | | | | | | | | | |
| 198 (2 RCTs) | very serious [d] | not serious | very serious [e] | very serious [c] | all plausible residual confounding would reduce the demonstrated effect | ⨁◯◯◯ VERY LOW | 3/98 (3.1%) | 7/100 (7.0%) | **RR 2.29** (0.61 to 8.59) | 31 per 1,000 | **39 more per 1,000** (from 12 fewer to 232 more) |

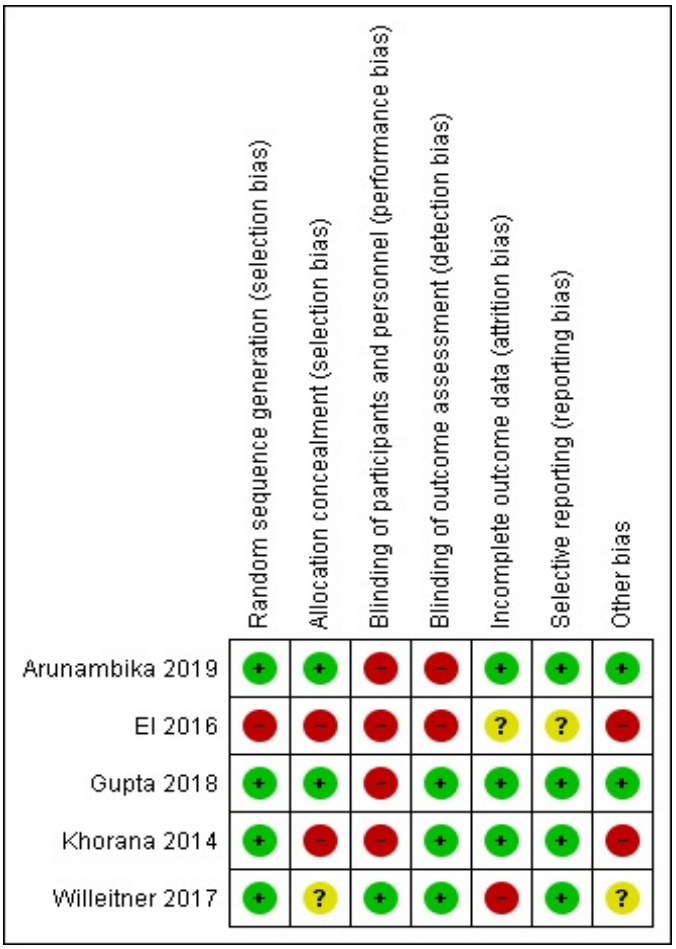
| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| *Definite NEC* | | | | | | | | | | | |
| 103 (2 RCTs) | very serious [f] | not serious | very serious [g] | very serious [c] | all plausible residual confounding would reduce the demonstrated effect | ⊕◯◯◯ VERY LOW | 6/53 (11.3%) | 1/50 (2.0%) | **RR 0.25** (0.04 to 1.39) | 113 per 1,000 | **85 fewer per 1,000** (from 109 fewer to 44 more) |
| *Duration of stay* | | | | | | | | | | | |
| 231 (3 RCTs) | very serious [h] | not serious | not serious | very serious [c] | all plausible residual confounding would reduce the demonstrated effect | ⊕◯◯◯ VERY LOW | 116 | 115 | - | - | MD 4.38 lower (7.39 lower to 1.37 lower) |
| *Time to full feeds* | | | | | | | | | | | |
| 83 (2 RCTs) | very serious [h] | not serious | not serious | very serious [c] | all plausible residual confounding would reduce the demonstrated effect | ⊕◯◯◯ VERY LOW | 43 | 40 | - | The mean time to full feeds was 0 | MD 1.29 lower (6.33 lower to 3.75 higher) |

***CI:*** *Confidence interval;* ***SMD:****Standardised mean difference;* ***MD:*** *Mean difference;* ***RR:*** *Risk ratio*

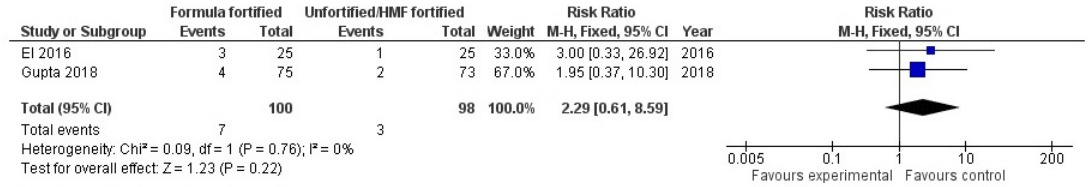
*Details: a. 4 studies (Arunambika 2019, El Sakka2016, Gupta 2018, Khorana 2014) were open labeled; Randomization and allocation concealment was improper in El Sakka 2016. b. Khorana 2014 and Arunambika 2020 favored control while other studies favored intervention. c. 95% confidence intervals are wide across studies. d. Included studies were open labeled. Randomization and allocation concealment was improper in El Sakka 2016. e. Definition of feed intolerance varied across studies. f. In Khorana 2014 allocation concealment and blinding was not clearly defined. In Willeitner 2017, allocation concealment was not explained properly and had incomplete outcome data bias. g. Definition of NEC varied across studies. h. Included studies were open labeled. Randomization and allocation concealment in not proper in El Sakka 2016.*



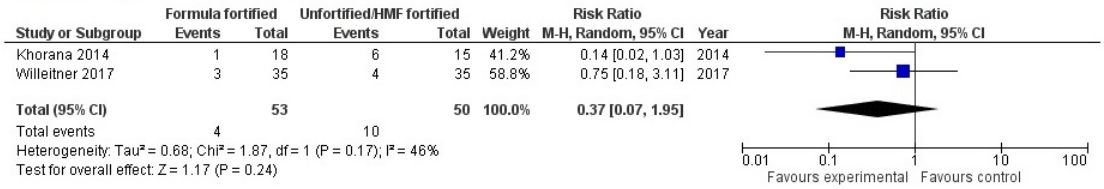

**Web Fig. 1** Risk of bias graph showing authors' judgement about each risk of bias item, presented as percentages across all included studies.



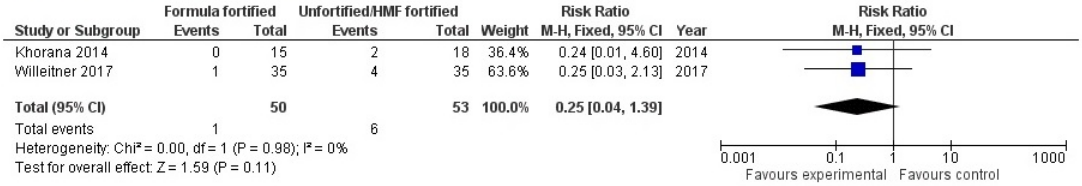

**Web Fig. 2**: Risk of bias summary for included studies, showing authors' judgements about each risk of bias item for each included study.



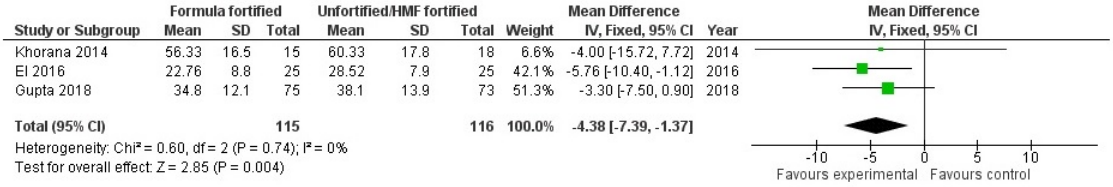

3a: Forest plot - Feed intolerance



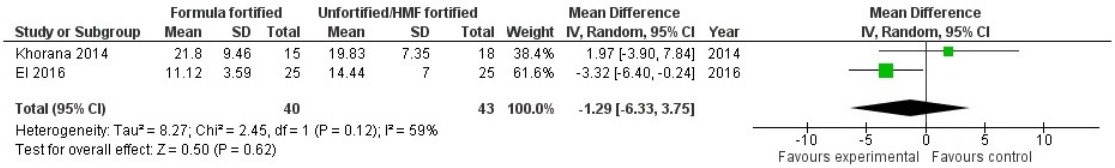

3b: Forest plot - Suspected NEC



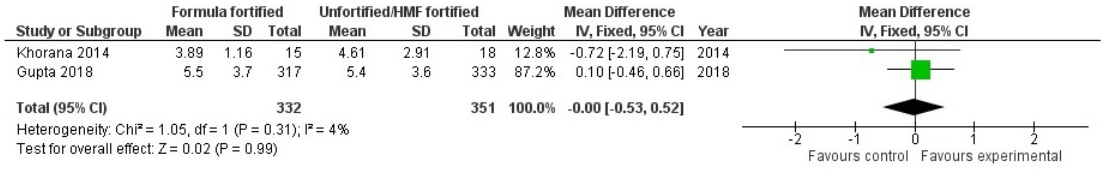

3c: Forest plot - Definite NEC



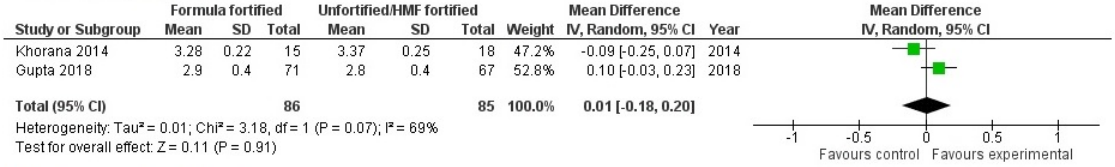

3d: Forest plot - Duration of stay




3e: Forest plot - Time to full feeds




3f: Forest plot - BUN




3g: Forest plot - Albumin

**Web Fig. 3** Forest Plot, Feed intolerance (3a), suspected necrotizing enterocolitis (NEC) (3b), definite NEC (3c), duration of stay (days) (3d), time to reach full feeds (days) (3e), blood urea nitrogen (BUN) (3f), albumin (3g)

REVIEW ARTICLE

# Psychogenic Nonepileptic Seizures in Children and Adolescents

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**Context:** Though psychogenic non-epileptic seizures (PNES) are seen commonly during evaluation of children and adolescents with epilepsy, the literature regarding developmental changes in PNES is limited. **Evidence Acquisition:** Literature search was conducted in PubMed. Key search terms included: Pseudoseizure* OR PNES OR [(non-epileptic or nonepileptic or psychogenic or non-epileptic attack disorder) AND (seizure*)], resulting in 3,236 articles. Filters included human, ages 1-18 years, English language and last 15 years (2004-2019), resulting in 533 articles. We reviewed 33 articles, which included 19 articles that involved children (1-18 years), with 10 or more children with PNES in their study group. 21 articles obtained in cross references that were outside the filter setting (including time frame and age range) were also reviewed, for a total of 54 articles. **Results**: Majority of the studies were retrospective. We detail clinical features, predisposing factors and appropriate workup for children and adolescents with possible PNES. There is no consensus regarding frequency of psychiatric comorbidities in children with PNES. No controlled trials of treatment of PNES in children are available, but cognitive behavioral therapy is the consensus for adult PNES. Outcome appears to be better in children with PNES. **Conclusions:** There is a need for be long-term prospective studies to document various clinical features and outcome of pediatric and adolescent PNES, and also the comorbid conditions.

**Keywords:** Psychogenic seizures, PNES.

Psychogenic nonepileptic seizures (PNES) are a common problem in children and adolescents. They pose difficulties to the pediatrician because of diagnostic uncertainties. PNES are frequently difficult to distinguish from epileptic seizures on clinical grounds. The consequences of misdiagnosing PNES as epileptic seizures are significant. Financial ramifications include the expense of inappropriate, unnecessary costly treatments and needless emergency room visits or hospitalizations. Risk for iatrogenic complications include the adverse effects of unnecessary medications and exposure to invasive procedures such as intubation for management of prolonged events (nonepileptic/pseudo-status epilepticus). Psychosocial sequelae include strain on interpersonal and family dynamics. Most importantly, misdiagnosis results in delay in initiating the much needed psychiatric treatment and may contribute to a poor outcome.

PNES has been described extensively in adults. There remains a relative paucity of literature in the pediatric age. There has been recent progress in understanding the etiology of PNES and in defining appropriate treatments. Unfortunately, most of these studies have been done in adults with PNES

Few studies have assessed the semiology of PNES exclusively in children and even fewer more recent studies have reported differences in the clinical features of PNES between younger children and adolescents, with respect to the semiology of the episodes and types of stressors [1,2]. Studies pertaining to PNES in children reported over the past 15 years have been listed in **Web Table I**.

In this review, we will discuss the clinical manifestations, predisposing factors, and appropriate workup for children and adolescents with possible PNES. We will review the recent literature on semiology, etiology, and treatment, particularly pertaining to children and adolescents.

## EPIDEMIOLOGY

### Definitions

Psychogenic nonepileptic seizures (PNES) are defined as paroxysmal events of altered movement or behavior that resemble epileptic seizures but are not due to cerebral neuronal dysfunction and are not associated with epileptiform abnormalities on the electroencephalogram (EEG) [3]. They are related to an underlying psychogenic process, thus differing from other paroxysmal nonepileptic events that are physiologic in origin.

A variety of terms have been used in the literature to describe PNES. Previously used terms such as

hysteroepilepsy, pseudoseizures and pseudoepileptic or nonphysiologic seizures are considered pejorative or inappropriate, and have been replaced by more contemporary terms such as nonepileptic attacks, nonepileptic attack disorder and psychogenic nonepileptic seizures (PNES).

In the DSM-5 [4], they are listed in the somatic symptoms disorder section as conversion disorder or functional neurological symptom disorder with attacks. The term dissociative convulsions is used in the ICD 10 [5]. The term PNES, is non-judgmental, and is recommended as the preferred term to be used [6].

### Demographics

PNES are common and seen in all age groups. The prevalence of PNES in the general population is estimated to be 2-33/100,000 [7]. They represent 5-20% of outpatient adult epilepsy clinics and 10%-20% of referrals to adult epilepsy centers [7]. However, reported estimates of the incidence or prevalence of PNES may represent an underestimation, as these reports include only cases for which the diagnosis was confirmed by video-electroencephalography (VEEG) [8].

There are no studies of prevalence or incidence of PNES in children. 3.5-20% of children undergoing VEEG monitoring [1,2,9] have PNES and it has been reported in 11-38% of children with all types of paroxysmal non-epileptic events [10,11].

PNES are seen in all age groups. The average age at diagnosis of PNES in children in India was 8 years [12] to 12 years [13], and the average delay in diagnosis was 5 months [14] to 3 years [13]. Overall, there is no significant difference in the demographics of age of onset and delay in diagnosis in the reports from India versus those from the Western world.

PNES are more common in adolescents than in children [15,16]. There is a female preponderance more apparent in adults and adolescents than in children [12,13,17]. Many of these children are given a diagnosis of epilepsy and are mistakenly placed on anti-seizure medications.

## CLINICAL DIAGNOSIS

A definitive diagnosis of PNES can be secured if the patient satisfies the 'rule of 2s' consisting of the following three criteria, which yields a positive predictive value of 85% [18,19]:

*i*) At least 2 PNES per week;

*ii*) Refractory to at least 2 antiepileptic medications; and

*iii*) At least 2 EEGs without epileptiform abnormalities.

Accurate and prompt diagnosis of PNES can be a challenging task, especially since a proportion of these patients also have epilepsy. A detailed history is critical and important part of the evaluation. The interview should be customized accordingly.

PNES share some unique common features. These include frequent attacks that have not responded to appropriate medication, specific triggers (e.g. presence of stressors), occurrence of events only when spectators are present and recurrence in a particular setting. A detailed description of the episode by an eye witness or the patient should be obtained. The ready availability of smart phones and other digital recording devices allow for easy acquisition of video recordings as a useful tool in early diagnosis. Observation of the episode at bedside, or in the clinic by the physician aids in differentiating PNES from epileptic seizures and other paroxysmal non-epileptic events.

History of provoking factors, triggers or stressors of the events and associated psychiatric, neurologic and medical disorders should be obtained. There may also be presence of varied symptoms suggesting somatization.

### Predisposing Factors (Stressors, Triggers)

A variety of risk factors have been identified as the etiologic basis of PNES. The most common risk factors in the pediatric age group are school related problems, seen in 21-46% of cases [1,14], reported more frequently in the adolescent age group [1,20] and exposure of the child to family dysfunction including parental divorce, sibling hostility and financial stress seen in 42-48% cases [1,14]. Interpersonal conflicts with teachers, peers and friends [1,20] are also seen frequently.

Physical and sexual abuse is reported less frequently in children, ranging from 0-32%, in contrast to a much higher frequency in adults [1,14,16,20,21]. Bereavement has been reported more frequently in adolescents [1]. One study reported a higher rate of suicidal attempts (13.5%) [16]. Self-related problems such as low self-esteem, body image issues and dependency, have also been reported [1,14].

### Associated Psychiatric, Neurologic and Medical Disorders

Psychiatric comorbidity is common in children, adolescents, and adults with PNES. In patients of all age groups with PNES, emotional problems have been reported with varying frequency, from 13.8% to 84% [1,12,14-16,22]. The range may be due to differences in assessment for psychiatric comorbidity. Only a limited

number of studies used structured interviews or standard measures.

Depression and anxiety are most common associated psychiatric disorders.in adults with PNES. There is controversy regarding the prevalence of these psychopathologies in children with PNES. In children, major depression ranges from 2.5% [14] to 45% [1,16,22-24]. It is more common in the adolescent age group [1]. Anxiety disorders have also been reported with varying frequencies from 16% [13] to 83% [24]. Bipolar and dysthymic disorder [21], adjustment disorder (8.8%) [14], panic disorder (2.5%) [14], post-traumatic stress disorder [24], separation anxiety and disruptive behavioral problems such as temper tantrums and aggressive behavior have also been reported [1]. Overall, it appears that, in Indian literature, the reported percentages for psychiatric disorders in children with PNES are lower [12-14]. This may be related to cultural differences, variability in referral patterns and limitations related to easy accessibility of psychiatric evaluations in children.

Concurrent epilepsy is seen commonly, occurring with varying frequency from 15-90 % [1,13,15,16] with higher rates noted in children younger than 12 years [1]. Family history of epilepsy is a common finding seen in 25%-43% of patients [1,15]. It is thought that observation of a seizure in a family member may serve as a behavioral model for the child to shape expression of their own conversion symptoms. Frequently, these patients are mistakenly started on anti-seizure medications, and a large percentage ranging from 35-79% were reported to be unnecessarily treated. [1,13,14,25].

Pseudo-status epilepticus may occur and was reported in one study in 13.5% children with PNES [1]. This is a serious problem as it can be associated with iatrogenic complications and considerable distress to the child and family.

Coexisting neurologic illness has been reported in almost half of the children (55%), most frequently cognitive dysfunction (39%) which was significantly more common in the younger children ($P$<0.0001). Less frequent associated conditions include attention deficit hyperactivity disorder (20.3%), headaches (19%) and past head trauma (10%) [1].

Medical comorbidities have been reported in 7.5% of patients [14]. Children with PNES have more associated medical illnesses than their siblings and have been noted to be on more prescribed as well as over the counter medications than their siblings, suggesting that chronic illness may also act as a stressor for inducing PNES. These children are also exposed to more lifetime adversities such as bullying and domestic or community violence. Parents of children with PNES reported more somatization as compared to parents of children with epilepsy suggesting that this becomes an intergenerational family communication model [20].

## ETIOLOGY

From the preceding two sections, one can see that PNES is a heterogeneous disorder with no uniform predisposing factors or comorbidity. Pathways exist for development of PNES. Some children may have significant pre-existing stressors whereas others may have no apparent etiology for PNES. Each child with PNES must be evaluated individually. Nevertheless, there is new information that helps improve understanding PNES. Reuber and Brown, et al. [26] proposed an integrated cognitive model. The motor manifestations of PNES are seen as an instinctual freeze or flight response or a learned motor pattern from experiencing or witnessing a seizure or seizure-like episode. These motor manifestations are triggered by threat or distress. The seizure-like episodes allow escape from the psychological distress and cause parasympathetic activation reducing stress. The seizure-like episodes thus become reinforcing. This neuropsychological model is supported by recent neuroimaging data that have shown changes in the limbic system and in the right inferior frontal cortex, a region involved in motor inhibition [27].

## EVALUATION

### Clinical

Differentiating PNES from epileptic seizures is the first and important step of the evaluation. Some of the major differences between the clinical features of nonepileptic and epileptic seizures has been summarized in **Table I**. PNES last longer than epileptic seizures in both adults and children [2,8]. Side to side head movements and disorganized, asynchronous, out-of-phase extremity movements suggest PNES. They have a gradual onset and termination, with preservation of consciousness during and immediately after events, even with generalized motor activity. Associated injury, tongue bite (usually involving the tip of the tongue) and urinary incontinence are infrequent. Negative emotions such as weeping, crying, or fear [2,13,14], may occur during and after the event, unlike the monotonous cry heard at the onset of some epileptic seizures. However, negative emotional signs have also been seen with epileptic seizures [28], and therefore does not categorically help with differentiating the two. The PNES episodes are often stereotypic, reported in up to 73% of the cases, suggesting that

**Table I Difference in Clinical Features of Psychogenic Nonepileptic Seizures and Epileptic Seizures**

| | *PNES* | *Epileptic seizure* |
|---|---|---|
| Duration | Prolonged (> 2 minutes) | Briefer |
| Semiology | Fluctuating (may be stereotypic) | Stereotypic |
| Onset | Usually gradual | Abrupt |
| Consciousness | Preserved | Altered (especially with generalized seizures)[a] |
| Onset | Usually gradual | Abrupt |
| Head movements | Frequently side to side | Usually unilaterally turned |
| Extremity | Out-of-phase, bizarre | In-phase, rhythmic movements |
| Emotional signs | Usually negative (crying) | Cry at onset |
| Eyes | Closed, resisting eye opening | Open |
| Pelvic thrusting | Infrequent in children, forward | Retrograde |
| Incontinence | Rare | May be present |
| Cyanosis | Absent | May be present |
| Related injury | Inconsistent with fall | Consistent with fall |
| Tongue bite | Infrequent (tip) | More common (lateral aspect) |
| Postictal | None, even after generalized movements | Present (may be absent with frontal lobe seizure) |
| Other features | Preictal pseudosleep | Absent |
| | Forced downward eye deviation | Absent |
| | Postictal whispering | Postictal headache |
| Reaction | Histrionic | Deeply concerned |

[a]*exception supplementary motor seizures.*

stereotypic nature of the episodes does not necessarily always suggest epilepsy [1,13,29].

Other features may also be seen. Commonly, eyes remain closed during the event whereas they are open in epileptic seizures [30], though this may not always be the case. It has been suggested that adolescents and adults who bring stuffed toys to the EMU, were more likely to be diagnosed with PNES and this has been referred to as the "teddy bear sign" [31]. However, this sign has not been found to be always reliable. Geotropic downward eye deviation, ictal stuttering [32], pre-ictal pseudo-sleep [13] and a postictal whispering tone [33] have also been noted.

The semiology of PNES in children differs from adults. Ictal eye closure, events lasting more than 2 minutes, postictal speech change, vocalization during the tonic clonic phase and tongue bite are seen more frequently in adults [29]. Pelvic thrusting is also more common in adults. This is rare in children, and when seen, occurs predominantly in the adolescent age group [13,22].

Varying descriptions of PNES in children are reported in the literature, ranging from unresponsiveness to disorganized motor activity. The features differ by age. Subtle motor activity (similar to hypokinetic or dialeptic seizures), commonly prolonged staring with unresponsiveness, is seen more commonly in children younger than 13 years of age. Prominent motor activity such as generalized arrhythmic jerking or flailing of extremities (similar to hyperkinetic seizures) is seen more frequently in adolescents and is similar to movements seen in adults [1,11,23,29,34]. The subtle behaviors noted in the younger children are more likely to be mistaken for epileptic seizures, thus contributing to a delay in diagnosis in the younger children as compared to the more overt, disorganized motor activity seen in adolescents.

One report further differentiates features of PNES in adolescents based on gender. Boys were more likely to demonstrate convulsive tonic-clonic like movements, whereas girls were more likely to experience atonic falls. Boys reported academic struggles significantly more frequently than girls, and girls more commonly reported difficulties with peer interactions. ADHD was more common in boys and major depression was more common in girls [22].

## Electroencephalography and Laboratory Studies

Prolonged VEEG remains the gold standard of

evaluation. Recording of the habitual event of clinical concern that is not associated with epileptiform abnormalities on the EEG, along with the appropriate historical data, points to a diagnosis of PNES. If the initial study is normal and induction techniques fail to elicit an event, it would be very reasonable to consider repeating the study.

There exists a controversy regarding the use of provocative techniques. These have included placebo induction with intravenous saline, tuning fork and use of skin patches. However ethical concerns have been raised, given that these techniques involve deception, and risk compromise of patient-physician trust. Hyperventilation, photic stimulation and verbal suggestion were deemed more appropriate because these techniques are also used to induce epileptic seizures [35].

Short term VEEG along with induction techniques may be a reasonable option, especially in areas with limited resources [14,36]. It is more cost-effective, and more time efficient. However, interictal epileptiform abnormalities could potentially be missed. Therefore, it is not diagnostic for assessment of co-existing epilepsy.

The following measures have been studied but may only be considered ancillary and are certainly not diagnostic. Biologic markers such as serum prolactin have been studied in adults, with mixed results. Elevated postictal serum prolactin levels, 10-20 minutes after an event have been reported following generalized tonic clonic seizures. However, lack of elevation is not conclusive of PNES, as normal levels are also seen with other seizure types such as focal seizures and absence seizures [37,38]. Similarly, alterations in serum lactate levels [39] and serum creatine kinase (CK) levels [40] have also been studied but are not used routinely. Several other biomarkers that are still being investigated including Neuron specific enolase (NSE) and serum NT-proCNP (a fragment of C-type natriuretic peptide) [41].

### Psychological Evaluation

The psychological assessment of the child with suspected PNES starts with a history, ideally taken form the parent and the child or adolescent separately. Questions about anxiety and depression are essential. Some children with PNES may have alexithymia, an inability to recognize emotions. Stress and trauma related disorder are covered with questions about discord at home, bullying or academic difficulties at school, and exposure to violence or displacement in the community. Screening instruments for emotional and behavioral problems such as the widely available Strengths and Difficulties Questionnaire, a free 25-item questionnaire normed for 4 to 16 year old children and available in multiple languages including Bengali, Hindi, Punjabi, Tamil, and Urdu, can be helpful. If academic difficulties are found, the child may need intellectual and achievement testing [42].

## TREATMENT

Following the diagnosis of PNES, there should be immediate involvement with a mental health professional such as a therapist, psychologist, or psychiatrist who is familiar with this condition. The diagnosis should be presented to the parents by the clinician (pediatricians/ pediatric neurologist), reassuring the family that a diagnosis has been made and that the child has a type of disorder that will not require anti-seizure medications but will need mental health treatment. A similar discussion occurs next with the child. When possible, the pediatrician should offer to continue to be involved in care of the child.

Treatment should be multidisciplinary and include the mental health professional, the child, parents, school, and a pediatrician/ pediatric neurologist [19,43-45]. The first step of treatment is providing psychoeducation regar-ding PNES to the family and school [19,44]. Many children diagnosed with PNES have a decrease in their daily functioning. Therefore, gradual increase in their participation in school, extra-curricular activities, social interactions, etc. to return to previous level of activity and functioning is recommended. In order to increase the patient's success in school, accommodations should be implemented to minimize school absences [44]. Both the school and the parents should be taught selective attention (ignoring the episodes) and be given specific instructions of how to react to the episodes. For example, once the school and parents, have understood the concept that there is no underlying organic cause for the PNES, they need not seek emergency medical care for every subsequent episode. However, they should make efforts to ensure the patient is safe and will not sustain injury during a PNES. Parents and the school should be encouraged to decrease conversations regarding the PNES and provide positive attention to adaptive functioning [44].

Cognitive behavioral therapy (CBT) has empirical support for treatment of adults with PNES though not children [19,44]. A controlled trial in adults with PNES, showed decrease in seizure number [46]. In contrast, a recent controlled study of 368 adults found no reduction in seizure number but improvement in quality of life and psychosocial functioning [47]. We think that CBT may be helpful in children and adolescents with PNES, particularly when associated with anxiety or depression. In individual therapy, the child learns cognitive and

behavioral strategies to reduce episodes. The patient is taught how the body and mind are connected and is provided psychoeducation regarding how thoughts influence emotions, behavior, and somatic symptoms. The patient learns to identify these emotions and the associated somatic symptoms. The patient is then taught relaxation skills and mindfulness strategies to manage distress. The therapist teaches the patient to identify unhelpful thoughts (automatic thoughts) and how to label negative thinking patterns (cognitive distortions). The patient then learns how to challenge and change these unhelpful thoughts to positive thoughts through the use of cognitive techniques. As the patient changes these thoughts, there is an increase in positive emotions, thereby decreasing PNES. In addition to challenging thoughts, patients learn problem solving, exposure and response prevention, and activity scheduling [44]. Through a combination of parent and school interventions, teaching the child distress tolerance skills and cognitive restructuring, and resuming previous levels of functioning, the PNES should usually decrease.

## OUTCOME

Though there are very few systematic studies reporting on the treatment of PNES in children, outcomes in children have been reported as more favorable than in adults [48-50]. According to a recent study, 36% of 90 children (5-18 years) with PNES who were followed up till 2 years, were symptom free at 6 months with sustained remission at 2 years. Another 33% did not achieve remission and this unfavorable outcome was attributed to delay in establishing their diagnosis and presence of comorbid epilepsy [50]. Chinta, et al. [49] reported that 35% were symptom free and an additional 50% experienced more than 50% reduction in frequency of symptoms on short-term follow-up of 3-6 months. Better outcome in these children was related to earlier diagnosis with lack of chronicity of complaints, as well as less severe associated psychiatric comorbidities.

## CONCLUSIONS

There are still unmet needs. There should be more comprehensive assessment of comorbidity in children with PNES. There seems to be heterogeneity in the predisposing and precipitating factors for PNES occurrence in children. This might dictate difference in treatments. Advanced neuro-imaging techniques have been used to study adults with PNES and other functional neurological disorders but there is limited information on changes in children and adolescents. There are currently no controlled trials of treatments for children with PNES. Finally, outcome appears to be better in children with PNES, but there needs to be long-term follow ups for both PNES and the comorbid conditions. Families also need additional study for factors that may provoke or maintain PNES in children or assist with satisfactory resolution.

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**WEB TABLE I Studies of Psychogenic Nonepileptic Seizures in Children in Past 15 Years (2004-2019)**

| *Author, y* | *No. (n/N)* | *Mean (range) age at onset, y* |
|---|---|---|
| Asadi-Pooya, et al. [25] (2019) | 51/51 | 12.3 (5-16) |
| Madaan [14] (2018) | 80/1987 | 10.5 (6-16) |
| Valente [16] (2017) | 53/53 | 12.81 (7-17) |
| Plioplys, et al. [24] (2016) | 55/55 | 14.3 (8.6-18.4) |
| Yadav, et al. [50] (2015) | 90/2097 | 14.3 (5-18) |
| Say, et al. [22] (2015) | 62/62 | 14.19 (11-18) |
| Dhiman, et al. [13] (2014) | 56/56 | 12.3 (2-17) |
| Yilmaz, et al. [51] (2013) | 54/765 | 8.8 (4-16) |
| Szabo, et al. [2] (2012) | 27/568 | 11.6 (8-18) |
| Kim, et al. [11] (2012) | 15/143 | Not available (6-19) |
| Kutluay, et al. [10] (2010) | 36/416 | 13.5 (6-17) |
| Salpekar, et al. [52] (2010) | 24/48 | 13.6 (10-17) |
| Verotti, et al. [23] (2009) | 36/36 | Not available (6-17) |
| Chinta, et al. [49] (2008) | 17/17 | 10.7 (6-14) |
| Kacinski, et al. [53] (2007) | 45/45 | Not available (11-19) |
| Patel, et al. [1] (2007) | 59/1967 | 12yr9mo (5-19) |
| Udall, et al. [54] (2006) | 12/223 | Not available |
| Vincentiis, et al. [15] (2006) | 21/69 | Not available (4-18) |
| Bhatia and Sapra [12] (2005) | 50/110 | Not available (6-11) |

*n: number of children with psychogenic nonepileptic seizures, N: number of children studied.*

# Low Osmolarity Oral Rehydration Salt Solution (LORS) in Management of Dehydration in Children

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**Justification:** The IAP last published the guidelines "Comprehensive Management of Diarrhea" in 2006 and a review in 2016. The WHO in 2002 and the Government of India in 2004 recommended low osmolarity rehydration solution (LORS) as the universal rehydration solution for all ages and all forms of dehydration. However, the use of LORS in India continues to be unacceptably low at 51%, although awareness about ORS has increased from a mere 14% in 2005 to 69% in 2015. Availability of different compositions of ORS and brands in market added to the confusion. **Process:** The Indian Academy of Pediatrics constituted a panel of experts from the fields of pediatrics, pediatric gastroenterology and nutrition to update on management of dehydration in children with particular reference to LORS and issue a current practice guideline. The committee met twice at CIAP HQ to review all published literature on the aspect. Brief presentations were made, followed by discussions. The draft paper was circulated by email. All relevant inputs and suggestions were incorporated to arrive at a consensus on this practice guideline. **Objectives:** To summarize latest literature on ORT and empower pediatricians, particularly those practicing in rural areas, on management of dehydration by augmenting LORS use. **Recommendations:** It was stressed that advantages of LORS far out-weigh its limitations. Increased use of LORS can only be achieved by promoting better awareness among public and health-care providers across all systems of medicine. LORS can also be useful in managing dehydration in non-diarrheal illness. More research is required to modify ORS further to make it safe and effective in neonates, severe acute malnutrition, renal failure, cardiac and other co-morbidities. There is an urgent need to discourage production and marketing all forms of ORS not in conformity with WHO approved LORS, under a slogan "One India, one ORS".

**Keywords:** *Diarrhea, Management, Oral rehydration therapy.*

Diarrhea is the second leading cause of death after pneumonia among children below 5 years of age in India [1, 2]. The 4th National Family Health Survey reported that 9.2% of under-5 children had diarrhea during the preceeding two weeks [3]. The situation remained same even after a decade, as the incidence was 9% in 2005 [4] Death in diarrhea is mainly from dehydration and its complications. Therefore, appropriate rehydration therapy remains the cornerstone in management. Advances in molecular technology have helped to better understand the etiology and pathophysiology of diarrhea. It helped conceptualizing and improving oral rehydration therapy.

WHO launched global diarrheal diseases control program with oral rehydration therpay (ORT) as its core strategy in year 1978 [5]. Being concerned with hypernatremia, especially in children having non-cholera diarrhea, single low osmolar ORS (LORS) formulation was recommended as an universal solution by WHO and UNICEF in a joint statement in which LORS was recommended as safe and effective to correct dehydration in diarrhea, including cholera in adults as well as children [6]. The Government of India followed the lead and approved the same composition of LORS as a single rehydration solution.

Multi-centric studies in India and Bangladesh established safety and efficacy of LORS in non-cholera as well as cholera related diarrhea without significant symptomatic hyponatremia [7]. The European Society for Pediatric Gastroenterology and Nutrition (ESPGHAN) as well as the North Americal Society for Pediatric Gastroenterology and Nutrition (NASPGHAN) committee also endorsed safety and efficacy of LORS for use in diarrhea [8].

## BACKGROUND

*Mechanisms of diarrhea*: There are four main mechanisms. *Secretory diarrhea*: Toxin induced active out-put of fluid into small intestine as in cholera and entero-toxogenic E. Coli (ETEC). It can result in severe

dehydration, metabolic acidosis and dyselectrolytemia due to rapid loss of fluid, bicarbonate and electrolytes, especially potassium [9].

*Osmotic diarrhea*: Mucosal damage leads to unabsorbed substances, mainly carbohydrates in small intestine. It results in high osmotic load and consequent passive movement of fluid and electrolytes into the lumen. An example is rotavirus-induced temporary lactose intolerance. Other etiological agents include norovirus, astrovirus and enteroviruses.

*Bloody diarrhea*: Diarrhea with visible blood in the stool is called dysentery and is associated with systemic symptoms like fever and crampy abdominal pain**.** Common infective causes include Shigella, Salmonella, enteroinvasive E.coli and Entamoeba histolytica, while non-infective causes include inflammatory bowel disease and milk protein allergy. The large bowel is predominantly involved and usually it does not cause dehydration.

*Malabsorptive diarrhea*: The classic examples are - diffuse mucosal disease, defects in pancreas and/or biliary system, celiac disease, giardiasis and cystic fibrosis. There is defective digestion or absorption of nutrients, minerals and vitamins, resulting in malnutrition or failure to thrive. Dehydration and electrolyte imbalance are seen only in prolonged and severe disease.

*Watery diarrhea*: It typically emanates from small bowel, either by abnormal secretory or osmotic process. Concentration of electrolytes in luminal contents remains in a state of equilibrium with that of blood. Any change in this bi-directional flow, either by increased secretion or decreased absorption or both, result in excess fluid entering large intestine. Diarrhea results if the fluid exceeds maximal absorptive capacity of colon [10].

*Basis for shifting to low-osmolar ORS (LORS)*: With improved infrastructure, water supply and better sanitation, incidence of cholera decreased considerably over time. On the other hand, Rotavirus diarrhea was recognized as a major etiological agent for acute diarrhea in children. Significantly lower stool electrolyte losses ($Na^+$, $K^+$, bicarbonate) in non-cholera diarrhea was recognized. Of particular concern was that sodium loss in rotavirus diarrhea was much less (52 mmol/L) than in cholera (90 mmol/L), resulting in a much higher incidence of hypernatremia with conventional ORS [9,10]. The earlier WHO ORS had poor acceptance by mothers for not reducing incidence of vomiting and stool volume [11]. LORS, having lower osmolarity (245 mOsm/L) than plasma (290 mMol/L), facilitated absorption of sodium and water faster **(Table I)**. Active absorption of glucose and amino acids was promoted. Not only LORS replaced fluid and electrolytes faster, it also decreased luminal volume by quick absorption, thus reducing chance of vomiting and stool volume across all ages. It also helped in reducing the need for unscheduled supplemental intravenous therapy [6] There were initial hesitation and resistance to change despite recommendations from the American Academy of Pediatrics (AAP), WHO, ESPGHAN and NASPGHAN to use LORS by parents and caregivers but the acceptance improved fast [12,13].

## PROCESS

The Indian Academy of Pediatrics constituted a panel of experts from the field of general pediatrics, pediatric gastroenterology and nutrition to update on management of diarrhea in children with particular reference to LORS. The committee met on August 5, 2018 and on June 23, 2019 in Mumbai, and reviewed all published literature and reports of expert bodies on the relevant aspects on managing dehydration with ORS. Brief presentations were made and followed by discussions. A draft practice guideline was compiled and circulated by email to all members. Several useful inputs were received which were incorporated to arrive at a consensus document. Finally the guideline was placed before the IAP Executive Body Meeting, 2020 which was approved.

## RECOMMENDATIONS

### *Rehydration in Watery Diarrhea*

Current management practice in diarrhea follows WHO

**Table I Electrolyte Composition of Plasma, Stool and Oral Rehydration Solution (ORS) [6,11]**

| | *Composition (mMol/L)* | | | | | | |
|---|---|---|---|---|---|---|---|
| *Composition* | *Na* | *K* | *Cl* | *$HCO_3$* | *Citrate* | *Glucose* | *Osmolarity* |
| Human plasma | 135 | 5 | – | 25 | – | 90 | 290 |
| Cholera stool | 105 | 25 | 30 | 30 | – | – | – |
| Non-cholera stool | 52 | 25 | 14 | 14 | – | – | – |
| Conventional ORS | 90 | 20 | 80 | – | 10 | 111 | 311 |
| Low osmolar ORS | 75 | 20 | 65 | – | 10 | 75 | 245 |
| ReSoMal | 45 | 40 | 76 | – | 7 | 125 | 300 |

**Key Recommendations**

*Strategies Need to be Operationalized Nation-wide for Increasing LORS Use*

- Ensuring uniform composition of ORS in market for Sachet and readymade solutions, with legally binding regulatory guidelines. Popularise the slogan, 'One country - one ORS.'
- Declaring LORS as a lifesaving drug
- Making LORS freely available at anganwadis, schools, kirana stores and pharmacies in moisture proof foil packs, clearly specifying that it confirms to WHO composition [35].
- Diarrhea management and use of ORS may be included in the school curriculum.
- Celebrating IDCF week in all education institutes and primary health-care facilities ass a mass movement like Pulse Polio Program.
- Persuading industries to take up diarrhea management, manufacturing, distributing and popularising LORS as their corporate social responsibility (CSR).
- Conducting education programs, refresher courses and workshops to improve and reinforce knowledge of basic health workers as well as practitioners from all systems of medicine.
- Provision for safe and wholesome drinking water at all nook and corner of the country [36].
- Social and electronic media be pressed into action to educate the public on ORS, particularly in the rural areas, with catchy slogans beaming and programs in local language by eminent personalities as brand ambassadors.
- Co-ordinated effort by public-private partnership while mitigating private sector risks for achieving public sector objectives in popularising and making ORS freely available.
- Evolving innovative strategies by experts for key behaviour change among stake holders to establish credibility of LORS for prevention and management of dehydration.

[6] and ESPGHAN [8] guidelines, focusing on oral rehydration therapy, continued feeding and zinc supplementation. WHO outlined treatment of dehydration in diarrhea according to grades of dehydration such as 'No dehydration', 'Some dehydration' and 'Severe dehydration' on specific clinical symptoms and signs [14,15]. Certain clinical signs are considered more specific and dependable. Of the ten commonly used signs to assess dehydration, prolonged capillary refill time, abnormal skin turgor, abnormal respiratory pattern, cool extremities, weak pulse and absence of tear have higher specificity [16] Management plan-A is the home management of diarrhea with very mild or no dehydration. LORS is advised at the rate of 10 mL/kg with each episode of watery stool. Breastfeeding is advised more frequently and for longer duration at each feed, if exclusively breastfeed. If not exclusively breastfed, food-based fluids such as soup, rice water or rice gruel, coconut water, yogurt and clean water offered frequently, apart from LORS. If on complementary feed, must continue what child had been taking from family pot. Plan-B is for mild to moderate dehydration or some dehydration. LORS is given as 50 to 100 mL/kg of bodyweight (Average 75 mL/kg) at the day care center or ORS corner over 3 to 4 hours; 12-25 mL/kg in the first hour in sips from cup and spoon, under supervision. To start with, 1-2 mL/kg may be given every 5 minutes to prevent vomiting and increased gradually as the child accepts more. In the event of vomiting, ORS is withheld for 15 to 30 minutes and then resumed. Current clinical condition, on-going loss and renal status are taken into account for fluid replacement within 4 to 6 hours, albeit at a slower rate in infants (Half of replacement fluid in first one hour for infants and in 30 minutes for children). Reassessment of hydration is done every four hours. Once dehydration is corrected, plan A management to continue. Mother is always counselled about red flag signs and when to return immediately.

*LORS for children with cholera:* LORS has been found to be safe and effective in correcting dehydration in a subgroup of 9% patients who were diagnosed as culture-proven cholera [7]. A systemic review by WHO on several studies conducted in children with cholera treated either with LORS or standard WHO ORS, reaffirmed the safety and efficacy of LORS. The mean serum sodium in children with cholera was found slightly lower after 24 hours (mean 131 mEq/L) than those having non-cholera diarrhea (mean 137 mEq/L), both being within acceptable range [6].

*Vomiting:* If the child is vomiting repeatedly, thorough clinical examination is warranted to rule out any organic or metabolic cause needing urgent intervention. Cases of recurrent vomiting, severe dehydration, dyselectrolytemia or sepsis should be hospitalized for investigations, intravenous fluid, close monitoring and early intervention. With persistent vomiting and/or increasing abdominal distension, sips of LORS can be given slowly, checking bowel sounds. Vomiting by itself is a cause of dehydration, worsening fluid loss as well as impairing oral rehydration. Oral ondansetron is considered safer for

children to arrest vomiting with 0.15 mg/kg/dose on as and when basis [17,18]. Usual feeding must continue which helps early mucosal repair and prevent malnutrition [8,14]. Administering zinc in diarrhea for two weeks, particularly in south-east Asia region where the soil is zinc deficient and people are mostly vegetarian, is recommended, although debated recently [19,20].

### *LORS for Special Situations*

#### Severe Acute Malnutrition (SAM)

Malnutrition is an underlying risk factor for diarrhea, causing of 61% of child deaths globally [1]. Diarrhea in children with SAM carries an 8-9 times higher mortality [2]. With improved access to better health care, deaths from diarrhea in hospitalized children now occurs only if malnutrition is co-existing. Decreased food intake, loss of appetite, poor absorption and increased nutrient requirement - all result in weight loss and delayed recovery from diarrhea, creating a vicious cycle. In these children, dehydration should not only be corrected quickly and nutritional rehabilitation should be simultaneously started with F-75 feeding [21]. There are additional risks of fluid retention, hypernatremia and cardiac failure if higher sodium containing fluids are used for rehydration. WHO recommends ReSoMal with low sodium for such children [22]. The Indian Academy of Pediatrics in 2006 suggested a modified rehydration fluid (mReSoMal) to provide $Na^+$ 45 mMol/L, $K^+$ 40 mMol/L, zinc, copper and magnesium [23]. Results of multi-centric studies by Alam, et al. [24] compared low $Na^+$ mRESoMal with standard WHO LORS in 130 children of 6 to 36 months age having acute diarrhea on SAM. 29% of subjects in mReSoMal group were found to be having hyponatremia in at 48 hours. Out of them, three had severe hyponatremia. On the other hand, only 10% developed hyponatremia in LORS group. Over hydration was reported in 5% and 12% of mReSoMal and LORS groups respectively [24]. In yet another RCT with 104 children, Kumar, et al. [25] used one sachet of LORS, 40 grams of sugar and 35 ml of potassium chloride solution added to 1700 ml of water to nearly match mReSoMal. Their other arm used LORS in one liter of water to which 15 ml of potassium chloride was added to take care of hypokalemia, commonly seen in SAM. Both types of fluid were found effective in correcting dehydration and hypokalemia, but the mReSoMal group took comparatively lesser time for correction and also had lesser incidence of hypernatremia [25]. Further, a systematic review of six RCTs, conducted in low resource settings in Asia, showed that LORS therapy took lesser time to rehydrate; with decreased stool volume and duration of diarrhea. None reported over-hydration or serious fatalities due to hyponatremia. WHO guidelines strongly recommend using ReSoMal universally but not supported by high quality evidence as certain RCTs shown an increased risk of hyponatraemia with WHO ReSoMal in Asian children. Therefore, more studies, especially from Africa were felt necessary [26]. Considering available data, either LORS dissolved in one litre of water with 15 mL of 20% Potassium chloride solution, or mReSoMal can be considered effective for rehydration as well as correcting basal hypokalemia associated in SAM with diarrhea.

#### Neonates and Infants

Larger body surface area to body mass ratio result in higher insensible water loss, besides immature kidneys. They are prone to asymptomatic hypernatremia with narrow safety window in intravascular vis-a-vis extra-vascular compartment. They quickly slip into severe dehydration and related complications. LORS can be administered above 2 months under supervision, while continuing breastfeeding [14]. There is insufficient evidence to recommend ORS below that age.

#### Severe Dehydration, Where Facility for Intravenous Fluid Therapy Is Not Available

In resource limited settings where IV treatment facility is either not feasible or it is not possible to access IV line due to edema or collapsed veins, sips of LORS or through NG tube at the rate of 20 mL/kg per hour for 6 hours (total 120 mL/kg) may be initiated. Reassessment every 1-2 hours is essential. Once the collapsed veins stand out, IV lines can easily be established. Enteral fluid therapy for 24 hours as compared to rapid IV fluid of 40-50 mL/kg in 4 to 6 hours found equally effective. Far less adverse events and shorter hospital stay are advantages [8].

#### Renal Failure

Azotemia occurs secondary to poor renal perfusion or acute tubular necrosis. A good amount of bicarbonate is lost in diarrheal which normally gets replenished by kidneys. Acidosis occurs due to base deficit and excess lactic acid production. Severe metabolic acidosis results in increased vomiting, deep but rapid breathing and altered sensorium. Enteral feeding with LORS in calculated amount under supervision can be considerd enroute to referral centre, pending intravenous fluid and appropriate corrections after assessing renal status [14,15].

#### Acute Febrile Illness

Fever often cause excessive fluid loss due to increased sweating, diminished thirst and poor water intake adversely impacting water-electrolyte balance. An

**Table II LORS Use Rates in Diarrhea Found in National Family Health Surveys (NFHS)-4 [3,4]**

| *Parameters related to Diarrhea* | *NFHS-4(2015-16)* | | | *NFHS-3 (2005-06)* |
|---|---|---|---|---|
| | *Urban* | *Rural* | *Overall* | |
| Prevalence in 2 week preceding survey | 8.2 | 9.6 | 9.2 | 9.0 |
| Cases in last 2 week who received ORS, % | 58.5 | 47.9 | 50.6 | 26.0 |
| Cases in last 2 week who received zinc, % | 23.7 | 19.1 | 20.3 | - |
| Cases in last 2 week taken to health facility, % | 74.1 | 65.8 | 67.9 | 61.3 |

additional 20% fluid intake is advised in all cases of fever irrespective of the cause. Fever is often associated with vomiting, particularly in small children. It can cause dehydration and electrolyte imbalance, warranting intravenous fluids and hospitalization. Early administration of LORS can prevent such eventualities [27].

*Dengue and other viral infections:* LORS is preferred for treatment of mild to moderate dehydration, rather than plain water in febrile dengue patients. Fruit juice or any other home based fluids, and ORS are encouraged, while continuing feeding [28]. Other viral infections, including Ebola and COVID-19 during fever cause dehydration and demand sufficient fluids, including ORS.

*Typhoid:* Dehydration is common in high fever, vomiting, poor feeding, loose stool, hepatitis and so on. Hypoglycemia, hypokalemia and hyponatremia occur frequently and oral or intravenous hydration are essential. LORS, home based fluids and appropriate diet are considered vital [29].

*Malaria:* A significant association was found between severity of dehydration and parasitemia. Prevalence of malaria-associated diarrhea was found in 61.7% of cases in Ghana. Parasite-positivity was associated with high fever and vomiting, causing dehydration. ORS was found invaluable in preventing dehydration and hypoglycemia [30].

*Heat stroke:* It results in refractory and prolonged hyperpyrexia (Core body temperature >41$^0$C), dehydration and dyselectrolytemia. Cool bath, sponging, ice packs, spraying cold water are important in management. In a Japanese study on 153 adult loaders at an airport cargo terminal in summer with 30$^0$C, the subjects were either given conventional ORS (Na 90 mMol/L) or their favorite beverages (Tea or coffee) on different days. Their fatigue score was found significantly lower on ORS intake days than on beverages days. The results suggested that intake of ORS during outdoor work in the hot environment can effectively prevent accidents and heat stroke [31]. These recommen-dations can safely be extrapolated, recommending LORS use in children.

### ORS Use in the Community

*Diarrhea alleviation through zinc and ORS therapy (DAZT):* This study was undertaken to understand ORS coverage to achieve reductions in diarrhea mortality, an operation research in three districts each of UP and Gujarat during 2010-14. Prescription trend in diarrhea, knowledge and practice of zinc therapy and ORS use by health care providers and family members were assessed. Structured, pre-validated questionnaires were administered to 127 healthcare providers and 43 home based care givers. Besides, 228 prescriptions from government health facilities were also analysed. It was found that Government functionaries dispensed ORS to the tune of 97% and zinc in 90% cases of diarrhea vs 79% and 71% respectively in the private sector [35].

*National Family Health Surveys (NFHS)*: The data show that ORS use in diarrhea increased from 14% in 2005 to 26% in 2010 and to 50% in 2015. Although the awareness and ORS access rate, a key to any diarrhea control program in community has improved over the last forty years, its actual utilization remains far below expected level [3,4] **(Table II)**. The disparity may be attributed to continued lack of public awareness, poor prescription rates of LORS, lack of perception on benefit by stake holders and practitioners. Besides, inadequate resource allocation, poor infrastructure and a general complacency are important factors.

**Table III Beverages Available in Market That are Unsuitable in Diarrhea**

| *Products* | *CHO (mmol/L)* | *Sodium (mmol/L)* | *Potassium (mmol/L)* | *Chloride (mmol/L)* | *Base (mmol/L)* | *Osmolarity (mosM/L)* |
|---|---|---|---|---|---|---|
| Fruit juice | 120 | 0.4 | 44 | 45 | - | 730 |
| Soft drinks | 112 | 1.6 | - | - | 13.4 | 650 |
| Sports/power drinks | 58.3 | 20 | 3.2 | 11 | - | 299 |

**Box I Suggested Research Priorities**

- Improve calorie content and palatability of LORS without increasing osmolality,
- Address safety concerns in neonates and young infants,
- Search for a better formulation of ORS in presence of severe acute malnutrition.

*The Intensified diarrhea control fortnight (IDCF):* This program was launched in 2014 from 28 July to 03 August every year under the Ministry of Health and Family Welfare under Government of India, with the goal to reduce U-5 childhood mortality further. It immensely helped in creating awareness on diarrheal disease and its treatment all over the country by involving all stakeholders from doctors to grassroot workers like ASHA and Anganwadi workers. Participation of NGOs, medical colleges, primary health centers, and professional bodies of pediatricians, dieticians and nurses ensured wide publicity for the program and its successful implementation.

*Barriers*: There are several reasons for low use of LORS, despite massive efforts by government and NGOs over years [33,34]. Lack of awareness among public and basic health workers at grass root level, coupled with non-availability of LORS in community are responsible [3]. Often pharmacies do not stock ORS for low profit margin. Many branded formulations are marketed in name of ORS do not conform to WHO composition [35]. These non-physiologic fluids have either low sodium content or high osmolarity, actually worsening diarrhea (**Table III**). Moreover, LORS is mostly available in powder form while ORS substitutes in market are in attractive liquid ready to drink packs, tablets and drops [35]. There is no regulatory mechanism insisting on a single, standard form of LORS sachets throughout the country. Palatability of ORS is poor owing to its citrate content, which however is vital for maintaining the pH. This taste factor forces parents to look for alternatives. Parents do insist on intravenous fluids without justifiable indication, expecting early cure but the hospitals do little to refuse such demand on commercial considerations.

## CONCLUSION

Reduced osmolarity ORS is adequate to manage dehydration in most children. Current use of ORS in diarrhea, as seen in last NFHS-4 is low, although awareness is considerably high. There are issues with use of LORS in severe acute malnutrition, for newborns and young infants, renal dysfunction. There exists scope for enteral feeding of LORS, where facilities for parenteral therapy not available or not possible. Not only diarrhea, LORS should be popularized as an effective remedy to combat dehydration due to any cause. Steps are urgently required to ensure availability and use of only the standard WHO LORS all over the country. Appropriate research priorities have been outlined.

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## CLIPPINGS

**Seasonal fluctuations in T4 and TSH measurement in NBS program** (*Int J Neonatal Screen. 2021;7:8)*

Newborn screening for hypothyroidism is universally practiced in most developed countries. This study from New York, USA evaluated the effect of seasonal changes in kits for NBS used on 2.4 million babies between 2008 to 2017. The measurement of T4 and TSH was based on fluroimmunoassay principle in dried blood spots. A higher level of TSH and T4 with higher false positive rate was seen in the colder months, indicating the effect of seasonal temperature variations in these kits. However, the number of confirmed hypothyroid cases remained the same irrespective of the season. They suggested the need to be aware of these fluctuations to optimize the recall rates in screen positive babies.

**Prediction of childhood obesity from BMI acceleration patterns** *(J Pediatr. Feb 2021. Epub ahead of print)*

This study from Israel analyzed BMI acceleration patterns among 417,915 adolescents. Electronic health records of children between 2002 to 2018 were retrieved to devise a model to predict obesity (BMI >95$^{th}$ centile). The model recorded the greatest acceleration in BMI at 2-4 years of age in obese adolescents and accurately predicted obesity at 5-6 years of age (AUC 0.803). Thus, anthropometric parameters during early childhood were concluded as important predictors of obesity at a later age.

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# AHA Pediatric Advanced Life Support Update 2020 - "More Breaths, Less Fluids, and a Focus on Recovery"

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Pediatric Advanced Life Support (PALS) guidelines are updated every five years and the new 2020 guidelines were issued recently. We briefly review the important changes in terms of rates of rescue breaths, timing of epinephrine, resuscitation in septic shock, use of extracorporeal therapies, and the new component in the chain of survival – recovery.

**Keywords**: *Extracorporeal therapies, Management, Resuscitation, Shock*.

The American Heart Association (AHA) 2020 guidelines with their changes for pediatric basic and advanced life support were issued recently [1]. We have discussed the changes made under three headings – pre-arrest, intra-arrest and post arrest care. This will make it convenient to compare these with the 2015 document, wherein a similar division was presented.

## PRE-ARREST CARE

The updated guidelines reaffirm the importance of prevention and preparedness for a cardiac arrest in children as the first component in the chain of survival.

### ECLS and ECPR in Children With Myocarditis

Newer evidence that has emerged since the last guidelines, shows that pre-arrest use of Extra Corporeal Life Support (ECLS) or Mechanical Circulatory Support (MCS) in patients with myocarditis may lead to better organ support and prevention of cardiac arrest. The survival to hospital discharge in patients with structurally normal hearts, receiving Extracorporeal Cardiopulmonary Resuscitation (ECPR) was 32% in adults. Myocarditis was a favorable prognostic marker for the use of ECPR in this study. Children with acute fulminant myocarditis had a 75% survival after ECLS/MCS either with recovery of native function (43.8%) or post cardiac transplant (31.3%) [2]. Retrospective analysis of the Extracorporeal Life Support Organization (ELSO) database showed a 61% survival to hospital discharge, 3% of these with heart transplantation. A recent German prospective registry showed weaning rates of 42% in children with myocarditis who received ECLS. Early transfer to ICU is also recommended for monitoring and initiation of therapy.

### Resuscitation in Septic Shock

*Type of fluid:* The newer guidelines have jumped into the raging debate between balanced and unbalanced crystalloids as initial fluid of choice in septic shock. Balanced crystalloids, with a composition closer to that of normal human plasma, were postulated to reduce the incidence of hyperchloremic metabolic acidosis and acute kidney injury (AKI). A retrospective matched analysis showed better survival at 72 hours and lower rates of AKI with balanced crystalloids although another similar analysis showed no difference [3,4]. A pilot RCT, that failed to demonstrate any difference, however, established the feasibility of further research in this area. While the physiological rationale for using balanced over unbalanced crystalloids seems sound, one cannot be recommended over the other, based on the current evidence.

*Volume of fluid:* The updated guidelines suggest that it is reasonable to administer fluids in smaller aliquots of 10-20 mL/kg with careful reassessment for both fluid responsiveness and overload after each bolus to titrate further therapy. There is a growing inventory of evidence beginning with the FEAST Trial, warning against the perils of overzealous fluid administration in septic shock. The last AHA update in 2015 had recommended cautious fluid resuscitation in setups with limited access to intensive care resources. The current 2020 update, however, recommends it uniformly, irrespective of availability of intensive care resources. On the contrary, the Survival

**Table I Summary of AHA Pediatric Advanced Life Support Update 2020 [1]**

| | *AHA 2010/2015* | *AHA 2020* | *COR* | *LOE* |
|---|---|---|---|---|
| *Pre-arrest care* | | | | |
| Use of ECLS and ECPR in children with Myocarditis | No Recommendation | Given the high risk of cardiac arrest in children with acute myocarditis with arrhythmias, heart block, ST-segment changes, and/or low cardiac output, early consideration for ICU transfer, monitoring and therapy is recommended | 1 | C-LD |
| | Venoarterial ECMO use may be considered in patients with acute fulminant myocarditis who are at high risk of imminent cardiac arrest | For children with myocarditis or cardiomyopathy and refractory low cardiac output, prearrest use of ECLS or MCS can be beneficial to provide end-organ support and prevent cardiac arrest | 2a | B-NR |
| | No Recommendation | Given the challenges to successful resuscitation of children with myocarditis and cardiomyopathy, early consideration of ECPR once cardiac arrest occurs, can be beneficial | 2a | B-NR |
| Resuscitation in Septic Shock | Providers should reassess the patient after every fluid bolus | Providers should reassess after every fluid bolus for fluid responsiveness and signs of volume overload | 1 | C-LD |
| | Either isotonic crystalloids or colloids can be effective as initial fluid for resuscitation | Either isotonic crystalloids or colloids can be effective as initial fluid for resuscitation | 2a | B-R |
| | No Recommendation | Either balanced or unbalanced solutions can be effective as fluid choice for resuscitation | 2a | B-NR |
| | Administration of an initial fluid bolus of 20 mL/kg to infants and children with shock is reasonable, including those with conditions such as severe sepsis, malaria and dengue | In patients with septic shock, it is reasonable to administer fluid in 10 mL/kg or 20 mL/kg aliquots with frequent reassessment | 2a | C-LD |
| | No Recommendation | In infants and children with fluid-refractory septic shock, it is reasonable to use either epinephrine or norepinephrine as an initial vasoactive infusion | 2a | C-LD |
| *Intra-arrest care* | | | | |
| Airway during CPR | In the prehospital setting it is reasonable to ventilate and oxygenate infants and children with a bag-mask device, especially if transport time is short | Bag-mask ventilation is reasonable compared with advanced airway interventions (SGA and ETI) in the management of children during cardiac arrest in OHCA | 2a | C-LD |
| Respiratory Rates with an Advanced Airway | If the infant or child is intubated, ventilate at a rate of about 1 breath every 6 to 8 seconds (8 to 10 times per minute) without interrupting chest compressions | When performing CPR in infants and children with an advanced airway, it may be reasonable to target a respiratory rate of 1 breath every 2–3 s (20–30 breaths/min), accounting for age and clinical condition. Rates exceeding these may compromise hemodynamics. | 2b | C-LD |
| | In the victim with a perfusing rhythm but absent or inadequate respiratory effort, give 1 breath every 3 to 5 seconds (12 to 20 breaths per minute), using the higher rate for the younger child | For infants and children with a pulse but absent or inadequate respiratory effort, it is reasonable to give 1 breath every 2 to 3 s (20-30 breaths/min) | 2a | C-EO |
| Use of Cuffed Endotracheal Tubes | Both cuffed and uncuffed endotracheal tubes are acceptable for intubating infants andchildren | It is reasonable to choose cuffed over uncuffed ETTs for intubating infants and children | 2a | C-LD |
| | No Recommendation | When a cuffed ETT is used, attention should be paid to ETT size, position, and cuff inflation pressure (usually <20–25 cm H2O) | 1 | C-EO |

*Table contd...*

**Table I** *continued*

| | *AHA 2010/2015* | *AHA 2020* | *COR* | *LOE* |
|---|---|---|---|---|
| Use of Cricoid Pressure | Apply cricoid pressure in an unresponsive victim to reduce air entry into the stomach | Cricoid pressure during bag-mask ventilation may be considered to reduce gastric insufflation. | 2b | C-LD |
| | | Routine use of cricoid pressure is not recommended during endotracheal intubation of children. | 3: No Benefit | C-LD |
| | Do not continue cricoid pressure if it interferes with ventilation or the speed or ease of intubation | If cricoid pressure is used, discontinue if it interferes with ventilation or the speed or ease of intubation. | 3: Harm | C-LD |
| Early Epinephrine | It is reasonable to administer epinephrine in pediatric cardiac arrest | For pediatric patients in any setting, it is reasonable to administer the initial dose of epinephrine within 5 min from the start of chest compressions. | 2a | C-LD |
| Use of Invasive BP monitoring during CPR | For patients with invasive hemodynamic monitoring in place at the time of cardiac arrest, it may be reasonable for rescuers to use blood pressure to guide CPR quality | For patients with continuous invasive arterial blood pressure monitoring in place at the time of cardiac arrest, it is reasonable for providers to use *diastolic* blood pressure to assess CPR quality. | 2a | C-LD |
| Opioid Related Cardiac Arrest | Naloxone reverses the respiratory depression of narcotic overdose | For patients known or suspected to be in cardiac arrest, in the absence of a proven benefit from the use of naloxone, standard resuscitative measures should take priority over naloxone administration, with a focus on high-quality CPR (compressions plus ventilation). | 1 | C-EO |
| *Post arrest care* | | | | |
| Targeted Temperature Management (TTM) | Continuous measurement of temperature during this time period is recommended | Continuous measurement of core temperature during TTM is recommended | 1 | A |
| | For infants and children remaining comatose after OHCA, it is reasonable either to maintain 5 days of continuous normothermia (36°C to 37.5°C) or to maintain 2 days of initial continuous hypothermia (32°C to 34°C) followed by 3 days of continuous normothermia | For infants and children between 24h and 18 yr of age who remain comatose after OHCA or IHCA, it is reasonable to use either TTM 32-34 C followed by TTM 36-37.5 C or only TTM 36-37.5 C | 2a | B-R |
| Hemodynamic Monitoring and Ventilation | When appropriate resources are available, continuous arterial pressure monitoring is recommended to identify and treat hypotension | When appropriate resources are available, continuous arterial pressure monitoring is recommended to identify and treat hypotension | 1 | C-EO |
| | After ROSC, we recommend that parenteral fluids and/or inotropes or vasoactive drugs be used to maintain a systolic blood pressure greater than fifth percentile for age | After ROSC, parenteral fluids and/or vasoactive drugs to maintain a systolic blood pressure greater than the fifth percentile for age are recommended | 1 | C-LD |
| | It may be reasonable for rescuers to target normoxemia after ROSC | It may be reasonable for rescuers to wean oxygen to target an oxyhemoglobin saturation 94-99% | 2b | C-LD |
| Neuromonitoring and Seizure Treatment | No Recommendation | When resources are available, continuous electroencephalography (EEG) monitoring is recommended for detection of non-convulsive seizures following cardiac arrest in patients with persistent encephalopathy | 1 | C-LD |
| | No Recommendation | It is recommended to treat clinical seizures following cardiac arrest | 1 | C-LD |
| | No Recommendation | It is reasonable to treat nonconvulsive status epilepticus following cardiac arrest in consultation | 2a | C-EO |

*Table contd...*

**Table I** *continued*

| | *AHA 2010/2015* | *AHA 2020* | *COR* | *LOE* |
|---|---|---|---|---|
| | | with experts | | |
| Prognostication following Cardiac Arrest | EEGs performed within the first 7 days after pediatric cardiac arrest may be considered in prognosticating neurologic outcome at the time of hospital discharge but should not be used as the sole criterion. | EEG in the first week post cardiac arrest can be a useful factor for prognostication, augmented by other information | 2a | B-NR |
| | The reliability of any one variable for prognostication in children after cardiac arrest has not been established. Practitioners should consider multiple factors when predicting outcomes in infants and children who achieve ROSC after cardiac arrest | It is reasonable for providers to consider multiple factors when predicting outcomes in infants and children who survive cardiac arrests | 2a | B-NR |
| Recovery | No Recommendation | It is recommended that pediatric cardiac arrest survivors be evaluated for rehabilitation needs | 1 | C-LD |
| | No Recommendation | It is reasonable to refer pediatric cardiac arrest survivors for ongoing neurological evaluation for at least the first year after cardiac arrest | 2a | C-LD |

*Class of recommendation (COR): 1: strong (Benefit>>>Risk); 2a: moderate (Benefit>>Risk); 2b: weak (Benefit³Risk); 3: no Benefit (Benefit=Risk); 3: harm (Risk>Benefit). Level of evidence (LOE):A-high quality; B-R: moderate from randomised trials; B-NR: moderate from nonrandomised data; C-LD: data with limitations of design or execution; C-EO: consensus of expert opinion.*

Sepsis Campaign (SSC) Guidelines 2020 recommend smaller volume boluses of 10-20 ml/kg, upto 40-60 mL/kg in the first hour of resuscitation, where intensive care resources are available. In settings with a lack of access to intensive care, SSC recommendations differ; in the absence of hypotension (compensated shock), fluid boluses are not recommended but if hypotension is present, 10-20 mL/kg bolus may be administered with close monitoring and utmost caution [5].

***Inotropes during septic shock***: It is reasonable to use either epinephrine or norepinephrine as a vasoactive infusion in septic shock. This is the first time the AHA has made such a recommendation for use of inotropes, specifically for septic shock. This is based on two important trials demonstrating superiority of epinephrine over dopamine in pediatric septic shock. The American College of Critical Care Medicine recommended use of either epinephrine or norepinephrine in septic shock depending on its 'cold' or 'warm' nature [6]. The distinctions into warm and cold shock have since been abandoned by newer guidelines [5]. Norepinephrine, however, has been found to be safe and effective as a first line agent in pediatric septic shock.

## INTRA-ARREST CARE

### Advanced Airway During CPR

It has been seen in one prospective and 2 retrospective studies that endotracheal intubation and bag-mask ventilation (BMV) have comparable outcomes in out-of-hospital cardiac arrest (OHCA) in children [7]. Similar comparative data is however not available for in-hospital cardiac arrest (IHCA).

### Respiratory Rates with an Advanced Airway

While formulating the previous guidelines, there was lack of evidence to support the use of respiratory rates different from those recommended in adults. Since then, newer evidence has emerged that higher respiratory rates may improve survival in children undergoing CPR, with a rider that overventilation may lead to hypotension [8]. Thus, rescue breaths should now be given at the rate of one breath every 2-3s (20-30/min) when an advanced airway is in place and while performing chest compressions.

### Cuffed Endotracheal Tubes (ETT)

The guidelines suggest that it may be reasonable to use cuffed over uncuffed ETT in infants and children. This is based on evidence that cuffed tubes improve ventilation and reduce the incidence of ETT changes, leading to lesser trauma [9]. Care should be given towards choosing the appropriate size and maintaining cuff pressures <20-25 cm H2O.

### Cricoid Pressure

Contrary to the previous guidelines, which recommended routine use of cricoid pressure (unless the maneuver

interferes with ventilation) in unresponsive children, the current guidelines recommend it in select cases primarily to prevent stomach insufflation. Routine use can hamper visualization during laryngoscopy and BMV. Newer data has shown that cricoid pressure during intubation and ventilation did not result in lower rates of regurgitation while decreasing success rates for first-attempt intubation.

### Early Epinephrine

It has been established in past guidelines that epinephrine has an important role in improving coronary and cerebral perfusion during CPR. The newer guidelines put a renewed emphasis on the timing of administration of epinephrine. Early (<5 min) administration of epinephrine from the start of chest compressions in pediatric cardiac arrest was associated with improved outcomes in multiple recent multicenter data [10,11].

### Use of Invasive BP monitoring during CPR

It has been suggested that in patients with invasive arterial BP monitoring lines in place, it is reasonable to use diastolic BP as a guide for quality of CPR. This recommendation stems from the evidence that a DBP >25 mmHg in infants and >30 mm Hg in children during CPR was associated with greater chances of survival to hospital discharge and better neurological outcome.

### Opioid Related Cardiac Arrest

Updated guidelines emphasize the importance of routine CPR protocol over naloxone use in opioid related cardiac arrest. This arises from lack of evidence for benefit of naloxone in opioid related cardiac arrest. Once CPR has been initiated as per protocol, it is reasonable to administer naloxone in suspected or confirmed opioid related cardiac arrests.

## POST-ARREST CARE

Achieving Return of Spontaneous Circulation (ROSC) is just the beginning for the healthcare providers. Following ROSC, the patient moves into the "Post Cardiac Arrest Syndrome" which includes ischemia and reperfusion injury to organs along with persisting pathophysiological derangement related to inciting trigger.

### Targeted Temperature Management (TTM)

The guidelines bring into the main fold, the focused update issued in 2019 about TTM. Continuous core temperature management is recommended for post cardiac arrest patients (Both IHCA and OHCA). Hyperthermia should be strictly prevented. Either hypothermia followed by normothermia or only normothermia had similar outcomes at 1 year in 2 pediatric RCTs [12,13] and hence either may be used.

### Hemodynamic Monitoring and Ventilation

The guidelines strongly advocate use of invasive arterial BP for post cardiac arrest monitoring when available and to maintain SBP >5$^{th}$ percentile for age. The recommendation comes from the fact that BP is labile in the period following ROSC and intermittent NIBP may be unreliable. While ventilating patients post ROSC, it has been re-emphasized that normoxemia and normocarbia should be maintained. Hyperoxemia (Spo2 100%) was not associated with better outcomes and therefore targeting SpO2 of 94-99% may be more prudent.

### Neuromonitoring and Seizure Treatment

When available, continuous EEG monitoring is recommended following ROSC as evidence has shown that non convulsive status epilepticus (NCSE) is common in these children. It has also been seen that children with clinical or non-convulsive seizures following ROSC have worse outcomes. However, no recommendation has been made regarding prophylactic use of AEDs in children without clinical or non-convulsive seizures.

### Prognostication Following Cardiac Arrest

Certain EEG patterns have been seen to be associated with favorable (Sleep spindles, normal background, reactivity) and poor (burst suppression, flat/attenuated) outcomes but the sensitivity and specificity are not high enough to recommend use of isolated EEG for prognostication [14]. Multiple factors including but not limited to EEG, neuroimaging and biomarkers should be taken into account for prognostication. In the absence of robust data, one should avoid being dogmatic while predicting outcomes following cardiac arrest.

### Recovery – A New Addition to the Chain of Survival

One of the major changes in the new guidelines is the updated chain of survival. It has been recognized that IHCA and OHCA have very different outcomes and different chains of survival have been formed for them. Both these chains now have a new sixth component which is 'recovery'. Cognitive, neuropsychological and physical impairments continue post discharge [15]. Ongoing assessment and support following hospital discharge is essential for improving long term outcomes in these children.

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# Seroprevalence to SARS-CoV-2 Among Healthcare Workers in an Exclusive Pediatric Hospital

Healthcare workers (HCWs), who are the first line in managing the pandemic of coronavirus disease (COVID-19), have been observed to be at a greater risk of infection [1]. Seroprevalence studies can provide relevant information on the proportion of people who have experienced a recent or past infection. Such studies performed among HCWs can provide information regarding the risk of exposure in a hospital setting, and also about the effectiveness of infection control strategies, including the proper use of personal protective equipment (PPE). Although, several studies have described the seroprevalence among HCWs [2-4], data from an exclusive pediatric hospital is lacking. We attempted to estimate the prevalence of IgG antibodies against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) among HCW, in a private pediatric hospital setting, and to correlate the seroprevalence based on their risk of exposure.

Our hospital is a tertiary care pediatric hospital and is a designated COVID treatment facility since March, 2020. There is regular surveillance of healthcare workers in our institute and quarantine of HCWs with exposure/symptoms were done as per guidelines by the Ministry of Health and Family Welfare [5]. This study was approved by the institutional ethics committee. HCWs who consented to the study were recruited. Sample collection was done between 30 July and 7 August, 2020. We used Indian Council of Medical research (ICMR) approved YHLO SARS- CoV-2 IgG antibody titer assay kit (Shenzhen YHLO Biotech Co. Ltd), and titers above 10 AU/mL were considered significant. Health care workers were split into two groups, High Risk and Low Risk [2]. HCWs in direct contact with a suspected or confirmed case of COVID-19 were categorized as high risk, and those not in direct contact were categorized under the low-risk group.

Out of the 466 HCWs in our hospital, 95 [41 doctors, 39 nurses, and 15 others (laboratory technicians, nursing orderlies)] participated in the study. Sixteen (16.8%) tested positive for SARS CoV-2 IgG antibodies. Of the 16 seropositive HCWs, 12 (75%) reported symptoms compatible with COVID-19 in the past, and 4 (25%) were asymptomatic. The majority of the seropositive were doctors ($n$=8, 19.5% of tested), followed by nurses ($n$=7, 17.9% of tested), with one laboratory person testing positive.

All 12 symptomatic HCWs underwent RT-PCR for SARS-CoV-2 and 9 were positive. Among the 95 HCWs participating in the study, 36 HCWs were grouped under high-risk. The proportion of those with positive serology was not significantly different between the two groups (13.8% vs 18.6%; $P$=0.75).

In our study, we noted 16.8% seropositivity to SARS-CoV-2 among HCWs. The seroprevalence rates may vary depending on various factors such as awareness and implementation of proper infection control strategies, access to PPE, and community prevalence of the virus. Interestingly we observed that the proportion of seropositivity among HCWs in a high-risk setting was not significantly different than those working in a low-risk setting. The seroprevalence of 16.8% to SARS CoV 2 among our HCWs is nearly similar to the recently reported community seroprevalence (21.5%) in Chennai [6]. We speculate that this might be because HCWs tend to take more precautions in a high-risk exposure setting and the strict implementation of the WHO protocol for PPE [7] in our SARI wards and fever triages. In an earlier observation by Hunter, et al. [8], HCWs with the most exposure to COVID-19 patients were not at higher risk for developing antibodies than HCWs with little to no work-related COVID-19 exposure.

There are a few limitations to our study. We included consenting HCWs in our study rather than by randomization, which could have resulted in those with prior symptoms or exposure volunteering to get tested. We did not assess the source of infection or contact in those who were seropositive. To the best of our knowledge, this is the first study on seroprevalence among HCWs in an exclusive pediatric hospital.

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# Digestive Tract Injuries Caused by Ingested Foreign Bodies Containing Magnets

We report on 16 children with ingestion of magnetic foreign bodies, who were identified by a medical record review of our hospital data for the time period between January, 2017 and May, 2018. Digestive tract wall was sandwiched in 13 (75%) children and 11 (74%) had gaptic intestinal perforation.

**Keywords:** *Toys, Unintentional injuries.*

The ingestion of magnetic foreign bodies in children often requires urgent management, which is quite often surgical [1]. A retrospective analysis was conducted on 16 patients (10 males), who were admitted to our hospital from January, 2017 to May, 2018, and presented with digestive tract injuries caused by magnetic foreign bodies ingestion. The age of these patients ranged within 2-9 years old (median: 4.6 ± 0.5 years old), with 6 (37.5%) infants and 6 (37.5%) infants.

An abdominal *X*-ray was used to detect the foreign bodies. Appropriate measures were taken for the removal of these foreign bodies. Two patients had removal using foreign body forceps, three patients underwent removal surgery with the use of magnetic attraction, (cylindrical magnets were bound at the end of the snare), and 11 patients underwent laparotomy and repair of the digestive tract.

The foreign bodies were all magnetic components, and 50% of these foreign bodies were buckyballs. The digestive tract walls of 13 patients (81.2%) were sandwiched by the attraction of magnetic foreign bodies, and were injured. Gastric intestinal perforations presented in 11 patients (68.8%), while one patient had 20 magnetic foreign bodies located in seven different sites of the small intestine, causing seven perforations in the small intestine.

Abdominal pain (31.2%), vomiting (12.5%), or both (37.5%) were the commonest complaint. In one patient, a tiny "gap" (**Fig. 1**) in the middle of the foreign body was noted by *X*-ray, and the small intestine walls were sandwiched by the attraction of two small magnetic foreign bodies, resulting in the perforation of the small intestine. All patients had a satisfactory outcome. Five patients underwent gastroscopic removal of foreign bodies and 11 patients underwent laparotomy for removal surgery.

With magnetic foreign bodies, gastrointestinal pressure necrosis between foreign bodies and the formation of fistula can occur [2]. The diagnosis of magnetic foreign bodies in the digestive tract is mainly dependent on the medical history, and the abdominal *X*-ray. Both anteroposterior and lateral *X*-ray films of the abdomen must be combined with careful examination, in order to determine whether a tiny 'gap' in its middle is present for the single metal foreign body in a fixed position of the digestive tract. Therefore, for multiple small adhesive metal foreign bodies revealed by an abdominal *X*-ray film, it should first be considered whether these are connected by the attraction among these magnetic foreign bodies, and whether the digestive tract walls are sandwiched by the attraction of these foreign bodies.

Upon magnetic approximation (when more than one foreign body is ingested), a considerable amount of force can result in inseparable magnetic attraction between bowel loops [3], which would rapidly result in the necrosis and perforation of the intestines.

Conservative treatment may be appropriate for patients who have ingested a single foreign body [4,5]. Although foreign body forceps under a gastroscope cannot effectively grasp these foreign bodies, based on the experience of the investigators, this can be performed as long as the connection point between the two magnetic foreign bodies formed by the magnetic attraction is grasped by the foreign body forceps.

Magnetic foreign body ingestion has typical features on the abdominal *X*-ray and early laproscopic/surgical intervention leads to a good outcome.

*Ethics approval:* Ethics Committee of The General Hospital of Ningxia Medical University; No. 2019-388.

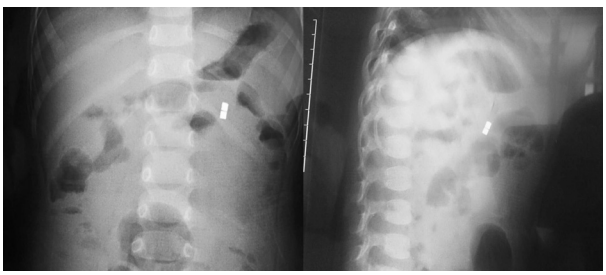

**Fig. 1** The anteroposterior and lateral *X*-ray films of the abdomen, showing a 'gap' among the small magnetic foreign bodies.

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# Pediatric ABO-incompatible Living Related Donor Liver Transplantation: Experience from Indian Subcontinent

We present our experience with pediatric ABO-incompatible liver transplantation in India. Data of patients <18 years of age undergoing ABO-incompatible liver transplantation our hospital between January, 2011 and November, 2018 were analyzed. Plasmapheresis was done pre-transplant till antibody titer was <16 units. Rituximab/Intravenous immunoglobulin was used for immunosuppression, in addition to standard drugs (mycophenolate mofetil, steroids, and tacrolimus). Out of 203 patients that underwent liver transplant during this period, 8 underwent ABO-incompatible liver transplantation; 4 (3 boys) had blood group O+ve. Median (range) age was 28 (7-91) mo, PELD score was 24.5 (14-42), and pre-transplant antibody titer range was 1:32-1024. Number of plasmapheresis sessions required ranged from 1-6. Post-operatively two patients had rise in antibody titer >64 requiring plasmapheresis. All 8 patients survived without rejection/biliary issues. Mean (range) of post-transplant hospital stay was 19.1 (13-22) d and follow-up period was 38.1 (7.1-84.4) mo. Pediatric ABO-incompatible liver transplantation can be successfully performed using plasmapheresis with optimal immune-suppression and vigilant post-op monitoring.

**Keywords:** *Immunoadsorption, Outcome, Plasmapheresis, Rituximab*.

Due to shortage of cadaveric organ donation, living donor liver transplantation (LDLT) is the primary form of liver transplantation (LT) in India. In the LDLT scenario, donors are restricted to family members and it is not always possible to find a healthy blood group-compatible donor in time. Liver transplant across the ABO blood group barrier is a promising approach in such patients. Antibody-mediated rejection along with biliary and vascular complications are the usual limiting factors in ABO-incompatible (ABOi) LT [1]. Determining optimal immunosuppression to avoid complications in ABOi LT is challenging. Desensitization with use of plasmapheresis and anti-CD20 monoclonal antibody (rituximab) plays an important role in successful outcome of ABOi LT [2]. Our aim was to study the course and outcomes of pediatric ABOi LT.

Data of all patients (<18 years) undergoing ABOi LDLT at our hospital, India between January, 2011 and November, 2018 were retrospectively analyzed. ABOi LT was performed only in cases where compatible related donors were unavailable even after exploring option of swap transplant between two families. Informed consent was taken for ABOi LDLT. Hospital liver transplant committee clearance was obtained. Demographic details, primary diagnosis and severity of liver disease using Pediatric end-stage liver disease (PELD) score were noted. Recipient and donor blood group and pre-transplant antibody titres were recorded. Surgical details and post-operative course including antibody titres, duration of ICU and hospital stay, incidence of hemolysis, rejection, biliary and vascular issues and infections were recorded.

For immunosuppression, all patients were started on mycophenolate mofetil (MMF) one week pre-transplant. After 2016, rituximab was added to the institutional protocol for pre-transplant preparation CD19 levels were in those receiving rituximab monitored before and post LT. Plasmapheresis was done pre-LT on alternate days to reduce antibody titre16. Prior to 2012, conventional plasmapheresis with AB blood group fresh frozen plasma was done. Thereafter with the availability of 2A column filter (Evaflux 2A column; Kawasumi Laboratories) at our institution, cascade plasmapheresis was performed. In cases where conventional/cascade plasma-pheresis failed to decrease antibody titre <16 or when urgent LT was needed, plasmapheresis with immune-adsorption technique using Glycosorb/ Adsopak filter was done. Prior to 2016, intravenous immunoglobulin (IVIG) was given for first five days post-operation to prevent antibody-mediated rejection. After 2016, IVIG was no longer used prophylactically, but reserved only for treatment of antibody-mediated rejection. Post-operatively triple immunosuppression with MMF, steroids and tacrolimus was administered. Target trough level of tacrolimus was 10-12 ng/mL in the first month post-LT. Antibody titers were closely monitored and the threshold to do plasmapheresis was a titer ≥64 for up to two weeks post-LT.

A total of 203 pediatric LDLT were performed at our institute during the study period; 8 these were ABOi LT. All 8 (3 males) patients had underlying cholestatic liver disease of which 5 had biliary atresia. Median (range) age was 28 (7-91), months and median (range) PELD score was 24.5 (14-42). Four of the 8 recipients had blood group O, two each had blood group A and B. Pre-transplant baseline antibody titer ranged from 32 to 1024 units. Mean (range) pre-transplant hospital stay was 12.5 (1-44) days. Number of pre-transplant plasmapheresis sessions ranged from 1-6 and was proportional to the initial titer. Conventional plasmapheresis was used in the first two patients, prior to 2012. Thereafter, cascade plasmapheresis was used for 5 cases; immune-adsorption was used in 2. One patient had high initial antibody titer of 1024 units which reduced to 256 units after two sessions of cascade plasmapheresis. There was a subsequent rise of antibody titer to 1024 units during an episode of sepsis. After recovery, three sessions of immune-adsorption plasmapheresis decreased antibody titer to 8. Another patient underwent ABOi transplant in limited preparation time with a domino graft using the explanted liver of a child of maple syrup disease undergoing liver transplant using immune-adsorption plasmapheresis. A single cycle decreased the antibody titer from 128 to 4 units. IVIG was used in first 3 cases, prior to 2016 and rituximab was used thereafter in 3 patients. Two of these were between 2-8 years with risk factors of high initial antibody titer (> 256) and re-transplantation.

Post-transplant, mean (range) ICU stay was 7.3 (5-11) days and post-transplant hospital stay was 19.1 (13-22) days. Post-operatively two patients had rise in antibody titer up to 256 (on sixth day) and 64 (on seventh day), respectively which required 4 and 3 sessions of cascade plasmapheresis to reduce antibody titer <32. One patient developed *E. coli* sepsis which responded to antibiotics. Two patients developed vascular complications. No biliary or bowel complications were noted. Two patients developed hemolysis (peripheral smear changes, LDH >1000 U/L and reticulocytes >2%) which resolved spontaneously. All eight patients survived without any evidence of acute/chronic rejection. The mean (range) follow-up period was 38.1 (7.1-84.4) months.

Pediatric graft survival rates were reported similar after ABOi LT and blood group compatible LT, whereas graft survival in adults after ABOi LT were lower [4]. Antibody-mediated rejection is a catastrophic complication which can occur in the first 2-4 weeks. The risk of ischemic cholangitis, bile leak, biliary stricture formation, and hepatic artery thrombosis is higher in ABOi LT [5]. The approach in ABOi LT is directed towards reducing antibodies pre-transplant and inhibiting its production post-transplant for at least 2-4 weeks by effective immunosuppression [6].

Plasmapheresis is the most effective way to control humoral antibody response to prevent rejection [7]. We used all the methods of plasmapheresis: conventional, cascade and immune-adsorption. IVIG inhibits complement and T-cell-mediated graft injury by FC-receptor-dependent B-cell apoptosis [3]. With availability of effective drugs like rituximab the use of prophylactic IVIG has become less relevant. However, IVIG is more effective for treating antibody-mediated rejection as exerts faster effects than rituximab [3]. We used CD19 as a surrogate marker for patients who received rituximab as it mirrors the expression of CD20 [8].

Recipient blood group O is the most susceptible group antibody-mediated rejection [9]. Although, half of our patients had blood group none of them developed antibody-mediated rejection or biliary complications probably due to optimal immunosuppression. The vascular complications seen in two patients were diagnosed early and timely surgical management prevented any permanent hepatic damage, thus emphasizing the importance of vigilant postoperative monitoring.

Our experience in pediatric ABOi LT suggests that it is a promising alternative in India when compatible graft donor is unavailable.

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# Fulminant Anti-MOG Encephalitis in a 5-Year-Old Child

Anti-myelin oligodendrocyte glycoprotein (MOG) antibody disease has been recently implicated as a major etiology for pediatric non-infectious encephalitis [1]. We report a case of acute fulminant encephalitis in a 5 year old child mimicking viral encephalitis, which was eventually diagnosed as anti-MOG antibody encephalitis leading to successful treatment with immunotherapy, thus highlighting this condition as an important differential diagnosis for acute viral encephalitis.

A 5-years-old previously normal Indian girl presented with intermittent fever for 4 days, headache for 2 days followed by encephalopathy. On admission, she was afebrile with normal vital signs and a Glasgow coma scale of 12. Subsequently her GCS deteriorated to 8 within 12 hours necessitating mechanical ventilation, and she developed signs of raised moderate intracranial hypertension and relative bradycardia. Neurological examination showed sluggish brainstem reflexes including pupillary and occulocephalic reflex, brisk deep tendon reflexes and bilateral extensor plantar response. There was no history of seizures, abnormal movements, visual disturbances, bowel or bladder dysfunction, or preceding altered sleep or behavior. There was no history of contact with tuberculosis or recent travel. There was no history of drug ingestion or any toxic exposure. The initial working diagnosis was acute febrile encephalopathy with differential diagnosis of viral encephalitis, acute demyelinating encephalomyelitis (ADEM) or neuro-metabolic disease with acute decompen-sation. Emergent neuroprotection measures and a combination of broad spectrum antimicrobials including Acyclovir were instituted.

MRI brain showed extensive asymmetrical high signal changes involving bilateral cortical regions along with ventromedial thalamus with extensive diffusion restriction suggestive of cytotoxic edema (**Fig. 1**). Post contrast study showed no evidence of parenchymal or leptomeningeal enhancement. MRI of spine and MR angiogram were normal. On day 2 of admission, patient developed focal seizures with EEG showing bilateral periodic lateralizing epileptiform discharges (PLEDs) highly suggestive of a neurotropic viral encephalitis. Due to the patient's clinical condition, cerebrospinal fluid (CSF) was deferred until the second week and was unremarkable including negative routine studies, culture and PCR for neurotropic viruses. The patient remained deeply encephalopathic and ventilator dependent for the next two weeks with no response to treatment including acyclovir. In the second week of admission she developed stereotypical flinging repetitive asymmetrical lower limb movements, prompting a trial of methyl prednisolone and immunoglobulins alongside autoimmune and lupus workup including CSF and serum anti-NMDAR (N-methyl-d-aspartate receptor) antibodies and oligoclonal bands which were negative. However, there was no significant clinical improvement. This led to the counselling of the family regarding an acute fulminant encephalitis with poor neurological outcome and need for tracheostomy. After review of the recently published case series by Armangue, et al. [1], serum anti-MOG antibodies (IgG) immunofluorescence test was sent and showed a positive titer of 1:40. Plasma Exchange was then instituted with a total of 5 cycles on alternate days. After the second cycle of plasma exchange itself, the patient showed rapid clinical improvement in the form of improved GCS and was successfully weaned off from ventilation. At the time of discharge two weeks later, the patient could walk unsupported and talk in simple sentences. On follow-up at 4 months after the illness, the patient remained clinically well, and had regained pre-illness developmental status. Her repeat anti-MOG titers were negative and repeat neuroimaging showed no active lesions.

During the last decade, anti-MOG antibodies have been implicated in a wide range of pediatric acquired demyelinating syndromes including optic neuritis, myelitis and ADEM [2]. However a recent multicenter observational study by Armangue, et al highlighted the widening spectrum of anti-MOG antibodies disease [1], demonstrating it as the commonest etiology of non-infectious, non-ADEM encephalitis in the pediatric age group, in addition to the well-established presentations mentioned above. Of the 116 anti-MOG antibody positive patients recruited in the study, 22 (19%) were diagnosed as encephalitis other than ADEM, thus making it an important differential diagnosis in pediatric encephalitis. The clinical presentation included encephalopathy, seizures, status epilepticus, fever, abnormal behavior, motor deficits, abnormal movements and brainstem-cerebellar dysfunction. MRI showed non-ADEM like findings including extensive bilateral cortical involvement (55%) with or without basal ganglia involvement and meningeal enhancement. Another recent publication by Budhram, et al. coined the term FLAMES based on a distinct radiological pattern of unilateral cortical FLAIR-hyperintense

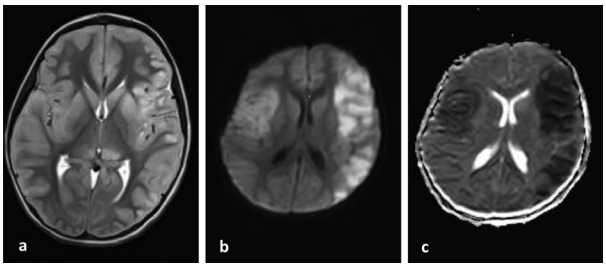


**Fig. 1** a) MRI T2-weighted image showing bilateral asymmetrical (left more than right) cortical high intensity signal with cortical swelling along with high signal in the ventromedial thalami, b) MRI diffusion weighted image (DWI) and c) apparent diffusion coefficient (ADC) images showing bilateral cortical cytotoxic edema in the form of diffusion restriction and corresponding drop in the ADC values.

lesions in adult anti-MOG encephalitis with seizures [3]. Our case had clinical features of encephalopathy, brainstem dysfunction and abnormal movements while MRI brain showed extensive bilateral cortical and basal ganglia involvement with diffusion restriction but without meningeal enhancement. These observations were in keeping with the findings described in the study by Armangue, et al. [1]

We conclude that anti-MOG encephalitis should be considered in a child with fulminant encephalitis with bilateral cortical involvement with diffusion restriction on MRI, with or without meningeal contrast enhancement. Steroids should be the first line treatment for anti-MOG encephalitis, and usually shows a good response. However, early plasma exchange should be considered in cases with inadequate response to pulse steroids.

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# Video-Assisted Thoracoscopic Surgery (VATS) in a 20-Day-Old Newborn With Empyema Thoracis

Pleural empyema is a known complication of bacterial pneumonia in childhood; however, it has been reported very rarely in neonatal population. The management of empyema in neonates has been either conservative with intravenous antibiotic therapy or conventional with tube thoracostomy [1]. Here, we report use of video-assisted thoracoscopic surgery (VATS) in a 20-day old neonate with staphylococcal pneumonia.

A 20-day-old male newborn was admitted to our hospital with a two-day history of fever, irritability and vomiting. The infant was born at term gestation via cesarean section with unremarkable antenatal history. Except for tachypnea, his clinical examination was normal. Chest *X*-ray done at admission was normal, and he was managed with intravenous fluids and antibiotics. Investigations revealed normal white cell counts but C-reactive protein (CRP) was significantly elevated (287.8 mg/L). Over next 24 hours, the infant developed respiratory distress with reducing oxygen saturation, and chest *X*-ray revealed dense homogenous opacification of left hemithorax. Ultrasonography of chest was suggestive of left lower lobe consolidation with no pleural collection. Infant was commenced on continuous positive airway pressure (CPAP) support. The blood culture grew methicillin-resistant *Staphylococcus aureus* (MRSA) and antibiotics were changed to intravenous vancomycin as per antibiotic sensitivity pattern. Chest *X*-ray on day 4 showed opacification of left hemithorax with heart and mediastinum pushed towards right. High resolution computed tomography (HRCT) of chest revealed gross mediastinal shift to the right with collapse of the lung medially and multiple septation running radially outwards dividing significant empyema collection in multiple loculi.

The infant underwent video-assisted thoracoscopic surgery (VATS); where evacuation of empyema fluid, debridement with breaking of multiple loculi and decortication of thickened parietal and visceral pleura was performed. Pus culture showed growth of MRSA with similar antibiotic sensitivity as in blood culture. Intercostal drain was removed on third post-operative day and infant was weaned from CPAP to room air. Infant had significantly improved left sided chest air entry and serial postoperative chest *X*-rays suggested well expanded lungs. Antibiotics were stopped after a total duration of 14 days. Investigations for primary immunodeficiency for the infant were normal. Both the infant's and parents' nasal swabs were negative for MRSA colonization. On follow-up, the infant was asymptomatic and chest radiograph after a month was normal.

Thoracoscopic decortication by early VATS as a first-line treatment for pediatric empyema has shown to reduce the mean length of hospital stay by around 6-8 days, compared to tube thoracostomy [2]. Even though VATS has been established as one of the standard modalities for the treatment of pleural empyema in pediatric population, its usage has not been reported for the same in neonates. The youngest child reported to undergo VATS for post-pneumonic empyema was older than one month, and it was used as a rescue measure for empyema that was refractory to medical response [3]. Non-availability of small sized instruments, technical limitations in suturing very small thoracic cavities and injury to surrounding tissues have been reported as an impediment for using VATS routinely in neonates [4,5]. However, we did not have any of above complications in our case. The conventional duration of antibiotic therapy in cases of empyema has been three to four weeks. In this case, we administered antibiotics for two weeks only as thoracoscopic debridement was performed early and there was clinical as well as biochemical recovery.

Staphylococcal empyema has become much more common after the introduction of pneumococcal conjugate vaccine for infants [6]. Considering the widespread prevalence of MRSA in children and adult population, more cases from neonatal population are likely to be reported as pneumococcal vaccination increases as well. We, herein suggest that while

intravenous antibiotics and catheter drainage remain the mainstays of treatment of neonatal empyema, VATS can be safely considered as a primary treatment modality to promote earlier recovery and shorten antibiotic therapy.

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# Varied Clinical Manifestations of LRBA Deficiency (Immune Dysregulation Disorder)

Inborn errors of immunity or Primary immune deficiencies (PIDs) are a heterogeneous group of disorders affecting several components of immune system, and the number of genetically defined PIDs is currently estimated to be more than 400 [1].

LRBA (LPS-responsive beige-like anchor protein) is a cytosolic protein which participates in polarized vesicle trafficking and turnover of CTLA4 (cytotoxic T-lymphocyte-associated protein 4) receptor in regulatory T (Treg) cells [2]. *LRBA* deûciency is an autosomal recessive disorder caused by biallelic mutations in the *LRBA* gene leading to immune dysregulation. The clinical spectrum has now been extended to include autoimmune lymphoproliferative syndrome (ALPS), cytopenia and lymphoproliferation [3]. Although initially categorized under common variable immunodeficiency (CVID), the International Union of Immunological Societies Expert Committee of Inborn Errors of Immunity has now classified LRBA deficiency under Diseases of immune dysregulation - Regulatory T cell defects [4]. In clinical practice immunodeficiency is always entertained in any recurrent infections but seldom in children presenting with autoimmunity and malignancy.

We present a series of three children with LRBA deficiency treated at tertiary care centres in Southern India. This case series is to make people aware of its presence in our community and its varied presentations.

**Case 1:** An 18-month-old male, born to consanguineous parents was evaluated for prolonged fever, respiratory distress, severe anemia, and thrombocytopenia for 4 months. He was undernourished, had generalized lymphadenopathy and hepatosplenomegaly. Blood picture was suggestive of hemolytic anemia and thrombocytopenia. Direct Coombs test was strongly positive and immunoglobulin profile was normal. Flowcytometry showed elevated double negative T cells consistent with ALPS. Imaging of lungs showed diffuse bilateral ground glass opacities consistent with interstitial lung disease. The child was treated with intravenous immunoglobulins and prednisolone with partial response. In view of inadequate response, PID was suspected and clinical exome sequencing done. It showed a homozygous two base pair deletion in exon 29 of the *LRBA* gene (chr4:151752970_151752971delAG; Depth:75x) resulting in a frameshift and premature truncation of the protein at codon 1576 (p.Ser1576Ter;ENST00000357115) – pathogenic. He was started on immunomodulators – sirolimus, hydroxychloroquine and fortnightly abatacept (10 doses in total). His transfusion requirements decreased along with regression of liver and spleen size. The child is awaiting a hemopoietic stem cell transplant (HSCT).

**Case 2:** A 5-year old male, born to consanguineous parents, was evaluated for chronic diarrhea since 7 months of age. His elder sibling had similar illness and had died at 3 years of age. Infectious causes for diarrhea were ruled out. Gut biopsy was suggestive of autoimmune enteropathy. He responded partially to steroids with intermittent flare up of enteropathy and hence, started on azathioprine. The child presented to us with abdominal distension, dyselectrolytemia and features of hyperperistalsis. Immunoglobulin profile was normal, while lymphocyte subset analysis showed reduced CD19+ B-lymphocytes. Clinical exome sequencing showed *a* homozygous missense variation in exon 6 of the *LRBA* gene (chr4:g.151837793T>C; Depth: 54x) resulting in substitution of Glycine for Aspartic Acid at codon 248 (p.Asp248Gly; ENST00000357115.3 – pathogenic; and another homozygous missense varation in exon 30 (chr4:g.151749420C>G; c.5083G>C; p.Val1695Leu) – of uncertain significance. The same mutations were detected in heterozygous state in his parents and elder sibling. The child underwent matched sibling HSCT successfully. Enteropathy resolved post-transplantation.

**Case 3**: A 5-year-old girl, born to consanguineous parents, was

evaluated for chronic diarrhea and failure to thrive since 3 months of age. She had a history of neonatal hepatitis at 2 months and infective spondylodiscitis at 11 months of age. Colonoscopic biopsy was suggestive of autoimmune enteropathy. Basic immunological workup including immunoglobulins and lymphocyte subsets were normal. Clinical exome sequencing showed a homozygous termination mutation in exon 4 of *LRBA* gene (c.544C>T; p.Arg182Ter) – (likely pathogenic). The child is awaiting HSCT.

This is likely the first reported case series of LRBA protein deficiency from our country. Our cohort of children presented with autoimmune enteropathy or cytopenia rather than severe infections. Since there was inadequate response to first line immunosuppression, clinical exome sequencing was done which confirmed the diagnosis. The mutation reported in patient 1, and one of the mutations in patient 2 (Val1695Leu) have been found to be novel on literature search. The other mutation in patient 2 and patient 3 have been previously reported [5,6].

There is no standard therapeutic approach to LRBA deficiency yet. Glucocorticoids and Sirolimus have been used historically. Recently, Abatacept, a CTLA4-fusion protein, has been introduced as a promising agent [7], which is used as a bridging option pending HSCT. HSCT appears to be an effective therapeutic option. Tesch, et al. [8] reported an overall survival 70.8% among 24 patients that underwent HSCT. Higher disease burden, longer duration before HSCT, and lung involvement were associated with poor outcome. A recent systematic review [9] of 109 cases of *LRBA* deficiency autoimmunity (82%), enteropathy (63%), splenomegaly (57%) and pneumonia (49%) as the most common clinical manifestations.

LRBA defects should be suspected in any child presenting with autoimmune manifestations of unusual severity, multisystem involvement, with presence of consanguinity, sibling death or unresponsiveness to first line immune suppressants. Early diagnosis and HSCT could be life-saving.

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## A Toddler With Pain Abdomen and Pica: The 'Concrete' Evidence!

30-month-old boy presented with intermittent pain abdomen of three days duration. There was no fever, vomiting, diarrhea, dysuria or constipation. In between the episodes of pain, the child was comfortable. Vital signs and systemic examination, including per rectal examination, was normal. A plain *X*-ray abdomen in erect posture revealed multiple, radio-opaque shadows in the small intestine, colonic and the rectal area. The opacities were of different size and shape but of uniform density (**Fig. 1**). On further querying, mother admitted that the child has a habit of scrapping the old wall and eating small pieces of loose concrete material that used to fall off! The child was given liberal amount of oral fluids, soft diet, oral lactulose after hospital admission. After passage of stools thrice, on day 3, repeat *X*-ray abdomen showed complete clearance of the shadows. He had mild microcytic hypochromic anemia. Peripheral blood smear did not show any basophilic stippling. Blood lead assay could not be carried out. Parents were counselled regarding Pica and the provided behavioral modification advice. Child has been under regular follow up since then and is thriving well.

In our case, the morphology of opaque shadows along with the history given by mother helped in a 'concrete' diagnosis! As seen in this case, during follow up, such clear corroborative diagnosis can motivate the parents to ensure all the preventive steps to avoid consumption of such materials by the child again.

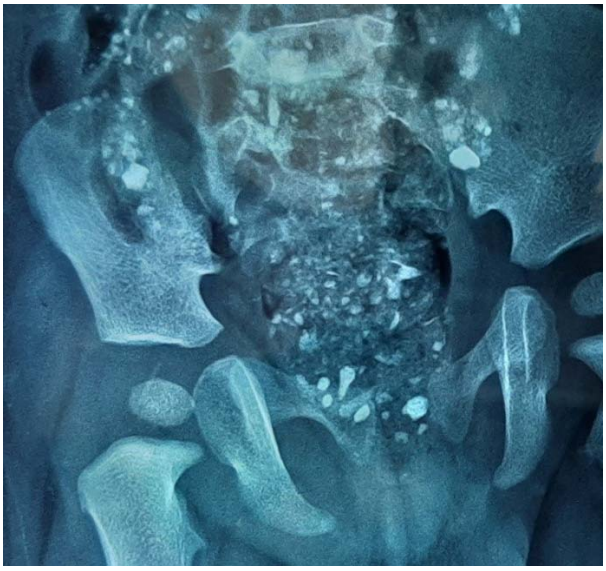

**Fig. 1** Multiple opacities of varying shapes and sizes but of uniform density *X*-ray abdomen.

We share the typical *X*-ray picture to remind the readers of this common condition which is frequently overlooked.

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## Bronchial Dieulafoy Disease with Recurrent Life-threatening Hemoptysis

Bronchial Dieulafoy's disease (BDD) denotes the presence of a vascular anomaly characterized by dilated, tortuous arteries in the bronchial submucosa, causing massive hemoptysis [1]. We present a child with life-threatening recurrent massive hemoptysis due to this disorder.

A 15-year-old boy, previously normal, had fainted after effortlessly expelling about 400 mL of blood and was intubated for airway protection. His hematological workup and coagulation and rheumatologic profile were normal. Upper gastrointestinal endoscopy demonstrated erosive gastritis, and he was treated for *H. pylori*. Ten days after discharge, he had an episode of drowsiness following a bout of cough with massive expectoration of blood. He was rushed to the emergency, where he was intubated, and received two units of packed cells. Computed tomography of chest and pulmonary angiography showed patchy haziness in right lower lobe with no evidence of pulmonary embolism. He was referred to our hospital where he had a bout of massive hemoptysis and became limp. He was resuscitated, intubated, shifted to the pediatric intensive care unit and again required blood transfusion. Bronchoscopy revealed blood clots in right lower lobe. Broncho-alveolar lavage was negative for tuberculosis and fungal infections.

The clinical sequence of repeated massive life-threatening hemoptysis and blood clot on bronchoscopy was suggestive of bronchial artery pathology, possibly bronchial Dieulafoy disease. Selective aortography was done through right common femoral artery, a 4F cobra catheter was advanced into descending thoracic aorta. Selective embolization of hypertrophic tortuous right bronchial artery was performed using poly-vinyl alcohol (PVA) particles of 300-500 μm with complete disappearance of the abnormal right bronchial artery [2]. After embolization, the boy had no further hemoptysis and was doing well on follow-up after 6 months.

The most important step in situation of massive blood loss through the mouth is to first establish that the child is indeed experiencing hemoptysis. The boy was initially treated as hematemesis, and differentiating hemoptysis from hematemesis is critical, as treatment strategies differ markedly [3]. Definition of massive hemoptysis has not been completely agreed upon and vary between 200 and 600 mL as a cut-off, ideally, a volume >200 mL warrants immediate investigation [4]. Although massive hemoptysis is relatively uncommon, mortality rates as high as 75% been reported. The common cause of death is asphyxia secondary to aspiration rather than blood volume loss. Selective bronchial artery embolization is an effective nonsurgical technique for immediate control of massive hemoptysis with a success rate around of 77 - 94% [5]. Bronchial dieulafoy disease is rare disease but known to cause massive hemoptysis, where bronchial artery embolization should be considered as the first-line treatment.

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# The Incompleteness of Incomplete Kawasaki Disease: A Customized Definition Is Needed for Indian Children

We read with interest the Indian Academy of Pediatrics position paper on Kawasaki disease in the journal [1]. It is indeed timely that this statement has come out amidst the coronavirus disease (COVID-19) pandemic and associated multisystem inflammatory syndrome in children. The authors have aimed to present this paper as a practice guideline specific to resource constrained setting like ours. In this context, we have the following comments:

In describing the definition for incomplete KD, the authors have presented the diagnostic approach, which is largely adapted from American Heart Association (AHA) scientific statement on Kawasaki disease [2]. While AHA algorithm considers evaluation for incomplete KD in children with fever ≥5 days and 2 or 3 compatible clinical criteria, the algorithm by Shenoy, et al. [1] triggers KD evaluation if fever ≥5 days is accompanied by less than four compatible clinical features. Although these two statements appear similar, this approach loses specificity by including children who present with fever and just one compatible clinical feature. Individually, the clinical features like rash, lymphadenopathy, conjunctival injection, oral or extremity changes are nonspecific and may occur with various childhood infections in India. This approach risks huge number of children with underlying infections being referred for echocardio-graphic evaluation.

Treatment with intravenous immunoglobulin is recommended if 3 of the 5 laboratory features (anemia for age, platelet ≥450×$10^9$/L, albumin <3 g/dL, elevated alanine aminotransferase, leucocyte count ≥15 ×$10^9$/L, urine >10 WBC/hpf) are met in a child lacking echocardiographic abnormalities. Compared to Western cohorts, these criteria should be carefully defined in a low- and middle-income setting like India, with a high prevalence of iron deficiency anemia [3] and associated thrombocytosis (present in up to a quarter of those with iron deficiency) [4]. Iron deficiency when associated with infection accounted for more than half of all cases of reactive thrombocytosis in Indian children [5]. Given these findings, the current definition is likely to overestimate the burden of incomplete KD in Indian children, risking increased cost and potentially delaying the diagnosis of underlying infections. For example, as per the algorithm, a child with undifferentiated fever ≥5 days due to measles or a rickettsial infection that has a rash, iron deficiency anemia (and associated thrombo-cytosis) and hypoalbuminemia (negative acute phase reactant) would be treated for Kawasaki disease even if the echocardiogram is normal. In the absence of a 'gold standard' for diagnosis, we believe that grading recommendations based on available quality of evidence may be more useful for the readers to make informed decisions [6].

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## AUTHORS' REPLY

We thank the readers for their query on the proposed algorithm for Incomplete Kawasaki disease in children [1]. This algorithm is indeed adapted from the American Heart Association [2], and we agree about the possible differential diagnoses that can be entertained in these children.

We wish to underscore that incomplete Kawasaki disease (KD) is considered in a child who is having ongoing high grade fever with less than four clinical criteria to satisfy a complete diagnosis. The algorithm raises the need for consideration of this diagnosis in a febrile child, particularly a young infant, with an unexplained persistent high grade fever for >5 days with systemic evidence for inflammation and with no other reasonable explanation for the same. The consequences of a delayed diagnosis of KD, especially the atypical presentation, can be devastating, as the risk of coronary aneurysms is higher in these patients. It bodes well to have a high index of suspicion for the same, and encourages the consideration for an echocardiogram in these children which can further aid the diagnosis and the decision to treat. A rickettsial illness can be a differential diagnosis; however, it would normally respond to appropriate therapy and is usually recognized in the endemic areas by the pediatricians. One must appreciate the fact that KD is a diagnosis of exclusion, and this is highlighted in Box 1 as 'exclusion of other diseases with similar findings' [1].

Children with incomplete KD have increased risk for coronary artery aneurysms and the current recommen-dations are meant to ensure a timely diagnosis in these children. Hence, referring children, especially young infants with fever and elevated inflammatory parameters for echocardiography seems prudent, all the more, when they do not respond to the first-line antibiotics. Anemia and thrombocytosis can be noted in iron deficiency and can act as confounders in the diagnosis of incomplete KD. At the moment, we do not have nationwide data to provide new criteria for diagnosis. Studies that collate nationwide data on KD are the need of the hour – these recommendations infact may pave the path for such studies, which shall in turn help us formulate revised definitions for incomplete KD in our setting.

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# Writer's Cramp and Psychosis: An Atypical Presentation of Systemic Lupus Erythematosus

A 9-year-old right-handed girl presented with handwriting deterioration and behavioral issues for five months. Initially, she had painful grip tightening and writing difficulty after writing about ten lines, which resolved on resting. After a month, symptoms emerged immediately on writing few words. Symptom fluctuation, weakness, sensory phenomena, ptosis, unresponsiveness, feeding /bladder/bowel/gait abnormalities were absent. Her behavioural problems included unprovoked crying/laughter, auditory hallucinations and tantrums. There was no history of fever, rash, jaundice, head-injury, toxin-exposure, abuse, family history of neurological illnesses. On examination, child's anthropometry, vital parameters, and systemic examination were normal. After writing 3-4 words, her grip tightened, wrist and interphalangeal joints got flexed, strokes coarsened and slowed, with inability to trace curves. Her left (non-writing) hand had mirror-posturing. Posturing resolved two minutes after discontinuing writing. Percussion myotonia, Trousseau sign and Chvostek's signs were absent. Child was diagnosed with Writer's cramp (WC) and psychosis.

Her hemogram, anti-Streptolysin O titer, electro-encephalogram, MRI brain and electromyography were normal. Workup for Wilson disease and autoimmune encephalitis was normal. ESR: 60 mm; Serum antinuclear antibody: strongly positive (speckled); anti double-stranded DNA (dsDNA) titer: 12 IU/mL (reference <5IU/mL); C3 and C4 levels: 30 mg/dL (reference: 80-200 mg/dL) and 12 mg/dL (reference: 15-40 mg/dL), respectively; anti-phospholipid antibodies: absent. She was diagnosed as SLE (European League Against Rheumatism/American College of Rheumatology criteria 2019 score:15) and administered intravenous methyl-prednisolone (30 mg/kg/day × 5 days) followed by maintenance oral prednisolone, hydroxychloroquine, trihexyphenydyl and physiotherapy. Dystonia and behavioural problems reduced significantly in three months.

WC is a rare task-specific limb dystonia reported in fourth fifth decade of life triggered by writing/typing/playing musical instruments and characterized by wrist flexion/extension, dystonic hand posturing, contralateral limb mirror dystonia and sensory trick phenomena [2]. The entity is extremely uncommon in childhood. The prominent differentials include essential tremor, primary writing tremor, neurodegenerative disorders (like Wilson disease), stroke, focal nerve entrapment and musculoskeletal issues [3]. Its patho-physiology is attributed to parietal-premotor pathway dysfunction secondary to etiologies such as genetic factors (*e.g*,: DYT -1 mutation) and hand trauma [2,4]. It is not reported with SLE. Antibody-mediated phenomena against basal ganglia and frontal lobe probably led to the index child's symptomatology. Treatments include pharmacological therapy, botulinum-toxin, physiotherapy, neurosurgery and assistive devices [3].

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# Heme Oxygenase-1 Deficiency

The oxidation of heme to biliverdin is facilitated by a stress-induced enzyme, heme oxygenase-1. This enzyme has antioxidant properties and plays an important role against inflammation. First case of heme oxygenase-1 deficiency was reported in 1999 [1]. The key features of heme oxygenase-1 deficiency, a recently described disorder, are hemolysis, generalized inflammation, bleeding diathesis, nephropathy and asplenia [1,2].

An 8-month old child presented with fever for 1-month. He was first of nonconcordant twins and the sibling was healthy. On examination, he had dysmorphic facies, frontal bossing, depressed nasal bridge and large ears. General examination revealed pallor and on systemic examination he had hepatomegaly. Investigation revealed increased inflammatory markers (raised CRP 84 mg/L and ferritin 3503 ng/mL), features of hemolysis (raised LDH 5253U/L, SGOT 210 units/L and SGPT 114 units/L), anemia and thrombocytopenia. He was worked up for bicytopenia (EBV, Parvo virus and CMV work up was negative). Other infections like tuberculosis and HIV were also ruled out. Bone marrow examination revealed few hemophagocytes. His initial USG abdomen revealed a normal spleen but on repeat CT abdomen after 6 months, spleen was not seen and only a small focal nodular calcified area within splenic fossa was seen. In view of the unclear primary diagnosis, hemophagocytes on marrow, increased liver enzymes and bicytopenia, he was started on oral steroids, pending further investigations. He improved, fever disappeared and liver enzymes returned to normal but he needed transfusion once in 2-3 months. However, on tapering steroids, fever reappeared and he became pale again. He was readmitted for investigations. USG showed absent spleen. Hemoglobin electrophoresis showed sickle cell trait.

In view of features suggestive of autoinflammatory disorder, transfusion dependent anemia, and auto-splenectomy, clinical exome sequencing was done, which revealed homozygous nonsense variation in exon 3 of *HMOX1* gene (OMIM*141250) which causes human heme hemoxygenase-1 deficiency, confirmed by Sanger sequencing. Both parents were asymptomatic heterozygous carriers of the pathogenic variation detected in our patient.

When comparing our case with previous cases published in literature, we found our child had delayed development, growth retardation and dysmorphic features as reported earlier. He presented with fever but did not have lymphadenopathy or rash as seen in earlier cases. Also, he did not have asplenia from the beginning but had autosplenectomy during the course of treatment. Laboratory features similar in our case to the previous cases were, features of hemolysis (raised LDH and SGOT), raised inflammatory markers (raised CRP, ferritin). Our case is different from previously reported cases as he did not have coagulation abnormalities, features of nephritis or abnormal lipid profile [1-3]. Subsequently our patient developed acute arterial stroke confirmed on MRI and later was transfusion dependent. He succumbed to his illness at the age of 3 ½ years at a peripheral hospital.

Human heme oxygenase-1 deficiency is a disease which is known to be associated with impaired stress hematopoiesis. This results in marked red blood cell fragmentation, intravascular hemolysis, coagulation abnormalities and endothelial damage. This leads to deposits in the kidney and liver. Clinical features include persistent hemolytic anemia, asplenia, nephritis, generalised erythematous rash, growth retardation and hepatomegaly. There is one case report of successful HLA matched stem cell transplantation in the literature.

Though a rare disorder if a patient presents with features of hemolysis, generalised 3 inflammation, bleeding diathesis, nephropathy and asplenia diagnosis of human heme oxygenase

1deficiency should be considered. All the features may not be present as our patient did not have coagulation abnormalities, features of nephritis or abnormal lipid profile. From our case, it is evident that the child had autosplenectomy, whereas literature suggests congenital asplenia.

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# Providing Medical Services Online to Children With Chronic Kidney Disease During the COVID-19 Pandemic

The coronavirus disease 19 (COVID-19) pandemic has thrown up unprecedented challenges for child care the world over [1-3]. In Kerala, government policy entails that children below 10 years are not allowed in public spaces. As government hospitals are handling the majority of COVID-19 cases, child-care areas and child-care professionals in these hospitals have been diverted to adult healthcare.

Thus, children with chronic kidney disease, who require regular follow-up, have been badly impacted. Being immunosuppressed, both due to the inherent nature of their disease, as well as their medications, they cannot attend regular outpatient services at the hospital. The pandemic has forced health professionals and patient-caregivers to find new ways to cope. Guidelines for the same have been published recently [4].

At our center, the follow-up clinic for pediatric renal disease was modified to adapt to the situation. Whatsapp was used to keep in touch with patients and caregivers. The social worker acted as liaison between caregivers and clinicians. Follow-up appointments were given as was usual in non-COVID times, acute problems were assessed via text- and voice-messages, and images, when necessary. Prescriptions were photographed and sent on Whatsapp, as were reports of laborstory investigations and recordings of weight, height and blood pressure. If face-to-face consultation was deemed necessary, it was fixed in the ward or the casualty, and duty resident informed, who also communicated findings via Whatsapp. This ensured that follow-up continued as regularly as in non-COVID times. We handled 633 visits during March-November, 2020 in this manner, as compared to 391 physical visits in whole of 2019.

Expensive or less easily procured drugs were made available using government schemes. This was not easy for children staying in other districts who needed to travel long distances to reach the hospital. In such cases, liaison was established with the Reproductive and Child Health (RCH) officer of those districts, or the doctors in peripheral rural hospitals, who went out of their way to make the drugs available locally.

Patient information material, as documents, pictures and videos were circulated, such as procedure for testing urine, balanced diet and exercise routines. A Google form was used to check compliance with drugs, immunization and life-style modifications.

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# Psychiatric Problems Among Adolescents With HIV

We read the recently published article by Pilania, et al. [1] with interest. Authors deserve appreciation for conducting a study on this novel and sensitive topic. However, we would like to draw attention towards following observations. Firstly, authors try to associate the CD4 count with depression, which is not part of the design and methodology of the study. Moreover, there are studies which paradoxically state better psychological status leads to high CD4 count [2,3]. Additionally, low prevalence of psychiatric illness in this study (12%) in comparison with most of the previous studies (up to 50%) [2,4,5], as described to be due to

lower stage of disease, could be of sampling error and data collection from non-heterogeneous population (predominantly stage 1). As per National Mental Health Survey 2016, prevalence of mental disorders in general population from urban area (aged 13-17 years) is 13.5% [6]. Lower prevalence of mental illness in index study compared to general population may not be taken as prerequisite to recommend a larger study. Most (88.1%) of cohort group had acquired HIV via vertical transmission suggesting long term illness; this might not substantiate author's explanation that adolescents were lacking in knowledge about their disease. Hence the low prevalence of the psychiatric illness cannot validate the above explanation. The index study is deducing partially informative data since the sample seems to be from very selected, population leading to questionable external validity. Hence, the study has doubtful implications, or minimal addition to existing knowledge.

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## AUTHORS' REPLY

The design and methodology of the study included the association of psychiatric problems in adolescents with various clinical factors including stage of the disease, which can be related to the CD4 count of the patients. The lower incidence of psychological problems in patients with high CD4 counts was also seen in various other studies [1].

Recently, a systematic review on prevalence of mental health problems in adolescent has also been published [2]. Since the study cohort was limited to tertiary-care center and most of the children were on HAART, it was difficult to reduce the skewing of the data. Also larger studies are needed to emphasize the need to integrate mental health in the care of adolescents living with HIV.

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## EXPERT COMMENTS

Pilania, et al. [1] have reported their observations on prevalence of psychiatric disorders in 101 consecutively enrolled adolescent patients with HIV. What this study brings out is, that prevalence of psychiatric disorders is similar to what is observed in apparently healthy urban Indian adolescents [2]. A possible explanation for this finding is that all their subjects were on anti-retroviral therapy (ART), nearly 3/4$^{th}$ of them for over 3 years. Thus, not surprisingly, 92% of them were in WHO stage 1 of the disease. Further information like their CD4 counts, viral load, nutritional status, are not given in the data, but most adolescents in this situation are expected to be having a good CD4 count, suppressed viral loads and body mass index in normal range, thus contributing to their overall wellbeing. A more appropriate conclusion from the study would have been that with early initiation and continued ART, adolescents with HIV do not have higher prevalence of psychiatric disorders as compared to age-matched peers. Any conclusion beyond this– trying to look for impact of factors like WHO clinical stage, age, socio-economic status, HIV status disclosure etc, on occurrence of psychiatric illness in these subjects is not possible from the data provided, which is primarily descriptive in nature. Calculation of odds ratios would have helped gain this information.

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# Position Paper on Kawasaki Disease in India: Pertinent issues

We read with interest the recently published IAP position paper on Kawasaki Disease (KD) [1]. We would like to highlight the following issues that require further consideration.

Under laboratory investigations, it is noted that serum levels of NT-Pro-BNP (N-terminal Pro-brain natriuretic peptide)>225 pg/mL can assist in the diagnosis of KD (86.5% sensitivity and 94.8% specificity to suggest myocardial dysfunction). However, in the subsequent section, authors mention that cut off values for

NT-Pro-BNP indicative of myocardial involvement are yet to be clearly defined. The AHA statement (2017) also states that this biomarker may not have sufficient discriminative ability [2]. It is notable that during childhood, NT-Pro-BNP is known to vary with age and therefore, it has been suggested that a single cut-off value based on ROC analysis would be inappropriate [3,4].

It is mentioned that it may take 36-48 hours for the fever to subside in IVIG responsive patients [1]. However, both this position paper and AHA statement define IVIG resistance as persistence or recurrence of fever 36 hours after the end of IVIG infusion. Several recent studies and the Japanese Society of Pediatric Cardiology and Cardiac Surgery guidelines suggest a 48-hour time frame for the same [5]. The 36-hour cut-off, when applied strictly, could potentially lead to over-diagnosis of IVIG resistance. This is a pertinent issue that needs further exploration, considering that the time taken for IVIG infusion itself can be variable (typically 12 hours in North America and 20-24 hours in Japan) [5]. AHA recommends IVIG infusion over 10-12 hours (as opposed to 12-24 hours recommended by authors).

There are certain variations in the definition of recurrence. Recurrent KD is defined as a repeat episode of KD after complete resolution of the first episode [1,2]. Acute illness in KD usually lasts for 4 to 6 weeks and several Japanese surveys have classified KD as recurrent if there is an interval of at least two months from the onset of the first illness to onset of the new episode [6].

In the paper, the available Indian data has not been critically evaluated. It is imperative to consider relevant local data to bring in the much needed Indian perspective. In the process, lack of good quality data on the disease epidemiology and the importance of a national registry could have been highlighted. Finally, a conflict of interest statement by the authors is missing.

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## AUTHORS' REPLY

We are in agreement with the author that NT Pro BNP is not a well established tool for the diagnosis of KD. As rightly pointed out, NT pro BNP varies with age and the values provided in the paper are from the study by Dahdah et al. [1]. It must be said that one should refer to age related upper limits of normal and it is also useful to keep in mind to avoid making diagnosis of Kawasaki disease just on the basis of NT Pro BNP alone. Though there has been a global effort to identify a suitable biomarker for KD diagnosis, but that still remains elusive. NT Pro BNP is presently an accessible tool in many centers and the facts relating to this tool has been added as an addendum in the paper.

Regarding the 36 hours (post intravenous immunoglobulin infusion) being the cut off for the diagnosis of IVIg resistance, it was more of an adaptation from the American Heart Association (AHA) guidelines [2]. It is important to keep in mind that this period is after the completion of IVIg infusion and the duration of the IVIg infusion (10-12 hours vs 12-24 hours) does not matter much. The longer infusion period would specially apply to the context of school-going children with the disease when a higher total dose of IVIg needs to be infused. It needs to be emphasized that in a disease like KD, it might be useful to overtreat rather than undertreat to prevent lifelong complications due to coronary aneurysms.

The definition of recurrent KD would essentially mean a recurrence after documented remission of the first episode of KD (clinically, echocardiography and laboratory). It goes without saying that this period would be at least for about 4 to 6 weeks.

This is a position paper on KD providing diagnostic and therapeutic guidelines for practising pediatricians across the country. We did not intend to highlight or analyze Indian data. Moreover, data on KD in India is predominantly emerging from few centres and not representative of the scenario in the whole country.

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### Memory B cells against COVID 19 persist and evolve with time

A study on the humoral responses in 87 patients with COVID 19 infections by Gaebler, et al. from the Rockefeller Institute is in the news. Both IgG and IgM antibodies levels against the spike protein Receptor Binding Domain (RBD) showed a decline at 1.3 and 6.2 months, though IgA levels were less affected. Further neutralising activity tested using viral assays showed a 5 fold decline.

In sharp contrast, the memory B cells against the RBD showed no decline or sometimes an increase in numbers. A clonal turnover of memory B cells was demonstrated at 6.2 months. Interestingly, the antibodies they expressed showed hypermutation with increased potency against mutations in the spike protein RBD.

The researchers then performed intestinal biopsies of 14 volunteers. They found persistence of viral genome in the intestinal mucosa in 50% of these asymptomatic patients. This fascinating data suggests that the virus may persist in various body tissues like the intestine where the virus continues to mutate and the memory B cells evolve to handle these mutations. Hence our protection after natural SARS CoV2 infection may be much longer lasting and evolving despite viral variation, much beyond 6 months as has been supposed earlier.
(bioRxiv *https://doi.org/10.1101/2020.11. 03.367391* (2020); *Nat Rev Immunol.* 2021)

### Guidelines to prevent infant food allergies

There have been several high impact RCT's published recently about strategies to prevent food allergies. They include LEAP (learning early about peanut allergy), EAT (enquiring about tolerance), STEP (starting time for egg protein) etc. They have resulted in a paradigm shift in the way we could potentially prevent food allergies.

The story starts in the 1990's when it was noticed that peanut allergy had doubled in children in Western countries over a period of 10 years. Peanut allergy starts early in life and is the leading cause of anaphylaxis and death in children in the West. Guidelines in UK (1998) and the US (2000) recommended that peanut be excluded from the diet of children upto 5 years of age, if they were at high risk for food allergies. But astute clinicans noticed that peanut allergy was 10 times higher in Jewish children brought up in UK versus those brought up in Israel. On questioning, they found that Israeli children were introduced to peanut much before 6 months of age. Hence it was hypothesized that perhaps early introduction to certain foods may help do develop tolerance to various foods.

This has been best studied for peanut allergy. In the LEAP study babies at high risk for allergies such as those with severe eczema and egg allergy were introduced to peanut paste between 4-6 months of age. Compared to controls in whom peanut was avoided till 5 years of age there was an 86% reduction in peanut allergy by 5 years. Since then there have been several well conducted studies which have borne out this hypothesis.

Hence the AAAAI (American Academy of Allergy, Asthma and Immunology) has brought out a consensus guideline about this. They have suggested that babies with severe eczema or family history of atopy may be introduced to peanut containing products between 4-6 months of age. All infants, even those without risk for allergies may be introduced to cooked egg products between 4-6 months. Other potentially allergic foods like soy, wheat, tree nuts and fish may also be introduced early. They suggest that introduction to a diverse foods early in infancy may be beneficial in preventing food allergies. They found no data to suggest that hydrolysed formulas could prevent food allergies.

Parents may introduce 1 food at a time every 3 days. Evidence for developmental readiness to handle complimentary foods include holding the head up when siting, showing interest in what others are eating and opening their mouth when food approaches.
(*Allergy Clin Immunol Prac. 2021*)

### Second generation COVID vaccines

So far the vaccine roll out has been fairly safe but several questions about the eventual effect of the COVID vaccines still remain. One scenario is that the vaccine recipient is protected but he becomes an asymptomatic carrier, continuing to spread the disease. More dramatically if the vaccine recipient subsequently contracts COVID19, could he develop a worse disease by a mechanism of antibody dependant enhancement? The mRNA vaccines have shown excellent protection but fall short in the requirement for extremely low temperatures in the cold chain. Scientists therefore are continuing to develop newer vaccines.

The Imperial College of London is developing a self amplifying mRNA vaccine which will not need a booster. Researchers in Maryland- based Novavax are developing a genetically engineered protein subunit vaccine with a saponin based adjuvant. Another unique approach is using nanoparticle of the receptor binding domain which has been shown to elect a ten times higher antibody response than when the entire spike protein is injected. Closer home, Bharat Biotech has received approval to conduct phase I trials on 75 individuals of its nasal vaccine which will also not require any booster.
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# Iron Deficiency Anaemia