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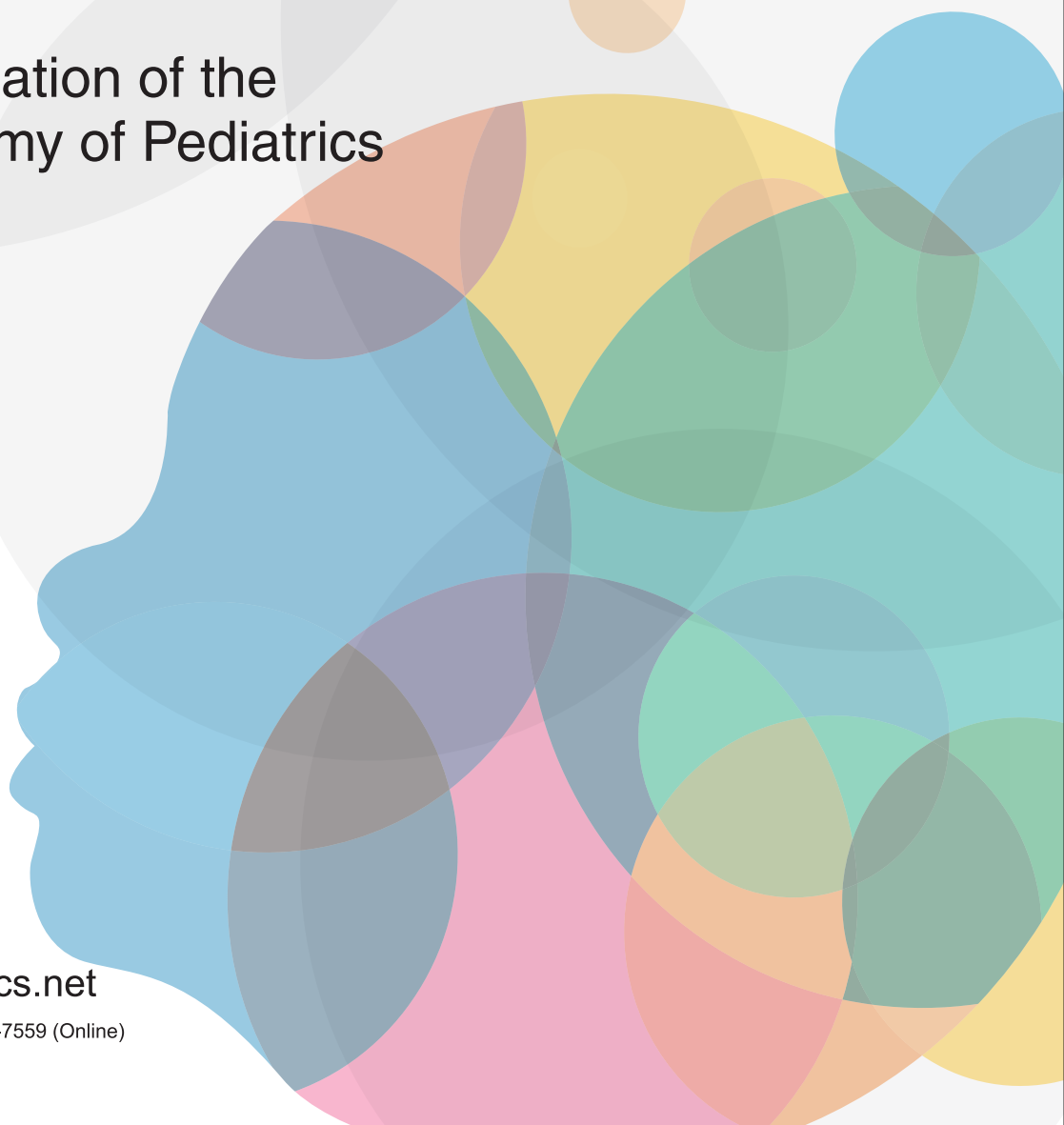

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Contents

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The Pandemic and India's Children

PIYUSH GUPTA
National President, Indian Academy of Pediatrics 2021
prof.piyush.gupta@gmail.com

Each generation has to go through an era-defining upheaval. Some of us lived through independence, some through wars, and some through the economic liberalization of the 90s. But, nothing could have prepared us for this. And in turn, it would have been nearly impossible to prepare our children for it. Nevertheless, they find themselves in the middle of a pandemic with death, illness, and misery all around.

The oft-repeated assertion entering the lay consciousness has been that children are safe from contracting coronavirus disease 2019 (COVID-19). Whereas a truer statement would be that they are relatively safer from severe forms of COVID-19. They are not immune to it, and the fact that the rate of mortality and severity is lower, does not mean we let our guard down. It also does not mean that we panic about a third wave where children are supposedly at dis-proportionate risk. This pandemic has exhibited a nonlinear, complex nature; all predictions, whether based on complex mathematical model or past experience or even just instinct, have gone for a toss. It would be prudent, therefore, to be cautious against any speculation or prediction unsubstantiated by hard evidence, especially regarding the third wave specifically targeting children. All this can result in is unwarranted mental trauma to children and their caretakers rather than preparing them for whatever lies in store. Usually, it is expected that every fresh wave would be weaker than the previous one, but the strange nature of the virus coupled with human behavior has belied this. The second wave took a heavier toll unlike expected and has given credence to the possibility that the third wave might be even stronger. The number of people infected has been much higher in the second wave; proportionately, the number of children infected has also increased. This remains a possibility even in the third wave but there is no logical reasoning or evidence to believe that the third wave will predominantly or exclusively affect children [1].

An Indian Council of Medical Research survey conducted in December, 2020 and January, 2021 showed that the percentage of infected children in the age group of 10-17 years was around 25%, the same as adults [2]. Almost 90% of infections in children are mild/asymptomatic. This indicates that while children are being infected like adults, they are not getting the severe form of the disease. As per data collected in the first two waves, even severe COVID infections in children are less likely to require intensive care unit management.

However, the impact of the pandemic on our children has not been limited to the actual disease, but a gamut of related issues and lifestyle changes forced upon them due to the pandemic. A recent study [3] found that almost one-third of children had developed psychosocial problems, presenting as symptoms of anxiety and depression. The underlying problems for these ranged from the fear of acquiring COVID-19 infection, not being able to attend school, to not being able to meet friends. The thing they missed the most, as well the activity they intended to engage in as soon as the lockdowns were over, was to meet friends [3].

One of the more serious side-effects of the pandemic has been the reduction in child hospital visits. Any pediatrician could attest to parents bringing their children more frequently to their doctors, either for immunization or even in cases of mild illnesses, than they would themselves. These used to provide a vital stopgap in diagnosis and management of chronic illnesses, which would have gone unnoticed, since routine check-ups are almost non-existent in a large part of the country. Now, due to COVID-19, there is an increasing reluctance in bringing children to the hospital. Sure, telemedicine has taken over, where it can, but there is no denying the value of an in-person visit for diagnosis of an underlying disease. Even routine immunization has suffered and can be disastrous in the long-term. The pandemic indirectly might end up aiding the proliferation of a host of other illnesses. Then there is the economic impact. Already marginalized communities have borne the brunt of it, with no jobs, no income, and savings exhausted. This will invariably have an impact on their child's nutrition, and in turn, their overall health.

School is an inextricable part of a child's life, as children spend at least a-quarter of their day in school or traveling to and from it. That is, until the pandemic necessitated stringent lockdowns. And with it, has come a simultaneous need and opportunity for a paradigm shift in education. We also are more cognizant of the possibility that attending school is more important for socialization

and associated skills, rather than mere learning. In view of this, and to take better care of our children's mental health, which has been tested time and again during the pandemic, there is an imminent need to redesign school curricula, as well as performance assessment systems.

A holistic approach to child mental health needs to be adopted by parents and teachers alike, who need to be sensitized into minimizing the stress on every child they are responsible for. Equating excellence in academic learning to self-worth merely serves to inflate an already competitive environment and does not work towards discipline and results. These positive traits need to be self-motivated if they are to sustain for the long term, and the self-motivation can only come from a place of mental calm and security. To build this, screen-time needs to be reduced for children. Yoga, meditation and other forms of exercise need to be taken up. Since we have already seen the psychological issues emanating from the pandemic, an exclusive helpline to combat adolescent suicide, is long overdue.

It is true that the pandemic has wrecked unprecedented misery and chaos everywhere, but it has also exacerbated existing problems like nutritional extremes. On one hand we are battling with nutritional issues such as malnutrition and anemia. The Comprehensive National Nutrition Survey (CNNS) India 2016-18 that included data on 112316 children and adolescents revealed that only 6% of infants are getting minimum acceptable diet, the prevalence of wasting (defined as low weight-for-height, indicating acute malnutrition) and stunting (defined as low height-for-age, indicating long-term malnutrition) in under-five children are 17% and 35%, respectively; and 41% of preschoolers, 24% of school-age children, and 28% of adolescents are anemic [4]. It is highly probable that the problem has worsened during the pandemic, especially amongst children belonging to underprivileged communities, who are strugg-ling to make ends meet. On the other hand, we have urban children living in economically stable households, who are at the other end of the nutritional extreme. In the last decade, options at home for junking have been on the rise, and in the lockdowns, have become ever-more prevalent [5].

Schools need to be reopened to provide a sense of normalcy. However, as an Indian Academy of Pediatrics (IAP) task force recently put forth in its guidelines [6], they should be opened "only when the local epidemiological parameters are favourable, the administration is equipped with adequate infrastructure and health care facilities, and the stakeholders (teachers, students, parents, and support staff) are prepared for the new normal. In the meanwhile, remote learning (media-based and /or otherwise) should reach to the last student to maintain uninterrupted education." School attendance will ensure that children are not leading a sedentary lifestyle, are mingling with peers, and developing a healthy immunogenic arsenal.

The speculation about a third wave which predominantly affects children should not be seen as a reason to panic, but an opportunity to better our pediatric healthcare infrastructure, which has historically been inadequate in normal times, let alone a pandemic. Not just district hospitals, but even many medical colleges in the country do not have intensive care services for children, be it trained specialists or essential equipment. The motto has to be – build in peacetime, so that we are ready for war. Healthcare, especially for children, has to become a priority if we are to handle a pandemic. This does not simply mean buying more ventilators, procuring more equipment or creating more beds. It also means making sure that the workforce responsible for our children is not scanty, starved, or scared.

The writing is on the wall for anyone to read. We can not think short-term and expect long-term gains. Dealing simply with COVID-19 will not erase decades of under-investment in child health. There needs to be a long-term commitment from all private and public stakeholders if we are to be prepared for the next era-defining upheaval.

Note: This article is excerpted with permission from the author's lead editorial published on 18 June, 2021 in the Outlook magazine titled "Our Penny stocks, Our Tomorrow." The full article can be accessed from the website of the magazine *https://magazine.outlookindia.com/story/collectors-issue-our-penny-stocks-our-tomorrow/ 304673*

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REFERENCES

1. Indian Academy of Pediatrics (IAP). IAP Viewpoint on the third wave of COVID-19 in India. Accessed 23 June, 2021. Available from: *www.iapindia.org*
2. ICMR sero survey: One in five Indians exposed to Covid-19. Accessed 23 June, 2021. Available from: *https://www.bbc. com/news/world-asia-india-55945382*
3. Shah S, Kaul A, Shah R, Maddipoti S. Impact of coronavirus disease 2019 pandemic and lockdown on mental health symptoms in children. Indian Pediatr. 2021;58:75-76.
4. Comprehensive National Nutrition Survey - National Health Mission. Accessed 23 June, 2021. Available from *https://nhm.gov.in*
5. Gupta P, Shah D, Kumar P, et al. Pediatric and Adolescent Nutrition Society (Nutrition Chapter) of Indian Academy of Pediatrics. Indian Academy of Pediatrics Guidelines on the Fast and Junk Foods, Sugar Sweetened Beverages, Fruit Juices, and Energy Drinks. Indian Pediatr. 2019; 56:849-63.
6. Ghate S, Parekh BJ, Thapar RK, et al. Indian Academy of Pediatrics Guidelines on School Reopening, Remote Learning and Curriculum in and After the COVID-19 Pandemic. Indian Pediatr. 2020;57:1153-65.

INVITED COMMENTARY

Management of Thalassemia: Blood and Beyond

RASHID MERCHANT

Thalassemia Clinic, Nanavati Max Super Specialty Hospital, Mumbai, Maharashtra. deandoc2000@hotmail.com

It has been almost a century since cases of thalassemia were reported by Cooley and Lee for the first time globally [1], and over eighty years since they were reported by Mukerjee and Coelho for the first time in India [2,3]. India has come a long way since then in reducing thalassemia-related morbidity and mortality, through collaborative efforts between government bodies, medical institutions and NGOs. Societies run by affected individuals and their parents have played an incredible role in spreading awareness and helping the thalassemia community navigate the medical and psychosocial complexity of adapting to the condition [4]. Numerous novel therapies, diagnostics, and approaches to management – stemming from decades of biomedical research – are on the horizon and hold great promise for improving the status-quo.

Though better management has substantially reduced morbidity and mortality, the consequent increase in life expectancy necessitates multidisciplinary care to mitigate the clinical sequelae of iron overload such as endocrine dysfunction and nutritional deficiencies. A study in this issue conducted by Bhat, et al. [5] suggests that low levels of vitamin C might be very common amongst individuals affected by transfusion-dependent thalassemia. In the study group a large majority of children were found to have vitamin C deficiency, which was in turn found to be associated with iron overload and higher oxidant levels. Supplementation with vitamin C in deficient patients led to a safe reduction of oxidant levels, which suggests that supplementation of vitamin C along with dietary counseling might reduce oxidative stress and thereby protect against myocardial damage. Another study in this issue by Singh and colleagues [6] reported that over a quarter of patients in their study cohort required hormone replacement therapy due to pubertal arrest/failure despite regular transfusions, intensive chelation, and regular follow-up. High systemic iron load was found to be the only statistically correlated determinant of pubertal arrest/failure, signaling the need for further personalization of iron chelation regimes based on genetic susceptibility to higher iron-overload.

Novel treatments such as Hb-F induction therapy and gene therapy are eagerly awaited as they have the potential to reduce the need for frequent blood transfusions and consequent iron overload. The use of hydroxyurea (hydroxycarbamide) has been studied in India [7], where the authors found a mixed response to hydroxyurea in cohorts of thalassemia intermedia and thalassemia major patients. The correlation of certain haplotypes with response to hydroxyurea supported the hypothesis that response was governed by variable genetic mutations, biochemical interactions, and γ/γ globin chain production. In a unique study included in this issue, Chandra and colleagues [8] report that over 75% of transfusion-dependent thalassemia patients in their cohort experienced a significant reduction in transfusion requirement and serum ferritin levels after administration of thalidomide over a 6-month period. Though encouraging, larger studies will be required to establish a safe and effective dosage regime, adverse event profile, drug interactions, and long-term effects of this intervention.

Repurposing drugs such as hydroxyurea and thalidomide might provide an effective way to reduce dependence on blood transfusions and iron chelation therapy; however, purpose-built novel therapies can take this a step further. Luspatercept, the activin II receptor trap, targets ineffective erythropoiesis and thereby decreases transfusion frequency in transfusion-dependent thalassemia patients – other similar therapies undergoing trials include Sotatercept and JAK2 inhibitors [9]. Minihepcidin, Ferroportin and TMPRSS6 inhibitors are promising novel therapies that improve iron dysregulation, especially in non-transfusion-dependent thalassemia patients [9]. In this issue, Soni [10] reviews gene therapy treatment strategies including gene insertion-based lentiviral vectors such as the recently EU-approved Zynteglo, and CRISPR-Cas9 based gene-editing of *BCL11A*, amongst others; these novel therapies will provide a long-awaited alternative to patients who do not have an HLA-matched donor for allogeneic hematopoietic stem cell transplant.

There is a high cost associated with thalassemia management, novel therapies, and allogeneic hematopoietic stem cell transplants, hence reducing the burden of thalassemia will simultaneously necessitate robust and multi-pronged preventive interventions including preconception, prenatal, and newborn screening. India's regional heterogeneity of *HBB* mutations has been studied by Colah, et al. [11] amongst others and has led to the establishment of such programs at various centers in India. It is important that such programs also include appropriate counselling to address fear and stigma associated with being diagnosed as a carrier and enable informed reproductive decision-making amongst high-risk couples [12].

In India, β-thalassemia poses a significant socio-economic burden [13]. The discoveries and innovations covered in this issue have the potential to greatly reduce this burden and improve the quality of life of affected individuals. Simultaneous public health interventions that improve access and adherence will be necessary to tap into this potential. Given India's underdeveloped reimbursement landscape, relatively low healthcare expenditure, and myriad public health priorities, successful implementation of new therapies, management strategies, and prevention programs will require structured and sustained collaboration between stakeholders across healthcare, government, social, and private sectors. All in all, innovative public health interventions coupled with cross-sector collaboration will enable translation of the exciting new research published in this issue and elsewhere into better outcomes for India's large thalassemia community.

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REFERENCES

1. Cooley T, Lee P. A series of cases of splenomegaly in children with anemia and peculiar bone changes. Trans Am Pediatr Soc.1925;37:29-30.
2. Mukherji M. Cooley's anaemia (erythroblastic or Mediterranean anaemia). Indian J Pediatr. 1938;5:1-7.
3. Coelho C. Erythroblastic anemia- Cooley's anemia. Med Bull Bombay. 1939;7:291-3.
4. Moirangthem A, Phadke S. Socio-demographic profile and economic burden of treatment of transfusion dependent thalassemia. Indian J Pediatr. 2018;85:102-7.
5. Bhat KV, Sharma R, Sharma S, et al. Vitamin C deficiency and oxidant levels in children with transfusion-dependent β-thalassemia. Indian Pediatr. 2021;58:631-4.
6. Singh P, Samaddar S, Parakh N, Chandra J, Seth A. Pubertal development and its determinants in adolescents with transfusion-dependent thalassemia.Indian Pediatr. 2021;58:611-6.
7. Italia K, Jijina F, Merchant R, et al. Response to hydroxyurea in β thalassemia major and intermedia: Experience in western India. Clinica Chimica Acta. 2009;407:10-15.
8. Chandra J, Parakh N, Sidharth, et al. Efficacy and safety of thalidomide in patients with transfusion-dependent thalassemia. Indian Pediatr. 2021;58:635-8.
9. Motta I, Bou-Fakhredin R, Taher A, Cappellini M. Beta thalassemia: New therapeutic options beyond transfusion and iron chelation. Drugs. 2020;15:1-11.
10. Soni S. Gene therapies for transfusion-dependent β-thalassemia. Indian Pediatr. 2021;58:667-74.
11. Colah R, Gorakshakar A, Nadkarni A, et al. Regional heterogeneity of beta-thalassemia mutations in the multiethnic Indian population. Blood Cells Mol Dis.2009;42:241-6.
12. Gupta V, Sharma P, Jora R, Amandeep M, Kumar A. Screening for thalassemia carrier status in pregnancy and pre-natal diagnosis. Indian Pediatr. 2015;52:808-9.
13. Verma I, Saxena R, Kohli S. Past, present and future scenario of thalassaemic care and control in India. Indian J Med Res. 2011; 134:507-21.

CLIPPINGS

Vaccines for human fungal diseases: Close, but still a long way to go! (*NPJ Vaccines. 2021;6: 33*)

The authors reviewed the progress in the development of anti-fungal vaccines. Various types of the vaccines (subunit formulations to live attenuated fungi) have been tested in mouse models and found to be protective against the common fungal pathogens (*Candida spp., Cryptococcus*, and *Aspergillus)* causing disease in humans. Superior efficacy was shown by novel adjuvants and delivery systems aimed at stimulating arms of the immune system critical for control of fungal invasion. Three vaccines have already undergone human trials as feasibility study and planned for further clinical trials in at-risk populations. Two recombinant *Candida* vaccines [PEV7-consists of recombinant aspartyl-proteinase 2 (Sap2), NDV-3, contains the recombinant N-terminus of *C. albicans* agglutinin-like sequence 3 protein] have reached human clinical testing and have shown encouraging results. A formalin-killed spherule (FKS) vaccine for coccidioidomycosis is the third anti-fungal vaccine studied in humans, and it demonstrated the feasibility of human trials of fungal vaccine. Apart from the individual vaccine, search for a pan-fungal vaccine, which can protect against majority of systemic fungal infections is going on. Though these vaccines are still far away from routine use but it gives a new hope for the high risk groups.

RAJESH KUMAR MEENA
raj.mamc@gmail.com

RESEARCH PAPER

Efficacy and Safety of Thalidomide in Patients With Transfusion-Dependent Thalassemia

JAGDISH CHANDRA,[1] NUPUR PARAKH,[1] SIDHARTH[1], NEHA SINGH,[1] SUNITA SHARMA,[2] MANISH GOEL,[3] HARISH PEMDE[1]

From the [1]Division of Pediatric Hematology, Department of Pediatrics; [2]Department of Pathology; and [3]Department of Community Medicine; Lady Hardinge Medical College and associated Kalawati Saran Children Hospital, New Delhi. Correspondence to: Dr Nupur Parakh, B-52, Ashoka Niketan, Opposite Vigyan Vihar, IP Extension II, Delhi 110 092. drnupurparakh@gmail.com

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Objective: To assess the efficacy and safety of thalidomide in children with transfusion-dependent thalassemia.

Methods: This prospective, single center, open-label study enrolled children aged 12-18 years, and who received thalidomide for a duration of 6 months at a starting dose of 2-3 mg/kg/day. Efficacy was assessed by reduction in transfusion requirement and rate of fall of hemoglobin. Efficacy was classified as major, moderate and minimal/no response depending on the reduction in transfusion requirement. Safety was assessed by adverse effects related to thalidomide.

Results: 37 children [mean (SD) age, 14.7 (1.8) years were included. Rate of fall of hemoglobin reduced from a mean of 1.0 (0.24) g/week pre-thalidomide therapy to 0.58 (0.26) g/week after 6 months of thalidomide (*P*<0.001). 19 children (51.3%) had major response and 12 (32.4%) had moderate response. In 13.5% and 32.4% children response was observed within the first and second month of therapy, respectively. 15 (40.5%) children remained transfusion - free for a median (IQR) time of 6 (3-10) weeks of thalidomide therapy. Mean serum ferritin (SD) decreased from 1758.9 (835.1) to 1549.6(1016.9) (*P*<0.001). Mean HbF (SD) showed an increase from 2.95(2.6) to 49.2(33.3) (*P*<0.001). In 32 children, 47 adverse events were observed. Common adverse events were constipation and neutropenia (mostly mild).

Conclusions: Thalidomide resulted in major/moderate response in majority of children with transfusion-dependent thalassemia with satisfactory adverse effect profile.

Keywords: *Hemoglobin F, Iron overload, Transfusion requirement*.

Coexistence of hereditary persistence of fetal hemoglobin (HbF) in patients with transfusion-dependent thalassemia (TDT) reduces the severity of the disease with several of them becoming non-transfusion dependent. This clinical benefit of increased HbF appears to be due to a decrease in the imbalance between β and non-β chains, resulting in reduction of ineffective erythropoiesis and hemolysis [1]. Based on these observations, many drugs including hydroxyurea, butyrate, 5-azacytidine etc have been studied as inducers of HbF for patients with thalassaemia and sickle cell disease (SCD) (2-6).

Thalidomide, a drug known for its immuno-modulating and anti-angiogenic properties, has recently been demonstrated to induce globin gene expression and to increase the proliferation of erythroid cells [7]. Experience with use in non-TDT (NTDT) and TDT is limited [8-10]. A recent study has shown major response (hemoglobin rise >2 g/dL) in 50% and 71 % at one month and three month of therapy, respectively in patients with NTDT [11].

In patients with TDT, a recent study showed mean hemoglobin increase from 8.9 g/dL to 10.5 (1.18) g/dL after 6 months of thalidomide treatment [12]. Ramanan and Kelkar from Pune have reported over 50% reduction in serum ferritin in 59 (50%) patients with thalassemia [13]. This study was thus undertaken to assess the efficacy of thalidomide in reducing transfusion requirement and iron overload and to assess its safety in patients with TDT.

Invited Commentary: Pages 609-10

METHODS

This prospective single-center open-label study was conducted in a tertiary care public hospital of India from October, 2019 to April, 2020. The Study included children with TDT aged 12-18 years enrolled from the thalassemia day care center, after detailed counselling regarding the study and explaining the adverse effects of use of thalidomide. Out of 37 patients, 4 had HbE-β-thalassemia, but were clinically behaving as TDT. No patient in this study was on hydroxyurea. Those having HIV, hepatitis C

or hepatitis B infection, known neurological problems, known chronic systemic disease, hypersplenism, and patients with vitamin B12 or folate deficiency were excluded. Post- pubertal girls were enrolled immediately after menstrual period. Ethical clearance was obtained from institutional ethics committee and approval of Drug Controller General of India (DCGI) was obtained for use of thalidomide for a new indication. A written consent was obtained from the parents/ caregivers and assent was obtained from the participating children.

The sample size was calculated using Epi Info (*https://www.openepi.com/SampleSize/SSMean.htm*). A sample size of 32 children was calculated considering the current mean packed red blood cell (RBC) requirement of 220 mL/kg/year and likely minimum 10% reduction in annual packed RBC requirement when thalidomide is provided. The sample size was computed considering the two tailed test with an alpha error of 0.05 and power of 80%. Considering a drop out of 15%, a final sample size of 37 children was enrolled in the study.

Detailed history and examination was done at baseline and during each follow up visit at 2-4 weeks interval. At follow visits, enquiries were made specifically for constipation, sedation and neurological symptoms. Baseline investigations included complete blood counts, absolute reticulocyte count (ARC) (using XN-1000 automated hematology analyzer, Sysmex Corporation). Prothrom-bin time (PT), activated partial thromboplastin time (aPTT) and d-dimer levels were performed on STA compact Stago automated coagulo-meter (Diagnostica Stago). These investigations were repeated every four weeks. Hemoglobin F (HbF) levels were estimated at baseline using Bio Rad Variant II (BIO RAD, US) and was repeated at the end of study at 6 months.

The goal of transfusion therapy was to keep pre-transfusion hemoglobin level between 9-10.5 g/dL. Thalidomide was started at dose of 2-3 mg/kg for 24 weeks [12]. Thalidomide was used in rounded-off value and different strengths were also created using empty capsules containing 25 mg drug. If the patient was showing response and was free of adverse effect, the same dose was continued. The dose was increased upto 3-4 mg/kg in cases with no response with initial dose if the drug was well tolerated (maximum dose given to patients was 3.7 mg/kg/day). Ecosprin was not given to any patient enrolled for the study, irrespective of dose of thalidomide, except for the patient who were splenectomized or transiently for patients with increased D-dimer, during monitoring.

The response to thalidomide therapy was also assessed as mean change in rate of fall of hemoglobin and transfusion requirement during study period. The levels of pre-transfusion hemoglobin, fetal hemoglobin, ARC and serum ferritin were also compared. Subjects with more than 50% reduction in transfusion requirement as compared to pre-study transfusion requirement were classified as having major response (Group 1); those with 25-50% reduction in transfusion requirement were classified as moderate response (Group 2), and those with less than 25% decrease in transfusion requirement were classified as minimal/no response (Group 3).

Statistical analysis: The response was statistically analyzed, using paired t test. In the three groups, response was compared using ANOVA test. Post-hoc analysis was also performed for finding out the statistically significant differences. *P* value of less than 0.05 was considered as statistically significant.

RESULTS

The flow of the study is shown in **Fig. 1**. The study included 37 children (M: F-2.36:1) with a mean age of 14.7 (1.82) years. **Table I** describes baseline parameters of study subjects. Notably, only one child was splenecto-mized, and none had HIV, Hepatitis B or HCV infection. Mean (SD) HbF level of the subjects was 2.95% (2.6%). Of the 33 children for whom information on mutation study was available, seven children had variable combination of β^0/β^0 mutations, 9 patients had severe β^+/severe β^+ muta-tions, and 5 patients were compound heterozygous for β^0 and severe β^+ mutations. Seven patients had variable combination of either severe β^+/ mild β^+ or compound heterozygous for β^0 mutations with second mutation being

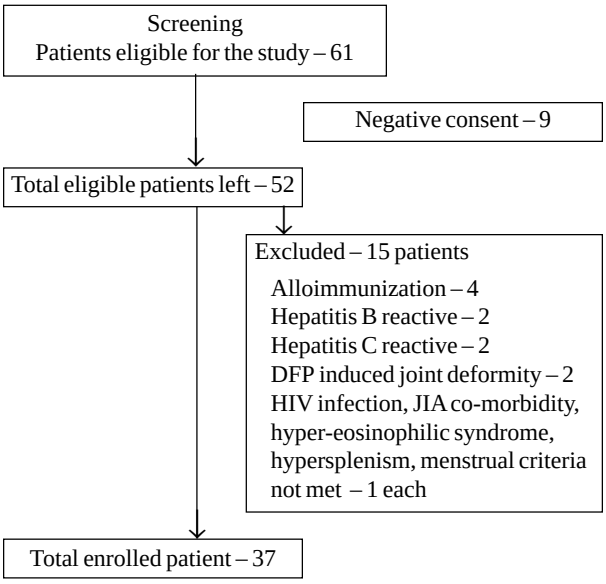

Fig. 1 Study flow chart.

Table I Baseline Characteristics of Children With Thalassemia Enrolled for the Study (*N*=37)

Characteristics	*Value*
Age (y)	14.7 (1.8)
M: F	26:11 (2.36:1)
Packed red cell received,[a] mL/kg	75.7 (12.3)
Pre-transfusion hemoglobin, mg/dL	9.45 (0.67)
Serum ferritin, ng/mL	1758.9 (835.1)
Absolute reticulocyte count	24076.8 (27781.3)
Fetal hemoglobin (%)	2.95 (2.6)
Mutations (*n*=33)[b]	
B^0/ Severe β^+	28
E β-double heterozygous	4
Thalidomide dose (mg/kg/day)	2.05 (0.35)
Chelation, no.	
Deferasirox	14
Deferasirox and deferiprone	23
Serum folate (ng/mL)	24.4 (14.9)
Serum vitamin B12 (pg/mL)	421.1 (147.2)

Data expressed as mean (SD) or as stated. [a]Six-month pre-study. [b]Mutation report of 4 patients were not available, and 1 patient did not have any of the common mutations.

uncommon Indian mutation which could not be detected. None of the subjects had low serum folate or vitamin B12 levels.

Table II describes changes observed in study parameters as compared to baseline parameters. Rate of fall of hemoglobin decreased from a mean of 1.0 (0.24) g/ week to 0.58 (0.26) g/week (*P*<0.001). Hemoglobin F levels showed a significant increase and serum ferritin decreased significantly (*P*<0.001). However, rise in mean ARC was statistically not significant. In 32/37 (86.5%) patients, the dose of drug was increased if they had tolerated the drug well without any evidence of adverse effects. In 27/37 (72.9%) patients, dose reduction was done for development of adverse effects on any follow up visit, but most of the adverse effects were either grade 1 or 2 [14].

Of the 37 patients recruited in the study, one child succumbed to dengue shock syndrome during second month of study period. In two children, therapy was discontinued due to withdrawal of consent and adverse effect in one case each. Nineteen children (51.3%) had major response while 12 children (32.4%) had moderate response; remaining 6 (16.2%) had minimal/no response. **Table III** shows that before intervention, the three groups were similar with respect to their mean pre-transfusion hemoglobin and mean packed RBC received in 6 months

Table II Patient Characteristics at Baseline and on follow-up in Children With Thalassemia Treated With Thalidomide

Characteristic	*Baseline*	*End of the study*
Hemoglobin, mg/dL (6 mo)	9.45 (0.67)	8.89 (0.6)
Transfusion requirement, mL/kg (for 6 mo)	75.7 (12.3)	38.9 (19.1)
ROF of hemoglobin, (g/wk)	1.0 (0.24)	0.58 (0.3)
Absolute reticulocyte count[a]	24076.8 (27781.3)	111518 (50236.8)
Serum ferritin, ng/mL	1758.9 (835.1)	1549.6 (1016.9)
Hemoglobin, F (%)	2.95 (2.6)	49.2 (33.3)

Data expressed as mean (SD). ROF- Rate of fall. All P<0.001 except [a]P=0.06.

preceding the study period. However, during the study period, the group with best response received 25.02 (10.37) mL/kg packed RBC compared to the group with minimal/ no response receiving 67.76 (16.31) mL/kg (*P*<0.001). Mean HbF in the group with best response was 66.9% (28.59%) while in group 3 it was only 16.62% (11.23%) (*P*<0.001). Although mean serum ferritin was significantly decreased, the fall in individual groups was not statistically significant.

In five children (13.5%) response was observed within first month of therapy, 12 more responded in the second month. Response was observed in third and fourth month of therapy in additional 5 (13.51%) and 6 (16.21%) patients, respectively. During the study period, 15 (40.5%) children remained free of transfusion for a median (IQR) time of 6 (3-10) weeks of thalidomide therapy. However, after stopping thalidomide therapy, all children have required transfusions after a median (IQR) of 24 (19-52) days.

A total of rest 32 children had 47 adverse events; constipation being the most common (14, 37.8%). Raised transaminases in two children were considered unrelated, as they were also receiving deferasirox. Other adverse effects included somnolence/sedation (*n*=3) and mild dizziness (*n*=5, in one child this necessitated discontinuation of therapy). One child developed acute kidney injury during study period. This child was also receiving deferasirox, but as renal injury occurred during the study period, thalidomide was discontinued. Neutropenia was observed in 10 children; however, only one child had absolute neutrophil count less than 500/mm^3, which required temporary cessation of thalidomide. D-dimer was elevated in 6 (16.2%) children but none had any features suggestive of thromboembolism. Infections occurred during study period in 8 subjects: pneumonia, 2; chicken-

Table III Study Parameters in Children With Thalassemia Based on Response to Thalidomide

Parameter	*Major response (n=19)*	*Moderate response (n=12)*	*Mild response (n=6)*
Pre-transfusion hemoglobin, mg/dL			
Pre-study	9.53 (0.61)	9.47 (0.67)	9.12 (0.87)
At the end of study[a]	9.2 (0.58)	8.65 (0.51)	8.39 (0.36)
pRBC received, mL/kg			
6 mo pre-study	73.96 (12.9)	77.8 (10.9)	77.1 (14.1)
6 mo during study[c]	25.0 (10.4)	48.8 (8.9)	67.8 (16.3)
Thalidomide, mg/kg/d	2.53 (0.36)	2.28 (0.49)	2.56 (0.35)
ROF of Hb (g/wk)[a]	0.45 (0.17)	0.67 (0.29)	0.8 (0.19)
Hemoglobin F (%)			
Pre-therapy	3.5 (2.2)	2.25 (3.0)	2.68 (3.0)
Post therapy[b]	66.9 (28.6)	39.74 (32.0)	16.62 (11.2)
Serum ferritin, ng/mL			
Initial	1648.8 (700.4)	1867.4 (1237.5)	1890.4 (640.6)
At the end of study	1314.4 (718.6)	1694.5 (1177.2)	1886.7 (1365.0)

Data expressed as mean (SD). Hb- Hemoglobin, pRBC-Pure red blood cell, ROF- Rate of fall. [a]*P<0.01,* [b]*P=0.001;* [c]*P<0.001.*

pox, 1; unclassified acute febrile illness, 3; and dengue infection in 2 (one of whom died of dengue shock syndrome). The patient who died of dengue shock syndrome, the starting dose of thalidomide was 1.6 mg/kg, upto maximum of 2.4 mg/kg in follow-up visit. That child was not splenectomized, never had neutropenia during the study period, and was on deferasirox alone. During the febrile period, thalidomide had been withheld.

All adverse events were grade1 to grade 2 except one episode of neutropenia (grade 3) and one episode of acute kidney injury (grade 4), necessitating temporary cessation of the drug. No female patient in our study population had any menstrual abnormality. One child withdrew from the study due to sedation interfering with his studies. This child was on starting dose of 2.4 mg/kg thalidomide which was reduced after grade 2 sedation.

Nerve conduction studies (NCV) were not performed routinely at baseline or after therapy. Only one child complained of mild tingling sensation, for which NCV was performed, and thalidomide was restarted as it was normal. His serum B12 and folate levels were normal.

DISCUSSION

Over the last decade, there has been an interest in use of thalidomide in patients with thalassemia syndromes. After initial isolated case reports, it was used with success in patients with NTDT. In TDT, the experience is limited and is now emerging. Jiskani and Memon [12] reported good response in 70 children but the extent of decrease in transfusion requirement was not commented upon. Yassin [15] described his results on 37 patients including adults and only 14 patients with TDT. He described response in over 75% cases. He also describes the fall in transfusion requirement in terms of 'units' of packed cells and not in mL/kg [15]. Other studies from India and China have also reported response in up to 70% patients [16-18].

The present study is exclusively on children with TDT. We included children above 12 years as FDA approval is restricted to 12 years or above [19]. We have demonstrated major and moderate response in 51% and 32 % patients with reduction in transfusion requirement coming up as early as first month of therapy. The response rates and timing of response are similar to earlier studies [15,16,20]. We assessed the weekly rate of fall of hemoglobin which decreased significantly, as well as a decrease in packed cell requirement. Of the responders, 15 patients remained transfusion free after a median (IQR) of 6 (3-10) weeks. However, all our patients have started requiring transfusions after stopping thalidomide.

The response to thalidomide is described to be by production of fetal Hb. There are experimental studies demonstrating increased HbF production with thalidomide and other related compounds [7,21,22]. Clinical studies have not looked at rise in HbF; though, we found a rise in HbF in those with major response. Thalidomide also seems to have effect on iron overload. We observed a modest but significant fall in mean serum ferritin; although, all the patients continued to receive chelation. This is in concordance with earlier observations [12,13,15].

Therapy with thalidomide was well-tolerated. Nag, et al. [16] observed constipation in over 40% cases. Shah,

WHAT IS ALREADY KNOWN

- Thalidomide induces globin gene expression and increases the proliferation of erythroid cells.

WHAT THIS STUDY ADDS?

- Thalidomide can be an effective drug to reduce transfusion requirement in children with transfusion-dependent thalassemia.

et al. [17] described thrombocytopenia in 66%, but their patients were also receiving hydroxyurea. Neutropenia was reported in 5% patients in another study [20]. However, we encountered neutropenia in 10 (27%) cases one of which was severe necessitating temporary cessation. Out of 10 study patients who developed low ANC during the study period, 6/23 patients were on DFX and DFP and 4/14 patients were on DFX. However, risk of development of neutropenia between the two group (on combined DFP and DFX and on DFX alone was not statistically significant (P=0.87). In a study by Naithani, et al. [23] on safety of deferiprone in children, neutropenia was observed only in 2/44 patients. However, as DFP can also cause neutropenia, children on deferiprone and thalidomide, they need a closer watch on their blood counts. One concern that we have is occurrence of infections in 8 subjects over the study period. It is unclear whether this is a chance occurrence or related to thalidomide administration. This is not described as a known adverse effect of thalidomide.

The study has certain limitations. We have not studied different doses. Moreover, follow up after stoppage of drug was not a part of the study.

Therapy with thalidomide is being looked at as an affordable alternative to transfusion therapy or at least to partially offset the transfusion needs [24]. Being relatively inexpensive and well tolerated also makes it a viable option. However, for a drug to be administered indefinitely there are certain questions which need to be addressed. First, studies are required to find out most effective and safe dose. Second, whether the drug should be continued in full doses or doses can be reduced after a response is obtained. Third, criteria for response need to be defined and applied uniformly. Role of intermittent therapy also needs to be explored. Safety under 12 years is also not been studied. Interactions with DFX and DFP- two commonly administered iron chelators also need to be studied. Larger studies to answer these issues are required before decision for long term routine use is taken. Till such time drug should be used under strict monitoring of the patients.

Ethics clearance: Institutional ethics committee, Lady Hardinge Medical College; No. LHMC/ECHR/2019/29 dated September 23, 2019. DCGI Clearance: F. No. 12-01/19-DC (Pt-208) dated November 20, 2019.

Contributors: JC: conceived and designed the study, drafted the manuscript; NP: reviewed the literature, collected the data, helped in drafting the manuscript; S and NS collected the data, SS supervised the laboratory work; MG: did statistical analysis: HP: helped in study design. All authors approved the final version of manuscript, and are accountable for all aspects related to the study.

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REFERENCES

1. Wood WG, Weatherall DJ, Clegg JB. Interaction of heterocellular hereditary persistence of fetal haemoglobin with beta thalassaemia and sickle cell anaemia. Nature. 1976;264:247-49.
2. Charache S, Terrin ML, Moore RD, et al. Effect of hydroxyurea on frequency of painful crises in sickle cell anaemia. N Engl J Med. 1995;332:1317-322.
3. Hajjar FM, Pearson HA. Pharmacologic treatment of thalassemia intermedia with hydroxyurea. J Pediatr. 1994; 125:490.
4. Candido EP, Reeves R, Davie JR. Sodium butyrate inhibits histone deacetylation in cultured cells. Cell. 1978;14: 105-13.
5. Ley TG, De Simone J, Anagnou NP. 5-azacytidine selectively increases gamma-globin synthesis in a patient with beta+ thalassemia. N Engl J Med. 1982;307:1469.
6. De Simone J, Koshy M, Dorn L, et al. Maintenance of elevated fetal hemoglobin levels by decitabine during dose interval treatment of sickle cell anemia. Blood. 2002;99: 3905-908.
7. Aerbajinai W, Zhu J, Gao Z, et al. Thalidomide induces ã-globin gene expression through increased reactive oxygen species-mediated p38 MAPK signaling and histone H4 acetylation in adult erythropoiesis. Blood. 2007;110: 2864-871.
8. Masera N, Tavecchia L, Capra M, et al. Optimal response to thalidomide in a patient with thalassemia major resistant to conventional therapy. Blood Trans. 2010;8:63-65.
9. Li Y, Ren Q, Zhou Y, et al. Thalidomide has a significant effect in patients with thalassemia intermedia. Haematology. 2018;23:50-54.
10. Ricchi P, Costantini S, Spaciano A, et al. The long term and extensive efficacy of low dose thalidomide in a case of untransfusable case of non-transfusion dependent thalassemia. Blood Cells, Molecules and Dis. 2016;57:97-99.
11. Ren Q, Zhou YL, Wang L, et al. Clinical trial on effect of

thalidomide on hemoglobin synthesis in patients with moderate thalassemia intermedia. Ann Haematology 2018; 97:1933-939.
12. Jiskani SA, Memon S. Effect of thalidomide in patients with β-thalassemia major. Hematology Trans International J. 2018;6:34-36.
13. Ramanan V, Kelker K. Role of thalidomide in treatment of beta thalassemia. J Blood Dis Med. 2017.
14. Ghobrial IM, Rajkumar SV. Management of thalidomide toxicity. J Support Oncol. 2003;1:194-205.
15. Yassin AK. Promising response to thalidomide in symptomatic β-thalassemia. Indian J Hematol Blood Transfus. 2020;36:337-41.
16. Nag A, Radhakrishnan VS, Kumar J, et al. Thalidomide in patients with transfusion dependent E-β thalassemia refractory to hydroxyurea: A single center study. Indian J Hematol Blood Transfus. 2020;36:399-402.
17. Shah S, Sheth R, Shah K, et al. Safety and effectiveness of thalidomide and hydroxyurea combination in β thalassemia intermedia and major: A retrospective pilot study. British J Haematol. 2020;188:e18-e21.
18. Yang K, Wu Y, Zhou Y, et al. Thalidomide for patients with β-thalassemia: A multicenter experience. Mediterranean J Hematol Infect Dis. 2020;12:e2020021.
19. Label (PDF) FDA. Accessed November 26, 2020. Available from: *www.accessdata.fda*
20. Mehta P, Yadav N, Soni P, et al. Experience with low dose thalidomide in transfusion dependent beta thalassemia in resource limited setting. Blood. 2019;134:963.
21. Parseval LAM, Verhelle D, Glezer E, et al. Pomalidomide and linelidomide regulate erythropoiesis and fetal hemoglobin production in human CD34+ cells. J Clin Invest. 2008;118;248-58.
22. Jalaji FMA, Fard AD, Hajizamani S, et al. Thalidomide is more efficient than sodium butyrate in enhancing GATA-1 and EKLF gene expression in erythroid progenitors derived from HSCs with B globin gene mutation. Int J Hematol Oncol Stem Cell Res. 2016; 10:37-41.
23. Naithani R, Chandra J, Sharma S. Safety of oral iron chelator deferiprone in young thalassaemics. Eur J Haematol. 2005;74:217-20.
24. Naithani R, Jeyaraman P, Mohapatra M. Alternative strategies in thalassemia: Focus on thalidomide. Indian J Hematol Blood Transf. 2020;36:27-28.

NOTES AND NEWS

RESEARCH PAPER

Low-Dose (0.05 Unit/kg/hour) vs Standard-Dose (0.1 Unit/kg/hour) Insulin in the Management of Pediatric Diabetic Ketoacidosis: *A Randomized Double-Blind Controlled Trial*

RAMACHANDRAN RAMESHKUMAR, PONNARMENI SATHEESH, PUNEET JAIN, JAGADEESH ANBAZHAGAN, SHILPA ABRAHAM, SEENIVASAN SUBRAMANI, NARAYANAN PARAMESWARAN, SUBRAMANIAN MAHADEVAN

From Division of Pediatric Critical Care, Department of Pediatrics, Jawaharlal Institute of Postgraduate Medical Education and Research, Puducherry.

Correspondence to: Dr R Rameshkumar, Associate Professor, Division of Pediatric Critical Care, Department of Pediatrics, Jawaharlal Institute of Postgraduate Medical Education and Research, Puducherry 605 006. krramesh_iway@yahoo.co.in

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Objective: To compare the efficacy of insulin infusion of 0.05 Unit/kg/hour vs 0.1 Unit/kg/hour in the management of pediatric diabetic ketoacidosis (DKA).

Design: Randomized, double-blind controlled clinical trial.

Setting: Pediatric critical care division of a tertiary care hospital from October, 2014 to July, 2018.

Participants: Children aged 12 years or younger with a diagnosis of DKA. Children with septic shock and those who had received insulin before enrollment were excluded.

Intervention: Low-dose (0.05 Unit/kg/hour) *vs*. Standard-dose (0.1 Unit/kg/hour) insulin infusion.

Outcome measures: The primary endpoint was time for resolution of DKA (pH ≥7.3, bicarbonate ≥15 mEq/L, beta-hydroxybutyrate <1 mmol/L). Secondary outcomes were the rate of fall in blood glucose until 250 mg/dL or less and the rate of complications (hypokalemia, hypoglycemia, and cerebral edema).

Results: Sixty patients were analyzed on an intention-to-treat basis (Low-dose group: *n*=30; Standard-dose group: *n*=30). Mean (SD) time taken for the resolution of ketoacidosis was similar in both groups [22 (12) vs 23 (18.5) hours; *P*=0.92]. The adjusted hazard ratio (95% CI) of the resolution of ketoacidosis was lower in the low-dose group [0.40 (0.19 to 0.85); *P*=0.017]. Mean (SD) rate of blood glucose decrease until 250 mg/dL or less reached [56 (41) vs 64 (65) mg/dL/hour; *P*=0.41] and time to achieve the target [4.2 (3.1) vs 4.8 (3.3) hours; *P*=0.44] were similar in both groups. Hypokalemia [30% vs 43.3%; *P*=0.28] and hypoglycemia [3.3% vs 13.3%; *P*=0.35] were lower in low-dose group. No child had cerebral edema, and no mortality occurred.

Conclusions: Time for resolution of ketoacidosis was similar in the low-dose and standard-dose insulin with a lower rate of therapy-related complications in the low-dose group. Hence, low-dose insulin infusion can be a safer approach in the management of pediatric DKA.

Keywords: *Cerebral edema, Complications, Hypokalemia, Outcome, Safety*.

***Trial registration**:* CTRI/2014/08/004823.

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Diabetic ketoacidosis (DKA) is a life-threatening complication in pediatric type 1 diabetes mellitus [1] with a mortality rate of up to 13% in developing countries [2-5]. Fluid and insulin are the cornerstones of DKA management. Although rehydration alone can cause a marked decrease in blood glucose, the objectives are to restore circulating volume and replace the electrolytes and the fluid deficits [1]. After the initial fluid resuscitation, insulin is essential to normalize the hyperglycemia, for the correction of acidosis by suppression of the lipolysis, ketogenesis, generation of bicarbonate from ketoacid metabolism, and to restore the normal cellular metabolism [1,6].

High dose (1 Unit/kg/hour) and bolus insulin strategy are no longer practiced after studies found that a similar therapeutic response could be achieved with a dose of 0.1 Unit/kg/hour with lower rates of adverse effects [7-10]. Limited studies have highlighted that lower insulin doses (0.03 to 0.05 Unit/kg/hour) could normalize the raised beta-hydroxybutyrate (BOHB) levels [11-14]. Recent guidelines recommend insulin infusion at a dose of 0.05 to 0.1 Unit/kg/hour, and low-dose insulin is considered safe and effective, despite not proving its superiority with controlled studies [1].

In low-middle income countries, patients may benefit from low-dose insulin because of associated comorbidities, such as malnutrition, high risk of therapy-related hypokalemia, and hypoglycemia [4]. With limited literature available on the effect of low-dose versus standard-dose insulin in the resolution of ketoacidosis, we hypothesized that low-dose (0.05 Unit/kg/hour) would be associated with early resolution of ketoacidosis, gradual decreases in blood

glucose (BG), and lower frequency of complications as compared to the standard-dose (0.1 Unit/kg/hour) insulin.

METHODS

The study was a randomized, double-blind, controlled clinical trial conducted in the division of pediatric critical care of a tertiary care academic institution from October 2014 to July 2018 after institutitional ethics committee approval. Consecutive children 12 years or younger who presented with DKA defined as hyperglycemia (blood glucose >200 mg/dL), acidosis (pH <7.3 or bicarbonate <15 mEq/L), and ketonemia (BOHB ≥3 mmol/L) or moderate or large ketonuria by urine dipstick test were enrolled [1]. Children with septic shock and those who had received insulin before enrollment were excluded. Informed written consent from parents or legally acceptable representative was obtained.

A web-generated, unstratified, block randomization sequence with variable block sizes (*www.sealed envelope.com*) was used to randomize the eligible children into two groups. A person not involved in the study performed the random number allocation. The allocation was concealed in serially numbered opaque, sealed envelopes (SNOSE), with three alphanumerical codes. The study drug was prepared by a nursing staff, who was not involved in patient care and was masked regarding the patient's identity. Each envelope contained a slip showing the instruction for the preparation of insulin (50 units of regular insulin in 50 mL of normal saline, 0.1 mL=0.1 U or 25 U of regular insulin in 50 mL of normal saline, 0.1 mL=0.05 Unit). Trial syringes were labeled with a random number and three alpha-numerical codes and study drug dose (0.1 mL/kg/hour). The study drug was prepared every six hours and given to nursing-sister responsible for medication administration. The participants, the treating team, those administering the medications, investigators, and research personnel who collected data, and study statisticians, were unaware of the treatment assignments. The treatment allocation was disclosed after the first draft of the results was finalized.

Continuous insulin infusion was administrated through a dedicated intravenous line using an infusion pump. The standard-dose group received regular insulin 0.1 U/kg/hour, and the low-dose group received regular insulin at 0.05 U/kg/hour. The resolution of ketoacidosis (pH ≥7.30, bicarbonate ≥15 mEq/L, and BOHB <1 mmol/L) was taken as the endpoint. After that, the child was shifted to regular subcutaneous insulin with an overlap time of 30 minutes with intravenous insulin.

Fluid volume was calculated as a sum of the deficit (85 mL/kg) and 48-hour maintenance fluid spread over 48 hours [1]. All children received 20 mL/kg of normal saline in the first hour of resuscitation. Children with evidence of hypoperfusion or hypotensive shock received an additional 20 mL/kg of normal saline for one hour. The bolus and other infusions were deducted from the total calculated volume to be infused. Normal saline was used for the first six hours and was changed to 0.45% saline based on serum sodium and effective osmolality. Dextrose (5%) was added to the hydrating fluid once the blood glucose level decreased to 250 mg/dL or less. If the blood glucose level approached 100 mg/dL or below despite dextrose concentration of 12.5%, the insulin infusion was tapered at the rate of 0.01 mL/kg/hour every half-hourly. Potassium chloride (40 mEq/L) was added to the rehydrating fluid after resuscitation and documentation of urine output. Continuous cardiac monitoring was done and potassium titrated to maintain a serum level of 3.5-5.5 mEq/L.

Capillary or venous blood glucose was monitored every half-hourly, and BOHB was monitored hourly by (Abbott Optium-H) ketone meter after calibration. Readings above the glucometer range (more than 500 mg/dL) or in the presence of poor peripheral circulation, capillary BG were counter checked by laboratory measurement using the hexokinase method. Urine ketone and glucose were monitored hourly by the dipstick method. Glycated hemoglobin (HbA1c) was done at admission. Serum electrolytes, blood glucose, urea, calcium, magnesium, phosphate, hematocrit, and venous blood gases and corresponding anion gap and effective osmolality were monitored two-hourly first six-hour, subsequently four-hourly till resolution of ketoacidosis. Vital signs, fluid (intake and output), continuous monitoring of the electrocardiogram, and neurologic assessment was performed hourly (or more frequently as indicated). A review of insulin therapy was performed for errors in dose, preparation, and infusion rate six-hourly and as indicated. Nutritional status was assessed using the World Health Organization standards [15].

The primary outcome was the time for resolution of ketoacidosis (pH ≥7.3, bicarbonate ≥15 mEq/L, beta-hydroxybutyrate <1 mmol/L). The secondary outcomes were the rate of decrease in blood glucose until the level reaches 250 mg per dL or less, the incidence of hypoglycemia, hypokalemia, and cerebral edema and/or worsening of cerebral edema. Hypokalemia was defined as serum potassium <3.5 mEq/L and/or suggestive electrocardiographic changes. Hypoglycemia was defined as a blood glucose level ≤60 mg/dL. Cerebral edema was diagnosed as per the criteria given by Muir, et al. [16].

With assumptions of the mean time for resolution of ketoacidosis in the standard-dose group as 20 hours and in the low-dose group as 16 hours [12,17], the sample size

was calculated with a common standard deviation of four hours, a two-sided alpha level of 5%, and 95% power. The sample size was 52 DKA episodes. Considering an attrition rate of 10%, we enrolled 60 participants.

Statistical analysis: The patient's data were analyzed according to their assigned group (Intention to treat). The normality of data was checked with the Kolmogorov-Smirnov *Z* test. Continuous variables between the groups were compared with Student *t*-test if normally distributed or Mann-Whitney *U* test if skewed data. The proportion was compared by the chi-square test (Fisher exact test if cell frequency was <5), and relative risk (95% CI) was calculated as appropriate. Cox proportional model adjusted a prior for age, weight, and severity of ketoacidosis was used to calculate the hazard ratio (95% CI) for resolution of ketoacidosis and blood glucose fall 250 mg/dL or less. Changes in continuous variables up to 24 hours were compared using repeated-measures analysis of variance (RM-ANCOVA), and missed values were handled by Last-Observation-Carried-Forward (LOCF) method. All tests were two-tailed, and a *P* value of less than 0.05 was considered statistically significant. Data analyses were performed using IBM-SPSS, version 20.0 (SPSS Inc) and Epi Info 7 (7.0.9.7, CDC).

RESULTS

The trial flow diagram is depicted in **Fig. 1**. Of the 69 children with DKA screened for eligibility, 60 were enrolled (30 in each group). No protocol violation was noted. Baseline characteristics were comparable (**Table I)**. New onset diabetes presenting as DKA was seen in 48.3% of children. Severe DKA was seen in 38.3% (*n*=23) of children. In all patients data, the mean (SD) blood sugar and BOHB change after the first hour of fluid bolus was –44.8 (72.2) and –0.3 (0.9), respectively. Mean (SD) fluid balance (percentage) at six hours of therapy was similar in the low-dose and standard-dose groups [0.15 (0.14) vs 0.10 (0.1); *P*=0.16].

Mean (SD) time taken for resolution of ketoacidosis was 22 (12) hours in low-dose and 23 (18.5) hours in the standard-dose groups (*P*=0.92) (**Table II**). Time taken for an individual parameter of ketoacidosis to achieve the end-points (pH ≥7.30, HCO3 ≥15 mEq/L, BOBH <1 mmol/L, and normal sensorium) was similar in the two groups (**Table II**). In sub-group analysis (severe DKA), there was no significant difference in mean (SD) time taken for the resolution of ketoacidosis in low-dose group vs standard-dose group [31.4 (11.3) vs 36.3 (21.4) h, *P*=0.52]. The hazard ratio of the resolution of ketoacidosis was significantly lower by 60% in the low-dose group than the standard-dose group [adjusted hazard ratio 0.40, 95% CI: 0.19 to 0.85; *P*=0.017] (**Fig. 2**). The hazard ratio of the resolution of BOHB (<1 mmol/L) was significantly lower by 65% in the low-dose group [adjusted hazard ratio 0.35, 95% CI: 0.17 to 0.74; *P*=0.006].

The mean (SD) rate of blood glucose fall per hour and the time taken until the level was 250 mg/dL was similar in the two groups (**Table II**). However, the mean (SEM) trend in fall of blood glucose until six hours and 24 hours was higher in the standard-dose group [by six hours, 43 (3.4), *P*=0.008; by 24 hours, 12.5 (1), *P*=<0.001] as compared to the low-dose group [by six hours, 29.6 (3.4); by 24 hours, 7.1 (1)]. The mean (SEM) trend in fall of BOHB until six hours and 24 hours was not significantly different between the standard-dose group [by six hours, 0.30 (0.06), *P*=0.81; by 24 hours, 0.17 (0.01), *P*=0.81] and the low-dose group [by six hours, 0.29 (0.06); by 24 hours, 0.16 (0.01)].

There was no difference in the proportion of patients who attained the blood glucose level of 250 mg/dL at six-hour in standard-dose and low-dose groups (76.7% vs 70%, RR=1.10, 95% CI: 0.81 to 1.19; *P*=0.56). The frequency of fall in blood glucose, more than 90 mg/dL/hour, was higher in standard-dose group (76.7%) as compared to low-dose group (60%) (RR=1.28, 95% CI: 0.90 to 1.82; *P*=0.17). The hazard ratio of achieving blood glucose 250 mg/dL or less by the end of six hours was 1.35 times higher in the low-dose group than the standard-dose group (adjusted hazard ratio 1.35, 95% CI: 0.72 to 2.54; *P*=0.35).

Hypoglycemia [RR (95% CI) 4.0 (0.47-33.7); *P*=0.35] and at least one episode of hypokalemia [RR 995% CI) 1.44 (0.73-2.8); *P*=0.28] was higher in the standard-dose group as compared to the low-dose group (**Table II**). None

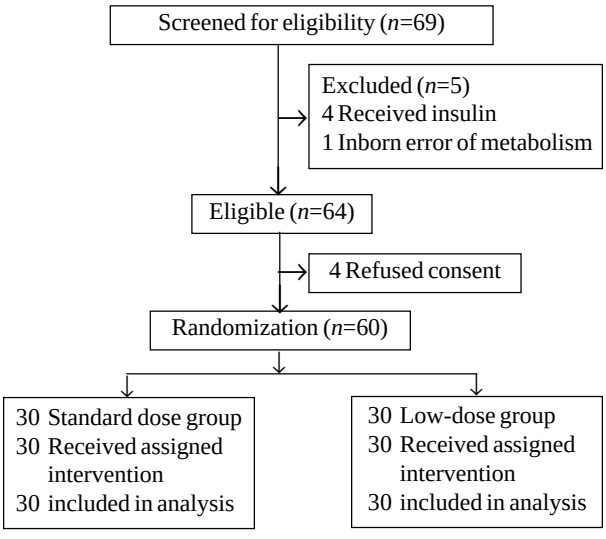

Fig. 1 Study flow chart.

Table I Baseline Characteristics in Children With Diabetic Ketoacidosis

Characteristic	*Low-dose group (n=30)*	*Standard-dose group (n = 30)*
Age, y	7 (3.6)	8.4 (3.2)
Body mass index, *z*-score	– 2.2 (1.6)	– 2.5 (1.5)
Malnutrition, *n* (%)	7 (23.3)	9 (30)
New onset DKA, *n* (%)	15 (50)	14 (46.6)
Established diabetes mellitus, *n* (%)	15 (50)	16 (53.4)
Duration of diabetes, mo	28 (29.6)	28.2 (25.4)
With previous DKA, *n* (%)	11 (73.3)	10 (62.5)
Duration of symptom, d	4.4 (5.7)	4.4 (4.7)
Severity, n (%)		
Mild	12 (40)	8 (26.7)
Moderate	8 (26.7)	9 (30)
Severe	10 (33.3)	13 (43.3)
Hemodynamic status, n (%)		
Compensated shock	3(10)	7 (23.3)
Hypotensive shock	1 (3.3)	1 (3.3)
m-GCS 8-14 at admission, *n* (%)	8 (27)	9 (30)
m-GCS score, median (IQR)	15 (14-15)	15 (14-15)
Hemoglobin A1c, %	13.5 (2.6)	13.3 (2.5)
Blood glucose, mg/dL	465.5 (105.6)	510.3 (113)
pH	7.15 (0.13)	7.10 (0.16)
Bicarbonate , mEq/L	8.9 (4.3)	7.1 (4.3)
PCO_2, mm Hg	20.6 (7.5)	19.2 (7.3)
Capillary BOHB, mmol/L	5.4 (1.4)	5.3 (1.4)
Blood urea nitrogen, mg/dL	11.5 (3.6)	12.8 (5.1)
Creatinine, mg/dL	1.0 (0.3)	1.1 (0.4)
Sodium, mEq/dL	137.5 (6.2)	138 (5.8)
Corrected sodium, mEq/L	143.5 (6.8)	144.6 (5.5)
Effective osmolality, mOsm/kg	292.4 (45.8)	304.4 (11.3)
Potassium, mEq/L	3.9 (0.7)	3.9 (0.7)
Anion gap	26 (7.4)	27.5 (5.6)
Lactate, mmol/L	1.8 (0.8)	2 (1)
Urine ketones, n (%)		
3+ (80 – 160 mg/dL)	19 (63.3)	19 (63.3)
4+ (>160 mg/dL)	11 (36.7)	11 (36.7)
Fluid received, mL/kg[a]	16 (10)	13.7 (5.6)
Blood glucose change, mg/dL[b]	– 61.4 (66)	– 28.3 (75.4)
Capillary BOHB change, mmol/L[b]	– 0.2 (1.0)	– 0.3 (0.9)
Duration of 0.9% saline therapy, h	5 (2.7)	6.3 (3.9)

Data presented in mean (SD) or as stated (%). DKA-diabetic ketoacidosis; m-GCS-modified Glasgow Coma Scale score; PCO_2 – partial pressure of carbon dioxide; BOHB – beta hydroxy hydroxybutyrate. [a]before starting insulin infusion; [b]after the initial hour of fluid resuscitation before starting insulin infusion. All P>0.05.

of the hypoglycemia patients encountered more than or equal to two episodes of hypoglycemia. The hypokalemia was more in malnourished children in the standard-dose group (*P*=0.31), and more children in the standard-dose group required a higher concentration of dextrose and tapering of insulin infusion at least once to counter the falling blood glucose *P*>0.005) None of the children required the increment of insulin infusion.

The mean (SEM) trend of fall in effective osmolality until six hours [0.53 (0.37) vs 0.70 (0.37); *P*=0.33] and 24 hours [0.54 (0.14) vs. 0.27 (0.14), *P*=0.45] was not significantly different between the standard-dose group and the low-dose group. No child had cerebral edema, and no mortality occurred in the study.

DISCUSSION

We found that the time taken for the resolution of ketoacidosis was similar in the low-dose insulin and standard-dose insulin groups. The hazard ratio of the resolution of ketoacidosis was lower by 60% in the low-dose group. The optimal insulin level for recovery of ketoacidosis is 20 to 200 micro Unit/ml, and it could be

Table II Outcome Measures in the Two Study Groups

Characteristic	*Low dose group (n=30)*	*Standard dose group (n=30)*
Primary outcome		
Time for resolution of DKA, h	22 (12)	23 (18.5)
Time for pH ≥7.30, h	13.4 (11.5)	17.1 (17.6)
pH	7.33 (0.02)	7.32 (0.03)
Time for bicarbonate ≥15 mEq/L, h	15.5 (11.7)	18.6 (18.7)
Bicarbonate, mEq/L	16.4 (1.4)	16.1 (1.3)
Time for BOHB <1 mmol/L, h	21.6 (11.8)	17.8 (9.8)
BOHB, mmol/L	0.76 (0.16)	0.73 (0.19)
Time for resolution of DKA (including normal sensorium), h	23 ((12.8)	23.1 (18.5)
Time for the normal sensorium, h	3.7 (11.1)	6 (17.4)
Secondary outcome		
Blood glucose decrease until the level reached ≤250 mg/dL, mg/dL/hour	56 (41)	64 (65)
Time to achieve blood glucose ≤250 mg/dL, h	4.2 (3.1)	4.8 (3.3)
Blood glucose, mg/dL	217 (29.8)	218.8 (32)
Hypokalemia[a]	9 (30)	13 (43.3)
Hypoglycemia[a]	1 (3.3)	4 (13.3)
Tapering of insulin infusion[a]	13 (43.3)	14 (46.7)

Data presented as mean (SD) *except [a]no. (%). DKA-diabetic ketoacidosis; BOHB-beta hydroxy hydroxybutyrate. All P>0.05.*

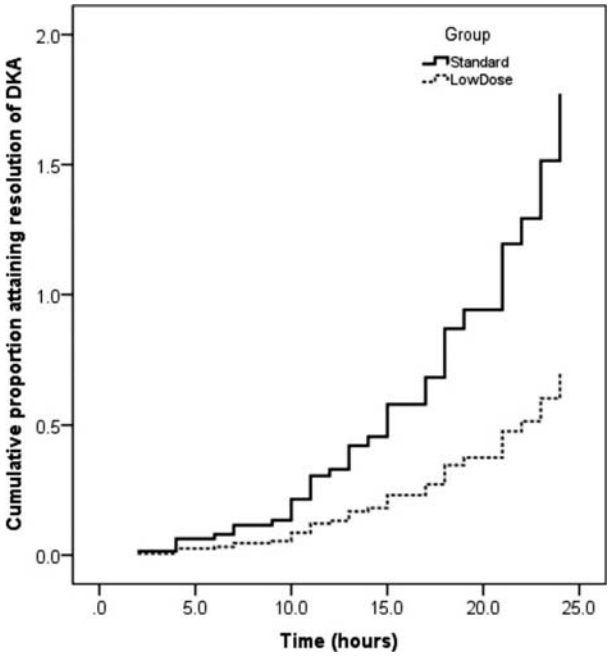

Fig. 2 Hazard curves plot for resolution of diabetic ketoacidosis (DKA).

achieved with a lower dose of insulin [18]. The standard-dose of insulin has been reported to achieve higher than optimal plasma insulin requisite level [6,18]. With this justification, lowering of insulin dose up to 0.025 U/kg/hour as compared with standard-dose was studied in pediatric DKA [12,14,19]. Puttha, et al. [12] found that a rise in pH at six hours and the median time for resolution of ketoacidosis (pH >7.3) was similar in the low and standard-dose groups [12]. Kapellen, et al. [19] reported that 0.025 vs 0.1 U/kg/hour was associated with a similar duration of acidosis [19]. A controlled study by Nallasamy, et al. [14] found that low-dose was not inferior to standard-dose insulin in the resolution of ketoacidosis. However, BOHB was not monitored as one of the endpoints of ketoacidosis [14].

The trend of fall in blood group up to 24-hour was significantly higher in the standard-dose group, although the reduction rate was within the reported range (36 to 90 mg/dL/h) as with other studies [12-14]. Nallasamy, et al. [14] reported that the rate of fall in BG until ≤250 mg/dL was similar in low and standard-dose groups [14]. Hence, any effective dose of insulin which achieves the optimal plasma insulin level (20 to 200 micro-unit/mL) could exhibit the desired therapeutic clinical response without affecting osmotic hemostasis [6,14]. Though we could not measure plasma insulin levels in our study, our results support the use of low-dose insulin to achieve clinically effective resolution of ketoacidosis and gradual reduction of BG.

Retrospective pediatric studies have reported rapid BG decrease with the use of insulin dose higher than 0.05 units/kg/hour [20] and the gradual decrease in plasma effective osmolality due to slower decrease in BG by using insulin dose of 0.05 U/kg/hour [13], despite the well-established fact that resolution of ketoacidosis and not BG determine the endpoint of DKA management [1]. The amount of insulin administrated in the first-hour and volume of fluid administrated over four-hour were associated with the risk of cerebral edema after adjusting for the severity of ketoacidosis in the management of DKA [21]. Our setting is complicated by delayed presentation, severe ketoacidosis, undernutrition, and high effective osmolality at presentation, where initial hours of therapy warrant a more cautious approach to preventing osmotic disequilibrium and cerebral edema. We found that fluid administration and change of effective osmolality was similar in both the groups. The frequency of fall of BG and tapering of insulin infusion despite maximum glucose concentration was higher in the standard-dose group. Nallasamy, et al. [14] found that more episodes of out of range fall of BG (> 90 mg/dL) in the standard-dose group in a study setting similar to ours. Hence, low-dose insulin after the first hour of fluid resuscitation is a safe approach in a setting where a gradual decrease in BG, effective osmolality and smooth resolution of ketoacidosis is desired.

Therapy-related complications, hypokalemia, and hypoglycemia were higher in the standard-dose group. Undernutrition, prolonged duration of illness, severe ketoacidosis, and osmotic diuresis likely contributed to hypokalemia in this study group. In addition to these factors, insulin dose also could have contributed to the lowering of potassium levels. In a similar setting, Nallasamy, et al. [14] and Moulik, et al. [22] reported the frequency of occurrence of hypokalemia was higher in the standard-dose group. Hypoglycemia was reported in higher proportion by previous authors [14,22] in the standard-dose group as compared to our study. Half-hourly monitoring of blood glucose could have contributed to the lower incidence of hypoglycemia in our study. However, the potentially beneficial effect of lowering the insulin dose cannot be ignored entirely.

We monitored the blood ketone (BOBH) as one of the endpoints of the study, which is in contrast to previous studies [9,12,14]. Unlike other studies, we followed until the resolution of ketoacidosis and collected and analyzed the 24 hours data. We also analyzed the factors unique to limited-resource settings, enabling the available study results to apply to the low and middle-income countries. The limitation of our study is that we could not enroll adolescents with DKA due to hospital admission policy during the study period.

WHAT IS ALREADY KNOWN?

- Insulin infusion at 0.05 Unit/kg/hour was comparable to 0.1 Unit/kg/hour with respect to a decrease in blood glucose and resolution of acidosis.

WHAT THIS STUDY ADDS?

- Low-dose (0.05 Unit/kg/hour) insulin infusion was comparable to 0.1 Unit/kg/hour insulin infusion for the resolution of ketoacidosis, decrease in blood glucose, and therapy-related complications in pediatric diabetic ketoacidosis.

We conclude that the time for resolution of ketoacidosis was similar in the low-dose and standard-dose insulin infusion, with a lower rate of therapy-related complications in low-dose insulin. Hence, insulin infusion at 0.05 Unit/kg/hour is a safer approach in the manage-ment of pediatric DKA.

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REFERENCES

1. Wolfsdorf JI, Glaser N, Agus M, et al. ISPAD Clinical Practice Consensus Guidelines 2018: Diabetic ketoacidosis and the hyperglycemic hyperosmolar state. Pediatr Diabetes. 2018;19:155-77.
2. Kanwal SK, Bando A, Kumar V. Clinical profile of diabetic ketoacidosis in Indian children. Indian J Pediatr. 2012;79:901-4.
3. Tiwari LK, Jayashree M, Singhi S. Risk factors for cerebral edema in diabetic ketoacidosis in a developing country: Role of fluid refractory shock. Pediatr Crit Care Med. 2012;13:e91-6.
4. Jayashree M, Singhi S. Diabetic ketoacidosis: Predictors of outcome in a pediatric intensive care unit of a developing country. Pediatr Crit Care Med. 2004;5:427-33.
5. Poovazhagi V. Risk factors for mortality in children with diabetic keto acidosis from developing countries. World J Diabetes. 2014;5:932-8.
6. Luzi L, Barrett EJ, Groop LC, Ferrannini E, DeFronzo RA. Metabolic effects of low-dose insulin therapy on glucose metabolism in diabetic ketoacidosis. Diabetes. 1988;37: 1470-7.
7. Alberti KG, Hockaday TD, Turner RC. Small doses of intramuscular insulin in the treatment of diabetic "coma". Lancet. 1973;2:515-22.
8. Kitabchi AE, Ayyagari V, Guerra SM. The efficacy of low-dose versus conventional therapy of insulin for treatment of diabetic ketoacidosis. Ann Intern Med. 1976;84:633-8.
9. Burghen GA, Etteldorf JN, Fisher JN, Kitabchi AQ. Comparison of high-dose and low-dose insulin by continuous intravenous infusion in the treatment of diabetic ketoacidosis in children. Diabetes Care. 1980;3:15-20.
10. Dunger DB, Sperling MA, Acerini CL, et al. ESPE/LWPES consensus statement on diabetic ketoacidosis in children and adolescents. Arch Dis Child. 2004;89:188-94.
11. Noyes KJ, Crofton P, Bath LE, et al. Hydroxybutyrate near-patient testing to evaluate a new end-point for intravenous insulin therapy in the treatment of diabetic ketoacidosis in children. Pediatr Diabetes. 2007;8:150-6.
12. Puttha R, Cooke D, Subbarayan A, et al. Low dose (0.05 units/kg/h) is comparable with standard dose (0.1 units/kg/h) intravenous insulin infusion for the initial treatment of diabetic ketoacidosis in children with type 1 diabetes-an observational study. Pediatr Diabetes. 2010;11:12-7.
13. Al Hanshi S, Shann F. Insulin infused at 0.05 versus 0.1 units/kg/hr in children admitted to intensive care with diabetic ketoacidosis. Pediatr Crit Care Med. 2011;12:137-40.
14. Nallasamy K, Jayashree M, Singhi S, Bansal A. Low-dose vs standard-dose insulin in pediatric diabetic ketoacidosis: A randomized clinical trial. JAMA Pediatr. 2014;168:999-1005.
15. de Onis M, Blossner M. The World Health Organization Global Database on Child Growth and Malnutrition: methodology and applications. Int J Epidemiol. 2003;32:518-26.
16. Muir AB, Quisling RG, Yang MC, Rosenbloom AL. Cerebral edema in childhood diabetic ketoacidosis: natural history, radiographic findings, and early identification. Diabetes Care. 2004;27:1541-6.

17. Kurup PM, Rameshkumar R, Soundravally R, Satheesh P. Capillary versus Serum b-hydroxybutyrate in Pediatric Diabetic Ketoacidosis. Indian Pediatr. 2019;56:126-9.
18. Soler NG, FitzGerald MG, Wright AD, Malins JM. Comparative study of different insulin regimens in management of diabetic ketoacidosis. Lancet. 1975;2: 1221-4.
19. Kapellen T, Vogel C, Telleis D, Siekmeyer M, Kiess W. Treatment of diabetic ketoacidosis (DKA) with 2 different regimens regarding fluid substitution and insulin dosage (0.025 *vs*. 0.1 units/kg/h). Exp Clin Endocrinol Diabetes. 2012;120:273-6.
20. Bradley P, Tobias JD. Serum glucose changes during insulin therapy in pediatric patients with diabetic ketoacidosis. Am J Ther. 2007;14:265-8.
21. Edge JA, Jakes RW, Roy Y, et al. The UK case-control study of cerebral oedema complicating diabetic ketoacidosis in children. Diabetologia. 2006;49:2002-9.
22. Moulik NR, Jayashree M, Singhi S, Bhalla AK, Attri S. Nutritional status and complications in children with diabetic ketoacidosis. Pediatr Crit Care Med. 2012;13:e227-33.

RESEARCH PAPER

Epinephrine Plus Vasopressin vs Epinephrine Plus Placebo in Pediatric Intensive Care Unit Cardiopulmonary Resuscitation: *A Randomized Double Blind Controlled Clinical Trial*

ABRAAR SHERIFF, RAMACHANDRAN RAMESHKUMAR, MUTHU CHIDAMBARAM, KAUSHIK MAULIK, ROUTHU SANTHOSH KUMAR, ATAHAR JAMAL, ROHIT BHOWMICK, NIRANJAN BISWAL, SUBRAMANIAN MAHADEVAN

From Division of Pediatric Critical Care, Department of Pediatrics, Jawaharlal Institute of Postgraduate Medical Education and Research (JIPMER), Puducherry.

Correspondence to: Dr Rameshkumar R, Associate Professor, Division of Pediatric Critical care, Department of Pediatrics, Jawaharlal Institute of Postgraduate Medical Education and Research (JIPMER), Puducherry 605 006.
krramesh_iway@yahoo.co.in

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Objective: To compare the efficacy of epinephrine plus vasopressin vs epinephrine plus placebo in the pediatric intensive care unit (PICU) cardiopulmonary resuscitation (CPR).

Design: Randomized, double-blind controlled clinical trial.

Setting: PICU in a tertiary care institute from February, 2019 to May, 2020.

Participants: Children aged one month to 13 years who required CPR during PICU stay. Patients in whom vascular access was not available or return of spontaneous circulation (ROSC) was achieved by defibrillation without epinephrine were excluded.

Intervention: Patients were randomized to receive vasopressin 0.1 mL per kg (=0.8 unit per kg) or placebo (0.1 mL per kg normal saline) in addition to epinephrine (1:10000) 0.1 mL per kg. The drugs were given as bolus doses every three minutes until the ROSC or up to a maximum of five doses, whichever was earlier.

Outcome Measure: The primary outcome was the proportion of patients who achieved ROSC. The secondary outcomes were survival rate and functional status (at 24-hour, PICU, hospital, and 90-day post-discharge), need for organ supports, length of stay (PICU and hospital), and adverse effect(s) of the study drugs.

Results: 90 patients (epinephrine plus vasopressin group, *n*=45 and epinephrine plus placebo group, *n*=45) were analyzed on intention-to-treat basis. There was no significant difference in the primary outcome between epinephrine plus vasopressin (*n*=25, 55.5%) and epinephrine plus placebo groups (*n*=24, 53.3%) (Relative risk 1.04, 95% CI 0.71 to 1.52). There was no significant difference in survival rate at 24-hour (*n*=7, 15.6% vs. *n*=8, 17.8%), at PICU, hospital, and 90-day post-discharge (*n*=1, 2.2% vs *n*=1, 2.2%). There was no difference in other secondary outcomes. No trial drug-related serious adverse events were observed.

Conclusion: A combination of epinephrine plus vasopressin did not improve the rate of return of spontaneous circulation in the pediatric intensive care unit cardiopulmonary resuscitation as compared with epinephrine plus placebo.

Keywords: *In-hospital cardiac arrest*.

Trial Registration: CTRI/2019/01/017200.

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Cardiac arrest is a dreadful event in pediatric intensive care unit (PICU). Cardiopulmonary resuscitation (CPR) involves chest compression and manual ventilation at appropriate intervals. Return of spontaneous circulation (ROSC) is the initial therapeutic goal in cardiac arrest and is a measure of initial success. Vasopressor medications are often used during CPR. These medications increase aortic diastolic pressure, thereby improving coronary perfusion pressure, which facilitates ROSC [1]. Epinephrine is the most widely studied and the first line vasoactive drug as per the pediatric advanced life support (PALS) guidelines [2]. Vasopressin, a potent vasoconstrictor, is well studied in adult cardiac arrest [3]. The recent advanced cardio-vascular life support guideline recommends that Vaso-pressin, combined with epinephrine, may be considered in adult cardiac arrest resuscitation [3]. Though the pediatric in-hospital cardiac arrest (IHCA) outcome has improved from 39% to 77% in high-income countries, data from low-and middle-income countries are lacking or are under-reported [2]. Animal studies, case series, and feasibility pilot studies have shown encouraging results for the use of vasopressin in pediatric cardiac arrest [4-6]. This study hypothesized that epinephrine plus vasopressin would be associated with a higher rate of ROSC as compared to epinephrine plus placebo in the pediatric intensive care unit cardiopulmonary resuscitation.

METHODS

This randomized, double-blind controlled clinical trial was undertaken in PICU of a tertiary care academic hospital from February, 2019 to May, 2020. Ours is a 19 bedded

level-III PICU, receiving critically ill children 24 hours a day throughout the year. Though our PICU is a predominantly medical ICU, it also receives complicated surgical and trauma patients. The PICU has facilities for providing multimodal hemodynamic and neuromonitoring, mechanical ventilation, and high-frequency ventilation. It is also equipped with an in-house blood gas analyzer with a co-oximeter module, osmometer, therapeutic plasma exchange, and renal replacement therapy. During the study period, the baseline mortality in our PICU was 20%, and the average length of PICU stay was six days, with a bed occupancy rate of 80%.

The trial was approved, and its progress was reviewed yearly by the institute ethics committee. Written informed consent was obtained from the parent/legally authorized representatives of all patients getting admitted to PICU at the time of transfer-in, stating that their child might be enrolled in the study if the child required CPR during the PICU stay. Children aged one month to 13 years, admitted in PICU, and who required CPR during their PICU stay were enrolled. Children who had a cardiac arrest outside of PICU and were shifted to PICU for post-cardiac arrest care were not enrolled. Children with either of the following conditions were also excluded (*i*) patients in whom vascular access was not available (*ii*) ROSC was achieved by defibrillation without the requirement of Epinephrine.

A computer-generated, unstratified, block randomization with variable block sizes of four, six, and eight was used with an allocation ratio of 1:1 by a person not involved in the study. Individual assignments were kept in serially numbered boxes. Each box contained ten identically looking one mL ampoules of either vasopressin or placebo (normal saline). The original label in each ampoule was removed and replaced by an opaque paper. Each box was serially numbered and allocated to the patient according to the random sequence. The serially-numbered trial drug boxes were kept in a separate place in PICU to avoid the wrong allocation in the stressful environment. Only one trial drug box was kept in the crash cart, which contained all the emergency drugs and equipment required for CPR. The nurses were instructed to open the trial drug box, which was kept in the crash cart during CPR. The investigator ensured the replacement of the trial drug box in the crash cart according to the serial number once the trial drug was used. Multiple simulation sessions were carried out and discussed before the start of the study. Injection normal saline (sodium chloride 0.9%, 1 mL, Serum Institute of India Pvt Ltd), injection epinephrine (Bioaderna, 1 mg per 1 mL, Health Biotech Ltd) and injection vasopressin (Vascel 20, 20 Unit per mL, CELON laboratories Pvt Ltd) were used in this study. The institute's central pharmacy supplied the trial drugs. The participants, treating team and nurses administering the medications, and the investigators, were unaware of the treatment assignments. The person who collected and entered the data into the datasheet and the study statistician were unaware of the treatment assign-ment throughout the analyses. The treatment assignment was disclosed, after the first draft of the result was finalized.

All patients received CPR in accordance with the PALS-2015 guidelines established by the American Heart Association (AHA) [2]. This includes the support of airway, breathing, including supplemental oxygen, evaluation of cardiac rhythm, high-quality CPR with minimally interrupted chest compressions, electrical defibrillation if appropriate, and medications except for the trial drugs. The resuscitation team members were trained to provide CPR as per the PALS 2015 guidelines [2]. The facility for standby extracorporeal membrane oxygenation (ECMO) is not available in the study site (PICU). Our hospital has no approved guidelines for 'Do not resuscitate' instructions. Epinephrine plus vasopressin group received intravenous epinephrine (1:10000) 0.1 mL per kg and vasopressin (1:1.5 dilution in normal saline) 0.1 mL per kg (=0.8 unit per kg; maximum dose of 5 mL, 40 unit). Epinephrine plus placebo group received intravenous epinephrine (1:10000) 0.1 mL per kg and placebo (1:1.5 dilution in normal saline) 0.1 mL per kg. The trial drugs were given as bolus doses, concurrently if two vascular accesses were available or within 10 seconds gap if one vascular access was available. The trial drugs were given at an interval of every three minutes until ROSC or a maximum of five doses, whichever was earlier. Three mL normal saline flush was given after adminis-tration of each dose of the trial drug. Subsequently, if needed, epinephrine was continued as per protocol. Post-resuscitation care was provided to the patients who achieved ROSC as per the unit protocol (from PALS-2015 guidelines) [2]. All patients were followed up until death or 90 day post-discharge. The functional status of the survivor was assessed by using the pediatric cerebral performance category (PCPC) scale and pediatric overall performance category (POPC) scale (lower the score, better the neurological outcome) [7]. Data regarding the cardiac arrest events and their outcomes were collected as per the Utstein style template and in the predesigned proforma [8-10].

The primary outcome was the proportion of patients who achieved ROSC. The secondary outcomes were (*i*) survival rate (at 24 hours, PICU, hospital, and 90-day of discharge), (*ii*) functional status (at PICU, hospital, and 90-day of discharge), (*iii*) need for organ support(s), (*iv*) length of stay in PICU and hospital, and (*v*) adverse effect(s) of the study drugs if any. ROSC was defined as the restoration of a spontaneous perfusing rhythm that results

in more than an occasional gasp, fleeting palpable pulse, or arterial waveform [2,3,10]. Sustained ROSC was defined as not requiring chest compressions for 20 consecutive minutes after obtaining ROSC and signs of perfusion [2,3,10]. The probability of adverse trial drug reaction was assessed by Naranjo algorithm [11].

The ROSC rate varies between 47% and 64.6%, as reported by previous studies [12,13]. We assumed that the primary outcome of interest in the control group was 50%. We calculated the sample size based upon the assumption of 30% improvement in the primary outcome by the intervention with 80% power at the 5% significance (two-sided) and 1:1 allocation. Thirty-nine patients were required in each group by calculation. With a 10% attrition rate, the final sample size was estimated as 86 [12-14]. The sample size was calculated using the software nQuery version 4.0.

Statistical analysis: Data were analyzed according to their assigned groups (intention to treat analysis). The distribution of data was checked with the Kolmogorov-Smirnov Z test. Continuous variables were compared between the two groups by Student's *t*-test for normally distributed or by the Mann-Whitney *U* test for skewed data. Proportions were compared by the Chi-square test (or Fisher's exact test if expected cell frequencies were less than five). Kaplan-Meier curve and log-rank test were used to analyze 'time to event' data followed by Cox proportional hazard regression analysis to adjust for the prespecified baseline factors (age, sex, and PRISM-III score). The relative risk and hazard ratio, with a 95% confidence interval, was calculated as appropriate. All tests were two-tailed, and a *P* value of less than 0.05 was considered statistically significant. IBM SPSS software 20.0 (IBM Corp) and Epi Info 7 (7.0.9.7, CDC) were used for data analysis.

RESULTS

The study flow is depicted in **Fig. 1**. Ninety patients were enrolled (epinephrine plus vasopressin, *n*=45, and epinephrine plus placebo *n*=45) after the screening of 118 patients. The baseline characteristics and clinical variables are described in **Table I**. The median (IQR) time to first cardiac arrest since admission was similar between groups [2 (1-7) vs 2 (1-5) day; *P*=0.75]. The most common (80%) arrest rhythm was pulseless electrical activity (PEA). Hemodynamic abnormality (67.8%) was the most common event that led to arrest, followed by respiratory events (23.3%). Respiratory failure was an underlying illness in 76 (84.4%) patients and sepsis in 60 (66.7%) patients. The median (IQR) duration of CPR was similar between groups [18 (10-30) vs 15 (6–30) minutes; *P*=0.96].

The proportion of patients who achieved ROSC was similar in epinephrine plus vasopressin group and epinephrine plus placebo group [RR (95% CI) 1.04 (0.71-1.52); *P*=0.83]. The time to achieve ROSC and the proportion of patients requiring ongoing CPR was similar between two groups during the first 30 minutes of CPR [Log rank *P*=0.99] **(Fig. 2)**. Among ROSC achieved patients (*n*=49), the median (IQR) time taken to ROSC was similar between two groups [10 (4-14) vs 6 (5-10) minutes, *P*=0.21]. The proportion of patients who underwent CPR beyond 30 minutes was also similar between two groups [RR (95% CI) 0.50 (0.19-1.35); *P*=0.16] and none achieved ROSC. There was no significant difference in the proportion of patients who achieved sustained ROSC in the study groups [44.4% vs 53.3%; *P*=0.40]. The survival to hospital discharge was similar in both groups [*n*=1 each]. Mean (SD) diastolic blood pressure (DBP) was similar in epinephrine plus vasopressin group as compared to epinephrine plus placebo group during CPR (38.1 (11.5) mm Hg vs 37.1 (13.4) mm Hg, *P*=0.77). There was no significant difference in the other secondary outcomes between study groups **(Table II)**. In epinephrine plus vasopressin group, one patient developed pulseless ventricular tachycardia which converted into asystole

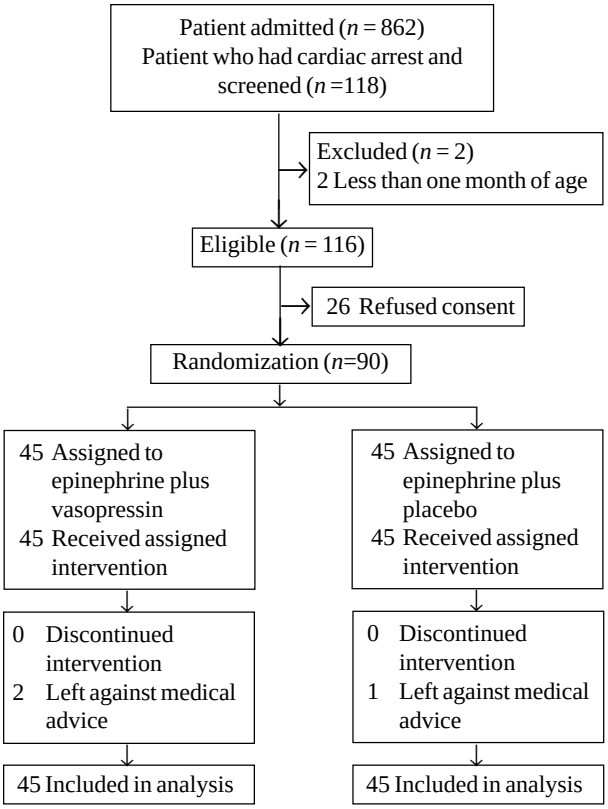

Fig. 1 Study flow chart.

Table I Baseline Characteristics and Clinical Variables of the Two Study Groups

Variables	*Epinephrine plus vasopressin group (n = 45)*	*Epinephrine plus placebo group (n = 45)*
Age, y[a]	2.5 (3.3)	3 (4.4)
Male: female	25:20	28:17
Body mass index[a]	– 2 (1.9)	– 1.9 (2.0)
Pediatric risk of mortality - III score[a]	19.6 (9.6)	18 (8.6)
Arrest rhythm		
Pulseless electrical activity	38 (84.5)	34 (75.6)
Asystole	6 (13.3)	10 (22.2)
Pulseless ventricular tachycardia	1 (2.2)	1 (2.2)
Events leading to arrest		
Hemodynamic abnormality	31 (68.9)	30 (66.7)
Respiratory events	11 (24.4)	10 (22.2)
Rhythm disturbance	3 (6.7)	5 (11.1)
Illness category		
Medical condition	40 (89)	42 (93.3)
Surgical condition	5 (11)	3 (6.7)
Diagnosis and underlying illness[b]		
Respiratory failure	38 (84.4)	38 (84.4)
Sepsis and shock[d]	37 (82.2)	23 (51.1)
CNS illness	19 (42.2)	22 (49)
Pneumonia	24 (53.3)	17 (37.8)
Congenital heart disease	7 (15.6)	10 (22.2)
Renal insufficiency	21 (46.7)	16 (35.6)
Hepatic insufficiency	21 (46.7)	14 (31.1)
Malignancy	5 (11)	4 (9)
Intervention in place at the time of event[c]		
Mechanical ventilation	44 (97.8)	43 (95.6)
$EtCO_2$ monitoring	44 (97.8)	43 (95.6)
Arterial line	37 (82.2)	34 (75.6)
Central venous access	43 (95.6)	42 (93.3)
Vasoactive drug infusion	40 (89)	39 (86.7)
Renal replacement therapy	8 (17.8)	6 (13.3)
Intervention done during CPR		
Sodium bicarbonate	14 (31.1)	23 (51.1)
Calcium gluconate	8 (17.8)	14 (31.1)
Atropine	1 (2.2)	2 (4.4)
Defibrillation	1 (2.2)	1 (2.2)
Doses of study drug[a]	3.6 (1.6)	3.5 (1.6)

Data in no. (%) or [a]mean (SD). CNS: central nervous system; SD: standard deviation; $EtCO_2$: end-tidal carbon dioxide; CPR: cardiopulmonary resuscitation; [b]Patient had one or more conditions; [c]had one or more interventions. Hence, the cumulative totals do not necessarily equal. Three patients also received EtCO2 monitoring after placement of endotracheal tube during CPR; [d]P=0.002.

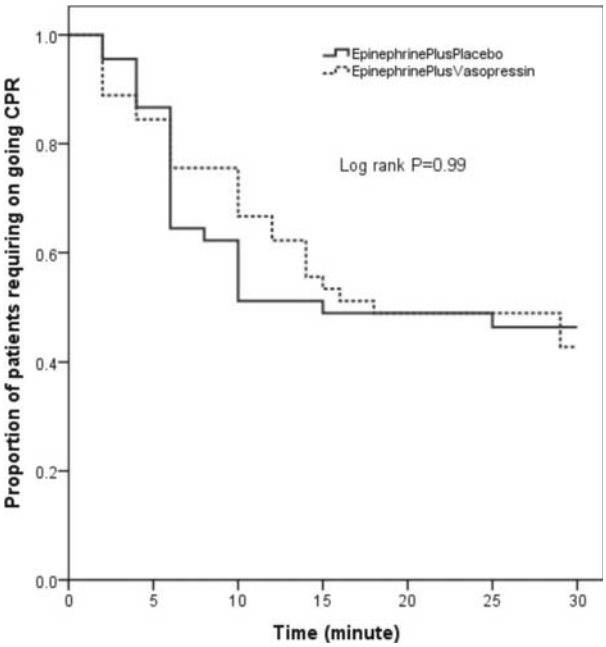

Fig. 2 Kaplan Meier curves showing time to return of spontaneous circulation (ROSC) and the proportion of patients requiring on-going cardio pulmonary resuscitation (CPR) between the two study groups.

during the third cycle of CPR. There were no serious trial drug-related adverse events observed.

DISCUSSION

This randomized controlled trial enrolled 90 patients who underwent CPR in PICU. We found no significant difference in the proportion of patients who achieved ROSC and survival rate between the Epinephrine plus Vasopressin group and Epinephrine plus Placebo group. In our study, the overall rate of achieving ROSC was 54.4%, and survival to hospital discharge was 2.2%. This observation contrasts with the data from high-income countries, where the rates were more than 80% and 40%, respectively [15]. The potential reasons could be the uniform reporting registries, universal healthcare programs, training of health care workers, and accessibility to extracorporeal membrane oxygenation (ECMO).

The outcome of a pediatric cardiac arrest depends upon many factors, including the initial presenting rhythm, the place of cardiac arrest, early recognition of the arrest, and the underlying conditions. In the previous studies done in high-income countries, asystole (55%) was the initial arrest rhythm, and respiratory failure was the common precipitating factor [1,9]. Nevertheless, they enrolled patients not only from PICU but also from the emergency department and general ward [1,9]. In contrast, this study

Table II Primary and Secondary Outcomes of the Study Groups

Variables	*Epinephrine plus vasopressin group (n = 45)*	*Epinephrine plus placebo group (n =45)*	*Relative risk (95% CI)*	*P value*
Primary outcome				
Proportion of patients achieved ROSC	25 (55.5)	24 (53.3)	1.04 (0.71-1.52)	0.83[a]
Proportion of patients achieved sustained ROSC	20 (44.4)	24 (53.3)	0.83 (0.54-1.28)	0.40[a]
Secondary outcomes				
Survival rate at 24 – hour	7 (15.6)	8 (17.8)	0.88 (0.35-2.21)	0.78[a]
At PICU discharge	1 (2.2)	1 (2.2)	1.00 (0.06-15.50)	1.00[c]
At Hospital discharge	1 (2.2)	1 (2.2)	1.00 (0.06-15.50)	1.00[c]
At 90-day post-discharge	1 (2.2)	1 (2.2)	1.00 (0.06-15.50)	1.00[c]
Functional status				
PCPC score – 1 (mild)	-	1 (2.2)	-	-
POPC score – 4 (severe)	1 (2.2)	-	-	-
Organ support therapy among patients achieved ROSC[a,b]				
Mechanical ventilation, h	1.5 (0.5-12)	5 (1.3-33)	-	0.07[b]
Vasoactive therapy, h	1.5 (0.4-8)	4 (1.3-19)	-	0.07[b]
RRT, h[c]	1 (1-14.3)	50 (1-137)	-	0.13[b]
PICU stay, h	1.5 (0.5-12)	5 (1.3-33)	-	0.08[b]
Hospital stay, h	1.5 (0.5-12)	5 (1.3-33)	-	0.08[b]

Data are presented as no.(%) except [a]median (IQR). ROSC: return of spontaneous circulation; CI: confidence interval; IQR: interquartile range; RRT: renal replacement therapy; PCPC: pediatric cerebral performance category; POPC: pediatric overall performance category. [b]25 in epinephrine plus vasopressin group and 24 in epinephrine plus placebo group; [c]seven in epinephrine plus vasopressin and six in epinephrine plus placebo group received RRT support after ROSC.

enrolled patients only from PICU, where stringent monitoring helped identify the arrest much earlier, before progressing to asystole. Similar to our study setting, Rathore, et al. [12] reported bradycardia (52.2%) and sepsis (71%) as the initial arrest rhythm and underlying diagnosis, respectively. They reported a higher ROSC rate (64.6%) and survival to hospital discharge (14%). However, only 21% of CPR occurred in PICU in that study. In our study, patients were enrolled only from PICU. So, the study population was different. Generally, PICU patients are sicker and the majority of them have multiple organ dysfunction requiring organ support. Also, the initial rhythm is an important factor in predicting the outcome; bradycardia rhythm with a pulse is more likely to recover than pulseless non-shockable rhythms [12].

At present, only a limited number of vasopressors are available for use in pediatric CPR, and insufficient data supporting their use [2]. The pediatric guidelines were extrapolated from adult clinical trials and animal studies. Vasopressin acts via the V-1 receptor in the arterial wall and increases the aortic diastolic pressure, thereby improving coronary perfusion pressure. In contrast to epinephrine, there are no β_1 mediated chronotropic and inotropic actions; hence it enhances the myocardial oxygen delivery and reduces the myocardial oxygen consumption during CPR and in the post-resuscitation period [1,16]. Another advantage of vasopressin includes the continuation of vasoconstrictive effects, even in severe acidosis, accompanying cardiac arrest. Hence, vasopressin can act as a better vasopressor during CPR, particularly in patients with sepsis-associated myocardial dysfunction and severe acidosis [16]. However, vasopressin has a longer duration of action than epinephrine, where the persistent vasoconstriction may worsen the myocardial dysfunction in the immediate post-resuscitation period. Post cardiac arrest myocardial dysfunction can be caused by various factors, including the underlying pre-arrest cardiac status, duration and quality of CPR, and the presence of other organ dysfunction(s). So, it is difficult to establish the causal relationship between post-cardiac arrest myocardial dysfunction and vasopressin use. However, no probable serious adverse event due to the trial drug was observed in this study.

The feasibility pilot study in pediatric cardiac arrest by Carroll, et al. [6] reported no significant difference in ROSC, survival to hospital discharge, and neurological

WHAT IS ALREADY KNOWN?

- Few studies have shown promising results of vasopressin use in pediatric in-hospital cardiopulmonary resuscitation.

WHAT THIS STUDY ADDS?

- The combination of cpinephrine and vasopressin did not improve the rate of return of spontaneous circulation, survival, and favorable neurological outcome as compared to Epinephrine alone.

outcome at discharge between vasopressin and control groups (who did not receive vasopressin). Nevertheless, they reported a higher survival rate at 24 hours in the vasopressin group. Their study was limited by non-randomization, small sample size, and addition of vasopressin only after non-response to epinephrine.

Similarly, Duncan, et al. [1] explored the use of vasopressin in pediatric in-hospital arrest from the American Heart Association National Registry of CPR data [1]. Patients who received vasopressin had a longer median arrest duration as compared to those who did not. They also noted that, on multivariate analysis, those who received vasopressin had a reduced ROSC; however, there was no difference in survival at 24 hours. Vasopressin was used as a "drug of last resort" for many of their patients [1], in contrast to our study, where it was used from the time CPR was initiated.

In comparison with an adult, children often present with a non-shockable rhythm, which requires high-quality chest compressions [15]. A systematic review that included 26 RCTs and 21704 participants found that vasopressin did not improve the ROSC rate but improved the survival to hospital admission compared to epinephrine [17]. However, the combination of epinephrine and vasopressin did not show any significant outcome benefits as compared to epinephrine alone [17]. However, most of the included studies were conducted over two decades back. Hence, these findings may not reflect the current practice in the growing era of extracorporeal life support (ECLS) availability.

Though all healthcare providers in our study have been trained in CPR, the intra- and inter-personal variations in chest compression were not monitored objectively. The temporal profiles of end-tidal carbon dioxide and DBP were not analyzed with the outcome of the study. Though our study found similar DBP in both the groups, the pediatric-specific target DBP during CPR is yet to be studied. However, evidence suggests that those who achieve DBP of 25 to 30 mm Hg during CPR have a higher chance of ROSC and survival [15]. Hence, goal-directed CPR targeting the end-tidal carbon dioxide and DBP needs to be considered in future study design. The availability of ECMO service during CPR or after achieving ROSC could have improved the survival to discharge. Recent studies showed that extracorporeal CPR (E-CPR) in pediatric cardiac arrest was associated with shorter resuscitation time and higher survival rate, ranging from 33-64% [18-20]. The AHA recommends considering E-CPR during in-hospital pediatric cardiac arrest, when standard resuscitation has failed, especially in a potentially reversible cause of cardiac arrest [2].

The study concludes that a combination of epinephrine and vasopressin did not improve the rate of return of spontaneous circulation, survival, and favorable neurological outcomes in pediatric intensive care unit cardiac arrest resuscitation as compared to epinephrine and placebo.

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Contributors: RR: had full access to all the data in the study and took responsibility for the integrity of the data and the accuracy of the data analysis; RR: Study concept and design; AS, MC, KM, RSK, AJ, RB: acquisition, analysis, or interpretation of data and drafting of the first manuscript; MC, RB, NB, SM: protocol development and revision of the manuscript; RR, SM: critical revision of the manuscript for important intellectual content; RR, NB: study supervision. RR: is the guarantor of the paper. All authors approved the final version of the manuscript.

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REFERENCES

1. Duncan JM, Meaney P, Simpson P, et al. Vasopressin for in-hospital pediatric cardiac arrest: results from the American Heart Association National Registry of Cardiopulmonary Resuscitation. Pediatr Crit Care Med.

2009;10: 191-5.
2. de Caen AR, Berg MD, Chameides L, et al. Part 12: Pediatric Advanced Life Support: 2015 American Heart Association Guidelines Update for Cardiopulmonary Resuscitation and Emergency Cardiovascular Care. Circulation. 2015;132:S526-42.
3. Panchal AR, Berg KM, Hirsch KG, et al. 2019 American Heart Association Focused Update on Advanced Cardiovascular Life Support: Use of Advanced Airways, Vasopressors, and Extracorporeal Cardiopulmonary Resuscitation During Cardiac Arrest: An Update to the American Heart Association Guidelines for Cardiopulmonary Resuscitation and Emergency Cardiovascular Care. Circulation. 2019;140:e881-e94.
4. Voelckel WG, Lurie KG, McKnite S, et al. Effects of epinephrine and vasopressin in a piglet model of prolonged ventricular fibrillation and cardiopulmonary resuscitation. Crit Care Med. 2002;30:957-62.
5. Mann K, Berg RA, Nadkarni V. Beneficial effects of vasopressin in prolonged pediatric cardiac arrest: a case series. Resuscitation. 2002;52:149-56.
6. Carroll TG, Dimas VV, Raymond TT. Vasopressin rescue for in-pediatric intensive care unit cardiopulmonary arrest refractory to initial epinephrine dosing: A prospective feasibility pilot trial. Pediatr Crit Care Med. 2012;13: 265-72.
7. Fiser DH, Long N, Roberson PK, Hefley G, Zolten K, Brodie-Fowler M. Relationship of pediatric overall performance category and pediatric cerebral performance category scores at pediatric intensive care unit discharge with outcome measures collected at hospital discharge and 1- and 6-month follow-up assessments. Crit Care Med. 2000; 28:2616-20.
8. Zaritsky A, Nadkarni V, Hazinski MF, et al. Recommended Guidelines for Uniform Reporting of Pediatric Advanced Life Support: The Pediatric Utstein Style. Ann Emerg Med. 1995; 26:487-503.
9. Reis AG, Nadkarni V, Perondi MB, Grisi S, Berg RA. A prospective investigation into the epidemiology of in-hospital pediatric cardiopulmonary resuscitation using the international Utstein reporting style. Pediatrics. 2002;109: 200-9.
10. Jacobs I, Nadkarni V, Bahr J, et al. Cardiac Arrest and Cardiopulmonary Resuscitation Outcome Reports: Update and Simplification of the Utstein Templates for Resuscitation Registries: A Statement for Healthcare Professionals from a Task Force of the International Liaison Committee on Resuscitation (American Heart Association, European Resuscitation Council, Australian Resuscitation Council, New Zealand Resuscitation Council, Heart and Stroke Foundation of Canada, Inter American Heart Foundation, Resuscitation Councils of Southern Africa). Circulation. 2004;110:3385-97.
11. Naranjo CA, Busto U, Sellers EM, et al. A method for estimating the probability of adverse drug reactions. Clin Pharmacol Ther. 1981;30:239-45.
12. Rathore V, Bansal A, Singhi SC, Singhi P, Muralidharan J. Survival and neurological outcome following in-hospital paediatric cardiopulmonary resuscitation in North India. Paediatr Int Child Health. 2016;36:141-7.
13. Donoghue AJ, Abella BS, Merchant R, et al. Cardiopulmonary resuscitation for in-hospital events in the emergency department: A comparison of adult and pediatric outcomes and care processes. Resuscitation. 2015;92: 94-100.
14. Ridgeon EE, Bellomo R, Aberegg SK, et al. Effect sizes in ongoing randomized controlled critical care trials. Crit Care. 2017;21:132.
15. Skellett S, Biarent D, Nadkarni V. What works in paediatric CPR? Intensive Care Med. 2018;44:223-6.
16 Agrawal A, Singh VK, Varma A, Sharma R. Therapeutic applications of vasopressin in pediatric patients. Indian Pediatr. 2012;49:297-305.
17. Finn J, Jacobs I, Williams TA, Gates S, Perkins GD. Adrenaline and vasopressin for cardiac arrest. Cochrane Database Syst Rev. 2019;1:CD003179.
18. Duncan BW, Ibrahim AE, Hraska V, et al. Use of rapid-deployment extracorporeal membrane oxygenation for the resuscitation of pediatric patients with heart disease after cardiac arrest. J Thorac Cardiovasc Surg. 1998;116: 305-11.
19. Morris MC, Wernovsky G, Nadkarni VM. Survival outcomes after extracorporeal cardiopulmonary resuscitation instituted during active chest compressions following refractory in-hospital pediatric cardiac arrest. Pediatr Crit Care Med. 2004;5:440-46.
20. Thourani VH, Kirshbom PM, Kanter KR, et al. Venoarterial extracorporeal membrane oxygenation (VA-ECMO) in pediatric cardiac support. Ann Thorac Surg. 2006;82:138-44; discussion 144-5.

RESEARCH PAPER

Vitamin C Deficiency and Oxidant Levels in Children With Transfusion-Dependent β-Thalassemia

VASUDEVA BHAT K,[1] RATNA A SHARMA,[1] SUJATA M SHARMA,[1] PRIYANKA JOSHI,[2] BINA F DIAS,[2] NIKITA SHAH,[1] MANINDER SETIA,[3] MAMTA V MANGLANI[1]

From Departments of [1]Pediatric Hematology-Oncology, and [2]Biochemistry, Lokmanya Tilak Municipal Medical College and General Hospital, Sion, Mumbai; [3]MGM Institute of Health Sciences, Navi Mumbai; Maharashtra.

Correspondence to: Dr Mamta V Manglani, Director, MCGM-comprehensive Thalassemia Care, Hematology-Oncology and BMT Centre, CCI Compound, Borivali (East), Mumbai 400 066, Maharashtra, India.
mmanglani@hotmail.com
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Objectives: To study vitamin C levels in children with transfusion-dependent β-thalassemia and correlate with age, transfusions received and iron overload; and to study the effect of administering vitamin C on its levels and Malondialdehyde (MDA) in deficient patients. **Methods**: This case-control study enrolled 100 children with transfusion-dependent β-thalassemia and 30 healthy controls. MDA levels before and after administration of vitamin C were performed randomly in 36 children with low vitamin C levels. **Results**: 81/95 (85.3%) study subjects vs none in control group, had low plasma vitamin C levels (*P*<0.001). Vitamin C levels were low in 64 of 71 (74.7%) subjects with dietary deficiency, while none of the 19 (63.3%) controls with dietary deficiency had low levels (*P*=0.04). Increasing serum ferritin values correlated with vitamin C deficiency (*P*=0.02). The mean level of MDA reduced (*P*<0.001) with vitamin C supplementation. **Conclusions**: Low levels of vitamin C are common in children with thalassemia. Dietary counseling along with supplementation with vitamin C, in those with low levels may prevent oxidative stress.

Keywords: *Iron overload, Oxidative stress, Thalassemia*, Scurvy, Supplementation.

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Children with transfusion-dependent β-thalassemia (TDT) require frequent blood transfusions resulting in iron overload [1]. Dietary vitamin C is destroyed through irreversible oxidation by ferric iron deposits, thus leading to its deficiency causing scurvy [2,3], given the fact that dietary deficiencies are common in Indian children [4]. However, the risk of vitamin C supplementation is that excess of vitamin C enhances iron absorption and also iron-mediated peroxidation of membrane lipids, causing an increased iron-induced membrane damage in cultured myocardial cells [2]. This study was thus designed to assess the plasma levels of vitamin C in Indian children with TDT and correlate them with various patient and disease factors, including overload and oxidant levels.

METHODS

This was a cross-sectional study conducted in the day-care thalassemia centre of a tertiary hospital between December, 2011 to May, 2012. All children (below 18 years of age), with TDT and receiving regular transfusions at the center were included in the study group. Any child who was already receiving vitamin C prior to enrolment was excluded. In the control group, 30 asymptomatic children who visited the pediatric outpatient department were enrolled.

A detailed history including age, gender, number of transfusions received till date (using record maintained by the patients), chelation therapy, dietary history and examination findings (with special reference to signs of scurvy) were entered in a predesigned proforma after taking informed consent. All children in the study group underwent the following investigations: complete blood count with RBC indices, liver and renal function tests, HIV antibody by ELISA, hepatitis B surface antigen (HBsAg), anti-HCV antibody, serum ferritin levels and baseline vitamin C levels prior to transfusion. Dietary assessment was done by oral questionnaire method by recalling food eaten in last 48 hours and during weekends [5] and comparing it with the ICMR food composition tables [6]. Nutritional assessment was done by calculating weight for height/mid upper arm circumference in less than 5 years, and as per body mass index in children more than 5 years using the WHO growth charts [7]. In the control group, a detailed dietary history with clinical examination was documented in the proforma, and their blood samples were collected for vitamin C estimation.

Invited Commentary: Pages 609-10

All children with low levels of vitamin C were administered vitamin C orally in therapeutic doses of 200

mg per day for a period of 15 days, while counselling to improve dietary content of vitamin C was also done. The dose 200 mg was chosen as non-heme iron absorption enhanced by vitamin C occurs above this dose [8]. In randomly selected children (*n*=36) with low levels of vitamin C, blood was also collected for oxidant malondialdehyde (MDA) levels prior to administration of vitamin C and prior to transfusion. Plasma vitamin C and MDA levels were repeated in these 36 children after completion of 15 days of oral administration of vitamin C.

Vitamin C estimation in plasma was done using 2, 6-dichlorophenol indophenol dye method [9]. A level of ≤0.3 mg/dL was considered as deficient according to this method. MDA estimation was done by modified method of Sadasavidu, et al. [10].

Sample size was calculated using Stata Version 15.1 (StataCorp) based on the 64% incidence of vitamin C deficiency in a previous study [12] of individuals with thalassemia. With an alpha of 0.05, power of 80%, and delta of 0.14, we estimated the sample size to be 98. Thus, we recruited 100 participants.

Data analyses: Data was entered in MS Excel (Microsoft Corp.) and converted to Stata Version 10 (Stata Corp) for analysis. The differences in the categorical outcomes were tested using the chi square test or Fisher exact test and the differences in means of the continuous variables were tested using the *t* test. We calculated the correlation coefficient (r) between vitamin C levels, MDA and ferritin levels. A *P* value of <0.05 was considered statistically significant.

RESULTS

A total of 100 children with TDT were enrolled. Of these, 95 were evaluable of which 61 (64.2%) were males (median age 9 years, IQR 7-13 years). In control group, of the 30 children enrolled, 16 (53.3%) were males (median age - 9 years, IQR 7.2-12 years). There was no statistically significant difference between the age and gender distribution in these two groups (*P*=0.56 and 0.29, respectively). The mean (SD) number of transfusions was 205 (111.5) and serum ferritin level was 4634.5 (2980.3) ng/mL. There was no statistically significant difference in the nutritional status between the study and control group (*P*=0.4); however, there were higher percentage of under-nourished children in the study group (90% vs 64%).

Bone pains (4 children) and gum bleeds (3 children) were seen only in the study group (*P*=0.69). Signs of scurvy were seen in 5 (5.3%) (Gum hypertrophy in 2 and typical skin changes in 3 children) of the children in study group whereas in none in control group (*P*=0.45). Eighty three children (87.4%) were on regular chelation, of which 54 (65.1%) children were on deferasirox, while 29 were receiving deferiprone (34.9%). Two (2.1%) children with TDT were HIV-1 infected, 18 (19%) were positive for anti-HCV antibodies and none were HBsAg positive. The mean (SD) value of vitamin C in study group was 0.2 mg/dL (0.1) and in controls was 0.8 (0.2) mg/dL (*P*<0.001).

Plasma vitamin C levels were low in 81 (85.3%) children in the study group, while all children in control arm had normal plasma vitamin C levels despite comparable dietary deficiency of vitamin C (*P*<0.001). **Table I** depicts the correlation of dietary deficiency with low plasma vitamin C levels in the 2 groups. Age (*P*=0.86), number of transfusions received (*P*=0.67), chelation (*P*=0.84), and associated infections (HIV, *P*=0.55, anti-HCV antibody positive, *P*=0.63) did not have any correlation with vitamin C levels, while increasing serum ferritin values correlated with vitamin C deficiency (r=0.3, *P*= 0.02) (**Fig.1**). There was a correlation between higher serum ferritin values and MDA levels done prior to administration of vitamin C (r=0.35, *P*=0.03). On administration of vitamin C, the mean (SD) levels of vitamin C rose from 0.2 (0.1) mg/dL to 0.8 (0.2) mg/dL in those with low plasma levels of vitamin C

Table I Vitamin C Dietary Deficiency and Plasma Levels in Children With Transfusion-Dependent Thalassemia and Controls

Dietary deficiency	*Study group, n=95*			*Control group,[a] n=30*
	Normal level n=14 (>0.3 mg/dL)	*Low level n=81 (≤0.3mg/dL)*	*Total*	*Normal level n=30 (>0.3 mg/dL)*
Present	7 (50)	64 (79)	71 (74.7)	19 (63.3)
Absent	7 (50)	17 (21)	24 (25.3)	11 (36.7)

All values in no. (%). [a]None had low vitamin C level. P=0.04 for low vitamin C levels in diet deficient children in study and control group.

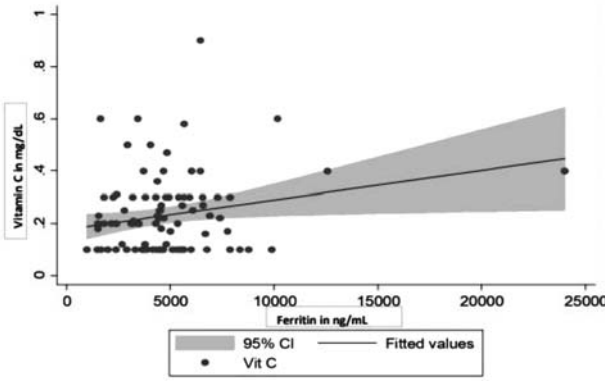

Fig. 1 Scatter diagram depicting increasing serum ferritin values significantly correlated with vitamin C deficiency.

WHAT THIS STUDY ADDS?

- Children with transfusion-dependent thalassemia are deficient in vitamin C and are more likely to develop scurvy, besides posing a risk of oxidative stress.

(P<0.001). The mean (SD) level of MDA dropped from 17.1 (7.0) nmol/mL to 8.1 (2.5) nmol/mL after 15 days of administration of vitamin C (P<0.001).

DISCUSSION

In addition to occasional case reports of scurvy occurring in children with thalassemia [3], a few studies have also described vitamin C deficiency in these children [11,12]. We determined the magnitude of vitamin C deficiency in Indian children with TDT and its impact on oxidant (MDA) levels. Clinical symptoms and/or signs of scurvy were seen in 7% of patients in the study group and none in the control group, and vitamin C deficiency was associated with iron overload and higher oxidant (MDA) levels.

Previous studies from various countries have reported vitamin C deficiency in 64-100% of patients with thalassemia [11-13], similar to 85.3% reported in this study. Hussien, et al. [12] reported suboptimal plasma levels of vitamin C in all children with TDT, despite a diet sufficient in vitamin C. We also found low levels of vitamin C in 70.8% of children with TDT without dietary deficiency, though it was higher in those with dietary deficiency (90.1%). In the control group, irrespective of dietary deficiency, all children had normal vitamin C levels, probably due to lower or no oxidant stress in them. Similar to our findings, a relation between iron overload and vitamin C deficiency has also been reported by Hussien, et al. [12].

The levels of oxidants and lipid peroxides are high in children with TDT due to the accumulation of free iron radicals and production of reactive oxygen species. A MDA higher level signifies peroxidative damage to lipid membranes in children with TDT [14,15]. Our results are similar to other studies done in patients with transfusion-dependent β-thalassemia, which have also found a marked imbalance in the oxidant and antioxidant status with reduction in the antioxidants and increase in the oxidant level with vitamin C deficiency [14,16]; although, few authors have not reported such an association [15].

A significant reduction in the MDA levels oxidant load was observed after administration of vitamin C, suggesting higher oxidative stress in children with vitamin C deficiency. This also confirmed that supplementation of vitamin C does not further increase the oxidative stress and hence is safe to be given in children who are deficient.

The present study had some limitations. Only plasma vitamin C and MDA levels were measured out of numerous antioxidants and oxidants that are present in the body. Iron overload was estimated using serum ferritin alone which may also be elevated due to infections and inflammation. Tissue iron overload was not estimated using T2*weighted magnetic resonance imaging.

Besides regular packed red cells and adequate iron chelation, maintaining vitamin C homeostasis is the key to reducing the oxidative stress, thereby protecting these children from myocardial damage and consequent mortality. Despite the fact that there was no statistically significant difference in nutritional status between the two groups, the proportion of undernourished children with TDT was higher; hence, improved dietary intake through counseling and supplementing vitamin C in those children with TDT with low plasma vitamin C levels, will improve outcomes in these children.

Ethics clearance: Institutional Ethics Committee of LTMM College and LTMG Hospital, Mumbai; No. PS/IECHR/DISS/105(11/10).
Contributors: VB: conducted the study and prepared the draft of the manuscript; RS: helped in management of the patients, monitored the outcomes and helped in analysing the results; SS: helped in collecting data and managing the patients; PJ: performed the tests in the laboratory; BD: supervised the laboratory testing of the samples and helped in correlating with the clinical findings; MS: helped in planning the study and did the statistical analysis; NS: helped in revising the draft; MM: conceptualized the study, guided throughout the study and finalized the draft. All authors approved the final version of manuscript, and are accountable for all aspects related to the study.
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REFERENCES

1. Verma IC, Saxena R, Kohli S. Past, present and future scenario of thalassaemic care and control in India. Indian J Med Res. 2011;134:507-21.
2. Darvishi KH, Emami ZA, Sharifi H, Jalali H. Is Vitamin C supplementation in patients with β-thalassemia major beneficial or detrimental? Hemoglobin. 2016;40:293-4.
3. Munni R, Mawaha R, Sethuraman G, Trehan A. Scurvey in transfusion dependent betathalassemia. Indian Pediatr. 1999:504-6.
4. Srihari G, Eilander A, Muthayya S, Kurpad AV, Seshadri S. Nutritional status of affluent Indian school children: What and how much do we know? Indian Pediatr. 2007:199-203.

5. Subar AF, Thompson FE, Kipnis V, et al. Comparative validation of the Block, Willett, and National Cancer Institute food frequency questionnaires: the Eating at America's Table Study. Am J Epidemiol. 2001;154: 1089-99.
6. Longvah T, Ananthan R, Bhaskarachary K, et al. Indian food composition tables. National Institute of Nutrition. Indian Council of Medical Research,2017.
7. WHO. The WHO Child Growth Standards. Accessed on October 9, 2020. Available from: *https://www.who.int/childgrowth/en/*
8. Kliegman R, Stanton B, St. Geme JW, Schor, NF et al. (2016). Nelson textbook of pediatrics (Edition 20.). Elsevier. p. 324.
9. VanderJagt DJ, Garry PJ, Hunt WC. Ascorbate in plasma as measured by liquid chromatography and by dichlorophenolindophenol colorimetry. Clin Chem. 1986;32:1004-6.
10. Sasikala M, Subramanyam C, Sadasivudu B. Early oxidative change in low density lipoproteins during progressive chronic renal failure. Ind J Clin Biochem. 1999;14:176.
11. Chapman RW, Hussain MA, Gorman A, et al. Effect of ascorbic acid deficiency on serum ferritin concentration in patients with beta-thalassaemia major and iron overload. J Clin Pathol. 1982;35:487-91.
12. Hussien JN A-KS, Al-Zuhairy SH. Role of vitamin C supplementation on iron overload and oxidative stress in beta thalassemia major patients in Maysan Province-Iraq. Kufa Medical J. 2017;17:111-27.
13. Wapnick AA LS, Krawitz P, Seftel HC, Charlton RW, Bothwell TH. Effects of iron overload on ascorbic acid metabolism. BMJ. 1968;3:704-7.
14. Naithani R, Chandra J, Bhattacharjee J, Verma P, Narayan S. Peroxidative stress and antioxidant enzymes in children with β-thalassemia major. Pediatr Blood Cancer. 2006; 46:780-5.
15. Gunarsih A, Amalia P, Boediman I. Variables associated with malondialdehyde level in thalassemia major patients. Paediatr Indone. 2012;52:125-31.
16. Ghone RA, Kumbar KM, Suryakar AN, Katkam RV, Joshi NG. Oxidative stress and disturbance in antioxidant balance in beta thalassemia major. Ind J Clin Biochem. 2008; 23: 337-40.

CLIPPINGS

Use of vaccines outside the cold chain or in controlled temperature may contribute to improving immunization coverage in low- and middle-income countries (LMICs) (*J Glob Health. 2021;11:04004*)

The universal guidelines recommend the maintenance of cold chain (+2°C to +8°C) to maintain the desired potency of the vaccine. Recently, WHO permitted the use of vaccines outside the cold chain as 'controlled temperature chain (CTC).' CTC usually involves a single excursion of vaccine into ambient temperatures not exceeding +40°C and for a specific number of days, just prior to administration. The present scoping review explored the use of CTC for increasing the immunization coverage in low- and middle-income countries (LMICs), with a focus on the timelines of the Global Vaccine Action Plan (2011-2020). A total of 173 original peer-reviewed articles were retrieved, of which 13 were included in the final review. Nine studies were from Africa, 3 from Asia and 1 from Pacific region. Non-randomized trails (4) were the commonest study type followed by randomized trials (3), simulation models, cross sectional studies (2 each) and a cohort study (1). Increased coverage, logistical ease and financial savings were the benefits of CTC found in the review without compromising with the potency of the vaccine. MenAfriVac and Gardasil are the two vaccines approved for use under CTC conditions. CTC helps in reaching large numbers of target population in far-off areas, especially in LMICs.

Can two different pneumococcal conjugate vaccines be used to complete the infant vaccination series? (*Expert Rev Vaccines. 2020;19:995-1010*)

This was a partially blinded randomized study done in 6-12 weeks old infants to assess the safety and immunogenicity during the regimens with interchangeability of 13-valent pneumococcal conjugate vaccine (PCV13) and completed with the pneumococcal non-typeable *Haemophilus influenzae* protein D-conjugate vaccine (PHiD-CV). Enrolled infants were supposed to receive 2 priming doses at 2, 4 months and a booster dose at 12-15 months and randomized into three arms for vaccination - PCV13 at priming and PHiD-CV at boosting (PPS); PCV13 then PHiD-CV at priming and PHiD-CV at boosting (PSS); or PHiD-CV at priming and boosting (SSS control). 294 infants were included in the vaccinated cohort at primary vaccination (out of 457); 267 and 257 completed the primary and booster vaccination phases, respectively. Grade 3 intensity adverse events occurring within 31 days after each primary vaccine dose (primary objective) were 8.7% (PPS), 11.4% (PSS), and 16.9% (SSS), respectively in the three study groups and none of them were considered vaccine related. Reactogenicity, safety and immunogenicity were assessed as secondary objectives, the immunogenicity of the two mixed PCV13/PHiD-CV regimens was mostly similar to that of a PHiD-CV-only series. Thus the study depicts the feasibility of interchangeability of 13-valent pneumococcal conjugate vaccine (PCV13) with the pneumococcal non-typeable *H. influenzae* protein D-conjugate vaccine (PHiD-CV).

RAJESH KUMAR MEENA
raj.mamc@gmail.com

RESEARCH PAPER

Pubertal Development and its Determinants in Adolescents With Transfusion-Dependent Thalassemia

PREETI SINGH, SUKLA SAMADDAR, NUPUR PARAKH, JAGDISH CHANDRA AND ANJU SETH*
From Department of Pediatrics, Lady Hardinge Medical College and Kalawati Saran Children's Hospital, New Delhi.

Correspondence to: Dr Anju Seth, Director Professor, Department of Pediatrics, Lady Hardinge Medical College and Kalawati Saran Children's Hospital, New Delhi 110 001, India. anju_seth@yahoo.com
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Objective: To assess pubertal development and its determinants in adolescents with transfusion-dependent thalassemia (TDT). **Methods:** In this cross-sectional study from a tertiary teaching hospital in Delhi, records of adolescents aged 17-19 years with TDT on regular transfusion at thalassemia day-care centre were reviewed. Pubertal development and its determinants were assessed. **Results:** Records of 58 (33 male) adolescents with TDT were reviewed. Among them, 42 (72.4%) had normal/delayed onset with spontaneous progression of puberty, while 16 (27.6%) had pubertal arrest/failure and received hormonal replacement therapy (HRT). Short stature was observed in all adolescents on HRT. Amongst other endocrinopathies, only hypoparathyroidism was found to be significantly higher in the HRT group. On multivariate analysis, serum ferritin (OR-1.005, 95% CI 1.002, 1.009) was observed to be the only significant determinant of pubertal arrest/failure. **Conclusion:** A significant proportion of adolescents with TDT continue to have pubertal arrest/failure. High systemic iron load is the key determinant for this.

Keywords: *Delayed puberty, Growth failure, Hypogonadotropic hypogonadism, Ferritin.*

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With the advent of intensive transfusion and robust chelation regimes, life expectancy has increased significantly in patients with transfusion-dependent thalassemia (TDT). Concurrently, endocrine dysfunction has emerged as an important cause of morbidity in adolescents and adults with TDT. Deranged pubertal development is amongst the commonest endocrinopathies observed in adolescents with thalassemia. Early recognition and timely management of these adolescents can not only help in optimizing growth and pubertal development but also improve their bone mineral density, quality of life and preserve fertility potential.

Children with TDT experience poor growth predominantly during the peri-pubertal phase or puberty. This is primarily due to transfusion associated iron overload affecting the growth hormone-insulin like growth factor axis (GH-IGF axis) and the hypothalamic-pituitary-gonadal (HPG) axis. Despite regular transfusions and optimal chelation therapy, the prevalence of pubertal disturbances in TDT still ranges between 30-70% [1-3]. In a previous study from our center, delayed puberty and/or hypogonadism were reported in 54.1% of children with TDT [4]. The key contributory factor is transfusion-mediated hemosiderosis in HPG axis that leads to hypogonadotropic hypogonadism (HH).

With improvement in the management of children with TDT, the prevalence of growth failure and pubertal disturbances have decreased. However, information on the prevalence and manifestations of pubertal disturbances in adolescents with TDT from India is scarce. We report the spectrum of pubertal disturbances and their determinants in adolescents with TDT.

Invited Commentary: Pages 609-10

METHODS

This cross-sectional study was conducted at the thalassemia day-care centre and pediatric endocrine clinic of a tertiary level teaching hospital in Delhi over a period of 3 months from June, 2019 to August, 2019. The records of all adolescents with TDT in the age group 17 to 19 years attending the center and on regular transfusion and chelation therapy (for at least previous 7 years) were reviewed. Information on annual transfusion requirement (ATR) and average ferritin (done at least biannually) levels over last 7 years were recorded. Anthropometric SD scores (SDS) were calculated according to age and sex-specific norms using IAP 2015 growth reference charts [5]. Mid-parental height (MPH) was calculated and the child's height within ±2SD of MPH was considered appropriate for the genetic potential. Children with height for age below 2 SDS were considered to have short stature. Pubertal development and progression over the previous years as assessed by sexual maturity rating (SMR) according to Tanner criteria was noted.

As a part of usual care protocol at our center, children with TDT after 10 years age undergo regular growth and pubertal assessment and an annual endocrine screen. The latter consists of thyroid function tests, fasting plasma glucose, oral glucose tolerance test (if indicated), serum calcium, phosphate, alkaline phosphatase, 25-hydroxy vitamin D and parathyroid hormone (if indicated by clinical or biochemical features). Bone age, luteinizing hormone (LH), follicle stimulating hormone (FSH), and sex steroids [estradiol (female) and testosterone (male)] are assessed in adolescents with delayed/arrested/failed puberty. Low FSH, LH and sex steroids for age, sex and pubertal stage indicate hypogonadotropic hypogona-dism. In those with equivocal results, gonadotropin-releasing hormone (GnRH) analog stimulation test is performed to evaluate the pituitary's ability to synthesize and secrete gonadotropins. In cases with arrested/delayed puberty, incremental age appropriate hormone replacement therapy (HRT) is started as per standard protocol. Screening for Hepatitis B, C, HIV and liver function is done in all children annually. ECG, echocardiography and MRI T2* imaging for cardiac and hepatic dysfunction is also performed to look for transfusional iron overload. A baseline DEXA (dual-energy *X*-ray absorptiometry) scan followed by annual/biennial screening of bone mineral density (in case of fragility fractures) is done on case to case basis.

Based on the onset and progression of puberty, the children recruited in this study were categorized into 4 groups: Group 1: Normal onset and progression of puberty, Group 2: Delayed onset and spontaneous progression of puberty after priming with low dose sex steroids for 3-6 months, Group 3: Normal/delayed onset with pubertal arrest, and Group 4: No spontaneous onset of puberty – pubertal failure.

Delayed puberty was defined as absence of gonadarche (testicular volume <4 mL) in males by 14 years and thelarche (appearance of breast bud) in females by 13 years. The failure of pubertal progression from one Tanner stage to the next over a period of 1 year was considered as pubertal arrest. Failure to achieve menarche by 16 years was defined as primary amenorrhea while the absence of menstrual cycles for >12 months after attaining menarche was considered secondary amenorrhea.

The study was approved by the institutional ethics committee and a written informed consent of the parent/guardian of the participants was taken. Consent/assent of the participants was also taken.

Statistical analysis: These were done using SPSS version 20.0. Categorical variables were analyzed using chi square test and continuous variables, using indepen-dent *t* test. Multivariate logistic regression was done to study factors determining pubertal failure and arrest.

RESULTS

The records of 58 adolescents with TDT were reviewed (**Table I**). Thirty four (58.6%) children in group 1 and 8 (13.8%) in group 2 did not require HRT i.e., non HRT group (*n*=42; 72.4%). Sixteen (27.6%) children [group 3; 7 (12.1%) and group 4; 9 (15.5%)] had pubertal arrest/failure and received HRT. All girls in HRT group had primary amenorrhea, except for one girl in group 3 with secondary amenorrhea. All children in the HRT group had short stature while in the non HRT group, 14/23 (60.9%) boys and 7/19 (43.8%) girls were short. When compared with the respective MPH, two-third of children fell short of their genetic potential in the HRT group. All children with delayed, failed and arrested puberty had delayed bone age i.e. < –2SD from chronological age.

The baseline and stimulated GnRH levels of LH, FSH, and estradiol (female) and testosterone (male) were assessed for subjects in group 2,3 and 4 (**Table II**). Both baseline and peak values of gonadotropins and sex steroids were significantly lower in group 3 and 4 as compared to group 2. No subject in this study had evidence of hypergonadotropic hypogonadism. There was no significant difference in endocrinopathies between HRT and non-HRT groups except for hypoparathyroidism, which was significantly higher in the former group **(Table III)**. On multivariate analysis of factors associated with failed/arrested puberty, the mean serum ferritin

Table I Anthropometric and Pubertal Characteristics of Children With Transfusion-Dependent Thalassemia

Characteristics	*Males (n=33)*		*Females (n=25)*	
	No HRT (n=23)	*HRT (n=10)*	*No HRT (n=19)*	*HRT (n=6)*
Age pubertal onset, y[a]	13.5 (1)	16.5 (1)	12 (1)	15.5 (1)
Menarche, y[b]	-	-	14.5 (1)	17.5 (1)
Height *z*-score[c]	– 2.09 (0.90)	– 3.29 (0.79)	– 1.57 (1.52)	– 4.25 (2.21)
Weight *z*-score[d]	– 1.81 (0.74)	– 2.76 (0.87)	– 1.87 (0.78)	– 2.70 (0.90)
BMI *z*-score	– 1.06 (0.88)	– 1.53 (0.85)	– 0.51 (0.94)	– 0.80 (0.81)
Final height - MPH, cm[e]	– 6.9 (5.9)	– 14.7 (8.6)	– 4.1 (9.5)	– 13.7 (6.5)

[a]P<0.001 for both sexes; [b]P=0.002; [c]P<0.01 for both boys and girls; [d]P=0.003 for boys and 0.036 for girls; [e]P=0.005 for boys and 0.04 for girls. MPH: mid parental height; BMI: body mass index; HRT: hormone replacement therapy.

(OR=1.005, 95% CI, 1.002, 1.009; P <0.05) remained the only significant predictor while mean ATR, gender and other co-morbidities like liver dysfunction, hypothyroidism, type I diabetes mellitus, hepatitis B/C failed to show any significant association.

DISCUSSION

This study describes the spectrum of pubertal disturbances in adolescents with TDT under regular follow-up. While nearly 60% subjects had spontaneous onset and progression of puberty, more than a quarter required HRT due to pubertal arrest/failure. All adolescents requiring HRT had short stature. Hypoparathyroidism and liver dysfunction were reported more in the HRT group. High iron overload was the only significant predictor of pubertal failure/arrest.

The main limitation of this study was that the dynamics of the genotype-phenotype interaction in the development of pubertal failure was not assessed. $\beta^o\beta^o$ compared to $\beta^o\beta^+$ and $\beta^+\beta^+$ phenotypes have been shown to have increased need of blood transfusions and thereby, higher iron overload and endocrinopathies [6]. Further, the evaluation of growth axis and its relative contribution to pubertal failure was not performed.

Growth failure is one of the most common complications observed in children with TDT. This study found short stature in all children on HRT. The pubertal failure/arrest significantly contributed to the reduced final heights in these children. This is in agreement with studies on growth failure in children with TDT reported from India [7,8] and globally [9].

Hypogonadism was found in 27.6% subjects which is lower than that reported in a previous study from our center [4] and literature published before 2005 [10]. The likely cause for this observation is use of regular transfusions, better chelation and monitoring in the current era. The variability in worldwide prevalence of hypogonadism [1-3] may be explained by discrepancy in definitions of pubertal failure, differences in study cohort

Table III Clinical Characteristics and Co-morbidities in Children With Transfusion-Dependent Thalassemia

Variables	*Non-HRT, n=42*	*HRT, n=16*
Males, *n* (%)	23 (54.8)	10 (62.5)
Pretransfusion hemoglobin, mg/dL	9.4 (0.4)	9.3 (0.5)
Annual transfusion requirement, mL/kg[b]	135.6 (11.3)	146.4 (17.2)
Serum ferritin, ng/mL[c]	2752.4 (1082)	5084.9 (1640.5)
SGOT, IU/L[c]	40.6 (15.7)	73.4 (30.7)
SGPT, IU/L	49.9 (30.2)	107.2 (63.7)
Hypothyroidism, *n* (%)[d]	5 (11.7)	4 (25)
Hypoparathyroidism, *n* (%)[b]	1 (2.3)	4 (25)
Impaired glucose tolerance/ Type I DM, *n* (%)	16 (38.1)	8 (50)
HCV positive, *n* (%)	4 (9.5)	2 (12.5)
MRI T2* liver score, ms	8.5 (7.2)	9.3 (7.3)
MRI T2* cardiac score, ms[a]	19.4 (11.2)	13.0 (5.6)
DEXA spine; SD scores[b]	–1.54 (1.64)	–3.06 (1.50)
DEXA hip; SD scores[c]	–1.38 (1.11)	–2.70 (0.96)

[a]P<0.05, [b]P<0.01, [c]P<0.001. 1 child in non-HRT group was hepatitis B positive, and 1 in HRT group was HIV-positive. SGOT: serum glutamic-oxaloacetic transaminase; SGPT: serum glutamic pyruvic transaminase; DM: diabetes mellitus; MRI: magnetic resonance imaging; DEXA: dual energy x-ray absorptiometry; HRT: hormone replacement therapy. [d]sub-clinical/avert.

Table II Gonadotropin and Sex Hormone Profile on Gonadotropin Releasing Hormone (GnRH) Analog Stimulation Test in Children With Transfusion-Dependent Thalassemia (*N*=24)

	Male, n=15		*Female, n=9*	
	Group 3 & 4, *n*=10	Group 2, *n*=5	Group 3 & 4, *n*=6	Group 2, *n*=3
LH (mIU/mL)				
Basal	0.36 (0.19)	0.80 (0.48)	0.45 (0.02)	0.90 (1.15)
Peak	0.92 (0.45)	2.50 (0.55)	1.10 (0.13)	2.22 (1.45)
FSH (mIU/mL)				
Basal	0.61(0.56)	1.3 (1.12)	0.85 (0.63)	2.23 (1.89)
Peak	0.94 (0.14)	3.8 (1.55)	1.5 (1.18)	4.35 (2.65)
Estradiol (pg/mL)				
Basal	-	-	2.75 (0.65)	9.80 (6.68)
Peak	-	-	4.23 (1.13)	26.33(8.55)
Total testosterone (ng/dL)			-	-
Basal	2.24 (0.66)	23.80 (5.77)	-	-
Peak	3.51 (0.68)	59.44 (6.50)	-	-

P<0.001 for all comparisons between Group 2 and Group 3 and 4. GnRH analog stimulation test was not performed in group 1 children who underwent spontaneous onset and progression of puberty.

WHAT THIS STUDY ADDS?

- Despite regular transfusion and intensive chelation, a significant proportion of adolescents with transfusion-dependent thalassemia continue to have pubertal arrest and failure.
- High systemic iron load is an important predictor of pubertal disturbances in these children.

and their genetic variability. Also, the design of this study would miss subjects who may develop hypogonadism and secondary amenorrhea as an adult.

The mechanism of hypogonadotropic hypogonadism is postulated to be pituitary iron deposition resulting in volume loss and failure of HPG axis [11]. Priming with low dose sex steroids improves the responsiveness of the pituitary to gonadotropin releasing hormones. It is an effective method of inducing physiological puberty, feasible even in low resource countries [12]. Therefore, it was used in adolescents in group 2 to jump-start the puberty.

The prevalence of other endocrinopathies in this study was consistent with the published literature [13]. Previous studies indicate male sex, high serum and tissue iron overload, genotype-phenotype interaction, severe clinical endocrinopathy as predictors of HH in children with TDT [14,15]. Our study showed no correlation with gender or endocrine dysfunction, while high serum ferritin levels had a significant association with pubertal failure/arrest. Further studies are required to assess the genotype - phenotype correlation in TDT, leading to a higher transfusion requirement and resultant increased systemic iron load. This will help design strategies to intensify chelation in subset of vulnerable children for the prevention of endocrinopathies and other comorbidities.

Ethics Clearance: Institutional Ethical Committee for Human Research, Lady Hardinge Medical College and associated hospitals; No. LHMC/ECHR/2018/29, dated 10 May, 2018.
Contributors: PS: planning, execution of the study, data analysis and writing the manuscript; SS: data compilation, data analysis and writing the manuscript; NP,JC: execution of the study and writing the manuscript; AS: planning, execution of the study, data analysis and writing the manuscript. All authors approved the final version of manuscript.
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REFERENCES

1. Toumba M, Sergis A, Kanaris C, Skordis N. Endocrine complications in patients with thalassaemia major. Pediatr Endocrinol Rev. 2007;5:642-8.
2. Raiola G, Galati MC, De Sanctis V. Growth and puberty in thalassemia major. J Pediatr Endocrinol Metab. 2003;16: 259-66.
3. Delvecchio M, Cavallo L. Growth and endocrine function in thalassemia major in childhood and adolescence. J Endocrinol Invest. 2010;33:61-8.
4. Sharma R, Seth A, Chandra J, et al. Endocrinopathies in adolescents with thalassaemia major receiving oral iron chelation therapy. Paediatr Int Child Health. 2016;36:22-7.
5. Indian Academy of Pediatrics Growth Charts Committee, Khadilkar V, Yadav S, Agrawal KK, et al. Revised IAP growth charts for height, weight and body mass index for 5- to 18-year-old Indian children. Indian Pediatr. 2015;52: 47-55.
6. Al-Akhras A, Badr M, El-Safy U, et al. Impact of genotype on endocrinal complications in b-thalassemia patients. Biomed Rep. 2016;4:728-36.
7. De Sanctis V, Soliman AT, Elsedfy H, et al. Growth and endocrine disorders in thalassemia: The international network on endocrine complications in thalassemia (I-CET) position statement and guidelines. Indian J Endocrinol Metab. 2013;17:8-18.
8. Pattanashetti MA, Pilli GS. Assessment of growth parameters in transfusion dependent thalassemics at a tertiary care hospital. Annals of Pathology and Laboratory Medicine. 2018;5:316-21.
9. Soliman AT, El Zalabany MM, Amer M, Ansari BM. Growth and pubertal development in transfusion- dependent children and adolescents with thalassaemia major. Hemoglobin. 2009;33:16-20.
10. Chatterjee R, Bajoria R. Critical appraisal of growth retardation and pubertal disturbances in thalassemia. Ann N Y Acad Sci. 2010;1202:100-14.
11. Mousa AA, Ghonem M, Elhadidy el HM, et al. Iron overload detection using pituitary and hepatic MRI in thalassemic patients having short stature and hypogonadism. Endocr Res. 2016;41:81-8.
12. Chatterjee R, Mukhopadhyay TN, Chandra S, Bajoria R. Sex steroid priming for induction of puberty in thalassemia patients with pulsatile reversible hypogonadotrophic hypogonadism. Hemoglobin. 2011;35:659-64.
13. De Sanctis V, Eleftheriou A, Malaventura C. Thalassaemia International Federation Study Group on Growth and Endocrine complications in thalassaemia. Prevalence of endocrine complications and short stature in patients with thalassaemia major: A multicenter study by the Thalassaemia International Federation (TIF). Pediatr Endocrinol Rev. 2004;2:249-55.
14. Albu A, Barbu CG, Antonie L, Vladareanu F, Fica S. Risk factors associated with hypogonadism in â-thalassemia major patients: Predictors for a frequent complication of a rare disease. Postgrad Med. 2014;126:121-7.
15. Belhoul KM, Bakir ML, Saned M-S, Kadhim AMA, Musallam KM, Taher AT. Serum ferritin levels and endocrinopathy in medically treated patients with â thalassemia major. Ann Hematol. 2012;91:1107-14.

RESEARCH PAPER

Pediatric Inflammatory Multisystem Syndrome Associated With SARS-CoV-2: A Retrospective Cohort Study From Argentina

MARIA TERESA ROSANOVA,[1] GUADALUPE PEREZ,[1] MARIA MARTHA KATSICAS,[2] ANA PAULA ARIAS,[1] MICELA PICOLLO, MARCELA PALLADINO,[3] CLAUDIA GONZALEZ,[3] NATALIA VELIZ,[3] ANA BUCHOVSKY,[4] ROBERTO LEDE[5], ROSA BOLOGNA[1]

From Departments of [1]Infectious Diseases Hospital Juan P Garrahan Combate de los Pozos 1881, [2]Rheumatology, Hospital Juan P Garrahan Combate de los Pozos 1881, [3]Clinics, 75, Hospital Juan P Garrahan Combate de los Pozos 1881, [4]Serology, Hospital Juan P Garrahan Combate de los Pozos 1881, [5]Universidad Abierta Interamericana (UAI), Avenida San Juan 961, Buenos Aires; Argentina.

Correspondence to: Dr Maria Teresa Rosanova, Chief of Clinics, Department of Infectious Diseases, Hospital Juan P Garrahan, Combate de los Pozos 1881 Buenos Aires, Argentina. maritesolcito@gmail.com
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Objective: To evaluate the differential characteristics of SARS-COV-2 associated inflammatory multisystem syndrome (MIS-C) in children. **Methods**: A retrospective cohort study was conducted. The definition of MIS- C was based on WHO criteria. Temporally related COVID-19 patients were included as controls. **Results**: 25 patients with MIS-C and 75 controls were included. Multivariate multiple logistic regression model of variables that showed to be significant in univariate analysis revealed that age ≥2 years (OR 24.7; 95% CI 1.03 -592.4; *P*=0.048), lymphopenia (OR 9.03, 95%CI 2.05-39.7; *P*=0.004), and platelet count <150x10^9/L (OR 11.7; 95% CI 1.88-75.22; *P*=0.009) were significantly associated with MIS-C. Presence of underlying disease seemed to reduce the risk of MIS-C (OR 0.06; 95% CI 0.01-0.3). **Conclusion**: MIS-C was more common in patients older than 2 years and in those with lymphopenia or thrombocytopenia. Underlying disease appears to reduce the risk of MIS-C.

Keywords:*Co-morbidity, Outcome, Lymphopenia, Thrombocytopenia*.

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Multisystem inflammatory syndrome (MIS-C), in association with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection, shares some clinical features with Kawasaki disease (KD), toxic shock syndrome (TSS), macrophage activation syndrome, and other inflammatory processes as in the so-called cytokine storm [1-5]. MIS-C is characterized by persistent fever, abdominal pain, vomiting, diarrhea, as well as mucocutaneous, cardiovascular, hematological, musculoskeletal, and neurological manifestations, among others [6-11]. After identification of MIS-C cases in Argentinean children with COVID-19, we conducted this study to evaluate the possible risk factors associated with MIS-C to allow clinicians to categorize patients who may require closer monitoring and interdisciplinary management.

METHODS

A retrospective cohort study was conducted at a tertiary pediatric referral center. Patients were identified from the electronic database of pediatric patients with confirmed COVID-19 seen between 19 April, and 31 October, 2020, at the Department of infectious diseases. This study was approved by the institutional research board.

Children with a diagnosis of MIS-C, as established by the World Health Organization (WHO) [12], were included in the study and defined as cases. For each case, three consecutive children with a positive reverse transcriptase-polymerase chain reaction (RT-PCR) for SARS-CoV-2 and no clinical or laboratory findings suspicions of MIS-C were selected as controls. Thrombocytopenia was defined as a platelet count <150×10^9/L and lymphopenia as a lymphocyte count <1×10^9/L on admission. C-reactive protein (CRP) <5 mg/L, B-type natriuretic peptide (BNP) <104 pg/mL, troponin <19 ng/L, and ferritin <200 ng/mL were considered to be within normal range. Cardio-vascular involvement was identified in the presence of any of the following: vasopressor requirement, an echo-cardiogram showing an abnormal ejection fraction, pericarditis or pericardial effusion, or elevated troponin or BNP levels. For both cases and controls, exclusion criteria were previous treatment with convalescent plasma or steroids, presence of any viral or bacterial co-infections, and outpatient status.

We analyzed epidemiological data (age, sex, co-morbidities, overcrowding defined as more than 4 people living in one room, and living in informal settlements);

virological data (RT-PCR of nasopharyngeal secretions and/or positive serology on admission); and clinical data (fever, respiratory distress, abdominal pain, diarrhea, vomiting, myalgia, dysgeusia, anosmia, conjunctivitis, rash, shock, intensive care unit (ICU) admission, mechanical ventilation requirement, oxygen therapy, or inotropic and vasopressors, and the length of hospital stay).

Laboratory tests on admission included leukocyte, lymphocyte, and platelet counts. In patients with suspected MIS-C, coagulogram, fibrinogen, ferritin, CRP, procalcitonin (PCT), liver and kidney function, lactate dehydrogenase (LDH), BNP, and troponin were analyzed (when available). Echocardiogram and radiographs were performed on admission and repeated depending on clinical features. Lung computed tomography (CT) scan and abdominal ultrasound were performed according to symptoms. Blood and urine cultures, PCR of nasopharyngeal swabs for influenza virus, respiratory syncytial virus, adenovirus, metapneumovirus, rhinovirus, and coronaviruses and serology for HIV, VDRL, HCV, HBV, CMV and EBV were also performed. Intravenous immunoglobulin (IVIG) and/or steroid use was documented. Outcome was defined as discharge or death.

Statistical analysis: Univariate analysis was performed to compare cases and controls. Odds ratio (OR) with a 95% CI was used for dichotomous variables. Chi-square or rank-sum test were used. Predictive factors for MIS-C were identified using a multiple logistic regression model including variables that were significant in univariate analysis. STATA 16 was used for statistical analysis. A value of $P<0.05$ was considered as significant.

RESULTS

Of the 533 children aged <18 years with COVID-19, 25 (4.7%) met the diagnostic criteria for MIS-C. In addition, 75 patients were included as controls. Median age of cases was 104 months (IQR 61-126) vs 78 months (IQR 18-139) in the control group. Underlying diseases were more commonly observed in the control group (*n*=52; 69%) than in MIS-C patients (*n*=7; 28%) Underlying diseases were cancer (18 controls; 24%), solid organ trans-plantation (18 controls; 24%), genetic disorders (4 controls; 5%), neurological disorders (4 controls; 5%, and 3 cases; 12%), congenital disorders (3 controls; 4%), obesity (3 controls; 4%), recurrent wheezing (3 cases; 12%), chronic renal failure (1 case; 4%), and others (15 controls; 20%).

Clinical manifestations of MIS-C patients and controls are shown in **Table I**. Nine MIS-C patients (36%) vs one control (1.3%) required intensive care unit (ICU) admission. Eight MIS-C patients (32%) had cardiac abnormalities consisting of myocarditis (*n*=3), pericarditis (*n*=1), left ventricular dysfunction (*n*=3) and coronary dilatation (*n*=1). Lymphopenia and thrombocytopenia were more common in MIS-C patients than controls. Median (IQR) CRP was 139 (122-248) mg/L, BNP 1116 (183-4857) pg/mL, troponin 2.5 (<1.5-79) ng/L, and ferritin 339 (191-611) ng/mL in MIS-C patients. At onset, PCR for SARS-Co-V2 was positive in 15 MIS-C patients (60%) and in all of those in the control group. IgM antibodies were positive in seven MIS-C patients (28%) and six controls (8%), while IgG antibodies were positive in 24 MIS-C patients (96%) and 22 controls (29%). In one MIS-C patient with a history of close contact with COVID-19 one month previously, both the PCR and antibody test were negative. All MIS-C patients

Table I Characteristics of Children With SARS-CoV-2 Infection With and Without MIS-C

Variable	*MIS-C n=25*	*Controls n=75*	OR (CI 95%)
Male sex	9 (36)	36 (48)	0.61 (0.21-1.69)
Age, mo[a]	104 (61-126)	78 (18-139)	–
Age ≥2 y	24 (96)	54 (72)	9.33 (1.19-73.4)
Living in a popular neighborhood	8 (32)	7 (9)	4.57 (1.23-16.8)
Asymptomatic	0	20 (27)	–
Fever	25 (100)	29 (39)	–
Abdominal pain	9 (36)	10 (13)	2.68 (0.8-8.2)
Diarrhea	14 (56)	9 (12)	9.33 (2.9-30.5)
Vomiting	12 (48)	13 (17)	4.40 (1.4-13)
Skin involvement	16 (64)	3 (4)	42.66 (9.1-254.8)
Conjunctival injection	9 (36)	0	–
Septic shock	8 (32)	0	–
Upper respiratory tract infection[b]	5 (20)	18 (24)	0.79 (0.2 -2.6)
Odynophagia	4 (16)	8 (11)	1.59 (0.3-6.7)
Tachypnea[c]	3 (12)	2 (3)	4.97 (0.5-61)
Headache	2 (8)	4 (5)	1.54 (0.1-11.5)
Myalgia	3 (12)	1 (1)	10.09 (0.7-536.6)
Anosmia	0	1 (1)	-

Values in no. (%) or [a]median (IQR). [b]Upper respiratory tract infection: cough, loss of appetite, sore throat, or nasal congestion, nasal stuffiness, rhinorrhea, anosmia; [c]Tachypnea (breaths/min): ≤2 mo: >60; 2-11 mo: >50; 1-5 y: >40; >5 y: >20. MIS-C: multi-system inflammatory syndrome associated with SARS-CoV-2 in children; PCR: polymerase chain reaction.

received IVIG, associated with steroids in 15 (60%). Median (IQR) length of hospital stay was 10 days (9-12) in cases vs 8 (4-11) days in controls (*P*=0.006) (**Table II)**.

Multivariate logistic regression analysis revealed that age >2 years (OR 24.71; 95%CI,1.03-592.42), lymphopenia (OR 9.03; 95% CI, 2.05-39.70), and thrombocytopenia (OR 11.73; 95%CI, 1.88-75.22) at diagnosis were significantly associated with MIS-C. Presence of underlying diseases (OR 0.06; 95%CI, 0.01-0.30) seems to reduce the probability of developing MIS-C.

DISCUSSION

In our series all MIS-C patients were older than 2 years, which is in agreement with previous finding that MIS-C does not affect infants younger than 1 year [1-8]. Potential age-related variability among MIS-C patients may result from differences in SARS-CoV-2 infection due to the likelihood of exposure or to differential nasal expression of angiotensin-converting enzyme 2, the entry receptor for SARS-CoV-2 [2-6]. Inequalities in access to health care, genetics-related risks, or overcrowding might be lines for future research. In our study, living with a person with COVID-19 and overcrowded living conditions were significantly associated with MIS-C in univariate analysis.

Several studies evaluating patients with MIS-C have found high rates of comorbidities [8,13,14]. In our series comorbidities were less frequently observed in MIS-C patients than in controls. A recent meta-analysis concluded that cancer patients undergoing treatment were not at a higher risk of developing more severe COVID-19 disease; however, the role of comorbidities in patients with COVID-19 needs further exploration [15]. Most MIS-C patients had a previous or concurrent laboratory-confirmed SARS-CoV-2 infection, supporting the hypothesis that MIS-C is an immune-mediated post-infectious synd-rome related to SARS-CoV-2 infection [6-11]. In our series, gastrointestinal symptoms were more frequent in MIS-C patients than in controls. Fever was observed in all MIS-C patients but not in all controls [8]. Similar to other series, shock and acute heart failure were more frequent in MIS-C patients than in controls [13].

MIS-C is associated with heart complications and inflammatory disorders triggered by SARS-CoV-2 with features similar to KD suggesting that this virus might be acting as an immunological trigger causing similar immune- mediated injury to the heart and coronary arteries comparable to KD [13]. Cardiovascular involvement was common in our MIS-C patients but not seen in any of the controls.

Low lymphocyte count, associated with poor outcome, was more common in MIS-C patients than in controls [14]. Levels of acute-phase reactants (CRP and PCT), may be high in patients with MIS-C [5,8]. Similar to other studies, all MIS-C patients received IVIG and combination with steroids in some of them [8]. As in previous studies on COVID-19 in children, ICU admission was more frequent in MIS-C patients [7-8], but no deaths were reported.

In conclusion SARS-CoV-2 associated MIS-C was more common in children older than 2 years and in those with lymphopenia or thrombocytopenia. The presence of underlying diseases seems to decrease the likehood to develop MIS-C; however, further studies are needed to confirm this observation and rule out that it was an incidental association in this study.

Ethics clearence: Hospital Juan P Garrahan; No. 1294, dated October 11, 2020.

Table II Laboratory Characteristics of Children With SARS-CoV-2 Infection With and Without MIS-C at Hospital Admission

Variable	*MIS-C, n=25 (%)*	*Controls, n=75 (%)*	*OR (CI 95%)*
White cells count[a]	10650 (6800-13680)	6840 (4350-10830)	–
Lymphopenia	15 (60)	14 (19)	4.8 (1.77-13.1)
Platelets count/L[a]	197000 (137000-246000)	294000 (210000-375000)	–
Thrombocytopenia	8 (32)	4 (5)	8.3 (2.24-31)
C-reactive protein, mg/L[a]	139.9 (122-248.5)	2.94 (0.77-22.8)	–
Abnormal liver enzymes	3 (12)	5 (7)	1.9 (0.3-10.7)
SARS-CoV-2 PCR	15 (60)	75 (100)	–
IgM SARS-CoV-2	7 (28)	6 (8)	4.47 (1.1-18.0)
IgG SARS-CoV-2	24 (96)	22 (29)	57.8 (8.1-2410.2)

Values in no. (%) or [a]median (IQR). Lymphopenia-lymphocyte count <1000/mm^3 on admission; Thrombocytopenia: platelet count <150000/mm^3. MIS–C -multisystem inflammatory syndrome associated with SARS-CoV-2 in children; PCR -polymerase chain reaction.

WHAT THIS STUDY ADDS?

- In Argentina, MIS-C was more common in children older than two years and those with lymphopenia or thrombocytopenia.
- Presence of underlying diseases seems to decrease the likelihood to develop MIS-C.

Contributors: MTR: conceptualized the study design; analyzed and interpreted the results, and wrote the manuscript; GP, MMK, AAP, MP, CG, NB, AB: recruited patients, collected demographic and clinical data analyzed and interpreted the results; RL,RB: conceptualized the study design- analyzed and interpreted the results, and commented on and revised the manuscript. All authors approved the final version of manuscript, and are accountable for all aspects related to the study.
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REFERENCES

1. Jones VG, Mills M, Suarez D, et al. COVID-19 and Kawasaki disease: Novel virus and novel case. Hosp Pediatr. 2020;10:537-40.
2. Zimmermann P, Curtis N. Coronavirus infections in children including COVID-19: An overview of the epidemiology, clinical features, diagnosis, treatment and prevention options in children. Pediatr Infect Dis J. 2020;39:355-68.
3. CDC. Multisystem inflammatory syndrome in children (MIS-C) associated with coronavirus disease 2019 (COVID-19). Accessed December 1, 2020. Available from: *https://www.cdc.Gov/coronavirus/2019-ncov/daily-life-coping/children/mis-c.html*
4. Royal College of Paediatrics and Child Health. Guidance*:* Paediatric multisystem inflammatory syndrome temporally associated with COVID-19. Accessed September 25, 2020. Available from: *https://www.rcpch.ac.uk/sites/default/files/2020-05/COVID19-Paediatric-multisystem-inflammatory syndrome-20200501.pdf.*
5. Henderson LA, Canna SW, Friedman KG, et al. American College of Rheumatology Clinical Guidance for Multisystem Inflammatory Syndrome in Children Associated With SARS–CoV2 and Hyperinflammation in Pediatric COVID-19: Version 1. Arthritis Rheumatol. 2020;72:1791-805.
6. Shobhavat L, Solomon R, Rao S, et al. Multisystem inflammatory syndrome in children: Clinical features and management-intensive care experience from a pediatric public hospital in Western India. Indian J Crit Care Med. 2020;24:1089-1094.
7. García-Salido A, de Carlos Vicente JC, Belda Hofheinz S, et al. Severe manifestations of SARS-CoV-2 in children and adolescents: From COVID-19 pneumonia to multisystem inflammatory syndrome: A multicentre study in pediatric intensive care units in Spain. Crit Care. 2020; 24:1-13.
8. Feldstein LR, Rose EB, Horwitz SM, et al. Multisystem inflammatory syndrome in US children and adolescents. N Engl J Med. 2020;383:334-46.
9. Jiang L, Tang K, Levin M, et al. COVID-19 and multisystem inflammatory syndrome in children and adolescents. Lancet Infect Dis. 2020; 20:e276-e288.
10. Radia T, Williams N, Agrawal P, et al. Multi-system inflammatory syndrome in children and adolescents (MIS-C): A systematic review of clinical features and presentation. Paediatr Respir Rev. 2020:S1526-1542.
11. WHO. Multisystem inflammatory syndrome in children and adolescents with COVID-19. Accessed November 25, 2020. Available from: *https://www.who.int/publications/i/item/ multisystem-inflammatory-syndrome-in-children-and-adolescents-with-covid-19*
12. Rodriguez-Gonzalez M, Castellano-Martinez A, Cascales-Poyatos HM, et al. Cardiovascular impact of COVID-19 with a focus on children: A systematic review. World J Clin Cases. 2020; 8:5250-283.
13. Huang I, Pranata R. Lymphopenia in severe coronavirus disease – 2019 27 38 (COVID 19): Systematic review and meta-analysis. J Intensive Care. 2020;8:1-10.
14. Yekedüz E, Utkan G, Ürün Y. A systematic review and meta-analysis: The effect of active cancer treatment on severity of COVID-19. Eur J Cancer. 2020;141:92-104.

RESEARCH PAPER

Agreement Between Non-Invasive (Oscillatory) and Invasive Intra-Arterial Blood Pressure in the Pediatric Cardiac Critical Care Unit

AMIT KUMAR,[1] JIGAR P THACKER, [1] MANOJ CHAUDHARY,[1] AJAY G PHATAK,[2] SOMASHEKHAR M NIMBALKAR[1]

From Department of [1]Pediatrics, Pramukhswami Medical College; and [2]Central Research Services, Charutar Arogya Mandal; Karamsad, Anand, Gujarat.

Correspondence to:
Dr Jigar P Thacker,
Assistant Professor, Department of Pediatrics, Pramukhswami Medical College, Shree Krishna Hospital, Karamsad 388 325, Anand, Gujarat.
thackerjigar@yahoo.co.in
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Objective: We assessed the agreement between non-invasive (oscillatory) blood pressure (NIBP) measurements and invasive intra-arterial blood pressure (IBP) in the pediatric cardiac critical care unit. **Methods:** Children with intra-arterial lines as per standard management protocol were enrolled. NIBP was measured every 4 hourly and the corresponding IBP reading was recorded. **Results:** A total of 839 brachial NIBP, 834 IBP femoral (IF), and 137 IBP radial (IR) readings were noted on 45 participants. The mean difference (95% CI) for agreement between NIBP and IF was -2.3 (-27.1, 22.5) mmHg for systolic, 0.9 (-21.3, 23.1) mmHg for diastolic and 0.3 (-23.3, 23.9) mmHg for mean BP. Similar results were found between NIBP and IR and between IF and IR. The interrater agreement [Kappa (95% CI)] was fair between NIBP and IF [0.54 (0.48, 0.61)], and IF and IR [0.62 (0.48, 0.76)] but lower between NIBP and IR [0.37(0.20, 0.55)] when values were classified as hypotensive, normotensive, and hypertensive. **Conclusions:** NIBP cannot replace but can supplement IBP in the pediatric cardiac critical care setting.

Keywords: *Accuracy, Comparison, Indirect blood pressure, Femoral blood pressure.*

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Blood pressure (BP) recording is an integral part of hemodynamic monitoring for quick therapeutic decisions in an intensive care setting. Non-invasive BP (NIBP) monitoring by an oscillatory automated device is an accepted modality in most clinical settings. Alternatively, invasive monitoring after placing an intra-arterial catheter is considered the gold standard as it provides continuous, reliable, and beat to beat monitoring of the BP [1,2]. Invasive BP (IBP) monitoring is the norm in pediatric post-cardiac surgery settings [3]. The NIBP measurement is affected by multiple factors including accuracy of the equipment, observer bias, position and movement of the arm, and the child's cooperation [4]. Determination of the appropriate BP cuff size is also crucial in pediatric patients. IBP reading is affected by movement artifact, altered pulse traveling in case of arterial dissection or stenosis, calibration errors, or overdamping and under-damping phenomena due to inappropriate dynamic response of the fluid-filled monitoring systems [5]. It is technically cumbersome and requires trained manpower to insert and maintain an arterial catheter, which may not be readily available at all facilities. IBP monitoring systems have known complications such as local tissue injury, excessive bleeding, hematoma formation, blood-stream infection, thrombosis or embolism, distal ischemia, and pseudoaneurysms of the vessels [6].

NIBP is easy, cost-effective, and avoids potential harms caused by invasive arterial line. Several studies have compared the accuracy of NIBP to IBP in intensive clinical care settings [3,7,8]. There are fewer such studies in the pediatric cardiac critical care setting [3]. This observational study assessed the agreement between NIBP by an automated oscillatory method with IBP in the pediatric cardiac critical care setting.

METHODS

This study was conducted in the pediatric cardiac critical care unit on post-operative patients who had undergone cardiothoracic surgeries. As per the protocol of the unit, the patient was induced on a radial arterial line by the anesthetic team in an operating room. Later, central venous and femoral arterial lines were inserted. Hemodynamics in the intra-operative and post-operative period were monitored via the femoral line. The radial line was not preferred for the peri-operative monitoring, particularly for the surgeries involving cardio-pulmonary bypass and was less stable in the pediatric age group due to frequent de-lining. The study was approved by the institutional ethics committed with a waiver of informed consent.

All consecutive patients with an intra-arterial line (radial and/or femoral) were selected for this study. The

decision of placing an arterial line was made by the treating team. All lines were placed without ultrasound guidance. Radial and femoral lines used in the same patient were connected to the same transducer via a three-way stopcock. The radial line was usually removed on the next day of surgery, whereas the femoral line was kept as long as required. NIBP measurement was done by the oscillatory method within one hour of admission to the post-operative care unit and then repeated every four hourly. Patients with contraindications to NIBP cuff application/inflation (arm injuries or wounds, limb edema) were excluded from the study. Patients with uncorrected or inadequately corrected coarctation/ interruption of aorta were also excluded. IBP reading was concurrently noted at each instance of NIBP recording in a patient. Age, weight, height, primary diagnosis, type of surgery/intervention, and current clinical condition of all participants were recorded.

IBP measurement was performed with an appropriately sized arterial catheter (Leader-Flex, Vygon, GmbH & Co) inserted into the radial/femoral artery and connected to a disposable pressure transducer (iPeX, B L Lifesciences Pvt Ltd) using rigid pressure tubing of identical length. In neonates, a 24 G cannula (Jelco) was used to obtain a radial line. The transducer was connected to the blood pressure module of the Drager, Infinity Vista XL (Dragerwerk AG & Co) bedside monitor. The catheter was flushed with heparinized saline (2 units/mL) at the rate of 3 mL/hour to prevent clotting. However, in neonates, the rate of flow was kept at 1 mL/hour via syringe pump to reduce their fluid intake. As both radial and femoral lines were connected to the same transducer by a three-way stopcock, the flush was entering one arterial line at a time. The IBP measurements were documented every hour by rotating the valve. Four hourly IBP values for invasive femoral (IF) and invasive radial (IR) were taken to coincide with the NIBP measurements. The pressure monitoring set had a continuous flush element pigtail that could be pulled to allow the rapid flush of the system. The transducer position was at the level of the patient's 4th intercostal space at the mid-axillary line, ensuring the absence of kinking or air bubbles in the tubing and transducer. Zeroing and fast-flush test to verify optimal damping was done in every nursing shift. Oscillatory BP (NIBP) in the upper limb was measured by using Drager, NIBP cuff (Dragerwerk AG & Co) of appropriate sizes and connected to the BP module of the Drager, Infinity Vista XL – Multi-para monitor via BP cable. The NIBP was measured in a different limb to that with the arterial line.

The blood pressure measurements by all the three methods were classified as hypotensive, normotensive, and hypertensive based on age and height by systolic value [9-11]. Fifth percentile cut off was used to label hypotension in our patient population [9].

Statistical analysis: Bland-Altman analysis was performed to assess agreement amongst NIBP, invasive femoral (IF), and invasive radial (IR) recordings. Kappa statistic with quadratic weights was used to assess agreement amongst the three methods at a crude level. The analysis was performed using STATA 14.2.

RESULTS

During a period of nine months, a total of 45 (33 male) patients [median (IQR) age 12 (4,84) months] were enrolled in the study. A total of 839 upper limb NIBP measurements, 834 femoral line, and 137 radial line blood pressure readings were available. The primary diagnosis and age group of the patients are mentioned in **Table I**. All the patients were on ventilatory and inotropic support during the initial post-operative period. The median (Q1,Q3) duration for ventilatory and inotropic support was 36 (7,74) and 57 (24,80) hours, respectively. None of the patients had pre-existing hypertension or features of vasculitis. The mean difference (95% CI) for agreement between NIBP and IF line was -2.3 (-27.1, 22.5) mm Hg for systolic, 0.9 (-21.3, 23.1) mmHg for diastolic and 0.3 (-23.3, 23.9) mm Hg for mean blood pressure. The mean difference (95% CI) for agreement between NIBP and IR line was -0.5 (-23.2, 22.3) mmHg for systolic, 2.1 (-19.6, 23.8) mmHg for diastolic and 2.3 (-19.1, 23.6) mmHg for mean blood pressure. The

Table I Age Group and the Primary Diagnosis of the Patients (*N*=45)

Characteristics	*No. (%)*
Age	
<1 mo	7 (15)
1-12 mo	20 (44)
1-5 y	5 (11)
5-10 y	7 (15)
10-18 y	6 (13)
Diagnosis[a]	
Ventricular septal defect	10 (22)
Atrial septal defect	9 (20)
Tetrology of fallot	8 (18)
Total anomalous pulmonary venous return	4 (09)
Double outlet right ventricle	4 (09)

[a]*Patent ductus arteriosis and transposistion of great arteries in two children each; and anomalous origin of left coronary artery from pulmonary artery, atrio-ventricular canal, coarctation of aorta,* single *right ventricle, tricuspid atresia, severe mitral regurgitation were seen in one child each.*

Table II Agreement in Classification of Blood Pressure by NIBP and IBP

	Invasive femoral	*Invasive radial*
Hypotensive	14/37	6/7
Normotensive	558/649	90/109
Hypertensive	103/147	5/20

Numerator denotes values agreed by NIBP and IBP and denominator denotes values by NIBP alone; IBP: invasive arterial blood pressure; NIBP: non-invasive blood pressure.

agreement between IF and IR line had a mean difference (95% CI) 0.3 (-21.5, 22.2) mm Hg for systolic, -0.6 (-15.8, 14.7) mmHg for diastolic and 0.7 (-13.7, 15.1) mm Hg for mean blood pressure.

The inter-rater agreement [Kappa with quadratic weights (95% CI)] for readings classified as hypotensive, normotensive, or hypertensive was fair between NIBP and IF [0.54 (0.48, 0.61)] and between IF and IR [0.62 (0.48, 0.76)] but slightly lower between NIBP and IR [0.37(0.20, 0.55)]. IF and IR classified 109/133 (81.2%) records correctly (6 hypotensive, 89 normotensive, 14 hypertensive). The correct classification between NIBP and IF and IR is shown in **Table II**.

DISCUSSION

The mean difference between NIBP and IBP among systolic, diastolic, and mean BP readings were marginal in the present study but wide 95% confidence limits made both these methods non-comparable and irreplaceable.

NIBP gave lower readings for systolic and higher for diastolic in comparison with both IF or IR as expected norms [1] but with a minimal difference, unlike wide mean difference reported earlier [8]. Higher systolic readings with NIBP were seen in few studies [3,12]. As per our unit policy of placing the femoral line for better stability, NIBP was compared against both invasive femoral (IF) and invasive radial (IR) unlike studies which compared radial lines only [5,7,8]. Both brachial NIBP and femoral IBP in this study represent central BP, unlike, radial readings which represents peripheral BP [8]. Physiologically, a slightly elevated lower limb BP reading than the upper limb is expected. The present study did not find significant difference between upper limb (NIBP or IR) and lower limb (IF) blood pressure, as also seen in a similar study [3].

IBP should be preferentially used when patient is hemodynamically stable or deviations can be detrimental in setting like pediatric cardiac intensive care [13]. Invasive and non-invasive (oscillatory) methods have entirely different principles. IBP monitoring has a column of fluid connecting an arterial catheter to a pressure transducer [1,7]. Oscillatory devices track oscillations of the pressure in a cuff during its progressive deflation. The maximal oscillation corresponds to MAP and systolic and diastolic readings are calculated which results in different accuracy with different devices [7]. Improper cuff size and poor cooperation and movement of the pediatric subject can affect NIBP reading [4].

Though the mean difference between IF and IR readings was insignificant, a wide range existed among; systolic, diastolic, and mean BP. Invasive measurements give different values depending upon the site of measurement [7,14] and state of shock where peripheral radial pressure value may not accurate [14].

Clinically significant discrepancies in systolic blood pressure values can be present between invasive and oscillometric non-invasive methods during hypotension [2]. Outside the normotensive range, the automated readings were higher during hypotension and lower during hypertension compared to the arterial BP [15]. However, such specific trends were not seen in this study. IBP reading is affected by underdamping/resonance phenomena in a significant number of events where NIBP measurement along with IBP is beneficial [5]. A clinician should remain cautious and check for the accuracy of both the instruments if in doubt; before undertaking any treatment [7]. Thus, the use of NIBP along with IBP results in lower use of vasopressors, transfusions, and antihypertensive when compared with IBP alone [16].

There are certain limitations to the study. This study enrolled participants from neonatal age to 18 years, representing diverse body mass and stature. The agreement between NIBP and IBP in age and height related subgroups was not assessed due to the small sample size. Almost all the patients were on inotropic support during the initial post-operative period, which might have affected the actual representation of BP readings. We could not separate the readings in the presence and absence of shock (compensated or non-compensated).

To conclude, the agreement between NIBP and Invasive BP readings was not optimal, while inter-rater agreement was fair for different categories of blood pressure. Considering IBP monitoring as the gold standard in the pediatric post-cardiac surgical setting, it cannot be replaced with NIBP, but rather should supplement with NIBP when in doubt.

Ethics clearance: Institutional ethics committee; No IEC/HMPCMCE/114/Faculty/11/ dated October 1, 2019. Considering the nature of the study, a waiver of informed consent was approved.
Contributors: JT: conceptualized and designed the study, guided

WHAT THIS STUDY ADDS?

- Non-invasive blood pressure measurement should supplement intra-arterial blood pressure measurement in pediatric cardiac critical care settings.

and supervise data collection, drafted the manuscript, and approved the final manuscript; SN: conceptualized and designed the study, critical review of the manuscript, and approved the final manuscript; AK; critical inputs for design of the study and manuscript preparation, drafting the manuscript, guided and supervise data collection, and approved the final manuscript; MC; helped in study planning and execution, continued onsite data collection and interpretation, and approved the final manuscript; AP: conceptualized and designed the study, data analysis, critical input for manuscript preparation, and approved the final manuscript.

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REFERENCES

1. Ward M, Langton JA. Blood pressure measurement. Continue Educ Anesth Crit Care Pain. 2007;7:122-6.
2. Lehman LW, Saeed M, Talmor D, Mark R, Malhotra A. Methods of blood pressure measurement in the ICU. Crit Care Med. 2013;41:34-40.
3. Ricci Z, Brogi J, De Filippis S, Caccavelli R, Morlacchi M, Romagnoli S. Arterial pressure monitoring in pediatric patients undergoing cardiac surgery: An observational study comparing invasive and non-invasive measurements. Pediatr Cardiol. 2019;40:1231-7.
4. Arafat M, Mattoo TK. Measurement of blood pressure in children: recommendations and perceptions on cuff selection. Pediatrics. 1999;104:e30.
5. Romagnoli S, Ricci Z, Quattrone D, et al. Accuracy of invasive arterial pressure monitoring in cardiovascular patients: An observational study. Crit Care. 2014;30;18:644.
6. Lin WQ, Wu HH, Su CS, et al. Comparison of continuous noninvasive blood pressure monitoring by TL-300 with standard invasive blood pressure measurement in patients undergoing elective neurosurgery. J Neurosurg Anesthesiol. 2017;29:1-7.
7. Ribezzo S, Spina E, Di Bartolomeo S, Sanson G. Noninvasive techniques for blood pressure measurement are not a reliable alternative to direct measurement: A randomized crossover trial in ICU. Scientific World Journal. 2014;2014:353628.
8. Krishna BV, Das S, Sen S. Correlation between blood pressure measurement by non-invasive and invasive methods in critically-ill children. Indian Pediatr. 2018;55:297-300.
9. de Caen AR, Berg MD, Chameides L, et al. Part 12: Pediatric Advanced Life Support: 2015 American Heart Association Guidelines Update for Cardiopulmonary Resuscitation and Emergency Cardiovascular Care. Circulation. 2015;132:S526-42.
10. Flynn JT, Kaelber DC, Baker-Smith CM, et al. Clinical practice guideline for screening and management of high blood pressure in children and adolescents. Pediatrics. 2017;140:e20171904.
11. Flynn JT. Neonatal hypertension: Diagnosis and management. Pediatr Nephrol. 2000;14:332-41.
12. Joffe R, Duff J, Guerra GG, Pugh J, Joffe AR. The accuracy of blood pressure measured by arterial line and noninvasive cuff in critically ill children. Crit Care. 2016;20:1.
13. Hayes S, Miller R, Patel A, et al. Comparison of blood pressure measurements in the upper and lower extremities versus arterial blood pressure readings in children under general anesthesia. Med Devices (Auckl). 2019;12:297-303.
14. Kim WY, Jun JH, Huh JW, Hong SB, Lim CM, Koh Y. Radial to femoral arterial blood pressure differences in septic shock patients receiving high-dose norepinephrine therapy. Shock. 2013;40:527 531.
15. Holt TR, Withington DE, Mitchell E. Which pressure to believe? A comparison of direct arterial with indirect blood pressure measurement techniques in the pediatric intensive care unit. Pediatr Crit Care Med. 2011;12:e391-4.
16. Wax DB, Lin HM, Leibowitz AB. Invasive and concomitant noninvasive intraoperative blood pressure monitoring observed differences in measurements and associated therapeutic interventions. Anesthesiology. 2011;115:973-8.

Breastfeeding and Coronavirus Disease 2019 (COVID-19) Vaccination: Position Statement of Indian Academy of Pediatrics Advisory Committee on Vaccination and Immunization Practices

SRINIVAS G KASI,[1] SHASHI KANT DHIR,[2] S SHIVANANDA,[3] SANJAY MARATHE,[4] KRIPASINDHU CHATTERJEE,[5] SUNIL AGARWALLA,[6] SANJAY VERMA,[7] ABHAY K SHAH,[8] SANJAY SRIRAMPUR,[9] SRINIVAS KALYANI,[10] HARISH KUMAR PEMDE,[11] S BALASUBRAMANIAN,[12] GV BASAVARAJA,[13] BAKUL J PAREKH,[14] REMESH KUMAR,[15] PIYUSH GUPTA[16]

From [1]Kasi Clinic, Jayanagar, Bengaluru, Karnataka; [2]Guru Gobind Singh Medical College, Faridkot, Punjab; [3]Fortis Hospital, Banneraghatta Road, Bengaluru, Karnataka; [4]Marathe Child Care Hospital, Nagpur, Maharashtra; [5]Gouri Devi Institute of Medical Science and Hospital, Durgapur, West Bengal; [6]MKCG MCH, Berhampur, Odisha; [7]Postgraduate Institute of Medical Education and Research, Chandigarh, [8]Dr Abhay K Shah Children Hospital, Ahmedabad, Gujarat;[9]Aditya Super speciality Hospital, Hyderabad, Telangana; [10]Niloufer Hospital, Osmania medical College, Hyderabad; [11]Lady Hardinge Medical College, New Delhi; [12]Kanchi Kamakoti Childs Trust Hospital, Chennai, Tamil Nadu; [13]IGICH, Bengaluru, Karnataka; [14]Bakul Parekh Hospital for Children, Mumbai, Maharashtra; [15]Apollo Adlux Hospital, Cochin, Kerala; [16]University College of Medical Sciences, New Delhi.

Correspondence to: Srinivas G Kasi, Convener, ACVIP of IAP, Kasi Clinic, 2nd Cross, 3rd Block, Jayanagar, Bengaluru, Karnataka. sgkasi@gmail.com

Justification: In India, till recently, breastfeeding women have been excluded from the coronavirus disease (COVID-19) vaccination program, rendering a significant population of the country, including frontline workers, ineligible to derive the benefits of the COVID-19 vaccine rollout. **Objective**: The objective of this recommendation is production of an evidence-based document to guide the pediatricians to give advice to breastfeeding mothers regarding the safety of COVID-19 vaccines in lactating women. **Process**: Formulation of key question was done under the chairmanship of president of the IAP. It was followed by review of literature regarding efficacy and safety of COVID-19 vaccines in breastfeeding women. The recommendations of other international and national professional bodies were also deliberated in detail. The available data was discussed in the ACVIP focused WhatsApp group. Opinion of all members was taken and the final document was prepared after achieving consensus. **Recommendations**: The IAP/ACVIP recommends the administration of COVID-19 vaccines to all breastfeeding women. The IAP/ACVIP endorses the recent recommendation of the Government of India, to consider all breastfeeding women as eligible for COVID-19 vaccination.

Keywords: *Lactation, Maternal, Protection, SARS-CoV-2*.

The development and implementation of coronavirus disease 2019 (COVID-19) vaccination program has been one of the recent and most prominent demonstrations of the power of modern science. As of 14 May, 2021, in India, a total of 13986142 (1 dose) and 39784951 (2 doses) have been administered [1,2]. Ever since the initiation of the COVID -19 vaccination program in India, pregnant and breastfeeding women had been excluded from the vaccination program [3]. Recently, the Government of India (GoI) has published a circular, recommending administration of COVID-19 vaccines in breastfeeding women [4]. Failure to include pregnant and lactating women in the phase 3 studies of the mRNA vaccines, Astra-Zeneca vaccine and Covaxin (Bharat Biotech Ltd) and consequent lack of safety data, were the cited reasons for excluding this group. If this cohort of pregnant and breastfeeding mothers continued to be excluded, a significant population of the country, including frontline workers, would have been left unprotected. Hence, the recent recommendation from the GoI is a welcome step.

OBJECTIVE

The objective of this recommendation is production of an evidence-based document to guide the pediatricians to give advice to breastfeeding mothers regarding the safety of COVID-19 vaccines in lactating women.

PROCESS

Formulation of key question was done under the chairmanship of President of the Indian Academy of Pediatrics (IAP). It was followed by review of literature regarding efficacy and safety of COVID-19 vaccines in breastfeeding women. The recommendations of other international and national professional bodies were also deliberated in detail. The available data was discussed in the Advisory Committee on Vaccines and Immunization Practices (ACVIP) focused WhatsApp group. Opinion of all members was taken and the final document was

prepared after the consensus and was approved by all members of the ACVIP (authors of the guidelines).

BREASTFEEDING AND COVID-19 VACCINES

The theoretical risk of COVID-19 vaccination in breastfeeding mothers and the potential harm to the infant is unknown. However, it is to be noted that none of the vaccines available for the COVID-19 contains live virus. There is no plausible biological mechanism to explain how an inactivated vaccine, given to the mother, would cause harm to a breastfed baby [5].

The COVID-19 vaccines presently available in India, i.e., Covishield and Covaxin are classified as inactivated (non-live) vaccines. Theoretically, administration of these vaccines to breastfeeding women should not render any harm to the breastfed infant. While the safety of adenovirus vectors in pregnancy and lactation is not established, wild adenoviral infections are present worldwide and have not been associated with teratogenic effects in the fetus or newborns [6]. With the exception of small pox and yellow fever vaccines no other vaccine is contraindicated during breast feeding [7].

Breast milk is a rich source of antibodies for the infant. Milk produced by infected mothers is a source of anti-SARS-CoV-2 IgA and IgG and neutralizes SARS-CoV-2 activity [8].

Studies have shown that maternal vaccination with the mRNA vaccine results in high titers of RBD-IgG binding antibodies and neutralizing antibodies as measured by the pseudovirus neutralizing tests (NT50), in maternal serum. High titers of RBD–IgG binding antibodies, neutralizing antibodies as measured by the NT50 and robust T-cell responses, as measured by ELISPOT and intra-cellular cytokine staining, have been demonstrated in the breast milk of mothers vaccinated with the mRNA vaccines [9]. In another study, involving six lactating women who received two doses of SARS-CoV-2 vaccine, significantly elevated levels of SARS-CoV-2 specific IgG and IgA antibodies in breast milk was observed, beginning at Day 7 after the initial vaccine dose, with an IgG-dominant response [10]. These SARS-CoV-2 specific immunoglobulins and products of the T-cell responses in breast milk may be protective for infants.

There is a paucity of data on immunological parameters in breastmilk following the administration of the AstraZeneca COVID-19 vaccine in lactating women. Thus, merely the absence of data should not exclude lactating women from getting the benefits of COVID-19 vaccination.

RECOMMENDATIONS FROM OTHER PROFESSIONAL BODIES

Many international and national recommending bodies have now recommended administration of COVID-19 vaccines in breastfeeding women (**Table I**).

While some have recommended the COVID-19 vaccines in breastfeeding women who are in the priority groups, after fully informing them about the benefits and risks of vaccination, some authorities have recommended the vaccine for all breastfeeding women. All have emphasized that breast feeding should be continued after vaccination.

Table I Recommendations for Coronavirus Disease 2019 (COVID-19) Vaccine for Lactating Women by Various National and International Bodies

Agency	*Recommendation*
World Health Organization [11]	Developmental and health benefits of breastfeeding should be considered along with the mother's clinical need for immunization against COVID-19. WHO does not recommend discontinuing breastfeeding after vaccination.
American College of Obstetricians and Gynecologists [12]	COVID-19 vaccines should be offered to lactating individuals similar to non-lactating individuals.
International Federation of Gynecology and Obstetrics [13]	Supports offering COVID-19 vaccination to pregnant and breastfeeding women.
Federation of Obstetric and Gynaecological Societies of India [14]	COVID-19 vaccine should extend to pregnant and lactating women. The very real benefits of vaccinating pregnant and lactating women seem to far outweigh any theoretical and remote risks of vaccination.
Australian and New Zealand health authorities [15,16]	Recommend COVID-19 vaccines in breast feeding women. They have emphasized that there are no concerns about their safety in breastfeeding women or their babies.
Italian Scientific Society [17]	Decision whether or not to administer the COVID-19 vaccine to the breastfeeding woman should be made after mutual agreement between her and the health professionals, considering specific health, social, familiar and work conditions.

IAP-ACVIP RECOMMENDATIONS

1. Breastfeeding is very beneficial in the first years of life for nutrition and the protection provided by it against infectious agents. This is of crucial importance in developing countries like India.
2. The benefits of COVID-19 vaccination should not be denied to breastfeeding women as the real benefits are much more than the "theoretical risks."
3. The IAP/ACVIP strongly recommends the administration of COVID-19 vaccines to all breastfeeding women.
4. The IAP/ACVIP endorses the recent decision of the GOI, to consider breastfeeding women eligible for COVID-19 vaccination.

Contributors: All authors were part of the IAP ACVIP team that formulated these guidelines. PG, BJP, GVB, and SGK: conceived the Guidelines, prepared the agenda, and executed administratively. PG and SGK: led the discussions and all the members actively participated. SGK, SKD, SV, HKP, AS reviewed the literature on national and international guidelines, SGK, SM, SKA, SS, SK reviewed the literature on safety of COVID vaccines in breast feeding women, S, KC, SK reviewed the literature on immunological parameters in breast milk. SGK, SKD wrote the first draft. The first draft was peer reviewed by PG, SBS. PG, BJK, PG, GVB, RK provided intellectual inputs and overall guidance at every step. PG, BJP, GVB provided the administrative support from the Indian Academy of Pediatrics and coordinated between the team and executive board members of the Academy. The final document was drafted by SGK and SKD; and edited by PG and SBS. All authors approved the final recommendations of the guidelines.

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REFERENCES

1. WHO Coronavirus (COVID-19) Dashboard. Accessed May 15, 2021. Available from: *https://COVID19.who.int*
2. India: WHO Coronavirus Disease (COVID-19) Dashboard With Vaccination Data. Accessed May 15, 2021. Available from: *https://COVID19.who.int*
3. COVID-19 vaccines operational guidelines. Ministry of health and family welfare. Accessed May 15, 2021. Available from: *https://www.mohfw.gov.in/pdf/ COVID19VaccineOG111Chapter16.pdf*
4. New Recommendations of NEGVAC accepted by Union Ministry of Health. Accessed May 21, 2021. Available from: *https://pib.gov.in/Press ReleasePage.aspx?PRID=1719925*
5. Drugs and Lactation Database (LactMed). Bethesda (MD): National Library of Medicine (US); 2006-. COVID-19 vaccines. [Updated 2021 Apr 19]. Accessed May 15, 2021. Available from: *https://www.ncbi.nlm.nih.gov/books/NBK565969/*
6. EMA, European Medicines Agency. Committee for Medicinal Products for Human Use (CHMP). Assessment Report – COVID-19 Vaccine AstraZeneca. EMA/94907/2021, 2021. Assessed January 29 2021 Available from: *https://www.ema.europa.eu/en/documents/assessment-report/COVID-19-vaccine-astrazeneca-epar-public-assessment-report _en.pdf*
7. Sachs HC, Committee On Drugs. The transfer of drugs and therapeutics into human breast milk: an update on selected topics. Pediatrics. 2013;132:e796-809.
8. Pace RM, Williams JE, Järvinen KM, et al. COVID-19 and human milk: SARS-CoV-2, antibodies, and neutralizing capacity. medRxiv [Preprint]. 2020 Sep 18.
9. Collier A-RY, McMahan K, Yu J, et al. Immunogenicity of COVID-19 mRNA Vaccines in Pregnant and Lactating Women. JAMA. 2021 May 13;
10. Baird JK, Jensen SM, Urba WJ, et al. SARS-CoV-2 antibodies detected in human breast milk post-vaccination. medRxiv. 2021 Mar 10;
11. Coronavirus disease (COVID-19): Vaccines safety. Accessed May 15, 2021. Available from: *https://www.who.int/news-room/q-a-detail/coronavirus-disease-(COVID-19)-vaccines-safety*
12. Vaccinating Pregnant and Lactating Patients Against COVID-19. Accessed May 15, 2021. Available from: *https://www.acog.org/en/clinical/clinical-guidance/practice-advisory/articles/2020/12/vaccinating-pregnant-and-lactating-patients-against-COVID-19*
13. COVID-19 Vaccination for pregnant and breastfeeding women. Accessed May 15, 2021. Available from: *https://www.figo.org/COVID-19-vaccination-pregnant-and-breastfeeding-women*
14. FOGSI position statement COVID vaccination for pregnant and breastfeeding women. The Federation of Obstetric and Gynecological Societies of India. Accessed May 15, 2021. Available from: *https://www.fogsi.org/COVID-vaccination-for-pregnant-bf-women/*
15. COVID-19 vaccination – Shared decision making guide for women who are pregnant, breastfeeding or planning pregnancy. Australian Government Department of Health. Accessed May 15, 2021. Available from: *https://www.health.gov.au/resources/publications/COVID-19-vaccination-shared-decision-making-guide-for-women-who-are-pregnant-breastfeeding-or-planning-pregnancy*
16. COVID-19 vaccines in pregnancy and breastfeeding. The immunization advisory centre. Accessed May 15, 2021. Available from: *https://COVID.immune.org.nz/faq/COVID-19-vaccines-pregnancy-and-breastfeeding*
17. Davanzo R, Agosti M, Cetin I, et al. Breastfeeding and COVID-19 vaccination: Position statement of the Italian scientific societies. Ital J Pediatr. 2021;47:45.

Consensus Guidelines on Management of Steroid-Resistant Nephrotic Syndrome

ANIL VASUDEVAN,[1] RANJEET THERGAONKAR,[2] MUKTA MANTAN,[3] JYOTI SHARMA,[4] PRIYANKA KHANDELWAL,[5] PANKAJ HARI,[5] ADITI SINHA,[5] ARVIND BAGGA,[5] EXPERT GROUP OF INDIAN SOCIETY OF PEDIATRIC NEPHROLOGY*

From [1]Department of Pediatric Nephrology, St. John's Medical College Hospital, Bengaluru; [2]INHS Asvini, Mumbai; [3]Maulana Azad Medical College, New Delhi; [4]Pediatric Nephrology Service, King Edward Memorial Hospital, Pune; [5]Division of Nephrology, Department of Pediatrics, All India Institute of Medical Sciences, New Delhi, India.

**List of expert group members provided in Annexure I.*

Correspondence to: Dr Arvind Bagga, Division of Nephrology, Department of Pediatrics, All India Institute of Medical Sciences, New Delhi 110 029, India. arvindbagga@hotmail.com

Justification: The management of steroid resistant nephrotic syndrome (SRNS) is challenging. These guidelines update existing 2009 Indian Society of Pediatric Nephrology recommendations on its management. **Objective:** To frame revised guidelines on diagnosis and evaluation, treatment and follow up, and supportive care of patients with the illness. **Process:** The guidelines combine evidence-based recommendations and expert opinion. Formulation of key questions was followed by systematic review of literature, evaluation of evidence by experts and two face-to-face meetings. **Recommendations:** Fourteen statements provide updated advice for managing steroid resistance, and underscore the importance of estimating proteinuria and baseline kidney function, and the need for kidney biopsy and genetic screening. Calcineurin inhibitors are recommended as most effective in inducing remission of proteinuria, the chief factor associated with long-term renal survival. Advice on managing allograft recurrence, congenital nephrotic syndrome, and monitoring and supportive care, including transition of care, are described. This revised practice guideline is intended to improve management and patient outcomes, and provide direction for future research.

Keywords: *Calcineurin inhibitors, Congenital nephrotic syndrome, Focal segmental glomerulosclerosis, Minimal change disease.*

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The prevalence of idiopathic nephrotic syndrome, characterized by proteinuria, hypoalbuminemia and edema, varies from 12-16 per 100000 children [1]. Majority of patients achieve remission of proteinuria following 4-6 weeks therapy with prednisolone. However, 10-15% patients do not achieve complete remission, and are termed steroid-resistant nephrotic synd-rome (SRNS) [2]. Renal histology shows focal segmental glomerulosclerosis (FSGS), minimal change disease and mesangioproliferative glomerulonephritis. Other patterns, including C3 glomerulopathy, membranous nephropathy and IgA nephropathy, and secondary causes of nephrotic syndrome are uncommon. The management of patients with SRNS is challenging. The illness is associated with unsatisfactory patient-reported quality of life, morbidity due to infectious and non-infectious illnesses, and side effects of therapy [2,3]. Patients with persistent proteinuria are at risk for progressive kidney failure [4].

Guidelines from the Indian Society of Pediatric Nephrology (ISPN) were first published in 2009 [5]. In view of recent evidence, the ISPN has proposed revision of these recommendations. The revised guidelines refer to patients with SRNS due to minimal change disease, mesangioproliferative glomerulonephritis and FSGS. These guidelines also address management of patients with post-transplant recurrence of FSGS and congenital nephrotic syndrome. Clinical practice recommendations, from the International Pediatric Nephrology Association (IPNA), on the illness were published recently [6].

PROCESS

Three work-groups were constituted to evaluate evidence on: (*i*) diagnosis and evaluation, (*ii*) treatment and follow up, and *(iii)* supportive care of patients with SRNS. The groups developed key questions, and reviewed and analyzed published studies. Quality of evidence was assessed and rated from A-D following the GRADE model [7], and is provided with each guideline. Each statement was assigned one of the two levels of guidance, recommendation or suggestion, indicating strength of the advice **(Web Table I)**. Ungraded statements (X) are like practice points, not supported by sufficient evidence.The work-groups discussed the evidence, through alternating breakout and plenary sessions, in New Delhi on 5 April 2019. Draft guidelines were discussed with members of the ISPN in Pune on 21 December 2019.

GUIDELINES

Table I compares the current and previous guidelines [5] and recent recommendations from the IPNA [6]. Given the challenges in management, we advise that a pediatric nephrologist be responsible for the diagnosis and management of children with SRNS.

Guideline 1: Diagnosis of Steroid-Resistant Nephrotic Syndrome (SRNS)

1.1 We recommend that steroid-resistance be defined in patients not showing complete remission of proteinuria, despite 6-weeks daily treatment with prednisolone. (1B)

1.2 We suggest similar definitions for initial and late (secondary) steroid-resistance (**Box I**). (X)

Rationale

Approximately 85-90% patients with idiopathic nephrotic syndrome respond to treatment with prednisolone, with complete remission of proteinuria and normalization of serum albumin [1]. There is lack of consensus regarding the minimum duration of daily prednisolone treatment before defining steroid-resistance. The International Study of Kidney Disease in Children (ISKDC) reported that, of patients who achieved remission, 94% did so within 4-weeks daily treatment and the rest during 4-weeks' alternate-day therapy [8]. Others found that 4-weeks and 6-8 weeks initial therapy results in remission in 90-92% and 87-93% patients, respectively [9-12]. While few experts suggest additional therapy with 3-doses of IV methyl pre-dnisolone before labeling steroid-resistance, this is not uniformly practiced [6,13,14].

Table I Guidelines on Steroid-Resistant Nephrotic Syndrome (SRNS): Current Indian Society of Pediatric Nephrology (ISPN), ISPN 2009 and International Pediatric Nephrology Association (IPNA) 2020

	Current ISPN	*ISPN 2009 [5]*	*IPNA 2020 [6]*
Definition: Duration of prednisone therapy	6 weeks daily	4 weeks daily	4 weeks daily; if partial remission, 2 weeks additional therapy (confirmation period)
Kidney biopsy	All; except if monogenic SRNS identified	All patients	All; except if monogenic SRNS identified
Genetic testing	Specific subsets of initial SRNS, congenital NS; not in late SRNS	Specific forms of initial SRNS	All patients with initial SRNS; not in late SRNS
Immunosuppression in monogenic SRNS	Not advised; may continue after counseling if partial remission	Not discussed	Not advised; may continue after counseling
Estimated GFR, mL/min/1.73 m^2	At diagnosis; q 3-6 months Avoid immunosuppression if eGFR<60	At diagnosis	At diagnosis; q 3 months Prefer MMF if eGFR <30 mL/min/1.73 m^2
First line: Calcineurin inhibitors (CNI)	Duration of therapy at least 2-year	Duration: 2-3 year	Duration: 1-2 year
Cyclophosphamide	IV cyclophosphamide may be used; oral not advised	IV therapy has low efficacy; oral not used	IV or oral cyclophosphamide
Indications for mycophenolate mofetil	(*i*) Prolonged CNI use and disease relapses; *(ii)* CNI-resistant SRNS	No recommendation	(*i*) eGFR<30 mL/min/1.73 m^2; (*ii*) CNI therapy for 1-yr; (*iii*) steroid sensitive relapses
Use of rituximab	(*i*) Prolonged CNI use and disease relapses; (*ii*) CNI-resistant SRNS; (*iii*) allograft recurrence	No recommendation	(*i*) CNI-resistant SRNS; (*ii*) allograft recurrence
Prednisone alternate day	Taper over 6-9 months	Taper over 1-1.5 yr	Taper and stop by 6 months
Statins; in addition to dietary advice	LDL cholesterol >160 mg/dL; >130 mg/dL if cardiovascular risk factors	Total cholesterol >200 mg/dL or LDL >130 mg/dL	LDL cholesterol >160 mg/dL; >130 mg/dL if cardiovascular risk factors
CNI-resistant disease	Rule out monogenic cause; consider rituximab or addition of MMF	Not discussed	Switch to MMF or rituximab; enroll in clinical trials
Renal transplantation	Evaluation of recipient, donor; managing recurrent FSGS	Not discussed	Evaluation of recipient, donor; managing recurrent FSGS

eGFR-estimated glomerular filtration rate; FSGS-focal segmental glomerulosclerosis; LDL-low density lipoprotein; MMF-mycophenolate mofetil; NS-nephrotic syndrome.

Box I Definitions Related to Nephrotic Syndrome

Nephrotic syndrome

Nephrotic range proteinuria (40 mg/m^2/h or > 1000 mg/m^2/day; spot Up/Uc ≥ 2 mg/mg; 3-4+ by dipstick); hypoalbuminemia (albumin < 3.0 g/dL); and edema

Steroid sensitive nephrotic syndrome

Complete remission within 6-weeks' treatment with prednisolone at a dose of 60 mg/m^2/day (2 mg/kg/day; maximum 60 mg/day)

Initial steroid-resistance

Failure to achieve complete remission after 6-weeks initial therapy with prednisolone (as defined above)

Late (secondary) steroid-resistance

Initially steroid-sensitive; steroid resistance in a subsequent relapse

Complete remission

Urine protein nil-trace by dipstick for 3 consecutive days, Up/Uc < 0.2, or 24-h protein < 100 mg/m^2/day

Partial remission

Urine protein 1+/2+ (dipstick), Up/Uc between 0.2-2, or 24-h urine protein 100-1000 mg/m^2/day; serum albumin ≥ 3.0 g/dL; and absence of edema

Non-response

Urine protein 3+/4+ (dipstick), Up/Uc ≥ 2, or 24-h urine protein > 1000 mg/m^2/day; albumin < 3.0 g/dL or edema

Relapse

Urine albumin 3+/4+ for 3 consecutive days, Up/Uc ≥ 2, or 24-h protein > 1000 mg/m^2/day, in a patient previously in partial or complete remission

Monogenic disease

Pathogenic or likely pathogenic variation, defined by American College of Medical Genetics and Genomics, in a gene associated with steroid-resistant nephrotic syndrome (Web Table II)

CNI-resistant disease

Non-response to cyclosporine or tacrolimus, given in adequate doses and titrated to optimal blood trough levels, for 6-months

Allograft recurrence of nephrotic syndrome

Persistent proteinuria (Up/Uc > 1) if previously anuric; or increase of Up/Uc by >1 if proteinuria at time of transplant (in absence of other apparent causes)

CNI-calcineurin inhibitor; Up/Uc-urine protein to creatinine ratio (mg/mg).

The previous version of this guideline defined SRNS as lack of complete remission despite 4-weeks therapy with prednisolone at a daily dose of 60 mg/m^2 [5]. The ISKDC and Kidney Disease: Improving Global Outcomes (KDIGO) proposed that steroid-resistance be defined following 8-weeks therapy [8,15]. Recent IPNA and KDIGO guidelines propose confirming steroid-resistance following 4-6-weeks' therapy with predniso(lo)ne, with or without additional therapy with three-doses of IV methylprednisolone [6,16].

In order to balance the benefits of extending therapy with steroid adverse effects, we recommend defining SRNS in patients who fail to show complete remission of proteinuria despite 6-weeks therapy with prednisolone at daily dose of 60 mg/m². Patients with steroid adverse effects may receive daily prednisolone for 4-weeks, followed by alternate-day therapy for the next 2-weeks. We do not advise therapy with IV methylprednisolone before making the diagnosis of SRNS.

We suggest similar definitions for initial (primary) and late (secondary) steroid-resistance (**Box I**). Initial resistance is lack of remission at the first episode of nephrotic syndrome. Patients who are steroid-sensitive initially but show steroid-resistance during subsequent relapse have late resistance. Systemic infections may be associated with persistent proteinuria and should be treated appropriately.

Guideline 2: Evaluation of Patients

We recommend the following in all patients with SRNS: Quantitation of proteinuria; serum creatinine; estimated glomerular filtration rate (eGFR); and kidney biopsy (***Box II)***. (1A)

Rationale

Nephrotic syndrome is characterized by nephrotic range proteinuria: ≥ 3+ by dipstick, proteinuria ≥ 40 mg/m^2/hr (> 1000 mg/m^2/day), urine protein to creatinine ratio (Up/Uc) ≥ 2 mg/mg; hypoalbuminemia (<3 g/dL); and edema [6]. All patients should be evaluated appropriately (**Box II**). Estimation of proteinuria, by Up/Uc in morning specimen or 24-hr protein excretion, at diagnosis and 6-monthly follow-up, helps determine response to therapy. Since 24-hr collection of urine is difficult to implement, Up/Uc is preferred. Parents are counseled regarding the importance of urinary dipstick analysis for home monitoring of proteinuria.

Response of proteinuria to therapy is an important determinant of renal survival [4,17,18]. Data from the PodoNet Registry on 1354 patients with SRNS shows that 10-year renal survival was highest (94%) in complete remission, 72% with partial remission and 43% with non-response [19]. Assessment of creatinine and eGFR at baseline and follow-up identifies acute kidney injury (AKI) secondary to hypovolemia, fluid loss, infections and drug toxicity, and CKD [20,21].

History and examination might help identify genetic and secondary forms of SRNS. History of deafness, developmental delay, seizures, family history of similar disorder and consanguinity, and syndromic features or extrarenal anomaly (e.g., genitourinary abnormality,

Box II Initial Evaluation of Patients with Steroid-Resistant Nephrotic Syndrome

Urinalysis, including microscopy

Spot urine protein to creatinine ratio; 24-h urine protein excretion

Complete blood counts

Blood creatinine, albumin, electrolytes, fasting glucose, glycosylated hemoglobin (HbA1c)

Total, low density and high-density cholesterol; triglycerides

Calcium, phosphate, alkaline phosphatase

Hepatitis B surface antigen; hepatitis C and human immunodeficiency virus antibodies

Ultrasonography of kidneys

Kidney biopsy (light, immunofluorescence, electron microscopy); avoided in selected patients*****

Investigations in selected children

Complement C3, C4; antinuclear antibody

Genetic tests: Initial steroid-resistance with: *(i)* onset during infancy; *(ii)* family history of steroid-resistance, *(iii)* extrarenal features, *(iv)* non-response to calcineurin inhibitors, *(v)* prior to transplantation

Biopsy may be avoided in patients with familial steroid-resistance or with extrarenal features, where genetic diagnosis is preferred; a biopsy is also not required in patients with congenital nephrotic syndrome (Web Box II).

microcoria, dystrophic nails and microcephaly) suggest a genetic etiology. History of joint pain, weight loss, alopecia, jaundice, rash or palpable purpura indicates a secondary cause.

All patients with SRNS should undergo a kidney biopsy before instituting specific treatment. Biopsies are examined by light, immunofluorescence and electron microscopy. An adequate biopsy should include the corticomedullary junction and have ~20 glomeruli to identify focal pathology like FSGS [22]. A biopsy is useful for: (*i*) identifying pathology, extent of interstitial fibrosis and glomerulosclerosis for diagnosis and prognosis; and (*ii*) excluding differential diagnosis and secondary causes of nephrotic syndrome. Repeat biopsy is required to assess calcineurin inhibitor (CNI) toxicity, progression of disease or change in pathology.

Chief histological diagnoses in children with SRNS include FSGS (40-50%), minimal change disease (25-40%) and mesangioproliferative glomerulonephritis (5-8%) [23]. Histology suggestive of FSGS is considered a risk factor for progression to CKD [15-17,24]. Around 10-15% patients show membranous nephropathy, IgA nephropathy or proliferative glomerulonephritis, which requires additional evaluation. A kidney biopsy is not necessary in patients with well described monogenic form of SRNS, known to be unresponsive to immunosuppression, e.g., congenital nephrotic syndrome, familial disease, or if a known genetic cause is already identified.

Screening for viral infections: Patients should be evaluated for hepatitis B and C, and HIV infections. Collapsing FSGS may be associated with HIV or parvovirus infection [25]. Those with positive serology are evaluated for viral load and extent of disease. Active infection may require the use of antiviral therapy.

Guideline 3: Indications for Genetic Studies

We recommend genetic studies in the following patients: congenital nephrotic syndrome; initial resistance during infancy; nephrotic syndrome with extrarenal features; familial steroid-resistance; non-response to therapy with CNI; and prior to transplantation. (1B)

Rationale

Approximately 20-30% patients with SRNS have pathogenic variations in genes encoding proteins of podocyte structure and function **(Web Table II)** [2]. Mutations in *NPHS1, NPHS2, WT1, COQ2, PLCE1* and *LAMB2* account for 50-60% of monogenic disease in children [26-28]. Genetic testing is useful as follows:

- Identification of causal variant enables diagnosis of monogenic disorders, and occasional phenocopies (e.g., Alport syndrome, Dent disease, cystinosis). Specific diagnosis allows counseling regarding progression of kidney disease and monitoring for extrarenal complications, e.g., patients with *WT1*, *LMX1B,WDR73* and *SMARCAL1* mutations [29].
- Patients with monogenic etiology have 4-fold risk of non-response to therapy with CNI (odds ratio, OR 4.00; 95% CI 2.52-6.51) and 3-fold risk of kidney failure (OR 2.87; 95% CI 2.22-3.72) **(Web Table III)** [18,26,28,30].
- Certain mutations respond to targeted therapy, e.g., coenzyme Q10 for defects in CoQ pathway, and eplerenone for *ARHGDIA* mutations [31,32].
- Compared to patients with no identifiable genetic cause, those with monogenic etiology have significantly lower risk for allograft recurrence [18,27,33].
- Diagnosis of a monogenic etiology assists in counseling for future pregnancies and antenatal diagnosis, and facilitates screening of live related renal transplant donors [34-36].

While IPNA guidelines suggest comprehensive genetic evaluation in all children with initial steroid-resistance [6], we suggest a focused approach. The likelihood of detecting a genetic cause is inversely related

to age at onset of the illness. A monogenic etiology was seen in 69%, 50%, 25%, 18% and 11% with disease presenting during the first 3 months, 4-12 months, 1-6 years, 7-12 year and 13-18 years, respectively [26]. Syndromic forms of the illness may be associated with specific mutations and characteristic phenotype (**Web Table II**). Family history of similar illness or consanguinity suggests a genetic cause in ~50-70% cases [26,27]. Although patients with an underlying genetic etiology are less likely to respond to therapy with CNI, few patients may occasionally show partial remission [37].

Siblings of patients with a monogenic cause may be screened for proteinuria by dipstick. There is no role for genetic screening in healthy children with family history of the disease. Since pathogenic mutations are not identified in patients with late steroid-resistance, genetic testing in these children is also not indicated [18,27].

The precise prevalence of monogenic variations in Indian patients with SRNS is unclear as studies are limited to small cohorts [38,39]. A nationwide study is in progress to determine the genetic basis of SRNS, and indications for testing may be revised in future.

Method of Genetic Testing

Causal variants in ~90 genes are associated with monogenic SRNS **(Web Table II)**. Most genes do not show a clear phenotype-genotype correlation. Next-generation sequencing (NGS) panels, incorporating multiple genes relevant to the phenotype, are feasible and less expensive, and provide higher diagnostic yield than Sanger sequen-cing. These panels include genes associated with other renal diseases that may have phenotype similar to SRNS. Clinical exome sequencing (Mendeliome gene panel), which includes all exons of genes listed in Online Mendelian Inheritance of Man (OMIM) database, facili-tates targeted gene analysis. In case a causative variant is not identified in the gene-panel, search for variants may be extended to remaining genes in the clinical exome. Whole exome sequencing might be considered for novel disease-causing genes. Sanger sequencing is preferred if a disease-causing mutation is highly likely in a specific gene, in context of extrarenal features or positive family history with known genetic cause. Sanger sequencing is essential to confirm variants detected on NGS, to screen parents to confirm segregation and for antenatal counseling.

Parents should be advised regarding risks and benefits of NGS, including limitation of insurance cover. Referral to genetic counselors might be necessary. Testing must be performed by certified and experienced laboratories, and pathogenicity of variants determined based on criteria proposed by the American College of Medical Genetics and Genomics [40].

Guideline 4: Therapy of Patients with SRNS

4.1 We recommend calcineurin inhibitors (CNI) as first-line therapy for patients with initial or late steroid-resistance. (1A)

4.2 We suggest continuing therapy with CNI for at least 24-months if partial or complete remission is achieved. (2C)

4.3 We suggest that CNI therapy should be withheld or discontinued for patients with AKI stage 2-3 or estimated glomerular function rate (eGFR) persistently below 60 ml/min/1.73m^2. (2C)

Rationale

Therapy aims to induce complete or partial remission, while avoiding medication-related toxicity. Long-term renal outcome in patients who achieve remission is significantly better when compared to non-responders [17-19,41]. Randomized controlled trials (RCT) and case series show that therapy with CNI (cyclosporine, tacrolimus) results in complete remission in 30-40% and complete or partial remission in 60-80% patients [2,3,18,41,42]. A Cochrane meta-analysis that compared cyclosporine to no treatment showed increased likelihood of complete or partial remission with the former (2 RCT; relative risk RR 3.50; 95% CI 1.04-9.57) at 6-months [43]. Similarly, therapy with CNI, compared to IV cyclophosphamide, was associated with higher rates of complete or partial remission (3 RCT; RR 1.98; 95% CI 1.25-3.13) [43]. While most reports do not show different outcomes between initial and late steroid-resistance [44-46], better outcomes in the latter have been reported [18]. The efficacy of tacrolimus and cyclosporin is comparable (2 RCT; RR 1.05; 95% CI 0.87-1.25), with no difference in nephrotoxicity or hypertension [43,47].

Similar to the IPNA and KDIGO guidelines, we recommend first-line use of CNI for patients with SRNS [6,16]. Tacrolimus is preferred to cyclosporine except in children who are unable to swallow tablets (cyclosporine is available as suspension), and patients with seizures or at risk for diabetes. Doses of tacrolimus and cyclosporine are titrated to achieve recommended trough levels, keeping in mind interaction with other medications **(Table II** and **Web Table IV)**. Low levels are associated with non-response and relapse, while high levels increase the risk for nephrotoxicity [48]. Lower levels may be targeted once sustained remission is achieved for 6-9 months [49,50]. **Fig. 1** provides an outline of the approach to management of SRNS.

Table II Dosing and Monitoring of Immunosuppressive Therapy

Medication	*Dose*	*Adverse effects*	*Monitoring*
First line therapy			
Tacrolimus	0.1-0.2 mg/kg/day in 2-divided doses; maximum initial dose 4 mg/day; trough level 4-8 ng/mL[a]	*Both:* Acute kidney injury, nephrotoxicity, hyperkalemia, hepatotoxic *Tacrolimus*: tremors, seizures, headache; diarrhea; glucose intolerance; hypomagnesemia	Screen for cosmetic side effects, tremors, diarrhea, hypertension Creatinine, potassium at 2-4 wk, then q 3-6 months
Cyclosporine	3-5 mg/kg/day in 2-divided doses; maximum initial dose 200 mg/day; trough level 80-120 ng/mL[a]	*Cyclosporine:* Gingival hyperplasia, hypertrichosis; hypertension; dyslipidemia	Liver function tests, glucose, uric acid, magnesium, lipids q 3-6 months
Prednisolone on alternate days	1.5 mg/kg for 4-wks; 1 mg/kg for 4-wks; taper to 0.3-0.5 mg/kg for ~6-9 months	Weight gain, Cushingoid features, glucose intolerance, hypertension, raised intraocular pressure, cataract, myopathy, osteoporosis	Blood pressure, screen for cosmetic effects; eye evaluation q 12 months Blood glucose q 6-12 months
Other agents[b]			
Cyclophosphamide	500-750 mg/m^2 IV; every month for 6-months	Leukopenia, hemorrhagic cystitis, vomiting, alopecia, risk of infections; gonadal toxicity, malignancies	Blood counts prior to infusion; withhold if total leukocyte count <4000/mm^3 Ondansetron, mesna prevent adverse effects
Rituximab	375 mg/m^2 every wk for 2-4 doses	Infusion reactions: Chills, fever, serum sickness, bronchospasm Neutropenia; *P. jirovecii* pneumonia; reactivation of hepatitis B, JC virus; acute lung injury; hypogammaglobulinemia	*Pre dose*: Blood counts, transaminases; hepatitis & HIV serology; IgG level *Post dose:* Monitor CD19, blood counts, IgG level
Mycophenolate mofetil	600-1200 mg/m^2/day in 2-divided doses	Leukopenia; liver dysfunction; pain abdomen, nausea, diarrhea; headache; warts; weight loss	Blood counts, liver functions q 3-6 months

[a]Dose titrated to blood trough level obtained 12-h after last dose; measure 2-wks after initiating therapy. Subsequently, if: (i) suspected drug toxicity, (ii) medications that affect levels (Web Table IV), or (iii) unsatisfactory response or relapses while on therapy. [b]Patients on intense immunosuppression (combination of calcineurin inhibitors and rituximab or mycophenolate mofetil) should receive prophylaxis with trimethoprim (5 mg/kg; 150 mg/m^{2}on alternate days).

Most patients who respond to CNI do so within the first 6-months of treatment [44,45,47,51]. Non-response to CNI is therefore considered in patients who continue to show nephrotic-range proteinuria, hypoalbuminemia or edema despite 6-months therapy. Patients showing non-response should be screened for significant genetic variations (see above), and considered for alternate management (Guideline 6).

Therapy with CNI is initially combined with prednisolone, administered at a dose of 1-1.5 mg/kg on alternate days for 4-6 weeks, and tapered over 6-9 months [6,44-46]. Following CNI-induced remission, ~60% patients may have steroid-sensitive relapses [44,45,52]. Relapses are treated with prednisolone (2 mg/kg/day until remission; tapered on alternate-days). Stoppage of steroid therapy might not be possible in patients with multiple relapses.

The duration of treatment with CNI for patients with partial or complete remission is not clear, with guidelines recommending minimum 12-months' therapy [6,16]. An RCT comparing continued therapy with tacrolimus vs switching to mycophenolate mofetil (MMF) at 6-months, found the former twice as effective in maintaining remission (90% vs 45%) [45]. In a retrospective study on 23 patients, therapy with cyclosporine for mean duration of 1.7 years could be successfully switched to MMF in 79% cases [52]. In view of the risk of relapse with early cessation of therapy, we suggest continuing therapy with CNI for 24 months or longer (**Fig. 1**), ensuring adequate dose and trough levels [49,51].

About 10-25% patients receiving prolonged CNI treatment are at risk of nephrotoxicity [53]. Risk factors for nephrotoxicity include presence of initial resistance, dose of CNI used, duration of heavy proteinuria, and hyper-

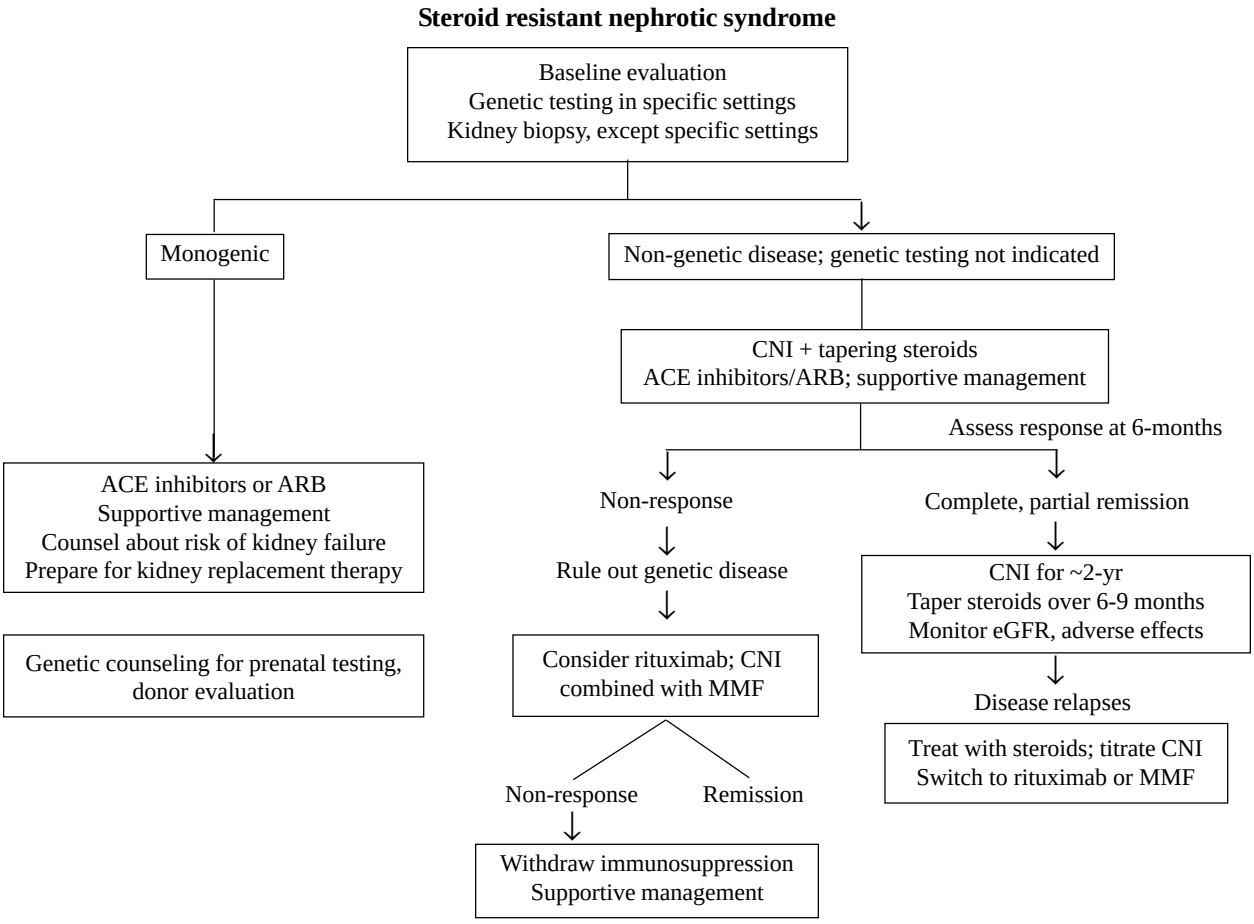

Fig. 1 Management of steroid-resistant nephrotic syndrome. Kidney biopsy is necessary, except in patients where genetic testing may obviate the need for biopsy (Box II). Patients with monogenic cause for steroid-resistance should not receive immunosuppression and are managed with angiotensin converting enzyme (ACE) inhibitors and supportive therapy. Patients with likely non-genetic disease are initiated on therapy with a calcineurin inhibitor (CNI) along with supportive care. Lack of remission despite adequate therapy with CNI for 6-months is an indication for genetic screening, if not performed earlier. Patients with CNI-resistant disease who do not show a monogenic defect may be treated with IV rituximab or combined therapy of CNI and mycophenolate mofetil (MMF). Immunosuppression is withdrawn in patients with continued non-response.

tension during therapy [48,53]. In order to balance the benefits and toxicity of CNI, we suggest individualizing therapy in children with partial or complete response at 24-months. Options include: *i*) discontinue therapy if patient has been in sustained remission; *ii*) continue CNI therapy; perform kidney biopsy if treatment is prolonged beyond 30-36 months, or if restarting treatment; *iii*) switch to IV rituximab or oral MMF in patients with CNI or steroid toxicity or steroid-sensitive relapses.

Risk factors for AKI in nephrotic syndrome include volume depletion, infections, nephrotoxic injury and steroid resistance [21,54,55]. We suggest withholding CNI during AKI [16,55,56]; treatment is restarted following recovery of kidney function. Therapy with CNI is avoided if eGFR is persistently <60 mL/min/1.73 m^2.

Guideline 5: Alternate Immunosuppressive Therapy

5.1 We suggest treatment with IV cyclophosphamide in patients with non-availability of CNI, either due to its cost or adverse effects. (2B)

5.2 We do not suggest the use of oral cyclophosphamide for therapy of patients with steroid-resistance. (2A)

Rationale

Studies utilizing IV cyclophosphamide (every month for 6-months) and tapering prednisolone show complete or partial remission in 10-50%, but with significant adverse effects [46,57,58]. Compared to CNI, IV cyclophosphamide is associated with lower rates of sustained

remission (RR 0.50; 95% CI 0.37-0.68) at 6-months [43]. A multicenter study compared the efficacy of cyclosporine (150 mg/m^2/day) for 48-weeks with IV cyclophosphamide (500 mg/m^2; 7-doses over 36 weeks) in patients with SRNS. While complete remission was low, 47% patients treated with cyclosporine and 6% with IV cyclophosphamide had partial response [57]. Another multicenter trial on 131 patients showed 6-month complete remission rates of 14.8% and partial remission rates of 31.1% with IV cyclophosphamide, as against 52.4% and 30.1%, respectively with tacrolimus [44].

Two RCT showed similar efficacy and safety of oral and IV cyclophosphamide in 61 children with steroid-resistance (RR 1.58; 95% CI 0.65-3.85) [58,59]. However, two other RCT found no difference in rates of remission in patients receiving oral cyclophosphamide with prednisone compared to prednisone (*n*=84; RR 1.06, 95% CI 0.61-1.87) [60,61]. Based on the above, we do not advise use of oral cyclophosphamide in patients with SRNS.

Guideline 6: Treatment of CNI-Resistant Nephrotic Syndrome

In patients with non-genetic forms of SRNS and non-response to therapy with CNI, we suggest additional treatment with either IV rituximab or oral MMF (**Fig. 1**). (2C)

Rationale

Approximately 25-35% patients with non-genetic forms of SRNS do not show complete or partial remission following 6-months' therapy with CNI [43]. The management of patients with non-response to CNI therapy is difficult, since they are at high risk of kidney failure [17-19]. Patients with initial steroid- and CNI-resistance should be screened for an underlying monogenic disorder. Those with no pathogenic or likely pathogenic variants in podocyte genes may be considered for additional immunosuppressive therapy, administered under close supervision.

While rituximab has shown promising results in patients with steroid-sensitive nephrotic syndrome, its efficacy in CNI-resistant SRNS is less satisfactory. In a systematic review (7 case series, one RCT; *n*=226) on efficacy of rituximab in steroid and CNI-resistant nephrotic syndrome, the mean number of rituximab doses was 3.1. Complete or partial remission was observed in 46.4%, with better response in minimal change disease (63.2%) than in FSGS (39.2%), and late-resistance (52.8%) compared to initial-resistance (40.8%) [62]. Similar findings of satisfactory response to rituximab in patients with late resistance are reported in a series from United Kingdom [18] and in a systematic review [63]. While less favorable outcomes were reported in a study from India, with remission in 29.3% of 58 patients with CNI-resistance, there was trend for better response in minimal change disease and late-resistance [64].

We suggest administering 2-doses of IV rituximab at a dose of 375 mg/m^2 at weekly interval, targeting CD19 count <5/µl or ≤1% of lymphocyte count. If CD19 target is not met, 1-2 additional doses may be repeated at weekly intervals (maximum 4 doses). In patients achieving complete or partial remission, repeat dose(s) of rituximab may be given following B-cell reconstitution, which typically occurs after 6-9 months. There is limited guidance regarding redosing with rituximab, and benefits should be balanced by the risk of side effects, including infusion reactions, serum sickness, neutropenia and hypogammaglobulinemia. Therapy with rituximab may be associated with reactivation of hepatitis B, *Pneumocystis jirovecii* pneumonia, severe lung injury and rarely, progressive multifocal leukoencephalopathy [65].

The efficacy of MMF in patients with SRNS is less satisfactory than in steroid-sensitive disease. In the PODONET cohort, monotherapy with this medication was not effective in 83% patients [19]. The efficacy of combination of CNI and MMF (600 to 1000 mg/m^2/day) has been reported in patients with CNI-resistant disease. Three case-series (*n*=168) on combined therapy for 6-12 months, show complete remission, partial remission and non-response in 11.8-47.7%, 8.7-38.2% and 43.5-58.8%, respectively [66-68]. There is limited data on the efficacy of treatment with adalimumab, abatacept, ofatumumab and adrenocorticotrophic hormone, oral galactose and LDL apheresis in patients with CNI-resistant SRNS. These therapies should only be used in context of clinical trials [69-71].

Intense immunosuppression is associated with risk of systemic infections. Patients receiving combined therapy with CNI and either rituximab or MMF should receive prophylaxis with cotrimoxazole (5 mg/kg trimethoprim on alternate days) for 3-6 months. **Table II** summarizes dosing, side effects and monitoring of children receiving immunosuppressive agents.

Guideline 7: Immunosuppressive Therapy With Pathogenic or Likely Pathogenic Variants

We do not recommend that patients with monogenic disease receive therapy with calcineurin inhibitors or other immunosuppressive agents. (1B)

Rationale

Patients with SRNS with pathogenic or likely pathogenic variations (monogenic disease, **Box I**) usually do not show complete or partial remission following therapy

with CNI. Analysis of pooled data (**Web Table III**; *n*=867) shows that compared to non-genetic disease, those with genetic forms of SRNS are not likely to respond to CNI (RR 4.00; 95% CI 2.52-6.51). Patients with monogenic forms of SRNS, irrespective of response are more likely to progress to kidney failure than those with non-genetic illness (RR 2.87; 95% CI 2.22-3.72).

The recent IPNA guidelines do not recommend that patients with monogenic disease receive immunosuppressive medications [6]. However, some patients with a genetic cause for steroid-resistance, especially those with *WT1* variants, might show partial remission following treatment with CNI [37]. The decision to continue therapy in such patients should follow counseling of parents regarding anticipated benefits (relief of edema, higher blood albumin) *vs* risks (therapy-related toxicity, infections) and cost of therapy. Targeted therapy is possible for specific mutations, e.g., coenzyme Q10 for defect(s) in CoQ10 pathway, eplerenone for *ARHGDIA*, and corticosteroids for mutations in genes of Rho/Rac/Cdc42 network [31,32].

Guideline 8: Angiotensin Converting Enzyme Inhibitors and Angiotensin Receptor Blockers

We recommend that all patients with SRNS should receive therapy with angiotensin converting enzyme (ACE) inhibitors or angiotensin receptor blockers (ARB) (**Table III**). (1B)

Rationale

Since proteinuria is a risk factor for progressive kidney disease, its reduction is important for renoprotection [72]. Use of ACE inhibitors is associated with 30-40% reduction in proteinuria in a dose- and time-dependent manner (16,43). ARB may be used as effectively **(Table III)** [73]. Dual blockade with ACE inhibitors and ARB further reduces proteinuria, but is associated with side effects such as hypotension, AKI and hyperkalemia, and is not recommended [74]. ACE inhibitors or ARB are avoided in patients with eGFR < 25 mL/min/1.73 m^2, and discontinued during vomiting, diarrhea or reduced oral intake. In patients with FSGS, sparsentan, that combines endothelin receptor type A blockade with angiotensin II inhibition, reduces proteinuria and hypertension more effectively than irbesartan [75]. We do not advise therapy with other medications that target the renin-angiotensin axis, including aliskrein, eplerenone and vitamin D analogs.

Table III Antihypertensive and Anti-proteinuric Medications

Medication	*Initial (maximum) daily dose*	*Interval*
ACE inhibitors		
Enalapril	0.08 (0.6) mg/kg	1-2 doses
Fosinopril	0.1 mg/kg (40 mg)	Once daily
Lisinopril	0.07 (0.6) mg/kg	Once daily
Ramipril	1.6 (6) mg/m^2/day	Once daily
Angiotensin receptor blockers		
Irbesartan	65 (150) mg; 150 (300) mg/day if ≥13-year	Once daily
Losartan	0.7 (1.4) mg/kg	Once daily
Olmesartan	10 (20) mg; 20 (40) mg if ≥35 kg	Once daily
Valsartan	1.3 (2.7) mg/kg	Once daily
Telmisartan	1 (2) mg/kg	Once daily
Sparsentan	200 (800) mg	Once daily
Calcium channel blockers		
Amlodipine	0.1 (0.6) mg/kg	1-2 doses
Nifedipine ER	0.2 (3) mg/kg	1-2 doses
Felodipine	2.5 (10) mg	Once daily
Thiazides		
Hydrochlorothiazide	1 (2) mg/kg	1-2 doses
Beta blockers		
Atenolol	0.5 (2) mg/kg	Once daily
Metoprolol	1 (6) mg/kg	1-2 doses
Labetalol	1 (12) mg/kg	2-3 doses
Alpha blockers		
Prazosin ER	0.05 (0.5) mg/kg	1-2 doses
Central alpha agonist		
Clonidine	5-7 (25) mcg/kg/day	2-3 doses

ER extended release.

SUPPORTIVE CARE AND MONITORING

Important aspects of supportive care are summarized in **Table IV**. Principles of management of edema, systemic infections and immunization are discussed in the revised ISPN guidelines on steroid-sensitive nephrotic syndrome, published recently [76].

Guideline 9: Thrombotic Complications

We do not recommend routine thromboprophylaxis in children with SRNS. (1C)

Rationale

The risk of thromboembolic complications in nephrotic syndrome is ~3% in children, compared to 25% in adults, with most events within the first 3-months of illness [77]. Risk factors for thrombosis include congenital nephrotic syndrome, heavy proteinuria, membranous nephropathy,

Table IV Supportive Care of Children with Steroid-Resistant Nephrotic Syndrome

Complication	*Pathophysiology*	*Management*
Thromboembolism	Urine loss of coagulation regulators; hepatic production of hemostatic proteins; lack of ambulation; dehydration; thrombocytosis; platelet aggregation	Prevention: Ensure ambulation, optimize hydration; remove central venous catheters, avoid arterial punctures; use compression stockings Treatment: Heparin, low molecular weight heparin; warfarin Preventive anticoagulation: If previous thrombosis, risk factors
Hypertension	Glomerular disease; high renin, aldosterone, epinephrine, norepinephrine; reduced atrial natriuretic peptide	Target blood pressure 50-75th percentile for age Lifestyle measures; restrict salt intake Angiotensin converting enzyme inhibitors (ACE-I), angiotensin receptor blockers
Acute kidney injury	Hypovolemia, medications (ACE-I, calcineurin inhibitors)	Supportive care: Attention to fluid and electrolytes; management of complications of acute kidney injury
Linear growth retardation	Exposure to glucocorticoids; malnutrition; adrenocortical suppression	Regular monitoring of height, height velocity; steroid minimization Limited evidence for growth hormone therapy
Obesity	Exposure to steroids; reduced physical activity	Monitor weight, body mass index; minimize steroids; modify lifestyle
Dyslipidemia	Increased low density lipoproteins (LDL) Reduced clearance of chylomicron, very LDL	Modify lifestyle (dietary change, physical activity, weight control) ≥ 8-yr-old with LDL cholesterol >160 mg/dL, or > 130 mg/dL with risk factors[a]: Atorvastatin 10-20 mg daily
HPA suppression	Corticosteroid therapy	Stress dose if receiving oral steroids >2-weeks within past 1-yr
Bone health	Urinary loss of vitamin D; osteoblast suppression, osteoclast induction	Vitamin D (400-800 IU); calcium (250-750 mg) supplements
Hypothyroidism	Urinary loss of thyroid binding globulin, transthyretin and albumin	No treatment if remission is expected; follow-up borderline levels Low free T4, TSH >10 mU/L: treat with thyroxine

HPA: hypothalamo-pituitary axis. [a]Risk factors: chronic kidney disease stage 3-5; blood pressure >90th centile for age; body mass index > 95th centile; family history of cardiovascular disease.

central venous catheters and coexisting heart disease [77]. Sites of thrombosis include the deep veins, cerebral sinus(es), renal veins and occasionally, arteries [78].

Routine use of prophylactic anticoagulants is not recommended [77]. Aspirin is less effective and is associated with risk of AKI [79]. Non-pharmacological measures such as ambulation, hydration and use of compression stockings are encouraged; central venous catheters and arterial punctures should be avoided [79,80].

Therapy aims to prevent extension of thrombi and reduce the risk of embolism. Thrombolysis followed by anticoagulation is considered in patients with life or limb-threatening thrombosis. While anticoagulation may be initiated with unfractionated heparin, this requires IV access and close laboratory monitoring, has less predictable pharmacokinetics and is associated with the risk of adverse effects (thrombocytopenia, anaphylaxis and osteoporosis) [80]. Use of low-molecular weight heparin is preferred [79,81]. Therapy is initiated with enoxaparin at a dose of 1.5 mg/kg/dose (<2-months age) or 1 mg/kg/dose (>2-months) subcutaneously, every 12-hr [81]. Long-term therapy may continue either with enoxaparin or warfarin (0.2 mg/kg/dose started concurrently with enoxaparin) for 3-months or until remission [80]. For warfarin the international normalized ratio (INR) for prothrombin time is targeted between 2.0 and 3.0. Children with recurrent throm-botic events require long-term anticoagulation [77,80].

Guideline 10: Cardiovascular Morbidity

We recommend strategies to minimize cardiovascular risk in patients with SRNS (X).

Rationale

Steroid resistance is associated with multiple cardio-

vascular risks, including hypertension, dyslipidemia, hypoalbuminemia, hypercoagulable state and steroid-induced obesity. Strategies to reduce this risk include minimizing residual proteinuria, managing hypertension, weight reduction to achieve BMI <85th centile for age, non-exposure to tobacco, and achieving target levels of lipids, fasting glucose (<100 mg/dL) and HbA1c (< 5.7%) [82].

Hypertension: Blood pressure should be measured at each visit. A study on Indian children with frequently relapsing disease showed clinic hypertension in 64%, ambulatory hypertension in 33%, white coat hypertension in 30% and increased left ventricular mass in 21% [83]. Systolic and diastolic blood pressures are targeted between 50-75th percentile for age and sex [84]. Lifestyle changes include increased intake of vegetables, fresh fruits, low-fat milk, legumes and nuts, and reduced salt and sweets. Pharmacotherapy is initiated with ACE inhibitor or ARB, in view of additional benefit of reducing proteinuria **(Table III)**.

Dyslipidemia: Children with nephrotic syndrome show high blood levels of cholesterol, triglycerides, apoB-containing lipoproteins (LDL, VLDL, IDL) and lipoprotein (*a*). While abnormalities resolve during remission, these might persist in patients with SRNS. Dyslipidemia aggravates glomerulosclerosis and proximal tubular damage and is associated with progression of CKD. Screening for dyslipidemia is advised in patients with SRNS, and those with steroid-sensitive disease and cardiovascular risk factors [82,85].

We advise reduced intake of trans-fats or saturated fats and sugar, and increased consumption of fruits, vegetables, legumes and whole grain cereals [85]. The CHILD-1 diet is the first step in children with dyslipidemia or risk factors for cardiovascular disease and includes restricting intake of saturated fat and cholesterol to <10% of daily calories and 300 mg, respectively. In case this is not effective, the respective restrictions are enhanced to 7% and 200 mg in the CHILD-2 diet [82,85]. Limiting leisure screen time to <2-hr/day, ensuring moderate physical activity for 1-hr/day, and vigorous physical activity at least 3 days a week are advised [85].

If lifestyle measures fail to correct dyslipidemia, therapy with statins is advised, especially if associated with risk factors for cardiovascular disease [85]. Therapy in children 8-year or older may begin with atorvastatin at 10 mg/day, with monitoring for adverse effects.

Guideline 11: Stress Dosing of Glucocorticoids

We recommend that patients, who have received oral corticosteroids for more than 2-weeks within the past one-year, should receive additional steroid dosing during conditions associated with physiological stress. (1D)

Rationale

Therapy for nephrotic syndrome involves high-dose prednisolone for 12-weeks for the first episode, 5-6 weeks for relapse, and prolonged alternate-day for frequent relapses and steroid-resistance. A systematic review reported that 269 of 487 (55.2%) children receiving corticosteroids for varied indications for more than 14-days had biochemical evidence of suppressed hypothalamo-pituitary axis (HPA) [86]. The duration of HPA suppression might last up to two years, and vary with dose and duration of treatment [87].

We recommend additional steroids in situations where physiological stress is expected (fever ≥38°C, inadequate oral intake, lethargy, dehydration, invasive surgery, dental surgery, trauma and large burns). Conditions such as uncomplicated viral infections, acute otitis media and fever post-immunization do not require stress dosing. In case of critical illness or surgery, hydrocortisone is administered parenterally at 100 mg/m^2, initially or preoperatively followed by 25 mg/m^2 every 6-hr. With less serious illness, hydrocortisone 30-50 mg/m^2/day or prednisolone 0.3-1.0 mg/kg in a single daily dose is given during stress and tapered thereafter [88].

Guideline 12: Monitoring of Patients

Children with SRNS are at risk for progression to stage 5 CKD, complications of the disease and adverse effects of medications [89-91]. Managing immunosuppressive therapies is a challenge due to the risk of infections, non-compliance and presence of co-morbidities. Patients require regular monitoring and careful follow up, and counseling regarding need for compliance with medications **(Table V)**.

Guideline 13: Transplantation

13.1 We recommend that kidney transplant be considered in all patients with SRNS and stage 5 CKD. (1B)

13.2 We recommend that genetic testing be performed before transplant to assist in donor selection and predict the risk of recurrence in allograft. (1B)

13.3 In a patient with prior allograft recurrence, the decision for retransplantation should be taken after discussing the risks and benefits with treating physicians, patient and family. (2C)

13.4 In patients with allograft recurrence, we suggest initiation of plasma exchanges, increasing the dose of CNI, with or without additional use of rituximab. (2B)

Table V Monitoring of Patients with Steroid-Resistant Nephrotic Syndrome

Parameter	*Frequency*
Home urine dipstick for protein	Daily for 1-2 weeks; 2-3 times/week until remission; once-weekly thereafter
Spot urine protein/creatinine ratio*	Baseline; 2-4 weeks; then every 6-12 months
Weight, height; growth velocity; body mass index	Every 3-6 months (frequent in infants and stage 3-5 chronic kidney disease)
Blood pressure Ambulatory blood pressure monitoring 2-D echocardiography	At each hospital visit Every 1-2 yr Annually, if hypertensive
Blood creatinine, electrolytes, albumin, eGFR	Baseline; 2-4 weeks; then every 3-6 months
Hemoglobin, glucose, calcium, phosphate, alkaline phosphatase, 25-hydroxyvitamin D; thyroid profile	Every 6-12 months with partial remission or non-response; every 12 months with complete remission; additional investigations may be required for stage 3-5 chronic kidney disease
Monitoring drug toxicity	See Table II
Fasting lipid profile	Every 6-12 months
Eye examination (cataract, glaucoma)	Annually, if receiving long-term steroids
Repeat renal biopsy	Calcineurin inhibitor therapy beyond 30-36 months; recommencing therapy for second course Non-recovery from acute kidney injury
Nutritional status and advice	Every 6 months; more frequent in infants, malnourished children, stage 3-5 chronic kidney disease
Immunization	Check and complete every 12 months, as appropriate

**24-hr urine protein estimation may be considered instead. eGFR estimated GFR (mL/min per 1.73 m^2) =* $\frac{0.413 \times height\ (cm)}{creatinine\ (mg/dL)}$

Rationale

Kidney transplantation is the definitive option for patients with SRNS and stage 5 CKD. Careful pre-transplant evaluation of recipient and donor is required. Genetic screening of the recipient is necessary, particularly if there is initial resistance or equivocal course of the illness, since it stratifies the risk for allograft recurrence and helps in donor screening. If inheritance pattern is autosomal recessive, a heterozygous carrier (parent) may be accepted as a donor with negligible risk of recurrence, except Afro-Caribbean donors with *APOL1* risk variant, or heterozygous R229Q variants in *NPHS2* [35,92]. Heterozygous carriers of pathogenic variants in *COL4A3* and *COL4A4* and women with variants in *COL4A5* should not be accepted as donors since they are at risk of kidney failure [93]. For autosomal dominant inheritance, individuals with same variant are not accepted as donors since they might show variable penetrance with late onset of disease.

FSGS recurs in the allograft in ~30% (range 6-50%) patients [94,95]. Recurrence is associated with allograft dysfunction and its loss in 40-60% patients, especially in those with persistent nephrotic range proteinuria [33,96]. Recurrence risk is highest in patients with late steroid resistance or recurrent nephrotic syndrome in a prior transplant (~80%), moderate with initial resistance and no identified genetic cause (~50%), and lowest with confirmed genetic mutation underlying SRNS (<5%) [18,97-100]. Patients with FSGS and kidney failure should be counseled about these risks.

Living-related transplantation is associated with better graft survival and is preferred for children in our country. While the risk of recurrence is minimally higher in children receiving live-related grafts, this is balanced by reduced risk of rejection and lower need for immunosuppression [100,101]. Live-related transplantation is therefore the first choice, except in patients with moderate to high risk of recurrence.

Nephrotic syndrome might recur occur within hours to days after transplant and is characterized by nephrotic range proteinuria and progressive hypoalbuminemia. Patients are monitored for recurrence by screening for proteinuria (Up/Uc ratio), initially daily and then with reduced frequency **(Web Box I)**. Recurrence is considered in patients with proteinuria and Up/Uc ≥1 mg/mg if anuric prior to transplant or increase of ratio by ≥1 in those with

proteinuria at transplantation [6]. Early onset graft dysfunction may be a feature of recurrent FSGS. Where feasible, an allograft biopsy is recommended to detect podocyte foot process effacement or segmental sclerosis that supports the diagnosis of recurrence. A biopsy may also help exclude other diagnosis in patients with lower degree of late-onset proteinuria or allograft dysfunction.

Multiple therapies have been used to prevent recurrence of nephrotic syndrome, including pre-transplant plasma exchanges, rituximab and lipoprotein apheresis. There is limited evidence that any of these strategies prevent allograft recurrence in the first kidney transplant [102,103]. Strategies for managing patients with allograft recurrence include combination of plasma exchanges with high-dose CNI and corticosteroids, with or without cyclophosphamide [104-107] **(Web Box I)**. Multiple reports show benefit from additional therapy with rituximab (2-4 doses of 375 mg/m^2, administered once every 1-2 weeks) [65,104]. Using these strategies, 60-70% patients with recurrent FSGS show complete or partial remission.

Guideline 14: Transition of Care

A significant proportion of patients continue to have active disease into adulthood [89]. These children will need to be cared for by 'adult' physicians and nephrologists, keeping with the policy of the Indian Academy of Pediatrics of caring for children upto 18 years [108]. Parallel to the change in medical caregiver, patients need to transition from care by parents to self-care. Transition should occur smoothly, without affecting patient health. Institution-specific protocols for transition of care should be based on standard guidelines [109].

Congenital Nephrotic Syndrome

Patients with congenital nephrotic syndrome present at birth or in first 3-months of life. Infants are born prematurely with large placenta, and show massive proteinuria, hypoalbuminemia and anasarca. Antenatal ultrasonography may show hyperechoic kidneys; amniocentesis reveals high alpha-fetoprotein. There may be dysmorphic features or comorbidities. Most patients develop kidney failure by the age of 2-8 years. Recommendations on genetic aspects and management were published recently [110,111].

Almost 70-80% patients with congenital nephrotic syndrome have a genetic cause; mutations in *NPHS1*, *NPHS2*, *WT1*, *LAMB2* and *PLCE1* account for ~90% cases [110,112]. Exome sequencing using an extended SRNS gene panel **(Web Table II)** is recommended. Results of screening have implications for genetic counseling. Rarely, the condition is secondary to intrauterine infec-tions with cytomegalovirus, rubella, toxoplasma and syphilis [111]. The role of kidney biopsy is limited and may be considered if a genetic diagnosis is not established.

Evaluation aims to confirm the diagnosis and identify complications, including poor growth, hypothyroidism, systemic infections and thromboembolism (**Web Box II**) [111]. Infants with *WT1* variants are monitored by ultrasonography for Wilms tumor every 3-6 months.

Management includes maintaining euvolemia, optimizing nutrition, and therapy of complications. Patients should receive high energy (110-120 Cal/kg) and protein (3-3.5 g/kg/d) diet, orally or by feeding gastrostomy. Supplements of thyroxine, vitamin D and calcium are required. Albumin infusions (0.5-1.0 g/kg) are advised in presence of hypovolemia (oliguria, prolonged capillary refill, tachycardia) or anasarca. IV furosemide (0.5-2 mg/kg) is given at the end of infusion, unless patient has features of hypovolemia. Monitoring of fluid status, creatinine, electrolytes and blood pressure are necessary during diuretic therapy [111].

After 4-weeks of life, judicious use of ACE inhibitors (**Table III**) with or without prostaglandin inhibitors (indomethacin, celecoxib) is effective in reducing the severity of proteinuria. Therapy with these agents and diuretics should be withheld during episodes of hypovolemia. Since infections are the chief cause of death, infants should receive all primary immunization and bacterial infections are treated promptly. Therapy with anticoagulants is considered in patients with history of thrombosis.

Unilateral or bilateral nephrectomies are not proposed routinely, and may be considered in patients with repeated episodes of hypovolemia or refractory edema, thrombosis and malnutrition [112]. Bilateral nephrectomy is advised, prior to kidney transplantation, in patients with *WT1* mutations or persistent nephrotic

Box III Research Priorities in Steroid-Resistant Nephrotic Syndrome

Determine genetic burden and genotype-phenotype correlation in Indian patients; models for evaluating functional significance of variants

Pathogenesis of non-genetic forms of the illness

Duration of therapy with calcineurin inhibitors; switching to less toxic medications

Treatment for patients who are non-responsive to therapy with calcineurin inhibitors

Prevention and therapy for recurrent focal segmental glomerulosclerosis

Improving quality of life and patient-centered outcomes.

range proteinuria. Kidney transplantation is the definitive treatment, but has ethical, technical and immunologic challenges.

CONCLUSIONS

Recommendations on management of SRNS, first proposed by the ISPN in 2009, have been revised based on systematic reviews, published studies and expert opinion. While there is better understanding regarding the genetic basis and management, important clinical issues require to be examined **(Box III)**. The management of the disease continues to be challenging, and patients not responsive to treatment with CNI are at risk of progressive kidney disease. We hope that the present guidelines will standardize therapies and improve the quality of care for these patients.

Note: Supplementary material related to this study is available with the online version at www.indianpediatrics.net

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REFERENCES

1. Noone DG, Iijima K, Parekh R. Idiopathic nephrotic syndrome in children. Lancet. 2018;392:61-74.
2. Tullus K, Webb H, Bagga A. Management of steroid-resistant nephrotic syndrome in children and adolescents. Lancet Child Adolesc Health. 2018;2:880-90.
3. Gipson DS, Trachtman H, Kaskel FJ, et al. Clinical trial of focal segmental glomerulosclerosis in children and young adults. Kidney Int. 2011;80:868-78.
4. Troyanov S, Wall CA, Miller JA, Scholey JW, Cattran DC. Focal and segmental glomerulosclerosis: Definition and relevance of a partial remission. J Am Soc Nephrol. 2005;16:1061-8.
5. Indian Society of Pediatric Nephrology, Gulati A, Bagga A, Gulati S, Mehta KP, Vijayakumar M. Management of steroid resistant nephrotic syndrome. Indian Pediatr. 2009;46:35-47.
6. Trautmann A, Vivarelli M, Samuel S, et al. IPNA clinical practice recommendations for the diagnosis and management of children with steroid-resistant nephrotic syndrome. Pediatr Nephrol. 2020;35:1529-61.
7. American Academy of Pediatrics Steering Committee on Quality Improvement and Management. Classifying Recommendations for Clinical Practice Guidelines. Pediatrics. 2004;114: 874-7.
8. The primary nephrotic syndrome in children. Identification of patients with minimal change nephrotic syndrome from initial response to prednisone: A report of the International Study of Kidney Disease in Children. J Pediatr. 1981;98:561-4.
9. Sinha A, Saha A, Kumar M, et al. Extending initial prednisolone treatment in a randomized control trial from 3 to 6 months did not significantly influence the course of illness in children with steroid-sensitive nephrotic syndrome. Kidney Int. 2015;87:217-24.
10. Nakanishi K, Iijima K, Ishikura K, et al. Two-year outcome of the ISKDC regimen and frequent-relapsing risk in children with idiopathic nephrotic syndrome. Clin J Am SocNephrol. 2013;8:756-62.
11. Bagga A, Hari P, Srivastava RN. Prolonged versus standard prednisolone therapy for initial episode of nephrotic syndrome. Pediatr Nephrol. 1999;13:824-7.
12. Hoyer PF, Brodeh J. Initial treatment of idiopathic nephrotic syndrome in children: Prednisone versus prednisone plus cyclosporine A: A prospective, randomized trial. J Am Soc Nephrol. 2006; 17:1151-7.
13. Murnaghan K, Vasmant D, Bensman A. Pulse methylprednisolone therapy in severe idiopathic childhood nephrotic syndrome. Acta Paediatr Scand. 1984;73:733-9.
14. Letavernier B, Letavernier E, Leroy S, Baudet-Bonneville V, Bensman A, Ulinski T. Prediction of high-degree steroid dependency in pediatric idiopathic nephrotic syndrome. Pediatr Nephrol. 2008;23:2221-6.
15. Kidney Disease: Improving Global Outcomes (KDIGO) Glomerulonephritis Work Group. KDIGO Clinical Practice Guideline for Glomerulonephritis. Kidney Int Suppl. 2012;2:139-274.
16. Rovin BH, Caster DJ, Cattran DC, et al; Conference Participants. Management and treatment of glomerular diseases (part 2): Conclusions from a Kidney Disease: Improving Global Outcomes (KDIGO) Controversies Conference. Kidney Int. 2019; 95:281-95.
17. Gipson DS, Chin H, Presler TP, et al. Differential risk of remission and ESRD in childhood FSGS. Pediatr Nephrol. 2006;21:344-9.
18. Mason AE, Sen ES, Bierzynska A, et al. Response to first course of intensified immunosuppression in genetically stratified steroid resistant nephrotic syndrome. Clin J Am Soc Nephrol. 2020;15: 983-94.
19. Trautmann A, Schnaidt S, Lipska-Ziêtkiewicz BS, et al. Long-term outcome of steroid-resistant nephrotic syndrome in children. J Am Soc Nephrol. 2017;28:3055-65.
20. Stevens PE, Levin A; Kidney Disease: Improving Global Outcomes (KDIGO) Chronic Kidney Disease Guideline Development Work Group Members. Evaluation and management of chronic kidney disease: Synopsis of the Kidney Disease: Improving Global Outcomes 2012 Practice Guideline. Ann Intern Med. 2013;158: 825-30.
21. Kushwah S, Yadav M, Hari P, Meena J, Sinha A, Bagga A. Incidence and determinants of acute kidney injury in patients with nephrotic syndrome. Asian J Pediatr Nephrol. 2019; 2:75-81.
22. Corwin HL, Schwartz MM, Lewis EJ. The importance of sample size in the interpretation of the renal biopsy. Am J Nephrol. 1988;8:85-9.
23. Gulati S, Sengupta D, Sharma RK, et al. Steroid resistant nephrotic syndrome: Role of histopathology. Indian Pediatr. 2006;43:6.
24. D'Agati VD, Fogo AB, Bruijn JA, Jennette JC. Pathologic classification of focal segmental glomerulosclerosis: A working proposal. Am J Kidney Dis. 2004;43:368-82.
25. Wenderfer SE. Viral-associated glomerulopathies in children. Pediatr Nephrol. 2015;30:1929-38.
26. Sadowski CE, Lovric S, Ashraf S, et al. A single-gene cause in 29.5% of cases of steroid-resistant nephrotic syndrome. J Am Soc Nephrol. 2015;26:1279-89.
27. Bierzynska A, McCarthy HJ, Soderquest K, et al. Genomic and clinical profiling of a national nephrotic syndrome cohort advocates a precision medicine approach to disease management. Kidney Int. 2017;91:937-47.
28. Trautmann A, Lipska-Ziêtkiewicz BS, Schaefer F. Exploring the clinical and genetic spectrum of steroid resistant nephrotic syndrome: The PodoNet Registry. Front Pediatr. 2018;6:200.
29. Landini S, Mazzinghi B, Becherucci F, et al. Reverse phenotyping after whole-exome sequencing in steroid-resistant nephrotic syndrome. Clin J Am Soc Nephrol. 2020;15:89-100.

30. Nagano C, Yamamura T, Horinouchi T, et al. Comprehensive genetic diagnosis of Japanese patients with severe proteinuria. Sci Rep. 2020;10:270
31. Montini G, Malaventura C, Salviati L. Early coenzyme Q10 supplementation in primary coenzyme Q10 deficiency. N Engl J Med. 2008;358:2849-50.
32. Gee HY, Saisawat P, Ashraf S, et al. ARHGDIA mutations cause nephrotic syndrome via defective RhoGTPase signaling. J Clin Invest. 2013;123:3243-53.
33. Morello W, Puvinathan S, Puccio G, et al. Post-transplant recurrence of steroid resistant nephrotic syndrome in children: The Italian experience. J Nephrol. 2020;33:849-57.
34. Hildebrandt F, Heeringa SF. Specific podocin mutations determine age of onset of nephrotic syndrome all the way into adult life. Kidney Int. 2009;75:669-71.
35. Lentine KL, Kasiske BL, Levey AS, et al. KDIGO Clinical Practice Guideline on the evaluation and care of living kidney donors. Transplantation. 2017;101:S1-S109.
36. Lipska BS, Iatropoulos P, Maranta R, et al. Genetic screening in adolescents with steroid-resistant nephrotic syndrome. Kidney Int. 2013;84:206-13.
37. Malakasioti G, Iancu D, Tullus K. Calcineurin inhibitors in nephrotic syndrome secondary to podocyte gene mutations: A systematic review. Pediatr Nephrol. 2021;36:1353-64.
38. Siji A, Karthik KN, Pardeshi VC, Hari PS, Vasudevan A. Targeted gene panel for genetic testing of south Indian children with steroid resistant nephrotic syndrome. BMC Med Genet. 2018;19:200.
39. Ramanathan ASK, Vijayan M, Rajagopal S, Rajendiran P, Senguttuvan P. WT1 and NPHS2 gene mutation analysis and clinical management of steroid-resistant nephrotic syndrome. Mol Cell Biochem. 2017;426:177-81.
40. Richards S, Aziz N, Bale S, et al. Standards and Guidelines for the Interpretation of Sequence Variants: A Joint Consensus Recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med. 2015;17:405-24.
41. Troost JP, Trachtman H, Nachman PH, et al. An outcomes-based definition of proteinuria remission in focal segmental glomerulosclerosis. Clin J Am Soc Nephrol. 2018;13:414-21.
42. Hamasaki Y, Yoshikawa N, Nakazato H, et al. Prospective 5-year follow-up of cyclosporine treatment in children with steroid-resistant nephrosis. Pediatr Nephrol. 2013;28:765-71.
43. Liu ID, Willis NS, Craig JC, Hodson EM. Interventions for idiopathic steroid-resistant nephrotic syndrome in children. Cochrane Database Syst Rev. 2019;11:CD003594.
44. Gulati A, Sinha A, Gupta A, et al. Treatment with tacrolimus and prednisolone is preferable to intravenous cyclophosphamide as the initial therapy for children with steroid-resistant nephrotic syndrome. Kidney Int. 2012;82:1130-5.
45. Sinha A, Gupta A, Kalaivani M, Hari P, Dinda AK, Bagga A. Mycophenolate mofetil is inferior to tacrolimus in sustaining remission in children with idiopathic steroid-resistant nephrotic syndrome. Kidney Int. 2017;92:248-57.
46. Mantan M, Sriram CS, Hari P, Dinda A, Bagga A. Efficacy of intravenous pulse cyclophosphamide treatment versus combination of intravenous dexamethasone and oral cyclophosphamide treatment in steroid-resistant nephrotic syndrome. Pediatr Nephrol. 2008;23:1495-502.
47. Choudhry S, Bagga A, Hari P, Sharma S, Kalaivani M, Dinda A. Efficacy and safety of tacrolimus versus cyclosporine in children with steroid-resistant nephrotic syndrome: A randomized controlled trial. Am J Kidney Dis. 2009;53:760-9.
48. Kim JH, Park SJ, Yoon SJ, et al. Predictive factors for ciclosporin-associated nephrotoxicity in children with minimal change nephrotic syndrome. J Clin Pathol. 2011;64:516-9.
49. Inaba A, Hamasaki Y, Ishikura K, et al. Long-term outcome of idiopathic steroid-resistant nephrotic syndrome in children. Pediatr Nephrol. 2016; 31:425-34.
50. Jahan A, Prabha R, Chaturvedi S, Mathew B, Fleming D, Agarwal I. Clinical efficacy and pharmacokinetics of tacrolimus in children with steroid-resistant nephrotic syndrome. Pediatr Nephrol. 2015;30:1961-7.
51. Büscher AK, Beck BB, Melk A, et al. Rapid response to cyclosporin A and favorable renal outcome in nongenetic versus genetic steroid-resistant nephrotic syndrome. Clin J Am Soc Nephrol. 2016;11: 245-53.
52. Gellermann J, Ehrich JHH, Querfeld U. Sequential maintenance therapy with cyclosporin A and mycophenolate mofetil for sustained remission of childhood steroid-resistant nephrotic syndrome. Nephrol Dial Transplant. 2012;27:1970-8.
53. Sinha A, Sharma A, Mehta A, et al. Calcineurin inhibitor induced nephrotoxicity in steroid resistant nephrotic syndrome. Indian J Nephrol. 2013; 23:41-6.
54. Sharma M, Mahanta A, Barman AK, Mahanta PJ. Acute kidney injury in children with nephrotic syndrome: A single-center study. Clin Kidney J. 2018;11:655-8.
55. Rheault MN, Zhang L, Selewski DT, et al. AKI in children hospitalized with nephrotic syndrome. Clin J Am Soc Nephrol. 2015;10: 2110-8.
56. Lombel RM, Gipson DS, Hodson EM; Kidney Disease: Improving Global Outcomes. Treatment of steroid-sensitive nephrotic syndrome: New guidelines from KDIGO. Pediatr Nephrol. 2013;28: 415-26.
57. Plank C, Kalb V, Hinkes B, et al. Cyclosporin A is superior to cyclophosphamide in children with steroid-resistant nephrotic syndrome: A randomized controlled multicentre trial by the Arbeitsge-meinschaft für Pädiatrische Nephrologie. Pediatr Nephrol. 2008;23: 1483-93.
58. Elhence R, Gulati S, Kher V, Gupta A, Sharma RK. Intravenous pulse cyclophosphamide - A new regime for steroid-resistant minimal change nephrotic syndrome. Pediatr Nephrol. 1994;8: 1-3.
59. Shah KM, Ohri AJ, Ali US. A randomized controlled trial of intravenous versus oral cyclophosphamide in steroid-resistant nephrotic syndrome in children. Indian J Nephrol. 2017;27:430-4.
60. International Study of Kidney Disease in children. Prospective, controlled trial of cyclophosphamide therapy in children with nephrotic syndrome. Report of the International study of Kidney Disease in children. Lancet. 1974;2:423-7.
61. Tarshish P, Tobin JN, Bernstein J, Edelmann CM. Cyclophosphamide does not benefit patients with focal segmental glomerulosclerosis. A report of the International Study of Kidney Disease in Children. Pediatr Nephrol. 1996;10:590-3.
62. Jellouli M, Charfi R, Maalej B, Mahfoud A, Trabelsi S, Gargah T. Rituximab in the management of pediatric steroid-resistant nephrotic syndrome: A systematic review. J Pediatr. 2018;197:191-7.e1.
63. Kamei K, Ishikura K, Sako M, Ito S, Nozu K, Iijima K. Rituximab therapy for refractory steroid-resistant nephrotic syndrome in children. Pediatr Nephrol. 2020;35:17–24.
64. Sinha A, Bhatia D, Gulati A, et al. Efficacy and safety of rituximab in children with difficult-to-treat nephrotic syndrome. Nephrol Dial Transplant. 2015;30:96-106.
65. Sinha A, Bagga A. Rituximab therapy in nephrotic syndrome: Implications for patients' management. Nat Rev Nephrol. 2013;9:154-69.
66. Wu B, Mao J, Shen H, et al. Triple immunosuppressive therapy in steroid-resistant nephrotic syndrome children with tacrolimus resistance or tacrolimus sensitivity but frequently relapsing. Nephrol (Carlton). 2015;20:18-24.
67. Okada M, Sugimoto K, Yagi K, Yanagida H, Tabata N, Takemura T. Mycophenolate mofetil therapy for children with intractable nephrotic syndrome. Pediatr Int. 2007;49:933-7.

68. Nikibakhsh AA, Mahmoodzadeh H, Karamyyar M, Hejazi S, Noroozi M, Macooie AA. Treatment of steroid and cyclosporine-resistant idiopathic nephrotic syndrome in children. Int J Nephrol. 2011;2011:930965.
69. Lee JM, Kronbichler A, Shin JI, Oh J. Current understandings in treating children with steroid-resistant nephrotic syndrome. Pediatr Nephrol. 2021;36:747-61.
70. Muso E, Mune M, Hirano T, et al. A prospective observational survey on the long-term effect of LDL apheresis on drug-resistant nephrotic syndrome. Nephron Extra. 2015;5:58-66.
71. Yu C-C, Fornoni A, Weins A, et al. Abatacept in B7-1-positive proteinuric kidney disease. N Engl J Med. 2014;370:1263-6.
72. van den Belt SM, Heerspink HJL, Gracchi V, de Zeeuw D, Wühl E, Schaefer F. Early proteinuria lowering by angiotensin-converting enzyme inhibition predicts renal survival in children with CKD. J Am Soc Nephrol. 2018;29:2225-33.
73. Webb NJA, Shahinfar S, Wells TG, et al. Losartan and enalapril are comparable in reducing proteinuria in children. Kidney Int. 2012;82:819-26.
74. Stotter BR, Ferguson MA. Should ACE inhibitors and ARBs be used in combination in children? Pediatr Nephrol. 2019;34: 1521-32.
75. Trachtman H, Nelson P, Adler S, et al. DUET: A phase 2 study evaluating the efficacy and safety of sparsentan in patients with FSGS. J Am Soc Nephrol. 2018;29:2745-54.
76. Sinha A, Bagga A, Banerjee S, et al; Expert group of Indian Society of Pediatric Nephrology. Steroid Sensitive Nephrotic Syndrome: Revised Guidelines. Indian Pediatr. 2021;58:461-81.
77. Kerlin BA, Ayoob R, Smoyer WE. Epidemiology and pathophysiology of nephrotic syndrome-associated thromboembolic disease. Clin J Am Soc Nephrol. 2012;7:513-20.
78. Suri D, Ahluwalia J, Saxena AK, et al. Thromboembolic complications in childhood nephrotic syndrome: A clinical profile. Clin Exp Nephrol. 2014;18:803-13.
79. Kerlin BA, Haworth K, Smoyer WE. Venous thromboembolism in pediatric nephrotic syndrome. Pediatr Nephrol. 2014;29:989-97.
80. Monagle P, Chan AKC, Goldenberg NA, et al. Antithrombotic Therapy in Neonates and Children: Antithrombotic Therapy and Prevention of Thrombosis, 9th edn. American College of Chest Physicians Evidence - Based Clinical Practice Guidelines. Chest. 2012;141:e737S-801S.
81. Dabbous MK, Sakr FR, Malaeb DN. Anticoagulant therapy in pediatrics. J Basic Clin Pharm. 2014;5:27-33.
82. Hari P, Khandelwal P, Smoyer WE. Dyslipidemia and cardiovascular health in childhood nephrotic syndrome. Pediatr Nephrol. 2020;35:1601-19
83. Sarkar S, Sinha A, Lakshmy R, et al. Ambulatory blood pressure monitoring in frequently relapsing nephrotic syndrome. Indian J Pediatr. 2017;84:31-5.
84. Lurbe E, Agabiti-Rosei E, Cruickshank JK, et al. 2016 European Society of Hypertension Guidelines for the Management of High Blood Pressure in Children and Adolescents. J Hypertens. 2016;34:1887-920.
85. Expert Panel on Integrated Guidelines for Cardiovascular Health and Risk Reduction in Children and Adolescents, National Heart, Lung, and Blood Institute. Expert panel on integrated guidelines for cardiovascular health and risk reduction in children and adolescents: Summary report. Pediatrics. 2011;128:S213-56.
86. Aljebab F, Choonara I, Conroy S. Systematic review of the toxicity of short-course oral corticosteroids in children. Arch Dis Child. 2016;101:365-70.
87. Ahmet A, Mokashi A, Goldbloom EB, et al. Adrenal suppression from glucocorticoids: Preventing an iatrogenic cause of morbidity and mortality in children. BMJ Paediatr Open. 2019; 3:e000569.
88. Liu D, Ahmet A, Ward L, et al. A practical guide to the monitoring and management of the complications of systemic corticosteroid therapy. Allergy Asthma ClinImmunol. 2013;9:30.
89. Kaku Y, Ohtsuka Y, Komatsu Y, et al. Clinical practice guideline for pediatric idiopathic nephrotic syndrome 2013: General therapy. Clin Exp Nephrol. 2015;19:34-53.
90. Foster BJ, Shults J, Zemel BS, Leonard MB. Risk factors for glucocorticoid-induced obesity in children with steroid-sensitive nephrotic syndrome. Pediatr Nephrol.2006;21:973-80.
91. Simmonds J, Grundy N, Trompeter R, Tullus K. Long-term steroid treatment and growth: A study in steroid-dependent nephrotic syndrome. Arch Dis Child. 2010;95:146-9.
92. Bierzynska A, Saleem MA. Deriving and understanding the risk of post-transplant recurrence of nephrotic syndrome in the light of current molecular and genetic advances. Pediatr Nephrol. 2018;33: 2027-35.
93. Gross O, Weber M, Fries JWU, Müller G-A. Living donor kidney transplantation from relatives with mild urinary abnormalities in Alport syndrome: Long-term risk, benefit and outcome. Nephrol Dial Transplant. 2009;24:1626-30.
94. Francis A, Didsbury M, McCarthy H, Kara T. Treatment of recurrent focal segmental glomerulosclerosis post-kidney transplantation in Australian and New Zealand children: A retrospective cohort study. Pediatr Transplant. 2018;22: e13185.
95. Kienzl-Wagner K, Waldegger S, Schneeberger S. Disease recurrence: The sword of Damocles in kidney transplantation for primary focal segmental glomerulosclerosis. Front Immunol. 2019;10:1669.
96. Vinai M, Waber P, Seikaly MG. Recurrence of focal segmental glomerulosclerosis in renal allograft: An in-depth review. Pediatr Transplant. 2010;14:314-25.
97. Dall'Amico R, Ghiggeri G, Carraro M, et al. Prediction and treatment of recurrent focal segmental glomerulosclerosis after renal transplantation in children. Am J Kidney Dis. 1999;34:1048-55.
98. Uffing A, Pérez-Sáez MJ, Mazzali M, et al. Recurrence of FSGS after kidney transplantation in adults. Clin J Am Soc Nephrol. 2020; 15:247-56.
99. Ding WY, Koziell A, McCarthy HJ, et al. Initial steroid sensitivity in children with steroid-resistant nephrotic syndrome predicts post-transplant recurrence. J Am SocNephrol. 2014;25:1342-8.
100. Koh LJ, Martz K, Blydt-Hansen TD; NAPRTCS Registry Investigators. Risk factors associated with allograft failure in pediatric kidney transplant recipients with focal segmental glomerulosclerosis. Pediatr Transplant. 2019;23:e13469.
101. Nehus EJ, Goebel JW, Succop PS, Abraham EC. Focal segmental glomerulosclerosis in children: Multivariate analysis indicates that donor type does not alter recurrence risk. Transplantation. 2013;96: 550-4.
102. Verghese PS, Rheault MN, Jackson S, Matas AJ, Chinnakotla S, Chavers B. The effect of peri-transplant plasmapheresis in the prevention of recurrent FSGS. Pediatr Transplant. 2018;22: e13154.
103. Shenoy M, Lennon R, Plant N, Wallace D, Kaur A. Pre-emptive rituximab and plasma exchange does not prevent disease recurrence following living donor renal transplantation in high-risk idiopathic SRNS. Pediatr Nephrol. 2020;35:1081-4.
104. Hansrivijit P, Ghahramani N. Combined rituximab and plasmapheresis or plasma exchange for focal segmental glomerulosclerosis in adult kidney transplant recipients: A meta-analysis. Int Urol Nephrol.2020;52:1377-87.
105. Kashgary A, Sontrop JM, Li L, et al. The role of plasma exchange in treating post-transplant focal segmental glomerulosclerosis: A systematic review and meta-analysis of 77 case-reports and case-series. BMC Nephrol. 2016;17:104.
106. Allard L, Kwon T, Krid S, et al. Treatment by immunoadsorption for recurrent focal segmental glomerulosclerosis after pediatric kidney transplantation: A multicentre French cohort. Nephrol Dial Transplant. 2018;33:954-63.

107. Cormican S, Kennedy C, O'Kelly P, et al. Renal transplant outcomes in primary FSGS compared with other recipients and risk factors for recurrence: A national review of the Irish Transplant Registry. Clin Transplant. 2018;32:e13152.
108. John TJ. IAP policy on age of children for pediatric care. Indian Pediatr.1999;36:461-3.
109. Watson AR, Harden P, Ferris M, Kerr PG, Mahan J, Ramzy MF. Transition from pediatric to adult renal services: A consensus statement by the International Society of Nephrology and the International Pediatric Nephrology Association. Pediatr Nephrol. 2011;26:1753-7.
110. Lipska-Ziêtkiewicz BS, Ozaltin F, Hölttä T, et al. Genetic aspects of congenital nephrotic syndrome: A consensus statement from the ERKNet–ESPN inherited glomerulopathy working group. Eur J Hum Genet. 2020;28:1368-78
111. Boyer, O, Schaefer, F, Haffner D, et al. Management of congenital nephrotic syndrome: Consensus recommendations by the ERKNet-ESPN Working Group. Nat Rev Nephrol.2021;17:277-89.
112. Joshi A, Sinha A, Sharma A, et al; NephQuest Consortium. Next-generation sequencing for congenital nephrotic syndrome: A multi-center cross-sectional study from India. Indian Pediatr. 2021;58: 445-51.

ANNEXURE I

List of Participants

Kamran Afzal, *Aligarh*; Indira Agarwal, *Vellore*; Vinay Agarwal, *New Delhi*; Kanav Anand, *New Delhi*; M Ashraf, *Srinagar*; Arvind Bagga, *New Delhi*; Sushmita Banerjee, *Kolkata*; Girish C Bhatt, *Bhopal*; Sudha Ekambaram, *Chennai*; Arpita Gogoi, *Dibrugarh*; Sanjeev Gulati, *New Delhi*; Pankaj Hari, *New Delhi*; Suprita Kalra, *New Delhi*; Kanika Kapoor, *New Delhi*; Priyanka Khandelwal, *New Delhi*; Sriram Krishnamurthy, *Puducherry*; Manish Kumar, *New Delhi*; Mukta Mantan, *New Delhi*; Jitendra K Meena, *New Delhi*; Kirtisudha Mishra, *New Delhi*; Amarjeet Mehta, *Jaipur*; OP Mishra, *Varanasi*; Aliza Mittal, *Jodhpur*; Saroj K Patnaik, *New Delhi*; Subal Pradhan, *Cuttack*; PK Pruthi, *New Delhi*; Sumantra Raut, *Kolkata*; Abhijeet Saha, *New Delhi*; Manisha Sahay, *Hyderabad*; Jyoti Sharma, *Pune*; Shobha Sharma; *New Delhi*; Jyoti Singhal, *Pune*; Aditi Sinha, *New Delhi*; Rajiv Sinha, *Kolkata*; Ranjeet Thergaonkar, *Mumbai*; Karalanglin Tiewsoh, *Chandigarh*; Susan Uthup, *Thiruvananthapuram*; Anand S Vasudev, *New Delhi*; Anil Vasudevan, *Bengaluru.*

Experts: Uma Ali, *Mumbai*; Amit K Dinda, *New Delhi*; Mohammed Faruq, *New Delhi*; Madhuri Kanitkar, *New Delhi*; Kumud Mehta, *Mumbai*; BR Nammalwar, *Chennai*; Kishore D Phadke, *Bengaluru*; Geetika Singh, *New Delhi*; RN Srivastava, *New Delhi.*

Web Table I Grading of Evidence [7]

Grade	*Quality of evidence*
A	Well designed and controlled studies; meta-analysis on applicable population; true effect lies close to the estimate of the effect
B	Studies with minor limitations; consistent findings from multiple observational studies; true effect is likely to be close to estimate of the effect, but there is a possibility that it is substantially different
C	Single, few or multiple studies with inconsistent findings or major limitations; confidence in the effect estimate is limited, the true effect may be substantially different from estimate of the effect
D	Expert opinion, case reports; very little confidence in effect estimate, true effect likely to be substantially different from estimate of effect
X	Situations where validating studies cannot be performed, and benefit or harm clearly predominates
Level	*Strength of recommendation*
1	"We recommend": Most patients should receive the recommended course of action
2	"We suggest": Different choices will be appropriate for different patients

Web Table II Gene List for Targeted Panel with Features of Steroid Resistant Nephrotic Syndrome (SRNS)

Gene	*Protein*	*Inheritance*	*Accession no; OMIM*	*OMIM phenotype*	*Key clinical features*
ACTN4	Actinin, alpha 4	AD	NM_004924; 603278	Focal segmental glomerulosclerosis (FSGS), type 1	Familial and sporadic SRNS (usually adolescent and adult)
ADCK4/ COQ8B	Coenzyme Q8B	AR	NM_024876; 615573	Nephrotic syndrome, type 9	FSGS or collapsing FSGS; one patient responded to coenzyme Q10
ALG1	Asparagine-linked glycosylation 1	AR	NM_019109; 605907	Congenital disorder of glycosylation, type Ik	Neurologic impairment and dysmorphic features
ANKFY1	Rabankyrin-5	AR	NM_001330063.2; 607927		Early onset illness
ANLN	Actin binding protein anillin	AD	NM_018685; 616032	FSGS, type 8	FSGS (onset between 9-70 years)
ARHGAP24	Rho GTPase-activating protein 24	AD	NM_001025616; 610586		FSGS
ARHGDIA	Rho GDP-dissociation inhibitor alpha	AR	NM_001185078; 615244	Nephrotic syndrome, type 8	Congenital nephrotic syndrome; SRNS early onset; diffuse mesangial sclerosis on biopsy
AVIL	Advillin	AR	NM_006576.3; 618594	Nephrotic syndrome, type 21	SRNS; diffuse mesangial sclerosis on biopsy
CD151	Tetraspanin (TM4)	AR	NM_004357; 609057	Nephropathy; deafness; SRNS; epidermolysis bullosa	Pretibial skin lesions, sensorineural deafness, lacrimal duct stenosis, nail dystrophy, thalassemia minor
CD2AP	CD2-associated protein	AD/AR	NM_012120; 607832	FSGS, type 3	FSGS
CLCN5	H^+/Cl^- exchange transporter 5	XR	NM_001127898.4; 300009	Dent disease; low molecular weight proteinuria, hypercalciuria	Failure to thrive; hypercalciuria, nephrolithiasis; low molecular weight proteinuria, albuminuria; FSGS
COL4A3	Type IV collagen α3	AR, AD	NM_000091; 120070	Alport syndrome 2, AR; Alport syndrome 3, AD	Alport syndrome; FSGS
COL4A4	Type IV collagen α4	AR	NM_000092; 120131	Alport syndrome 2, AR	Alport syndrome; FSGS
COL4A5	Type IV collagen α5	XLD	NM_000495; 301050	Alport syndrome 1, XL	Alport syndrome; FSGS

COQ2	Coenzyme Q2	AR	NM_015697; 609825	Coenzyme Q10 deficiency, primary, 1	Mitochondrial disease; isolated SRNS
COQ6	Coenzyme Q6	AR	NM_182476; 614647	Coenzyme Q10 deficiency, primary, 6	Early SRNS; sensorineural deafness; ataxia, facial dysmorphism; FSGS, diffuse mesangial sclerosis
CRB2	Crumbs cell polarity complex component 2	AR	NM_173689; 616220	FSGS, type 9	SRNS
CUBN	Cubilin	AR	NM_001081; 261100	Megaloblastic anemia	Megaloblastic anemia; proteinuria
DGKE	Diacylglycerol kinase, epsilon	AR	NM_003647; 615008	Nephrotic syndrome, type 7	
DLC1	DLC1 Rho GTPase activating protein		NM_182643.3; 604258		Child and adult steroid sensitive illness and SRNS; partial CNI response
E2F3	E2F transcription factor 3		NM_001949.4; 600427		FSGS, mental retardation; also with partial deletion of chromosome 6
EMP2	Epithelial membrane protein 2	AR	NM_001424; 615861	Nephrotic syndrome, type 10	Childhood SRNS; steroid sensitive illness also reported
FAT1	FAT tumor suppressor homolog 1	AR	NM_005245.4; 600976		SRNS, tubular ectasia, hematuria
FN1	Fibronectin	AD	NM_212482.3; 601894	Glomerulopathy with fibronectin deposits 2	Proteinuria, hematuria; glomerulomegaly, fibronectin positive subendothelial, mesangial deposits
GAPVD1	GTPase-activating protein, VPS9-domain protein 1		NM_001282680.3; 611714		Early-onset SRNS
INF2	Inverted formin 2	AD	NM_022489; 613237	FSGS, type 5	Isolated SRNS; Charcot-Marie-Tooth neuropathy with FSGS
ITGA3	Integrin α3	AR	NM_002204; 605025	Interstitial lung disease; epidermolysis bullosa	Congenital, SRNS; interstitial lung disease; epidermolysis bullosa (congenital)
ITGB4	Integrin β4	AR	NM_000213; 147557	Epidermolysis bullosa; pyloric atresia	Epidermolysis bullosa (junctional); pyloric atresia; FSGS
ITSN1	Intersectin-1	AR	NM_003024.3; 602442		Congenital, SRNS; steroid sensitive illness reported
ITSN2	Intersectin-2	AR	NM_019595.4;		Steroid sensitive illness

			604464		(minimal change) or membranoproliferative glomerulonephritis
KANK1	KN motif ankyrin repeat domain-containing protein 1	AR	NM_015158.3; 607704		Steroid sensitive illness
KANK2	KN motif ankyrin repeat domain-containing protein 2	AR	NM_015493; 617783		Steroid sensitive illness; steroid dependence; hematuria
KANK4	KN motif ankyrin repeat domain-containing protein 4	AR	NM_0181712.4; 614612		SRNS; hematuria
KIRREL1	Kin of IRRE-like protein 1	AR	NM_018240.7; 607428		SRNS
LAGE3	EKC/KEOPS complex subunit LAGE3	XR	NM_006014.4; 301006	Galloway-Mowat syndrome 2	Early-onset SRNS; FSGS; microcephaly, gyral abnormalities; delayed development
LAMB2	Laminin, beta-2	AR	NM_002292; 614199	Nephrotic syndrome, type 5; ocular anomalies	Pierson syndrome; SRNS, microcoria, neurodevelopmental delay
LCAT	Phosphatidylcholine-sterol acyltransferase	AR	NM_000229.2; 245900	Norum disease	Proteinuria, renal failure, anemia, corneal lipid deposits
LMNA	Prelamin-A/C	AD	NM_170707; 151660	Lipodystrophy type 2, partial	Familial partial lipodystrophy; FSGS
LMX1B	LIM homeobox transcription factor 1β	AD	NM_002316; 602575	Nail-patella syndrome	FSGS; SRNS, mild ridging to hypoplasia of nails, absent, hypoplastic patella; glaucoma
MEFV	Pyrin	AD/AR	NM_000243.2; 608107	Familial Mediterranean fever	Fever, pericarditis, pleuritis, arthralgia; nephrotic syndrome
MAFB	Transcription factor MafB	AD	NM_005461.5; 166300	Multicentric carpotarsal osteolysis syndrome	Proteinuria, end stage kidney disease; skeletal disorders; mental retardation; minor facial anomalies
MAGI2	Membrane-associated guanylate kinase inverted 2	AR	NM_012301.4; 617609	Nephrotic syndrome, type 15	SRNS; FSGS
MYO1E	Myosin IE	AR	NM_004998; 614131	FSGS, type 6	FSGS; collapsing FSGS
MYH9	Myosin-9	AD	NM_002473; 155100	Macrothrombocytes, granulocyte	MYH9-related disease; Epstein, Fechtner

				inclusions; nephritis, deafness	syndromes: nephritis, deafness, thrombocytopenia, giant platelets
NEU1	Sialidase-1	AR	NM_000434.4; 256550	Sialidosis, type I/II	SRNS; FSGS; hepatomegaly, corneal clouding, cherry red spots (nephrosialidosis)
NPHS1	Nephrin	AR	NM_004646; 256300	Nephrotic syndrome, type 1	Congenital, SRNS
NPHS2	Podocin	AR	NM_014625; 600995	Nephrotic syndrome, type 2	Congenital, SRNS
NUP85	Nucleoporin, 85-kDa	AR	NM_024844.5; 618176	Nephrotic syndrome, type 17	SRNS; FSGS
NUP93	Nucleoporin, 93-kDa	AR	NM_014669; 616892	Nephrotic syndrome, type 12	SRNS; FSGS
NUP107	Nucleoporin, 107-kDa	AR	NM_020401; 616730	Nephrotic syndrome, type 11 Galloway-Mowat syndrome-7	SRNS
NUP133	Nucleoporin, 133-kDa	AR	NM_018230.3; 618177; 618349	Nephrotic syndrome, type 18 Galloway-Mowat syndrome-8	Isolated FSGS
NUP160	Nucleoporin, 160-kDa	AR	NM_015231.2; 618178	Nephrotic syndrome, type 19	SRNS
NUP205	Nucleoporin, 205-kDa	AR	NM_015135; 616893	Nephrotic syndrome, type 13	Early onset SRNS
NXF5	Nuclear RNA export factor 5	XR	NM_032946; 300319		FSGS co-segregating with heart block
OCRL	Inositol polyphosphate 5-phosphatase	XR	NM_000276; 309000	Lowe syndrome	FSGS; absence of proximal tubular dysfunction reported
OSGEP	Probable tRNA N6-adenosine threonylcarbamoyltransferase	AR	NM_017807.4; 617729	Galloway-Mowat syndrome 3	SRNS
PAX2	Paired box protein 2	AD	NM_003987; 616002	FSGS, type 7	FSGS without extrarenal manifestations
PDSS2	Decaprenyl diphosphate synthase subunit 2	AR	NM_020381; 610564	Leigh syndrome	Mitochondrial disorder; proteinuria
PLCe1	Phospholipase C, epsilon-1	AR	NM_016341; 610725	Nephrotic syndrome, type 3	Congenital, SRNS

PMM2	Phosphomannomutase 2	AR	NM_000303; 212065	Disorder of glycosylation, type Ia	Psychomotor retardation, peripheral neuropathy with SRNS
PODXL	Podocalyxin	AD	NM_005397; 602632		FSGS
PTPRO	Protein-tyrosine phosphatase, receptor-type O	AR	NM_030667; 614196	Nephrotic syndrome, type 6	SRNS
SCARB2	Lysosome membrane protein 2	AR	NM_005506; 254900	Myoclonic epilepsy, 4; renal failure	Progressive myoclonic epilepsy; SRNS; FSGS
SGPL1	Sphingosine-1-phosphate lyase 1	AR	NM_003901.4; 617575	Nephrotic syndrome, type 14	Primary adrenal insufficiency, neurologic abnormalities; SRNS
SMARCAL1	SMARCAL1	AR	NM_014140; 242900	Schimke immunoosseous dysplasia	Spondyloepiphyseal dysplasia; immune deficiency, neurological features; FSGS
SYNPO	Synaptopodin	AD	NM_007286; 608155		Sporadic FSGS (promoter mutations)
SYNPO2	Synaptopodin-2	AR	Not available		Congenital childhood onset, SRNS
TBC1D8B	TBC1 domain family, 8B	XR	NM_017752.3; 301028	Nephrotic syndrome, type 20	Early-onset SRNS with FSGS
TNS2	Tensin 2	AR	NM_170754.3; 607717		Steroid dependence (minimal change, FSGS, diffuse mesangial sclerosis)
TP53RK	EKC/KEOPS complex subunit TP53RK	AR	NM_033550.4; 617730	Galloway-Mowat syndrome 4	Early onset SRNS
TPRKB	EKC/KEOPS complex subunit TPRKB	AR	NM_001330389.1; 617731	Galloway-Mowat syndrome 5	Early-onset SRNS
TRPC6	Transient receptor potential channel, subfamily C member 6	AD	NM_004621; 603965	FSGS, type 2	Familial and sporadic SRNS (chiefly adult)
TTC21B	Tetratricopeptide repeat protein 21B	AR	NM_024753; 613820	Nephronophthisis 12	Late onset FSGS; tubulointerstitial fibrosis and tubular atrophy; Joubert syndrome
WDR4	tRNA (guanine-N7-) methyltransferase subunit WDR4	AR	NM_001260475.1; 618347	Galloway-Mowat syndrome 6	Early-onset SRNS

WDR73	WD repeat domain 73	AR	NM_032856; 616144	Galloway-Mowat syndrome 1	SRNS
WT1	WT1 transcription factor	AD	NM_024426; 256370	Nephrotic syndrome, type 4	Isolated SRNS; Frasier & Denys-Drash syndromes
XPO5	Exportin 5	AR	NM_020750; 607845		Childhood SRNS
ZMPSTE24	CAAX prenyl protease 1 homolog	AR	NM_005857; 608612	Mandibuloacral dysplasia, type B lipodystrophy	FSGS; skeletal anomalies, dysplastic nails; skin pigmentation; calcified skin nodules
APOL1	Apolipoprotein L-I		NM_003661; 612551	FSGS, type 4	G1, G2 risk alleles: Susceptibility to FSGS; end stage kidney disease in African, Hispanic Americans

OMIM Online Mendelian Inheritance in Man; AR autosomal recessive; AD autosomal dominant; CNI calcineurin inhibitors; XR X-linked recessive, XL X linked
Phenocopy genes (OMIM no.; phenotype): NPHP4 (606966; nephronophthisis 4); CLCN5 (300009; Dent disease 1); CTNS (219800; cystinosis); DGKE (615008; hemolytic uremic syndrome); NPHP13 (614377; nephronophthisis 13); GLA (301500; Fabry disease); FN1 (601894; glomerulopathy with fibronectin deposits 2); PAX2 (120330; papillorenal syndrome); COL4A3 (104200; Alport syndrome); COL4A4 (203780; Alport syndrome); COL4A5 (301050; Alport syndrome); AGXT (259900; primary hyperoxaluria type 1); FAT4 (612411; Van Maldergem syndrome 2); WDR19 (614377; nephronophthisis 13).

Web Table III Corticosteroid Response and Kidney Failure in Children with Genetic and Non-Genetic Forms of Steroid-Resistant Nephrotic Syndrome

Author, yr [Ref]	*Genetic cause, %**	*Complete, partial remission*		*Kidney Failure^*	
		Non-genetic, N	*Genetic, N*	*Non-genetic, N*	*Genetic, N*
Trautmann, 2018 [28]	373/1554 (24%)	159/387	10/74	113/501[^1]	116/241[^1]
Landini, 2020 [29]	37/64 (57.8%)[$1]	13/17	1/19[$2]	3/6[^2]	11/25[^2]
Nagano, 2020 [30]	69/230 (30%)	41/158	2/37	79/158[^3]	52/69[^3]
Mason, 2020 [18]	81/271 (29.9%)	69/149	9/26	41/149[^4]	16/26[^4]
Total[#]	1086/3902 (27.8%)[#]	282/711 (39.7%)	22/156 (14.1%)	236/814 (29.0%)	195/361 (61.5%)
Genetic versus non-genetic disease		*Odds ratio*	*95% confidence interval*		*P*
Non-response		4.00	2.52, 6.51		<0.0001
Kidney failure		2.87	2.22, 3.72		<0.0001

Only includes reports based on next-generation sequencing; latest or largest report for units with multiple papers

**Congenital nephrotic syndrome not excluded, except by Trautmann et al*

[#] Includes 526 of 1783 families tested by Sadowski et al [26]

[^] Numbers at [1]last follow up; [2]at 10-yr; or extrapolated from Kaplan Meier analysis, at [3]last follow up or at [4]10-yr

[$1] Includes and [$2]excludes 18 patients with phenocopies

Web Table IV Important Drug Interactions of Cyclosporine and Tacrolimus

Medication	*Effect*	*Management*
Drugs that decrease levels		
Anticonvulsants: Phenytoin, carbamazepine, phenobarbitone	Enzyme induction leads to lower levels; risk of non-response or relapse	Increase medication by 30%; monitor trough levels following change of dose or discontinuation of anticonvulsant
Antibiotics: Rifampin; caspofungin (only with tacrolimus)		Monitor trough levels following addition, change of dose or discontinuation of medication
Drugs that increase levels		
Erythromycin, clarithromycin Fluconazole, ketoconazole, voricanazole	Enzyme inhibition results in high levels and risk of nephrotoxicity	Monitor trough levels following addition, change of dose or discontinuation of medication Monitor serum creatinine, electrolytes, liver function tests
Diltiazem, verapamil		
Pharmacodynamic interactions		
Aminoglycosides, amphotericin B, nonsteroidal anti-inflammatory drugs	Risk of nephrotoxicity	Avoid if alternative options are available Monitor creatinine and electrolytes frequently
HMG-CoA reductase inhibitors	Myalgia, rhabdomyolysis	Start with low dose of statins; monitor for toxicity
Nifedipine, amlodipine, phenytoin (only with cyclosporine)	Higher incidence and severity of gingival hyperplasia	Avoid long-term combined use; change to alternative agent Dental and oral hygiene; regular dentist visits

Web Box I Management of Allograft Recurrence of Nephrotic Syndrome

Monitor proteinuria by urine protein to creatinine (Up/Uc) ratio
Daily for 1 week; weekly for 4-weeks; monthly for 1-yr; then every 3-6 months
Renal biopsy, especially if low grade proteinuria or graft dysfunction
Treatment of Recurrence
Plasma exchange
Membrane filtration or centrifugation based; heparin or citrate anticoagulation
Replacement fluid: 5% albumin; fresh frozen plasma
Schedule: Plasma exchange 1.5 times plasma volume (60-75 mL/kg) per session on alternate days for 2-weeks; single volume (40 mL/kg) once per week for 4-6 weeks
Medications
IV methylprednisolone 250 mg/m^2/day for 3 days; taper to previous dose of oral prednisolone
Increase dose of calcineurin inhibitors: Tacrolimus trough 8-12 ng/mL; cyclosporine trough 150-200 ng/mL
Rituximab 375 mg/m^2 two doses, one-week apart
Add angiotensin converting enzyme inhibitor once allograft function established with stable estimated GFR
Consider therapy with oral cyclophosphamide for 3 months in place of mycophenolate mofetil

Recurrence: Urine protein to creatinine ratio (Up/Uc) ≥1 mg/mg if anuric before transplant; or increase in Up/Uc by ≥1 mg/mg if proteinuria at time of transplant

Web Box II Evaluation of Patients with Congenital Nephrotic Syndrome

Extra-renal features: Dysmorphic features, eye, urogenital abnormalities; large placenta
Urinalysis; urine protein to creatinine ratio
Complete blood counts
Blood creatinine, protein, albumin, electrolytes, calcium, phosphate
Transaminases, alkaline phosphatase, 25-hydroxyvitamin D
Lipid profile, free thyroxine, thyroid stimulating hormone
Renal ultrasonography
Kidney biopsy: Not necessary, except if a genetic diagnosis is not established
Identifying the cause
Exome sequencing (*Web Table* II)
Serology for intrauterine infections (TORCH), syphilis, hepatitis B and C, HIV
Karyotyping (infants with ambiguous genitalia, extra-renal features)

REVIEW ARTICLE

Gene Therapies for Transfusion-Dependent β-Thalassemia

SANDEEP SONI

From Division of Pediatric Bone Marrow Transplant, Department of Pediatrics, University of California, San Francisco, CA, USA. Correspondence to: Dr Sandeep Soni, Associate Clinical Professor, Division of Pediatric Bone Marrow Transplant, Department of Pediatrics, University of California, San Francisco, CA, 94158, USA. sandeep.soni@ucsf.edu

β-Thalassemia is one of the most prevalent monogenic diseases usually caused by quantitative defects in the production of β-globin, a component of adult hemoglobin (α2β2), leading to severe anemia. Technological advances in genome sequencing, stem cell selection, viral vector development, transduction and gene-editing strategies now allow for efficient *ex-vivo* genetic manipulation of human hematopoietic stem cells that can lead to a meaningful clinical benefit in thalassemia patients. In this perspective, the status of the gene-therapy approaches available for transfusion-dependent thalassemia and early results of clinical trials are discussed. It is highly anticipated that gene therapies will soon become a treatment option for patients lacking compatible donors for hematopoietic stem cell transplant and will offer a suitable alternative for definitive treatment of β-thalassemia, even in young children.

Keywords: *BCL11A, Gene-editing, Hematopoietic stem cell transplantation, Lentivirus vectors.*

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β-thalassemia is a monogenic disorder characterized by reduced or absent synthesis of the β-globin chain, one of the main components of adult hemoglobin (HbA, α2β2). Several hundred mutations (both point mutations and deletions) are now described in the human β-globin gene (*HBB* gene cluster on chromosome 11, **Fig. 1**) or its regulatory elements, leading to decreased (β^+ genotype) or absent (β^0 genotype) synthesis of the β-globin [1]. This results in a relative increase in the unattached α chains (α/β-chain imbalance) that form insoluble hemi-chromes in the erythrocyte progenitors. The hemi-chromes damage the erythrocyte membrane, leading to severe intramedullary erythrocyte apoptosis (ineffective erythropoiesis) and severely shortened red blood cell (RBC) life span due to extra-medullary hemolysis, leading to severe anemia (low hemoglobin, Hb) [2,3].

The phenotype of β-thalassemia is variable depending upon the reduction (α^+/β^+) or complete absence of β-globin chain synthesis (β^0/β^0) and other genetic variables like co-inheritance of α- and γ-mutations, as well as co-inheritance of other hemoglobinopathies (e.g. HbE, Lepore and sickle hemoglobin) [4,5]. Some mutations also alter the fetal hemoglobin (HbF, α2γ2) to HbA switch and may lead to higher production of HbF into adulthood (hereditary persistence of fetal Hb, HPFH) resulting in less severe anemia [6,7]. Therefore, though the severity of thalassemia can be usually predicted based on the mutation analysis of the *HBB* cluster, other genetic factors may modify the actual phenotype and transfusion requirements.

Although the switch from γ- to β-globin synthesis begins before birth, complete replacement of the HbF by HbA occurs in the postnatal period. Consequently, infants with severe β-globin chain abnormality become transfusion-dependent around 6 months of age, when levels of HbF decrease significantly. Based on their transfusion needs, β-thalassemia patients are classified as transfusion-dependent thalassemia (TDT) or non-transfusion-dependent thalassemia (NTDT), although these definitions are also fluid, as some NTDT patients may need regular transfusions as they become older [8].

β-thalassemia has a high global incidence, especially in Asia (northern and eastern India) and Eastern Mediterranean regions. The conventional management of patients affected by the severe form of the disease relies on chronic and regular blood transfusions (every 3-4 weeks) to maintain nadir hemoglobin at or above 9 g/dL

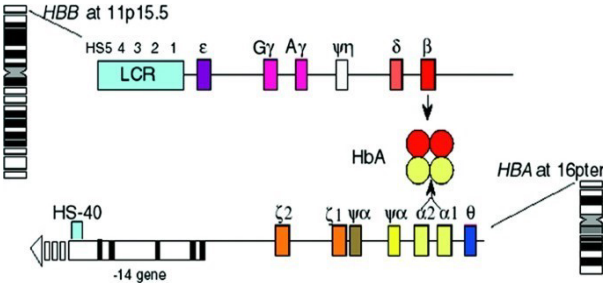

Fig. 1 *HBB* gene cluster (β-globin gene) and *HBA* gene cluster (α-globin gene); transcribed globin proteins combine to make adult hemoglobin (HbA, ∝2β2).

along with iron chelation therapy, to prevent the toxicities of iron overload [3,9].

Currently, the only curative therapy is allogeneic hematopoietic stem cell transplant (HSCT) from an HLA-matched sibling or unrelated donor or cord blood unit (located through national bone marrow donor registries), with good outcomes [10]. HSCT is recommended in relatively younger patients, prior to development of increased iron overload in organs (especially, liver and myocardium) and when suitable HLA-matched donors are available to decrease the risks of toxicity and graft-versus host disease (GVHD). Disease-free survival exceeds 85%, depending on patients' age, HLA-matching and clinical factors like iron overload, liver fibrosis and hepatomegaly [11,12]. Full matched sibling donor HSCT in younger children (<16 years of age) is considered standard of care, while alternative donor HSCT (from mis-matched unrelated or haploidentical donors) are still experimental, and are not devoid of complications like rejection, viral reactivations, and graft versus host disease (GVHD) [13-15].

GENE THERAPY FOR THALASSEMIA

The era of genome sequencing, understanding of the *HBB* gene cluster and its strict regulation and control, along with advancements in vector development and gene-editing platforms, has provided new options for the treatment for thalassemia patients. The expression of β-like genes is regulated by a locus control region (LCR) via looping-mediated interactions with the globin promoters, therefore these LCR and promoter regions are essential for the globin gene expression [16]. The complete understanding of the switch from γ-globin to β-globin production during infancy, and the control of this switch by various transcription factors (TF) has provided new targets for gene-modifications. Speckle-type POZ protein (SPOP), globin transcription factor 1 (GATA-1) and B-cell Leukemia/Lymphoma 11A (BCL11A) are now recognized as important TFs, that bind to specific sites in the *HBB* gene and control the switch from production of HbF to HbA [17-19].

Advances in vector development, transduction of human stem and progenitor cells (HSPCs) and various gene-editing tools, provide a new hope for availability of curative options soon, making gene-therapy one of the most promising treatment options.

The goal of current gene therapy strategies is to induce production of β- or γ-globin, thereby decreasing the levels of unattached α-globin chains, to restore the alpha/non-alpha globin ratio in RBCs. This should lead to correction of ineffective erythropoiesis and improved RBC lifespan (decreased hemolysis), with larger number of erythrocytes with higher hemoglobin surviving longer

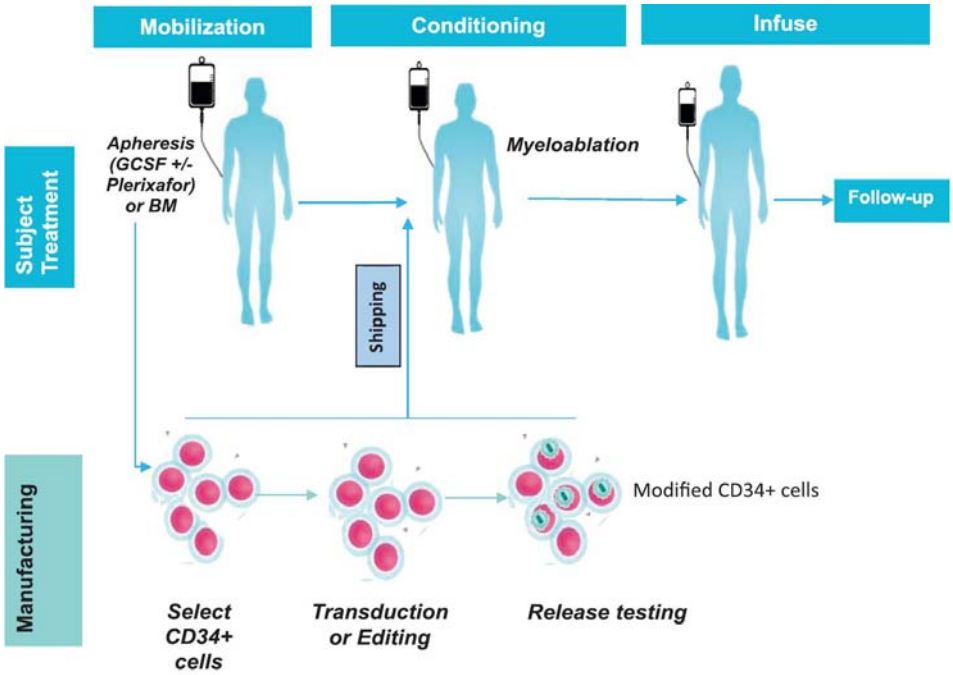

Fig. 2 Overview of treatment plan for gene-modified human stem and progenitor cells.

in the peripheral blood, leading to the correction of anemia and reduction in transfusion needs [20-23].

The common treatment schema of patients undergoing autologous gene modification and infusion is shown in **Fig. 2**. Major steps in the treatment are explained below.

Stem cell mobilization and collection: G-CSF and plerixafor mobilized HSPCs (HPC-Apheresis) are obtained in TDT patients by apheresis procedure as the starting material for gene-modification. Plerixafor is added upfront in the collection protocols, as it leads to efficient mobilization of large number of stem cells in the periphery and decreases the number of collection days and procedures needed for adequate number of stem cells to be collected for gene modifications [24,25]. Adequate HSPCs can be collected from the TDT patients using this combination, despite G-CSF dose reductions recommended in post-splenectomy patients to avoid hyperleukocytosis. HSPCs are collected via the leukapheresis procedure and large volumes of blood (~15-20 L of recirculated blood volume for adults or approximately four total blood volumes in younger patients) should be cycled per day, based on patient tolerability. Average HPC-A collections over 2-3 days in thalassemia patients can yield approximately 15-50×10^6 CD34+/kg (based on experience from early clinical trials) which are adequate for manufacturing and for storing a small fraction (≥2 ×10^6 CD34+/kg cells) as an unmanipulated 'back-up', as a safety precaution in case of non-engraftment with modified HSPCs. The collected HSPCs undergo CD34+ enrichment process, prior to undergoing gene-modification.

Myeloablation: Efficient myeloablation of the expanded erythroid pool in the bone marrow of the TDT patients is essential to create adequate space in the bone marrow niches for adequate engraftment of gene-modified HSPCs, as the gene-modified HSPC do not have a selective survival advantage in thalassemia over the non-gene-modified HSPC. Busulfan is currently the best agent to achieve myeloablation, as the dose can be tailored for each patient based on first dose pharmacokinetics to achieve a standardized target dose range required for myeloablation and to avoid excessive extra-medullary toxicity and lymphodepletion.

Infusion of gene-modified stem cells: The gene-modified HSPCs are usually cryopreserved in 5% dimethyl sulfoxide (DMSO) solution. Once the final product meets all the release criteria (sterility, viability, purity, and % gene-editing frequency or vector copy number [VCN] for gene-insertions), and minimum cell dose criteria (> 2-3× 10^6 CD34+/kg) needed for hematopoietic engraftment, the cryopreserved cells are transported to the treatment site. The cells are thawed and infused intravenously as per standard infusion procedure for autologous stem cell transplants.

Post-transplant care: Care in a specialized BMT unit is recommended, as these patients do become neutropenic and need transfusion support (packed red blood cells and platelet transfusions). Close monitoring and supportive care for busulfan related side-effects, especially mucositis, nausea, infections and veno-occlussive disease (VOD) of liver is recommended. Patients are discharged once they achieve neutrophil engraftment, can eat, drink, and retain their prophylactic medications. Since busulfan is myeloablative but does not cause severe lymphopenia, infection prophylaxis is only recommended for a short period post-transplant. Currently, a 15 years follow up is required for all gene-therapy trials as dictated by regulatory agencies in US and EU. This long follow up is required to ascertain the durability and safety of these experimental approaches.

Currently, the gene-therapy approaches can be divided into two broad groups viz., gene-insertion, and gene-editing approaches.

Gene-Insertion

This involves insertion of a lentiviral or retroviral vector, that contains the whole regulatory machinery and the β-or γ-globin producing genes, into autologous HSPCs 'ex-vivo', and then infusing these modified HSPCs back to the patient after myeloablation [26-28]. Though conceptually straightforward, the field has techno-logically advanced only recently, where the vectors (packaged with the large *HBB* gene and its regulatory elements- promoter, enhancer and parts of LCR) can now be produced at a large scale, achieve high levels of purification and potency to transfect large number of 'non-proliferating' human stem cells to provide clinical meaningful responses [22,29]. For a long-lasting correction and life-long production of erythrocytes (with the hope of one-time curative treatment), the insertions are done in HSPCs (CD34+ enriched population, Milteyni), which includes the long-term repopulating subsets of stem cells. For gene insertion into stem cells, the globin producing genes are placed under the control of an erythroid-specific promoter, so that the transcription of the inserted genes can only occur in erythroid precursors, and not in white blood cells or platelets, which are also derived from the modified hematopoietic stem cells [30].

There are multiple designed lentiviral vectors in clinical trials now for β-thalassemia (**Web Table I**). Once a significant number of HSPCs have been transduced and infused back to a patient, it is expected that the

erythrocyte progenitors derived from these modified stem cells will produce enough β- (or γ) globin (depending on the insertion) to combine with α-chains and reduce the α/β imbalance.

Risks of Gene-Insertion

Since the vector insertions into the stem cells occur randomly and remains largely an uncontrolled process, there is a small risk that some insertions into human stem cells can occur near proto-oncogenes and can stimulate clonal proliferation leading to leukemia/myelodysplastic syndrome (MDS) [31-33]. With the new optimized and self-inactivating (SIN) lentiviral vectors, the insertions into the human stem cells occur 'semi-randomly' i.e. lentivirus insertions occur at preferential sites in the transcription units of human genome, but still lead to polyclonal reconstitution, compared to retroviral vectors that were associated with high risk of insertional mut-agenesis [34]. All clinical trials currently perform integration site analysis to monitor patients of any emerging clonal population. Currently, regulatory agencies require all patients treated with gene therapies to be followed for a total period of 15 years, to clearly establish the incidence of this risk. Fortunately, till date, none of the patients treated with lentiviral vectors have developed any leukemia or MDS related to lentiviral vector insertions [35].

Results of gene-insertion clinical trials: All patients treated recently have tolerated the conditioning regimen with myeloablative doses of busulfan without any unexpected toxicity. Approximately 10% of patients are reported to have developed mild to moderate veno-occlusive disease (VOD) of the liver related to underlying liver fibrosis but have responded to supportive care or defibrotide treatment. In the early Phase 1/2 trials, all patients had engrafted, though efficacy analysis of the first few patients treated with BB305 lentiviral vector, showed variable responses and total hemoglobin production. This variability is expected, as patients with β-thalassemia have large genetic heterogeneity due to varied mutations in the *HBB* cluster and various genetic modifiers and therefore, the level of hemoglobin required to become transfusion independent is variable. The initial results of two concurrent trials (HGB 204 and 205 using BB305 vector), show an average production of 4-5 g/dL of HbA^{T87Q} from the gene-insertions (HbA^{T87Q} is the gene-insertion derived HbA that can be detected separately from transfusion derived HbA by HPLC due to presence of one amino-acid substitution: Threonine at 87 position instead of Glutamine) [30]. An increase of hemoglobin by ~5 g/dL is enough to lead to transfusion independence in HbE/β-thalassemia and β^0/β^+ patients, but only leads to decrease in transfusion requirements in β^0/β^0 patients, where there is a need for higher levels of hemoglobin production to become transfusion independent [22]. Ninety percent (18/20 with >3 months follow up) of non-β^0/β^0 patients treated show rapid rise in gene-derived hemoglobin (HbA^{T87Q}) production post-treatment, maintaining total hemoglobin levels of >9 g/dL (mean 11.6 g/dL; range 9.3-13.3g/dL), with transfusion independence [36]. Based on early encouraging results and safety profile, the lentiglobin gene therapy (Zynteglo) was conditionally approved in EU in June, 2019 for TDT patients with non-β^0/β^0 genotype who are ≥12 years of age (this is still not approved by FDA in US). The results for the β^0/β^0 patients are still under study (HGB 212 trial, NCT 03207009), but do show variable results with 8/11 patients followed for >3 months maintaining hemoglobin above 9 g/dL, though it is still early to comment on durability of the outcomes at this stage [37].

Gene-Editing

Availability of new tools and techniques in the last few years is leading to a rapid development of gene-editing approaches to ameliorate the anemia in thalassemia patients. Last few years have seen advances in availability of different engineered nucleases – zinc-finger nucleases (ZFN), transcription activator-like effector nucleases (TALENS), and clustered regularly interspaced short palindromic repeats (Crispr)-associated-nuclease 9 (Crispr-Cas9), which are nucleases that act like mole-cular scissors and cut the human DNA at precise locations [38-40]. These nucleases differ in their precision, specificity, efficiency, and ability to make single versus double stranded edits in the target sequence of DNA. The major differences between gene-insertion and gene- editing platforms are highlighted in **Table I**.

Of these techniques, Crispr-Cas9 is the most appealing, as it leads to precise double stranded breaks in the DNA helix, using a pre-designed 42-nucleotide guide sequence (Crispr guide), which has bases complimentary to the target site of the desired break in the DNA [41-43]. The guide carries the Cas-9 nuclease to the target location in the genome to make small edits. Electroporation of Cas9 nuclease and single guide RNA (sgRNA) as a ribonucleo-protein (RNP) complex leads to efficient delivery of genome editing material into HSPCs [44].

BCL11A (the TF that controls the switch from HbF to HbA and functions as a repressor of HbF) provides an excellent target for gene-editing approaches for hemo-globinopathies [45,46]. By suppressing BCL11A TF, it is postulated that HbF production can be triggered again in thalassemia patients to a sufficient degree to ameliorate anemia and avoid transfusions.

Table I Pros and Cons of Gene-Insertion vs Gene-Editing

Parameter	*Gene insertion*	*Gene- editing*
Insertions/Edits	Semi-random	Precise edits
Efficiency	Moderate	High for NHEJ
Therapeutic efficacy based on	Transduction efficiency (VCN)	In-del and NHEJ efficiency
MOA	Produce HbA or F	Produce HbF or Recreate HPFH
Delivery	Lentivirus vectors transduction	Extrapolation of Crispr- Cas9 nuclease + sgRNA or mRNAs encoding specific ZFN
Regulation of gene expression	Vector to provide promoters and regulatory elements of the *HBB* gene	Uses endogenous regulation
Safety	• Recombinant HIV • Insertional oncogenesis • Safety data can be generalized for all lentiviral vectors	• No risk of HIV • off-target' activity • Safety data is specific for the guide/ mRNA and cannot be generalized
Cost of goods	High (viral vector manufacturing)	Low (for NHEJ)

VCN-vector copy number (average insertion of the gene in HSPCs); In-del-small insertions-deletions at the site of edit that can be analyzed by PCR as a marker of editing efficiency; NHEJ-non-homologous end-joining; MOA-Mechanism of Action; sgRNA-specific guide RNA (Crispr); off-target-an unintended site of gene-edit, especially in a functional gene, ZFN-zinc finger nucleases.

Making specific deletions in the erythroid specific enhancer region of the *BCL11A* gene is a promising approach that is being explored currently [47,48]. Two programs to treat TDT are using either ZFN or Crispr-Cas9 platforms to make small deletions in the erythroid specific enhancer region of the *BCL11A* gene located on Chromosome 2. The major advantage of these platforms is that they do not directly make edits in the *HBB* gene, as they target the *BCL11A* gene, allowing the endogenous regulation and sustained production of the globin proteins to continue. These clinical trials are currently recruiting patients.

Another approach to increase HbF production is to recreate the mutations seen in patients with HPFH by making gene edits in the *HBB* gene. This is achieved by: *i*) creating small deletions e.g. in the γ-δ intergenic region leads to significant enhancement of the γ-gene expression [6]; *ii*) creating small deletions in the area of *HBB* cluster where BCL11A binds (e.g. CCAAT box region), so the effect of TF can be inhibited [49,50]; and *iii*) creating point mutations in the β-globin promoter region that can also lead to over expression of the mutated gene [51].

Pre-clinical studies are currently ongoing using Crispr-Cas12 platform to perform edits in the CCAAT box of the *HBB* gene, which overlaps with the BCL11A TF binding site, to increase the levels of HbF. This approach requires a higher degree of precision ('on-target' activity), so as not to disrupt the endogenous production of globin proteins. Both the gene-insertion and gene-editing methods, now scaled to human applications, are in multiple clinical trials now (**Web Table I**).

Pros and Cons of Gene-Editing Strategy

The main advantage of gene-editing (especially Crispr-Cas9 or Cas12) platform is the high efficiency and precision of the gene-edits made in the defined DNA locus [52].The main drawback of gene-editing nucleases is that they can make unintended edits in other parts of the genome, what is called 'off-target' activity [53,54]. Despite their design for accurate target gene editing, unintended off-target interactions between nucleases and genome sequences can still occur. There are multiple cell based and in-vitro assays and computational strategies designed to assess the off-target activity of the guides and nucleases and to predict their functional importance during pre-clinical assessments [55-58]. The goal of these pre-clinical assessments is to define the efficiency of 'on-target' editing and ascertain risks of 'off-target' activity (if any) of a Crispr guide.

In addition to potential off-target activity, chromosomal rearrangement events can also occur, due to double stranded breaks induced during gene-editing [59]. Therefore, serial karyotype analysis is also important during follow-up to analyze chromosome instability of gene-editing platforms.

The assessment of on-target, off-target and genotoxicity assays done in the gene-editing platforms is specific to the guide and the nuclease used to make the gene edits. Unlike the gene-insertion trials using

lentiviral vectors, the safety profile of the gene-editing techniques cannot be generalized, as it is specific for the guide and nuclease. Therefore, it is essential to keep this caveat in mind when comparing adverse events of one gene-editing clinical trial with another.

Long-term assessments of safety in clinical trials is still the gold standard compared to the computational models for analyzing off-target activity currently available [55-57], as detection of an 'off-target' site activity for a guide does not necessarily mean it will lead to a clinically meaningful adverse event.

Results of gene-editing clinical trials: Gene editing is currently undergoing phase I trials in humans. The results of the first patient (β^0/IVS-1-110 genotype) treated with Crispr-Cas9 gene editing (CTX001 product) at 12-months post-treatment show that the patient is transfusion independent, with total hemoglobin level of 12.7 g/dL (12.4 g/dL of Hb-F), and 99% erythrocytes in peripheral blood expressing high levels of HbF (F cells) [60].

Therefore, it is essential to recognize that long-term safety, durability, with continued transfusion independence and improvement in quality of life with no further requirement for chelation therapy, will decide which of these platforms lead to optimal risk-benefit ratio for acceptability.

PEDIATRIC PERSPECTIVES

Most of the Phase I human clinical trials of gene therapy are initiated first in adult patients (>18 years of age) who can understand the risks and benefits of these approaches clearly and consent to the experimental treatment. Once safety is established in the initial cohort of adult patients, the age can be lowered to include younger patients. Currently, lentiviral gene insertion trials are enrolling patients >12 years of age and the goal is to follow similar regulatory strategy for other gene-editing trials, once the initial safety data is available. The younger age limit needs to be established, as it is not the busulfan toxicity, but the risks of HSPCs collection via apheresis procedures in very young patients (currently safety is established for patients >20 kg without requiring any blood priming or other safety precautions).

Since the 'off-target' effects of many of these gene-editing strategies and the risk of insertional oncogenesis may require a longer duration of follow-up in pediatric patients to establish safety, therefore it is expected that for many of these new experimental trials it may take longer time for safety to be established prior to approval in younger patients.

It is also expected that younger patients may tolerate busulfan myeloablation much better than older patients with organ dysfunctions related to iron overload, although the issue of fertility cryopreservation needs to be discussed with individual families as part of the consent process (as infertility is a common long-term toxicity of busulfan and sperm or egg cryopreservation options may be limited in younger patients compared to adults). It is to be noted that the risks of infertility also exist with allogeneic HSCT where chemotherapy based conditioning regimens are utilized.

It is also important to note that correction of ineffective erythropoiesis is an important treatment goal for young patients, other than transfusion independence, to avoid complications of NTDT later in life.

Hence, it is envisioned that gene therapy may provide an alternative option of treatment for younger patients with TDT, especially in patients who lack well matched (HLA) family donors and in countries where large national bone marrow donor registries or cord blood banks do not exist.

CONCLUSIONS

Recent advances in whole genome sequencing, an understanding of the control and regulation of *HBB* gene along with improvements in vector biology and manufacturing, availability of new gene-editing nucleases that can lead to sufficient degree of gene modifications in HSCs to achieve meaningful clinical benefit, has recently led to multiple active clinical trials in patients. The early data from these experimental trials looks promising with potential to lead to a long-term durable transfusion independence and one therapy has already been approved in EU for TDT patients >12 years of age for non-β^0/β^0 patients. There is a hope that with the continued analysis of safety, durability and with continued refinement of manufacturing with improved efficiencies, gene therapies could potentially address the global health burden of β-thalassemia.

Note: Supplementary material related to this study is available with the online version at *www.indianpediatrics.net*

Competing interests: The author is also employed by Crispr Therapeutics Inc. that sponsors the CTX001 thalassemia trial. Only publicly available information has been provided and the manuscript was not influenced in any way by this relationship. Part of the text in this manuscript was adapted for pediatrics audience from previously submitted reviews to other journals by the author.

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REFERENCES

1. Giardine B, Borg J, Viennas E, et al. Updates of the HbVar database of human hemoglobin variants and thalassemia mutations. Nucleic Acids Res. 2014;42:D1063-9.
2. Rund D, Rachmilewitz E. Beta-thalassemia. N Engl J Med.

2005;353:1135-46.
3. Rachmilewitz EA, Giardina PJ. How I treat thalassemia. Blood. 2011;118: 3479-88.
4. Thein SL. Genetic modifiers of beta-thalassemia. Haemato-logica. 2005;90:649-60.
5. Danjou F, Anni F, Galanello R. Beta-thalassemia: From genotype to phenotype. Haematologica. 2011;96:1573-5.
6. Bernards R, Flavell RA. Physical mapping of the globin gene deletion in hereditary persistence of foetal haemoglobin (HPFH). Nucleic Acids Res 1980;8:1521-34.
7. Forget BG. Molecular basis of hereditary persistence of fetal hemoglobin. Ann NY Acad Sci. 1998;850:38-44.
8. Musallam KM, Rivella S, Vichinsky Rachmilewitz EA. Non-transfusion-dependent thalassemias. Haematologica. 2013; 98: 833-44.
9. Cao A, Galanello R. Beta-thalassemia. Genet Med. 2010; 12: 61-76.
10. Angelucci E, Matthes-Martin S, Baronciani D, et al. Hematopoietic stem cell transplantation in thalassemia major and sickle cell disease: indications and management recommendations from an international expert panel. Haemato-logica, 2014;99: 811-20.
11. Lucarelli G, Isgrò A, Sodani P, Gaziev J. Hematopoietic stem cell transplantation in thalassemia and sickle cell anemia. Cold Spring Harb Perspect Med. 2012;2: a011825.
12. Locatelli F, Kabbara N, Ruggeri A, et al. Outcome of patients with hemoglobinopathies given either cord blood or bone marrow transplantation from an HLA-identical sibling. Blood. 2013; 122:1072-8.
13. Sodani P, Isgrò A, Gaziev J, et al. Purified T-depleted, CD34+ peripheral blood and bone marrow cell transplantation from haploidentical mother to child with thalassemia. Blood, 2010;115: 1296-302.
14. Bertaina A, Merli P, Rutella S, et al. HLA-haploidentical stem cell transplantation after removal of alphabeta+ T and B cells in children with nonmalignant disorders. Blood. 2014;124: 822-6.
15. Soni S, Breslin N, Cheerva A. Successful unrelated umbilical cord blood transplantation for class 3 beta-thalassemia major using a reduced-toxicity regimen. Pediatr Transplant. 2014; 18:E41-3.
16. Kim A, Dean A. Chromatin loop formation in the beta-globin locus and its role in globin gene transcription. Mol Cells. 2012;34:1-5.
17. Lan X, Khandros E, Huang P, et al. The E3 ligase adaptor molecule SPOP regulates fetal hemoglobin levels in adult erythroid cells. Blood Adv. 2019;3:1586-97.
18. Masuda T, Wang X, Maeda M, et al. Transcription factors LRF and BCL11A independently repress expression of fetal hemoglobin. Science. 2016;351:285-9.
19. Sankaran VG, Menne TF, Xu J, et al. Human fetal hemoglobin expression is regulated by the developmental stage-specific repressor BCL11A. Science, 2008; 322:1839-42.
20. Breda L, Carla Casu, Sara Gardenghi, et al. Therapeutic hemoglobin levels after gene transfer in beta-thalassemia mice and in hematopoietic cells of beta-thalassemia and sickle cells disease patients. PLoS One, 2012;7: e32345.
21. Bank A, Dorazio R, Leboulch P. A phase I/II clinical trial of beta-globin gene therapy for beta-thalassemia. Ann N Y Acad Sci. 2005;1054:308-16.
22. Thompson AA, Walters MC, Kwiatkowski J, et al. Gene therapy in patients with transfusion-dependent beta-thalassemia. N Engl J Med. 2018;378:1479-93.
23. Roselli EA, Mezzadra R, Frittoli MC, et al. Correction of beta-thalassemia major by gene transfer in haematopoietic progenitors of pediatric patients. EMBO Mol Med, 2010;2:315-28.
24. Yannaki E, Karponi G, Zervou F, et al. Hematopoietic stem cell mobilization for gene therapy: superior mobilization by the combination of granulocyte-colony stimulating factor plus plerixafor in patients with beta-thalassemia major. Hum Gene Ther. 2013;24: 852-60.
25. Yannaki E, Papayannopoulou T, Jonlin E, et al. Hematopoietic stem cell mobilization for gene therapy of adult patients with severe beta-thalassemia: results of clinical trials using G-CSF or plerixafor in splenectomized and nonsplenectomized subjects. Mol Ther. 2012;20:230-8.
26. Cavazzana M, Mavilio F. Gene Therapy for Hemoglobinopathies. Hum Gene Ther. 2018.
27. Dong A. Rivella S, Breda L. Gene therapy for hemoglobinopathies: Progress and challenges. Transl Res. 2013;161: 293-306.
28. Malik P, Arumugam PI. Gene therapy for beta-thalassemia. Hematology Am Soc Hematol Educ Program. 2005: p. 45-50.
29. Negre O, Eggimann AV, Beuzard Y, et al. Gene therapy of the beta-hemoglobinopathies by lentiviral transfer of the beta (A(T87Q))-globin gene. Hum Gene Ther. 2016;27:148-65.
30. Payen E, Colomb C, Negre O, Beuzard Y, Hehir K, Leboulch P. Lentivirus vectors in beta-thalassemia. Methods Enzymol. 2012; 507:109-24.
31. Ginn SL, Liao SVL, Dane AP, et al. Lymphomagenesis in SCID-X1 mice following lentivirus-mediated phenotype correction independent of insertional mutagenesis and gammac overexpression. Mol Ther. 2010;18:965-76.
32. Hacein-Bey-Abina S, Garrigue A, Wang GP, et al. Insertional oncogenesis in 4 patients after retrovirus-mediated gene therapy of SCID-X1. J Clin Invest. 2008; 118:3132-42.
33. Moiani A, Paleari Y, Sartori D, et al. Lentiviral vector integration in the human genome induces alternative splicing and generates aberrant transcripts. J Clin Invest. 2012;122: 1653-66.
34. Ronen K, Negre O, Roth S, et al. Distribution of lentiviral vector integration sites in mice following therapeutic gene transfer to treat beta-thalassemia. Mol Ther. 2011;19:1273-86.
35. Kanter J, Walters MC, Hsieh M, et al. Outcomes for initial patient cohorts with up to 33 months of follow-up in the Hgb-206 Phase 1 Trial. Blood. 2018;132:1080-80.
36. Thompson AA, Walters MC, Kwiatkowski JC, et al. Northstar-2: Updated safety and efficacy analysis of lentiglobin gene therapy in patients with transfusion-dependent β-thalassemia and non-â0/â0 genotypes. Blood. 2019;134:3543-3543.
37. Lal A, et al. Northstar-3: Interim results from a phase 3 study evaluating lentiglobin gene therapy in patients with transfusion-dependent β-thalassemia and either a β0 or IVS-I-110 mutation at both alleles of the HBB Gene. Blood. 2019; 134:815-15.
38. Gilles AF, Averof M. Functional genetics for all: engineered nucleases, CRISPR and the gene editing revolution. Evodevo. 2014;5:43.
39. Palpant NJ, Dudzinski D. Zinc finger nucleases: Looking toward translation. Gene Ther. 2013;20:121-7.
40. Scharenberg AM, Duchateau P, Smith J. Genome engineering with TAL-effector nucleases and alternative modular nuclease technologies. Curr Gene Ther. 2013; 13:291-303.
41. Hoban MD, Bauer DE. A genome editing primer for the hematologist. Blood. 2016; 127:2525-35.
42. Rossin EJ, Wu DM. CRISPR-based gene editing: A guide for the clinician. Int Ophthalmol Clin. 2017;57:151-64.
43. Komaroff AL. Gene editing using CRISPR: Why the Excitement? JAMA. 2017;318: 699-700.
44. Dever DP, Porteus MH, The changing landscape of gene editing in hematopoietic stem cells: A step towards Cas9 clinical translation. Curr Opin Hematol, 2017;24: 481-88.
45. Bauer DE, Kamran SC, Lessard S, et al. An erythroid enhancer of BCL11A subject to genetic variation determines fetal hemoglobin level. Science. 2013;342:253-7.
46. Psatha N, Reik A, Phelps S, et al. Disruption of the BCL11A

erythroid enhancer reactivates fetal hemoglobin in erythroid cells of patients with beta-thalassemia major. Mol Ther Methods Clin Dev.2018;10:313-26.
47. Bauer DE, Orkin SH. Hemoglobin switching's surprise: the versatile transcription factor BCL11A is a master repressor of fetal hemoglobin. Curr Opin Genet Dev. 2015;33:62-70.
48. Sankaran VG, Xu JNY Orkin SH, Transcriptional silencing of fetal hemoglobin by BCL11A. Ann NY Acad Sci. 2010; 1202:64-8.
49. Alhashem YN, Vinjamur DS, Basu M, et al. Transcription factors KLF1 and KLF2 positively regulate embryonic and fetal beta-globin genes through direct promoter binding. J Biol Chem. 2011;286:24819-27.
50. Ikonomi P, Noguchi CT, Miller W, et al. Levels of GATA-1/GATA-2 transcription factors modulate expression of embryonic and fetal hemoglobins. Gene. 2000;261:277-87.
51. Traxler EA, Yao Y, Wang YD, et al. A genome-editing strategy to treat beta-hemoglobinopathies that recapitulates a mutation associated with a benign genetic condition. Nat Med. 2016: 22:987-90.
52. Lin MI, Wang J, Tan Y, et al. Re-creating hereditary persistence of fetal hemoglobin (HPFH) to treat sickle cell disease (SCD) and β-thalassemia. Blood. 2016;128:4708-4708.
53. Zhang XH, Tee LY, Wang XG, Huag QS, Yang SH. Off-target effects in CRISPR/Cas9-mediated genome engineering. Mol Ther Nucleic Acids. 2015;4: e264.
54. Lazzarotto CR, Nguyen NT, Tang X, et al. Defining CRISPR-Cas9 genome-wide nuclease activities with CIRCLE-seq. Nat Protoc. 2018;13:2615-2642.
55. Cho SW, Kim S, Kim Y, et al. Analysis of off-target effects of CRISPR/Cas-derived RNA-guided endonucleases and nickases. Genome Res. 2014;24:132-41.
56. Cradick TJ, Qiu P, Lee CM, Fine EJ, Bao G, et al. COSMID: A web-based tool for identifying and validating CRISPR/Cas off-target sites. Mol Ther Nucleic Acids.2014;3:e214.
57. Kim D, Bae S, Park J, et al. Digenome-seq: Genome-wide profiling of CRISPR-Cas9 off-target effects in human cells. Nat Methods. 2015;12:237-43.
58. Tsai SQ, Joung JK. Defining and improving the genome-wide specificities of CRISPR-Cas9 nucleases. Nat Rev Genet. 2016;17:300-12.
59. Long J, Hoban MD, Cooper AR, et al. Characterization of gene alterations following editing of the beta-globin gene locus in hematopoietic stem/progenitor cells. Mol Ther. 2018;26: 468-79.
60. Corbacioglu S, Chapin J, Chu-Osier N, et al. Efficacy results with a single dose of autologous crispr-cas9 modified cd34+ hematopoietic stem and progenitor cells in transfusion-dependent β-thalassemia and sickle cell disease. EHA Meeting Abstract. S280.
61. Marktel S, Scaramuzza, S, Cicalese MP, et al. Intrabone hematopoietic stem cell gene therapy for adult and pediatric patients affected by transfusion-dependent β-thalassemia. Nat Med.2019;25:234-41.

Web Table I Listing of Current Gene Therapy Clinical Trials for TDT in North America and EU

Trial NCT number	*Sponsor*	*Phase*	*Patient population*	*Vector or platform/DP name*	*MOA*	*References*
NCT 01745120 and 02151526	bluebird bio (HGB-204 and 205)	1/2	All TDT	SIN Lentiviral vector, BB305 ($\beta^{A\text{-}T87Q}$)	Increase HbA^{T87Q} levels	Thompson et.al, NEJM [24]
NCT 02906202	bluebird bio (HGB-207)	3	TDT with non-β^0/β^0 genotypes	SIN Lentiviral vector, BB305($\beta^{A\text{-}T87Q}$) with process improvements	Increase HbA^{T87Q} levels	Thompson et.al, 2019 [32]
NCT 03207009	bluebird bio (HGB-212)	3	β^0/β^0 genotypes	SIN Lentiviral vector, BB305($\beta^{A\text{-}T87Q}$) with process improvements	Increase HbA^{T87Q} levels	Lal et.al, 2019 [33]
NCT 02453477	IRCCS, San Rafael and Orchard Therapeutics	1/2	All TDT (6-35 years)	SIN Lentiviral vector (GLOBE)-β^A-globin; OTL-300	Increase HbA levels	Marktel et.al, [51]
NCT 03432364	Bioverative and Sanofi	1/2	All TDT (18-40 years)	Gene-editing of erythroid specific region of BCL11a with ZFN; ST 400	Increase HbF	No publication
NCT 03655678	Crispr Therapeutics and Vertex (CTX001-111)	1/2	All TDT (18-35 years)	Crispr-Cas9 gene-editing of erythroid specific BCL11a region; CTX001	Increase HbF	Corbaciogl u et.al, 2020 [60]
Pre-clinical	Editas Medicine	TBD	TDT	Crispr-Cas12 gene-editing of β-globin locus that binds BCL11a	Recreate HPFH	No publication

Framework to Incorporate Leadership Training in Competency-Based Undergraduate Curriculum for the Indian Medical Graduate

SUMITA SETHI,[1] HENAL SHAH,[2] AVINASH SUPE[3]

From Department of [1]Ophthalmology, BPS GMC for Women, Khanpur, Sonepat, Haryana; Department of [2]Psychiatry, Topiwala National Medical College and BYL Nair Charitable Hospital, Mumbai, Maharashtra; and [3]Department of Surgical Gastroenterology and Medical Education, Seth GS Medical College and KEM Hospital, Mumbai, Maharashtra.

Correspondence to: Dr Sumita Sethi, Coordinator Medical Education Unit, BPS GMC for Women, Khanpur, Sonepat, Haryana. sumitadrss@rediffmail.com

The new competency-based curriculum recognized the importance of leadership skills in physicians and has outlined competencies that would lead to attaining this goal. To prepare the Indian medical graduates as effective healthcare leader, there is no universal approach; it is desirable that the institutes organize the leadership competencies into an institutional framework and integrate these vertically and horizontally in their curriculum in a longitudinal manner. We describe the rationale for incorporating formal leadership training in the new competency-based undergraduate curriculum and propose a longitudinal curricular template utilizing a mixed/multi-modality approach to teach and apply leadership competencies.

Keywords: *Competency-based medical education, Physician leader*.

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The recently revised Graduate Medical Education regulations (GMER) recognized 'leader and member of the health care team and system' as one of the roles for the Indian medical graduate (IMG) [1]. With a vision to develop an IMG who is globally relevant, this was a desirable step. It was aligned to Accreditation Council for Graduate Medical Education (ACGME), which requires students to demonstrate the ability to 'work effectively as a member or leader of a healthcare team or other professional group' [2]. While broad outlines are provided in the curriculum, steps to implement the competencies and achieve goals is largely the responsibility of each institute. We herein describe the rationale for the inclusion of a formal, culturally sensitive leadership training in undergraduate medical education, and provide overarching principles of designing an institutional framework for incorporating leadership training in Indian medical colleges under the new competency-based curriculum (CBME).

THE FRAMEWORK

Leadership Competencies

The first and foremost step is to identify the desired leadership competencies and outcomes; these will then serve as the basis for creating course objectives and further guide the institutional framework and all subsequent details like content and delivery of leadership training. Many leadership competencies are already described in the new curriculum [3]; however, these are not comprehensive and institutes may need to reframe and expand them to precisely describe the leadership competencies for their students. Ideally a complete set of leadership competencies should include self-management competencies (exploration and management of self to develop greater self-awareness and emotional intelligence), team management competencies (under-standing principles of working collaboratively and leading teams in multi-professional environments), ability to work with healthcare systems and other focused leadership competencies (e.g., leading change, setting realistic goals) and behavior or transfer of learning based competencies (e.g., demonstration as successful team leader in actual conditions, networking) [4-8].

Teaching Learning Methods

Once competencies have been identified and defined; these will then guide the learning experiences that will be used to deliver the leadership training. Methodologies described for leadership training are vast, methods such as group discussions and collaborative work, interactive lectures, sharing narratives, presentations, demons-trations, use of media clips and role play activities have been used previously [9-13]. Based on an extensive literature review of teaching learning methods in leadership and teamwork training [5,6,9-14] and from our experience of introduction of institutional student leadership program [15], we propose the following methodology for teaching learning of leadership and teamwork principles:

Activities designed to enable an exploration of self: 'Who you are is how you lead' [5,13]; it is of foremost importance that a leader knows and understands himself well so that he can identify areas for improvement [4]. The leadership journey for the student will require an in-depth understanding of self so that one can constantly learn from own experiences and deal with the volatile, unpredictable, complex and ambiguous (VUCA) nature of heathcare system [16]. We suggest tools such as SWOT analysis (for self-exploration of one's strength and weakness), changing 'self-talk' (for building self-image and improving self-confidence, reflective writing (for developing deeper knowledge of self) etc. in form of small group interactive discussions to generate awareness of self and for developing attributes like strong emotional intelligence and resilience.

Activities designed to understand leadership and teamwork principles: Ability to work with others in a team has been identified as an essential skill for a leader [7]. We suggest tools such as Myers Briggs type inventory (MBTI); small group interactive activities aiming at highly specific team related skills like Color blind, Mission to Burundi; games based on group dynamics and stages of team building; role plays based on difficult conversations, conflict management, communication and negotiation skills to help them learn about the underlying principles of team management, group dyna-mics and common barriers to effective team working [17,18]. Use of appreciative leadership principles of inquiry, illumination, inclusion and inspiration as a method of positive strength-based leadership to create change would be a useful model [19].

Experiential learning: Team-based experiential learning activities have been accepted to be the most effective for practicing leadership skills [7,14]. Students are asked to identify an issue or concern in clinical, community or educational setting and execute its solution through a standard framework that includes defining the problem, communicating with team members and stakeholders, preparing a timeline, deciding a solution to the problem and implementation strategy. However, before designating any assignment as team task, it is important to understand the concept of 'task interdependence' i.e., the extent to which team members depend on one another for task completion; if a task is insufficiently complex and can be completed by an individual working alone, then it should not be labelled as a team task [20]. Some examples of team based experiential learning tasks are student leadership activities like leading a team for a seminar or a competition, leading and participating in inter-professional teams in hospitals or rural or mobile units, participating in audits and utilizing clinical practice guidelines to plan comprehensive effective patient care in multi-disciplinary settings.

Reflective practice: Reflecting on an experience and subsequent analysis facilitate incorporation of behavioral changes into practice, help in exploring its relevance to past personal experiences and identifies opportunities in future to achieve more desirable outcome [17,18,21]. Equally important is the concept of team reflexivity; there is evidence that regular team reflexivity helps in improving organizational outcomes in healthcare [22].

Clinical and community postings: Not every opportunity for teaching of leadership skills needs to be formal and explicit; there are certain very informal and readily available opportunities in our medical curriculum which can be well utilized. Clinical care rounds are the most commonly identified curricular approach in literature towards teaching leadership and teamwork by specifically demonstrating the roles, responsibilities and interactions among members of multidisciplinary teams in fulfilling needs of patients [23]. Similarly, much of leadership and teamwork content can also be folded in the form of community healthcare responsibilities by providing an opportunity to appreciate teamwork principles associated with patient management and safety challenges in community settings. Structured reflections could be obtained to understand how the students benefitted from the clinical and community postings.

Opportunity for networking and near peer assisted teaching learning: Peer networking refers to a network of like-minded individuals who can support, encourage and offer opportunities to each other to learn and develop and also to take on new leadership roles [24,25]. Networking with senior leaders provide a wide range of contacts, offers an entirely diverse range of perspectives, and can provide powerful supplementary teaching mechanisms for leadership development [13,14].

We believe participants in leadership training will learn best through multi modal learning strategies involving active participation. Institutes need to identify methodology for leadership training in alignment to the respective learning objectives and availability of institutional resources. Readers are referred to some other publications for more detailed discussion of teaching learning methodology for leadership [10,18].

Assessment Methods

The assessment plan should focus on leadership competencies pre-identified and defined in the institutional framework. During the clinical/community postings, students can be asked to reflect on any one incident wherein team-based care had a positive effect on patient care and another incident where dysfunctional team collaboration and failure of effective communication

amongst team members and leader resulted in a major lapse in patient care. While the students are learning to reflect on an experience, it is important to make them understand to go beyond a mere description of events; instead, they should analyze and gather critical evidence of learnings from the event and how they will apply these learnings for their development as a leader. Students should be encouraged to undertake various change initiatives in hospital and community settings; these can be discussed in the student leadership cell, highlighting the key areas of teamwork and deliberating on the leadership challenges that were involved. These can be assessed by reflective writing assignments and scored by a rubric, with a pre-decided score designated for a particular level of competency. E-portfolio can be used for the whole documentation process including various reflective writing sessions, experiential learning activities with critical analysis and comments for satis-factory performance, record of student's participation in other leadership activities like student organizations and community participation.

An important point to ensure is that students are being assessed on 'doing' in addition to 'learning' of leadership traits. During the implementation of leadership program at our institute, the participants completed at least one team based experiential learning assignments in hospital and/or community settings with multisource feedback on the assignments [15]. These were presented in the student leadership cell and critically analyzed by a panel of faculty members; those who performed exceptionally well were felicitated by institutional student leadership awards. **Table I** describes a few leadership competencies from the document [3] and suggests the corresponding teaching learning and assessment methods. These are just suggestions and it is up to the institute to decide how to approach the particular competency. If required, any of the validated leadership assessment instruments readily available in literature may be utilized [26], ensuring that it is aligned with the institutional framework and the pre-decided leadership model.

Evaluation

We suggest a mixed method design including both quantitative and qualitative methods of evaluation. Qualitative methods of evaluation like focus group discussions, structured interviews or interactive feedback sessions are helpful in understanding of students' perspectives and the underlying factors, which makes the whole learning process effective. In our leadership program, students shared their leadership journey though reflections written at the end of each session which were later qualitatively analyzed through content analysis [15]. Questionnaire-based feedback usually target participants' perceptions (Kirkpatrick level-1) and thus may not truly represent effectiveness of the program; targeting level-2 (learning of leadership skills) and 3 (transfer of learned skills to real life situations) is desirable. This can be well achieved through evaluation of the experiential learning activities and ensuring long term follow up for concrete results like changes in organizational practices.

The Timetable

Three block experiences can be created and incorporated in the timetable vertically and horizontally in the CBME viz., block-1 for introduction to basic teamwork and leadership principles, block-2 for experiential learning through clinical/community postings and electives and block-3 for networking and mentoring.

Block-I: Introduction to key leadership and teamwork principles: Extracurricular hours in phase-I and II can be utilized for introducing participants to key self-management and team management principles longitudinally through methodology as described earlier. Timings and duration of individual sessions can be decided by the institute; however, group size should not exceed more than fifteen students to ensure an effective interaction of all participants. Sessions of self-management should precede those of team management, following the basic principle that one needs to manage 'self' first and then 'others'. Reflective practice needs to be initiated early and practiced throughout; sufficient opportunities for this are already available in the curriculum e.g., small group teaching activities such as problem-based learning sessions and tutorial/seminar presentations can be explored as opportunities for leadership training from the first year onwards. Anatomy dissection teams are their first professional exposure to teamwork and a good opportunity to illustrate basic principles of group dynamics. Discussions can be initiated on how to define roles and responsibilities of members, identify one's own leadership style, establish team goals, lay down strategies for improved team performance, illustrate success and frustrations within the team etc. Similarly, in second professional year, when the clinical postings are initiated, a pharmacology session can be integrated with clinical case discussion wherein the student learns the use of available literature in pharmacology to plan an effective multidisciplinary treatment plan for the patient.

Block-2: Experiential learning through clinical and community postings and elective posting: Further leadership training can be continued as an optional 4-weeks elective (block-1) through the leadership cell; since students will also be continuing their clinical and community postings, there will be lots of opportunities for

Table I Competencies for Leadership Role of Indian Medical Graduate

Learning objectives	*Suggested teaching learning methodology*[a]	*Suggested assessment methods*
3.2.1 Work effectively and appropriately with colleagues in an inter-professional health care team respecting diversity of roles, responsibilities, and competencies of other professionals.		
Participants will be able to appreciate their own leadership style and that of the team members and how to use this to manage team, identify individuals' type preferences and capitalize on their leadership strengths in leading and working successfully with others.	Myers-Briggs Type Indicator (MBTI) (Workshop in designated extracurricular hours)	Formative assessment of reflections and narrative writing using rubrics
Participants will be able to attend and observe multidisciplinary team meetings to understand principles of effective working of an interprofessional team in a clinical setting.	Clinical placement and observation of role models and experiential posting	Formative assessment of reflections and narrative writing using rubrics
Participants will be able to have a hands-on experience of working in/leading an interprofessional team effectively.	Experiential learning assignment (clinical setting)	Multi-source formative feedback; formative assessment of discussions and presentations in the Student Leadership Cell.
3.2.2 Recognize and function effectively, responsibly, and appropriately as a health care team leader in primary and secondary health care settings		
Participants will be introduced to effective small work group and its stages of formation; define an effective team and recognize where group performance is more effective than individual work; describe the stages of team work and identify actions that move the groups through various stages.	Team building and group dynamics (Workshop in designated extracurricular hours)	Formative assessment of reflections and narrative writing using rubrics.
Participants will be able to understand principles of leading healthcare team effectively in primary and secondary health care settings.	Community placement and observation of role models and experiential posting	Formative assessment of reflections and narrative writing using rubrics
Participants will be able to have a hands-on experience of leading healthcare team effectively in primary and secondary health care settings.	Experiential learning assignment (community setting)	Multi-source formative feedback
3.2.3 Educate and motivate other members of the team and work in a collaborative and collegial fashion that will help maximize the health care delivery potential of the team.		
Participants will be able to appreciate difficult conversations and principles of Conflict management using a specific model to resolve conflict (e.g. HEAL-IT model)	Small group interactive activities/ role play on difficult conversations, conflict management (Workshop in designated extracurricular hours)	Formative assessment of reflections and narrative writing using rubrics
Participants will network with peers and near peers with shared objectives for activities and responsibilities within the college, involvement with social groups and organizations; aimed to provide students with an opportunity to develop experience of leadership, and to understand how effective leadership will have an impact on the system and benefit patients as they move from learner to practitioner	Near peer assisted learning, role modelling and networking through student leadership cell	Team reflexivity with feedback from supervising faculty
3.2.4 Access and utilize components of the health care system and health delivery in a manner that is appropriate, cost effective, fair and in compliance with the national health care priorities and policies, as well as be able to collect, analyze and utilize health data.		
Participants will be able to understand national health care priorities and policies in relation to community needs	Participating in national groups/ societies, interaction with senior students who have leadership positions in the field, mentoring by a senior leader in the field	Formative assessment of reflections and narrative writing using rubrics

Contd..

Table I *contd.*

learning objectives	*Suggested teaching learning methodology*[a]	*Suggested assessment methods*
Access and utilize components of the health care system and health delivery in a manner that is appropriate, cost effective, fair and in compliance with the national health care priorities	Experiential learning assignment (based on data audit)	Multi-source formative feedback
Participants will be able to collect, analyze and utilize health data and have an opportunity to influence the decision-making process	Near peer assisted learning, role modelling and networking through student leadership cell	Team reflexivity with feedback from supervising faculty

NMC: National medical commission. [a]*Sessions on self-management and reflective writing to precede any session on team management.*

reinforcement and application of what has been learnt. Specific modules can be developed in community health or chronic illness or in emergency medicine with pre-defined learning objectives e.g., the chronic diseases modules can be used to understand the importance of working with other health professionals, while at the same time, having a particular health care professional as patient's care coordinator. Small group discussions, student presentations and reflections may be used and students can be given exercises addressing leadership and teamwork directly related to the modules. Above all, this is the most appropriate time for students to 'do' what they have learnt, they should complete at least two team based experiential learning assignments during the elective posting; examples and assessment methods of team based experiential learning assignments have already been discussed.

Block-3: Networking and near peer assisted learning: In third professional year part-2, students with particular interests can attend activities held by the leadership cell outside curricular hours and undertake activities for bringing changes in organizational practices, they also continue networking and mentoring the new participants. Members of the club can meet once in a month or fortnightly to discuss and deliberate on various teamwork and leadership related issues.

The Rationale

Medical students have always been expected to evolve as physician leaders and take on leadership roles from the beginning of their professional career; it is ironical that the traditional undergraduate medical curriculum did not address leadership training formally. Recognition of 'leader and member of healthcare team' as a role for the IMG in the new CBME curriculum is a much-desired move towards ushering in formal leadership training; however, there are a few questions that need to be addressed before planning leadership training. The first and foremost is 'whether leadership can be taught'; if yes, what leadership models will guide the whole process? What will be the goals for the program and what will be the most effective learning experiences to achieve them? When should the training be initiated and how will the leadership competencies be assessed? We have tried to address these questions while proposing this longitudinally incorporated framework for leadership training. Yes, leadership does consist of a series of definable skills that can be well taught; while a few may have inherent characteristics that make them better leaders, adequate training and experiences could create successful leaders [7,27]. Different models and theories like transformational leadership, authentic leadership, servant leadership, self-leadership and appreciative leadership have their own characteristics [7,26,28-30]; it is important to develop feasible models for various branches of health-care, in different regions of the country for respective institutions. Another important point to consider is 'when' and 'how' to introduce this training; one school of thought is that once negative perceptions develop as a result of negative role modelling during clinical postings, it becomes more difficult to change; other school of thought is that such training will be effective only when sufficient clinical experience has been gained [17]. There is no approach which is specifically 'right' or 'wrong'; it is essential that the institute has a clarity for the specific aims of the program and design the framework accordingly. Having an institutional commitment is desirable; Janke, et al. [8] emphasized the importance of weaving leadership development into the mission and goals of the institute; including financial support, support of administrators responsible for resource management, in-organization recognition awards and appropriate faculty development and reward systems [8].

Leadership training is not just meant to prepare students for particular leadership roles; instead, it is targeted to develop strong personal and professional values and a range of non-technical skills such as communication skills, strong emotional intelligence, negotiation skills, etc. which will allow them to lead across professional boundaries and influence many facets of life including

healthcare [4]. Any one-time opportunity for development of leadership will not be sufficient and the importance of providing continuous opportunities for practicing leadership skills, networking and mentoring cannot be over emphasized. There are student bodies, student clubs, community and other group activities in almost all medical schools; these opportunities can be explored and utilized for formal leadership training. Chaudry, et al. [31] proposed a medical leadership society at medical schools as an easier to implement solution to cater to the growing demand for leadership training for students who demonstrate a special interest in leadership. We suggest the introduction of student leadership cells with opportunities for networking and peer mentoring to keep them engaged in leadership activities in different stages of professional development.

Challenges and Limitations

Some institutes may already have one or the other formal or informal leadership program in place; however, if such training is being introduced for the first time in the institute, many challenges would be expected. Hiring professional managers or trainers might work as a one-time solution to initiate the program but if it has to run as an institutional program, it is important that faculty members are sufficiently trained. There will be requirement of faculty development activities targeting leader-ship skills to help the faculty develop as trainers as well as role models. In the initial phase of introduction of leadership training, not having sufficient number of trained faculty in the institute will be a major limitation. Under such circumstances, if training is made compulsory for all students, it will tend to inherently dilute the quality and the whole drive because there will simply be too many students and too less trainers. On the other hand, if only a few students are included, the whole concept of including leadership as a core competency for students will not be fulfilled. As an intermediate solution, we suggest utilizing near peer mentoring through the institutional leadership cell till the faculty development program on leadership is completed. Furthermore, we will be mistaken by assuming that any one-time course will make our students evolve as leaders; to achieve this goal, longitudinal integration of leadership training in the curriculum is to be ensured. This will require meticulous planning and involvement of all the stakeholders i.e., members of curriculum committee and medical education unit and other faculty members. Another major challenge will be evaluation of the program. As discussed earlier, only quantitative form of evaluation will not be sufficient and more of qualitative information targeting higher Kirkpatrick's levels of learning will be required; this will create severe time limitations. Above all, long-term follow-up and evaluation will be needed to provide concrete results in the form of change in organizational practice as a result of leadership training.

CONCLUSION

The new competency-based curriculum not only addresses a well-recognized gap in our medical undergraduate training by recognizing the role of 'leader' for the IMG but also provides scope for formal leadership training in the already crowded undergraduate curriculum, through dedicated extracurricular hours and electives. It is exciting to propose a formal framework for explicit leadership training including team training, community and clinical experiences, student leadership opportunities, experiential learning, mentoring and networking. The framework can be finalized by the institute itself according to its own desired competencies, preferred teaching methods and available resources. We believe that this framework could be aligned with the current curriculum, without stretching either the time or the resources. It is of foremost importance to have institutional commitment and develop a supportive atmosphere, conducive for the students to evolve as leaders and for faculty as role models through administrative and financial support, appropriate allotment of resources, and training and incentives for faculty.

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REFERENCES

1. Medical Council of India. Regulations on Graduate Medical Education 2019 - Addition as Part II of the Regulations on Graduate Medical Education, 1997; E-gazette. Accessed Dec 2, 2019. Available from: *mciindia.org/documents/Gazette/GME-06.11. 2019*
2. The Accreditation Council for Graduate Medical Education (ACGME). The outcome project 2014. Accessed February 12, 2019. Available from: *www.acgme.org.*
3. National Medical Commission: Competency based Undergraduate curriculum for the Indian Medical Graduate 2018. Accessed January 7, 2018. Available from: *https://www.nmc.org.in/information-desk/for-colleges/ug-curriculum*
4. Hargett CW, Doty JP, Hauck JN, et al. Developing a model for effective leadership in healthcare: A concept mapping approach. J Healthc Leadersh. 2017;9:69-78.
5. NHS Institute for Innovation and Improvement and Academy of Medical Royal Colleges (NHS III). Medical leadership competency framework: enhancing engagement in medical leadership. 3rd edition. 2010 NHS Institute for Innovation and Improvement and Academy of Medical Royal Colleges.
6. Webb AM, Tsipis NE, McClellan TR, et al. A first step toward understanding best practices in leadership training in undergraduate medical education: A systematic review. Acad Med. 2014; 89: 1563-70.
7. Varkey P, Peloquin J, Reed D, et al. Leadership curriculum in undergraduate medical education: A study of student and faculty perspectives. Med Teach. 2009;31:244-250.
8. Janke KK, Nelson MH, Bzowyckyj AS, et al. Deliberate integration of student leadership development in doctor of pharmacy programs. American J Phar Edu. 2016;80:2.
9. Shulman LS. Signature pedagogies in the professions. Daedalus.

2005;134:52-9.
10. Jenkins DM. Exploring signature pedagogies in undergraduate leadership education. Journal of Leadership Education. 2012; 11:1-27.
11. Russell SS. An overview of adult-learning processes. Urologic Nursing. 2006;26:349-52.
12. Sethi S. Leadership development programs in undergraduate medical education: Understanding the need, Best practices and Challenges. *In*: Jespersen EA, editor. Exploring the opportunities and challenges of medical students. NOVA Publishers; 2019. p.32-57.
13. Till A, Mc Kimm J, Swanwick T. Twelve tips for integrating leadership development into undergraduate medical education. Med Teach. 2018;40:1214-20.
14. Warren OJ, Carnall R. Medical leadership: Why it's important, what is required, and how we develop it. Postgrad Med J. 2011;87:27-32.
15. Sethi S, Chari S, Shah H, Aggarwal R, Dabas R, Garg R. Implementation and evaluation of leadership program for medical undergraduate students in India. *In*: Abstract book of Annual conference of AMEE (Association for Medical Education in Europe): The virtual conference; 2020 September 7-9.p.166.
16. Till A, Dutta N, McKimm J. Vertical leadership in highly complex and unpredictable health systems. British J Hosp Med. 2016;77: 471-5.
17. Banerjee A, Slagle JM, Mercaldo ND, et al. A simulation-based curriculum to introduce key teamwork principles to entering medical students. BMC medical education. 2016;16:295.
18. Shah H, Ladhani Z, Morahan PS, et al. Global leadership model for health professions education Part 2: Teaching/learning methods. The J Leadership Educ. 2019;18:193-203.
19. Orr T, Cleveland-Innes M. Appreciative leadership: Supporting education innovation. International Review of Research in Open and Distributed Learning. 2015;16:235-41.
20. West MA, Lyubovnikova J. Illusions of team working in health care. J Health Organ Manag. 2013;27:134-42.
21. Sandars J. The use of reflection in medical education: AMEE Guide No. 44. Medical Teacher. 2009;31:685-95.
22. Schippers MC, Edmondson AC, West MA. Team reflexivity as an antidote to team information-processing failures. Small Group Research. 2014;45:731-69.
23. O'Connell MT, Pascoe JM. Undergraduate medical education for the 21st century: Leadership and teamwork. Fam Med. 2004;36: S51-6.
24. Gaufberg EH, Batalden M, Sands R, et al. The hidden curriculum: what can we learn from third-year medical student narrative reflections? Acad Med. 2010;85:1709-16.
25. Chen TY. Medical leadership: An important and required competency for medical students. Ci Ji Yi Xue Za Zhi. 2018;30:66-70.
26. Houghton JD, Dawley D, DiLiello TC. The abbreviated self-leadership questionnaire (ASLQ): A more concise measure of self-leadership. Int J Leadership Studies. 2012;7:216-32.
27. Clyne B, Rapoza B, George P. Leadership in undergraduate medical education: Training future physician leaders. R I Med J. 2015;98:36-40.
28. Trastek VF, Hamilton NW, Niles EE. Leadership models in health care-case for servant leadership. Mayo Clinic Proceedings. 2014;89:374-81.
29. Stoller JK. Developing physician leaders: A perspective on rationale, current experience, and needs. Chest. 2018;154:16-20.
30. Judge TA, Bono JE. Five-factor model of personality and transformational leadership. Journal of Applied Psychology. 2000;85: 751-65.
31. Chaudry A, Amar Sodha AN. Expanding management and leadership education in medical schools. Adv Med Educ Pract. 2018;9:275-78.

NOTIFICATION FOR FELLOWSHIP COURSE IN PEDIATRIC INTENSIVE CARE, PEDIATRIC EMERGENCY MEDICINE AND PEDIATRIC PULMONOLOGY 2021 – 2022 SESSION

The Institute of Child Health and Hospital for Children, Egmore,Chennai is conducting one year post doctoral fellowship courses in **Pediatric Intensive Care, Pediatric Emergency Medicine and Pediatric Pulmonology** under the Tamilnadu Dr MGR Medical University. These courses will impart specialised knowledge in all aspects of pediatric intensive care and pediatric emergency medicine. The pediatric intensive care and pediatric emergency medicinetraining will empower the candidates to independently manage critically ill children and help establish pediatric intensive care and pediatric emergency medicine departments at other medical colleges and in district hospitals. The Pediatric Pulmonology training will empower the candidates to manage Pediatric pulmonology cases and Bronchoscopy.

The eligibility for the course is MD(Pediatrics) / Diploma in National Board (Pediatrics)

Name of course (Post doctoral fellowship)	No of seats	course duration
1. Pediatric Intensive Care	2	1 year
2. Pediatric Emergency Medicine	2	1 year
3. Pediatric Pulmonology	2	1 year

Out of the two seats one will be allotted to service candidate and one will be allotted to private candidate in each course. If seat is not filled up in one category the seat will be allotted to eligible student from other category.

The course fee is Rs 50,000 [Rupees Fifty Thousand only] for all candidates. This should be paid at the time of admission, as demand draft favouring "The Director and Superintendent", Institute of Child Health and Hospital for Children, Egmore, Chennai. 600008.

Selected service candidates will be deputed for the course and they will be eligible for pay and allowances as per GO. MS. No.156 dt 22.6.2011.

The application form can be downloaded from the Tamilnadu Dr MGR medical university website *www.tnmgrmu.ac.in* The rules and regulations of the fellowship course and eligibility criteria are clearly given in above mentioned website.

The filled up application along with a DD for Rs1000 in favour of "The Director and Superintendent, Institute of Child Health and Hospital for Children" should be sent to the following address and superscribed as

'APPLICATION FOR FELLOWSHIP IN PEDIATRIC INTENSIVE CARE, PEDIATRIC EMERGENCY MEDICINE AND PEDIATRIC PULMONOLOGY

Director and Superintendent, Institute of Child Health & Hospital for Children,
Halls Road, Egmore, Chennai 600008.

"**Service candidates should submit theapplication through proper channel**."

Last date for submission of application : **10/08/2021 at 5 pm**
The date of entrance exam will be : **23/08/2021 at 10 am**

Venue: Institute of Child Health and Hospital for Children, Egmore, Chennai 600008.

Course commencement : 8th **September, 2021**

The qualifying examination will be of 3 hours duration with total 100 MCQ.
Pediatric Intensive care from General Pediatric and Pediatric Intensive care.
Pediatric Emergency Medicine from General Pediatric and Pediatric Emergency Medicine.
Pediatric Pulmonology from General Paediatric and Pediatric Pulmonology.

For further details please contact

For Pediatric Intensive care, Dr V Poovazhagi	:	9840033020	*poomuthu@gmail.com*
Emergency Medicine, Dr R Ezhilarasu	:	9841021018	*elilarasu74@gmail.com*
Pulmonology, Dr Sarath Balaji	:	9443697844	*sarath1731@gmail.com*

Levels of Aminotransferases Among Schoolchildren in Jaipur, Rajasthan

We did cross-sectional study for normal values of amino-transferases in school children aged 2- 18 years. Median (IQR) AST and ALT values in study subjects were 30 (27- 34) U/L and 23 (19-29) U/L. We also provided age-and sex-related percentiles of aminotransferases of children. We observed a peak of median AST serum values in the age group 6-8 years followed by continuous decline with increasing age. While in ALT, we observed maximum values in age group 2-5 years followed by continuous decline. There was a statistically significant difference in values of amino-transferases between sexes.

Keywords: *Aspartate aminotransferase, Alanine aminotransferase, Normal values.*

Several studies have suggested that the upper limit of normal aminotransferases should be revised [1,2]. In the past seven years, several approaches have been made to establish new reference intervals or thresholds for liver enzymes in children [3-7], but most of these were for Western population. With the assumption that the current reference range for amino-transferases may need revision, we conducted this study to evaluate the normal values of aminotransferases in school children aged 2-18 years.

This school-based cross-sectional study was carried out in Jaipur in the year 2019 after institutional ethics committee clearance. Three schools were selected randomly from rural areas and two schools from urban areas of Jaipur, Rajasthan. Study population included children aged 2-18 years, after parental written consent. A total of 590 children and adolescents were initially screened. During the screening, participants were asked a comprehensive questionnaire regarding their basic demographic information, medical history including current history of febrile illness, medication use (including ayurvedic, growth and apetite stimulators) and social information which included age, sex and history of previous liver disease. Clinical history and general physical examination was done based on a predefined proforma. Height, weight and triceps skin fold thickness (by skinfold caliper) were measured. Five milliliter of non-fasting venous blood sample was collected and processed within 4 hours. We excluded 149 study subjects (active viral upper respiratory infection, 14;HBsAg positive, 5; IgAtTG positive, 7; those with aminotransferses values >3 standard deviation, 16; BMI less than 10th percentile, 50; BMI 90th percentile, 57) [7]. Finally, aminotransferases levels of 441 subjects (165 males) were analyzed.

Student *t* test and ANOVA (one-way analysis of variance) test followed by post hoc test were used for comparing the difference between the various groups. Pearson correlation was conducted to examine the relationship between aminotransferase levels and various parameters like age, sex, body mass index (BMI), triceps skin fold thickness etc.

Mean (SD) age of study subjects was 12.3 (7.4) years, and for analysis, we divided study subjects to different age groups (2-5, 6-8, 9-11, 12-15 and 16-18 years) with 22, 57, 77, 195, and 90 study subjects in each age group, respectively. Median (IQR) AST and ALT values in study subjects were 30 (27- 34) U/L and 23 (19-29) U/L (**Table I**). However, Poustchi, et al. [3] reported median ALT for boys and girls to be 16 U/L and 13 U/L which were quite lower than our median ALT values. The difference between sexes was statistically significant, similar to previous studies [4,7]. We found upper limit of normal (97th percentile) AST and ALT to be 44 U/L and 40 U/L, which were somewhat similar to as described by England, et al. [4] 40 and 35, respectively, but were higher than those reported by Dehghani, et

Table I Aspartate Aminotransferase and Alanine Aminotransferase (ALT) Percentiles Values Among School Children (*N*=441)

Study Population	*Aspartate aminotransferase levels (IU/L)*			*Alanine aminotransferase value (IU/L)*		
	3rd	*Median (IQR)*	*97th*	*3rd*	*Median (IQR)*	*97th*
All children[a]	21	30 (27-34)	44	15	23 (19-29)	40
Male	23	32 (28-34)	46	17	27 (22-32.3)	42
Female	21	29 (26-33)	43	15	21 (18-27)	38
Age group[b c]						
2-5 y	26	32 (27-36)	42.8	18	31 (24-33.7)	40.8
6-8 y	25.7	33 (29-37)	47.3	17.5	27 (22-31.5)	41.3
9-11 y	23.3	30 (27-33)	43	16	26 (21-32)	41.7
12-15 y	20.9	29 (26-32)	45.1	15	21 (18-27)	40.1
16-18 y	20.7	29 (26-33)	43.3	14.7	22 (18-27)	38.3

[a]*P<0.01 for comparison between males and females for both AST and ALT,* [b]*P=0.01 for comparison between 6-8y and 9-11y age-group for AST and* [c]*P=0.02 for 9-11y vs 12-15y for ALT.*

al. [5] (29 and 21, respectively).

We observed peak of median AST serum values in age group 6-8 years followed by continuos decline with increasing age. However in a study by Bussler, et al. [7], the AST serum values were showing peak at age group 1-3 years followed by continuos decline with increasing age. While in ALT, we observed maximum values in age group 2-5 years followed by continous decline and we did not find any peak around puberty. The initial decrease in ALT has also been described by previously [4], and apart from the missing ALT peak in early puberty in boys, Zierk, et al. [6] presented similar patterns of ALT with age. However, others reported initial fall in ALT with increasing age followed by peaking around puberty [7].We found that both AST and ALT were significantly negatively related to age (P<0.001). Bussler, et al. [7] showed that AST also decreases with increasing age, with no significant effect of age on ALT. Reverse association of ALT increase with increasing BMI, with weak negative association with AST was previously reported [7], but we did not observe such an association.

We provide age- and sex-related percentiles of aminotransferases of children from a limited data set from a single center in Northern India. In addition to the small sample size, our sample was not equally distributed between males, females and different age groups, so it was not representative of the population. Also, our data cannot be generalized to others parts of the country. We did not use ultrasound or fibroscan to exclude pediatric non-alcoholic fatty liver disease (NAFLD). We didnot do C-reactive protein levels to exclude occult sepsis. Tanner staging was not done to see effect of puberty on transaminases. We did not take into account the other factors like timing of day, effect of exercise and day to day variation of aminotransferases. However, our findings underscore the need for large multi-centric studies to document normal aminotransferase levels children.

Contributors: SN, GKG: Concept and designed the study; SR, SS, GKG: Analyzed data and drafted the manuscript; SR, KD: Collected the data and helped in data analysis.

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SANDEEP RATRA,[1] KAPIL DHINGRA,[1] SAHIL SHARMA,[2] GAURAV KUMAR GUPTA,[1*] SANDEEP NIJHAWAN[1]

Department of [1]Gastroenterology, SMS Medical College and Hospital, Jaipur, Rajasthan; [2]Health and Family Welfare Department, Kapurthala, Punjab.

[*]kumarggauravpgi@gmail.com

REFERENCES

1. Prati D, Taioli E, Zanella A, et al. Updated definitions of healthy ranges for serum alanine aminotransferase Levels. Ann Intern Med. 2002;137:1-10
2. Kariv R, Leshno M, Beth-Or A, et al. Re-evaluation ofserum alanine aminotransferase upper normal limit and its modulating factors in a large-scale population study. Liver Intr. 2006;26: 445-50
3. Poustchi H, George J, Esmaili S, et al. Gender differences in healthy ranges for serum alanine aminotransferase levels in adolescence. PLoS One. 2011;6:e21178.
4. England K, Thorne C, Pembrey L, Tovo P-A, Newell M-L. Age- and sex-related reference ranges of alanine aminotransferase levels in children: European paediatric HCV network. J Pediatr Gastroenterol Nutr. 2009;49:71-7.
5. Dehghani SM, Erjaee A, Haghighat M, Imanieh MH, Ahmadi R. Upper limits of normal aminotransferases in children in Southern Iran. J Compr Ped. 2014;5:15274.
6. Zierk J, Arzideh F, Rechenauer T, et al. Age- and sexspecific dynamics in 22 hematologic and biochemical analyses from birth to adolescence. Clin Chem. 2015;61:964-73.
7. Bussler S, Vogel M, Pietzner D, et al. New pediatric percentiles of liver enzyme serum levels (alanine aminotransferase, aspartate aminotransferase, gamma glutamyl transferase): Effect of age,sex, body mass index and pubertal stage. Hepatology. 2018;68: 1319-30.

Clinical Profile of Adolescents With Delayed Puberty

One year study on forty-eight adolescents with delayed puberty revealed etiology of constitutional delay, hypogonadotrophic hypogonadism (HH), hypergonadotrophic hypogonadism, chronic systemic disease, hypothyroidism and sex reversal in 14(29.2%), 13 (27%), 12 (25%), 5 (10.4%), 3 (6.3%) and 1 (2.1 %) cases, respectively. Earlier presentation, male preponderance, significant normal variants and utility of GnRH analogue testing observed.

Keywords: *Hypogonadism, Pubertal disorder, Thelarche.*

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Delayed puberty has heterogeneous etiology in adolescents. Data on delayed puberty are available from the Western world [1-3] and from some parts of India [4]. Hence, we conducted this study between June, 2017 and May, 2018 to describe the clinical, biochemical and radiological profile of adolescents with delayed puberty in a tertiary care hospital in Southern India.

After approval from the institutional ethics committee, adolescents referred to an endocrine clinic with delayed puberty or delayed sexual maturity rating were recruited. Delayed puberty was defined as absence of thelarche by 13 years or no menarche 5 years after thelarche (girls) or no progression of secondary sexual characters for 18 months after onset of puberty [5], or no testicular enlargement (≥4mL) by 14 years (boys). Details of age, sex, history of pubertal onset, growth, systemic disease, family history of delayed puberty and previous illnesses were retrieved. Anthropometric measurement and sexual maturity rating (SMR) assessments were performed on girls with minimal clothing in complete privacy with a female staff nurse and mother, for boys in the presence of father. Breast stage and pubic hair (in girls) and testicular volume (using Prader orchidometer) and gonadal stage (in boys) were classified into stages described by Tanner [6].

Subjects underwent baseline biochemical evaluation for systemic diseases, thyroid profile, bone age assessed using the Greulich Pyle atlas in those with short stature, and ultrasound to assess pelvic organs in girls [7]. Hypothalamo-pituitary gonadal (HPG) axis was assessed by luteinizing hormone, follicle stimulating hormone and serum estradiol (in girls), and serum total testosterone (in boys). Serum estradiol >10pg/mL and testosterone >25ng/dL was considered as pubertal onset. Those with inconclusive LH (<0.65 IU/L) and FSH (<1.2 IU/L) underwent gonadotrophin analogue (GnRHa) stimulation test to distinguish hypergonadotrophic hypogonadism (HH) from constitutional delay of growth and Puberty (CDGP) [8,9]. Children with HH also underwent MRI brain. Human chorionic gonadotrophin stimulation test (hCG) was performed to assess leydig cell function in males with dysgenic gonads. Pubertal induction with testosterone (50 mg intramuscularly) and estrogen (2.5 mcg ethinyl estradiol or 0.25 mg estradiol valerate on alternate days) was done in hypogonadism and subjects were followed-up.

A total of 48 adolescents (27 males) with mean age (SD) of 15.5 (1.0) years (males, 15.3 year and females 15.8 years) were studied. Delayed sexual maturation, no progress of maturation and no menarche, was noted in 77.1%, 10.4% and 12.5%, respectively. The etiology was CDGP, HH, hypergonado-trophic hypogonadism, chronic systemic disease, primary hypothyroidism and sex reversal in 14(29.2%), 13(27%), 12(25%), 5(10.4%), 3(6.3%) and 1(2.1%) cases, respectively.

GnRH analogue testing was performed in twelve subjects (68% had flat gonadotrophin response suggesting HH and 34% had normal pubertal response suggesting CDGP). An increment in height *z*-score of +0.4 was seen in three subjects with CDGP treated with intramuscular testosterone (owing to significant psychological distress) vs +0.3 in eight with spontaneous puberty after 12 month follow-up. Pubertal progression was noted in all on follow-up. One patient each with Crohns disease, type-1 diabetes mellitus, congenital adrenal hyperplasia (CAH), systemic lupus erythematosus (SLE) and primary immunodeficiency had delayed puberty where control of the primary disease was the primary strategy. Oral estrogen was initiated in one adolescent with SLE, steroid dose modification in CAH and thyroxine replacement in primary hypothyroidism.

Thirteen (27%) subjects (10 boys) had HH (9 with panhypopituitarism). The etiological profile was non-syndromic in 9. Kallman syndrome 2, Prader Willi syndrome 1 and Bardet Biedl syndrome 1. Multiple pituitary hormone replacement (sex hormone, growth hormone, cortisol, thyroxine and desmopressin) and pubertal induction, resulted in height *z*-score increment of +0.6 and +0.15 in subjects with multiple pituitary hormone deficiency and isolated HH, respectively.

Hypergonadotrophic hypogonadism was seen in 12 adolescents (median age 15.4 years; 8 females) with mean (SD) height SDS of –3.2 (0.9). These included Turner syndrome in 5, Klinefelter syndrome in 2, bilateral anorchia in 1, primary gonadal dysgenesis in 3 and post intracranial tumor therapy in 1. Three subjects with Turner syndrome had co-morbidities (solitary left kidney, horse shoe kidney and aortic valve abnormality in one subject each). One adolescent with delayed puberty had complete androgen insensitivity synd-rome and was initiated on oral estrogen.

In our series, normal variants predominated among the cases. A similar prevalence of 17.9% to 68% of normal variants was observed earlier [1,4]. Puberty being an important milestone in southern India leads to increased health seeking behavior of families. Boys with delayed puberty have low self-esteem and reduced peer contact leading to earlier health seeking behavior [10]. Hence, we observed a higher male preponderance in HH. Need for pituitary hormone replacement in majority of subjects with HH and significant co-morbidities in few with hypergonadotrophic hypogonadism, highlights the need for detailed systemic evaluation in delayed puberty.

Ethics clearance: Mehta Multispeciality Hospitals IEC; No. IRB-MCH/10/2017, dated April 4, 2018.
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AARTHE SELVARAJ,[1] HEMCHAND KRISHNA PRASAD,[1]* KANNAN NARAYANASAMY,[1] ANAND THIAGARAJAN,[2] NEDUNCHELIAN KRISHNAMOORTHY[1]
Departmentss of [1]Pediatrics and [2]Radiology, Mehta Multispeciality Hospitals India Pvt Ltd, Chennai, Tamil Nadu.
**pediatricendocrinology.mehta@gmail.com*

REFERENCES

1. Sedlmeyer IL, Palmert MR. Delayed puberty: Analysis of a large case series from an academic center. J Clin Endocrinol Metab. 2002;87:1613-20.
2. Toublanc JE, Roger M, Chaussain JL. Etiologies of late puberty. Horm Res. 1991;36:136-40.
3. Reindollar RH, Byrd JR, McDonough PG. Delayed sexual development: A study of 252 patients. Am J Obstet Gynecol. 1981;140:371-80.
4. Bhakhri BK, Prasad MS, Choudhary IP, Biswas K. Delayed puberty: Experience of a tertiary care centre in India. Ann Trop Paediatr. 2010;30:205-12.
5. Stanhope R, Preece MA. Management of constitutional delay of growth and puberty. Arch Disease Child. 1988;63:1104-10.
6. Marshall WA, Tanner JM. Variations in pattern of pubertal changes in girls. Arch Dis Child. 1969;44:291-303.
7. Khadilkar VV, Khadilkar AV, Kinare AS, Tapasvi HS, Deshpande SS, Maskati GB. Ovarian and uterine ultrasonography in healthy girls between birth to 18 years. Indian Pediatr. 2006;43:625-30.
8. Harrington J, Palmert MR. Clinical review: Distinguishing constitutional delay of growth and puberty from isolated hypogonadotropic hypogonadism: Critical appraisal of available diagnostic tests. J Clin Endocrinol Metab. 2012;97:3056-67.
9. Prasad HK, Khadilkar VV, Jahagirdar R, Khadilkar AV, Lalwani SK. Evaluation of GnRH analogue testing in diagnosis and management of children with pubertal disorders. Indian J Endocrinol Metab. 2012;16:400-05.
10. Lee PD, Rosenfeld RG. Psychosocial correlates of short stature and delayed puberty. Pediatr Clin North Am. 1987;34:851-63.

Clinical Profile and Outcome of COVID-19 Among Immunocompromised Children

This retrospective study describes the clinical profile, risk of infection and outcome of coronavirus disease-19 in immunocompromised children. It was found that children on immunosuppressant medication has 2.89 times increased risk of infection (*P*=0.01). Disease manifestation was asymptomatic (*P*=0.01) or mild with predominant gastrointestinal symptoms (*P*=0.02) without alteration in immunosuppressive treatment regime.

Key words: *COVID-19, Children, Immunocompromised, Outcome.*

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection in children manifests as mild to moderate disease with low fatality [1]. There is limited information about susceptibility to SARS-CoV-2 infection, clinical profile and outcome among immunocompromised children in India. Published literature has revealed milder disease and favorable outcome without discontinuation of immunosuppressive medication in these children [2-6]. We, herein describe the clinical features and outcome of immunocompromised children diagnosed with SARS-CoV-2 infection at our center.

This was a retrospective analysis of data from 1 June to 31 October, 2020 at a tertiary care center of a teaching hospital. The analysis was approved by institutional ethics committee. All children admitted during the study period were screened for SARS-CoV-2 infection and those who were diagnosed with coronavirus disease 19 (COVID-19) were classified into immunocompromised and immunocompetent. All immunocompromised children in the present study were either on anticancer drugs or immunosuppressive therapy for kidney diseases, and fulfilling criteria for immunocompromised state [6]. The variables extracted from the hospital records were age, gender, presenting symptoms, complications, severity of illness, diagnosis, laboratory investigations, imaging findings, treatment and outcome. The diagnosis of COVID-19 was made by positive reverse transcription polymerase chain reaction (RT-PCR) test for SARS-CoV-2. The severity of illness was classified as per guidelines given by Ministry of Health and Family Welfare, Government of India [7], and children were treated as per unit protocol without alteration in immunosuppressive treatment regime. Proportions were compared using Fisher exact test.

Data of 409 children were extracted during the study period; 162 (396%) of these were immunocompromised. Of the 409 children, 26 (6.3%) were diagnosed with SARS-CoV-2. Data of one child was incomplete and he was excluded from the analysis. The proportion of SARS-CoV-2 positivity in immunocompromised and immunocompetent children was 16 (9.9%) and 9 (3.6%), respectively [OR (95% CI) 2.89 (1.24-6.73); *P*=0.01]. Comorbidities were present in 184 (44.9%) children, mainly malignancy in 78 (18.9%) and nephrotic syndrome (NS) in 67 (16.3%). The clinical profile and outcome of children with COVID-19 is shown in **Table I**. All children with severe COVID-19 disease had features of sepsis and shock; however, 3 children had acute respiratory distress syndrome (ARDS). Only 12 children with COVID-19 underwent blood biochemistry, and reports revealed anemia (*n*=6), leucopenia (*n*=5), neutrophil-lymphocyte ratio>3 (*n*=5) and thrombocytopenia in 3 children. High resolution computed tomography of chest showed ground glass opacities in two and air-bronchogram in three children. One child presented with severe abdominal pain and computed tomography of abdomen was suggestive of pancreatitis, he also had elevated amylase (217µ/L) and lipase (365µ/L).

Table I Clinical Profile and Outcome of Children with SARS-CoV-2 Infection

Variables	*Children with COVID-19*	
	Immuno-competent (n=9)	*Immuno-compromised (n=16)*
Comorbidity[a]	5	16
Severity of illness		
Asymptomatic[b]	0	8 (50)
Mild	0	3 (18.7)
Moderate	5 (55.5)	3 (18.7)
Severe (*n=6*)		
Septic shock	4 (44.4)	2 (12.5)
ARDS	2 (22.2)	1 (5.9)
Clinical features		
Fever	9 (100)	7 (43.7)
Cough and difficulty in breathing	8 (88.8)	3 (18.7)
Diarrhea and vomiting	0	7 (41.1)
Convulsion and altered sensorium	3 (33.3)	0
Abdominal pain	2 (22.2)	5 (29.4)
Treatment[d]		
Hospitalization	9 (100)	8 (50)
Oxygen	4	2
Vasopressor requirement	4	2
Died	2 (20)	2 (12.5)

Data presented as no. or no. (%). [a]Immunocompetent:1 each with thalassemia, Wilson disease, diabetes, epilepsy and congenital hydrocephalous with shunt; Immunodeficient: 8 children with nephritic syndrome, 2 with Non-Hodgkin lymphoma and 1 each of Hodgkin lymphoma, acute lymphoblastic leukemia, acute myeloid leukemia, myelodysplastic syndrome, aplastic anemia and retinoblastoma, [d]Only one immunocompetent patient required ventilation.

Immunocompromised children had a significantly higher risk of SARS CoV-2 infection which may be due to the need of frequent hospital visits for their medications (chemotherapy), which exposed them to get infection. However, they had a lower hospitalization rate [8 (50%) vs 9 (100%), *P*=0.01] as compared to immunocompetent children possibly because of day care

treatment protocol. The occurrence of disease was lesser in immunocompromised children, with most being either asymptomatic or with mild disease, and with complete recovery. It is possibly due to weaker immune response under the influence of immunosuppressants. Similar observations were reported in a survey conducted in 25 countries (>200 children tested and 10000 at risk) among children on anticancer treatment [8] and in a systematic review of 16 articles (100 adults, 10 children) by Minotti, et al. [5], which concluded that these children had asymptomatic or mild disease, and had a favorable outcome. Severe disease manifestations and require-ment of intensive care, respiratory support, and inotropes were comparable between the two groups. Overall mortality was 16% and this might be a reflection of high incidence of non-COVID sepsis and associated complications in these patients. In a study on 113 children with kidney diseases receiving immunosuppressive medications from 30 countries; authors found that only 9.7% had severe grade of disease [3]. Features of relapse, or new organ involvement (pancreas) or new onset glomerulonephritis have been seen in children with nephrotic syndrome in present study and this might be like other viruses, SARS Co-V-2 infection may also precipitate relapses or infect new organ [9,10].

The present study has few limitations, one being retrospective analysis and small sample size, and short term follow-up. Additionally, admitting policy kept on changing during the study period depending on government guidelines. We conclude that children with immunosuppressant medication are at an increased risk of SARS Co-V-2 infection, and disease manifestations may be asymptomatic or mild with predominantly gastrointestinal symptoms.

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SUNIL KUMAR RAO,* ASHUTOSH KUMAR, RAJNITI PRASAD, VINEETA GUPTA, OM PRAKASH MISHRA
Department of Pediatrics,
IMS-BHU, Varanasi,
Uttar Pradesh.
**10111drsunilrao21@gmail.com*

REFERENCES

1. Meena J, Yadav J, Saini L, et al. Clinical features and outcome of SARS-CoV-2 infection in children: A systematic review and meta-analysis. Indian Pediatr. 2020;57:820-26.
2. Marlais M, Wlodkowski T, Vivarelli M, et al. The severity of COVID-19 in children on immunosuppressive medication. Lancet Child Adolesc Health. 2020;4:e17-18.
3. Marlais M, Wlodkowski T, Al-Akash S, et al. COVID-19 in children treated with immunosuppressive medication for kidney disease. Arch Dis Child. 2020 Dec 21;archdischild-2020-320616 (Online ahead of print).
4. El Dannan H, Al Hassani M, Ramsi M. Clinical course of COVID-19 among immunocompromised children: A clinical case series BMJ. Case Rep. 2020;13:e237804.
5. Minotti C, Tirelli F, Barbieri E, et al. How is immunosuppressive status affecting children and adults in SARS-CoV-2 infection? A systematic review. J Infection. 2020;81: e61-66.
6. Vasudevan A, Mantan M, Krishnamurthy S, et al. Managing children with renal diseases during the COVID-19 pandemic. Indian Pediatr. 2020;57:641-51.
7. Government of India, Ministry of Health and Family Welfare Guidelines on clinical management of COVID-19. Directorate General of Health Services (EMR Division); p. 3-5. Accessed December 23, 2020. Available from: *https://www.mohfw.gov.in/pdf/ Guidelines on Clinical Management of COVID 1912020.pdf*
8. Hrusak O, Kalina T, Wolf J, et al. Flash survey on severe acute respiratory syndrome coronavirus-2 infections in paediatric patients on anticancer treatment. Euro J Cancer. 2020;132:11-16.
9. Alvarado A, Franceschi G, Resplandor E, et al. COVID-19 associated with onset nephrotic syndrome in a pediatric patient: Coincidence or related conditions? Pediatr Nephrol. 2021;36:205-07.
10. Harambat J, Allard L, Godron-Dubrasquet A. Relapse rate of nephrotic syndrome in the time of COVID-19. Pediatr Nephrol. 2021; 36:211-12.

CLIPPINGS

A comparison of post-vaccination hepatitis B surface antibody level on the large and appropriate for gestational age infants (*Clin Exp Vaccine Res. 2021;10:47-51*)

Hepatitis B infection is the most common cause of chronic hepatitis leading to cirrhosis and carcinoma. Perinatally acquired hepatitis B infection has the highest rates of progression from acute to chronic disease compared to an infection at later age. Hepatitis B vaccination is important in reducing the global burden of the disease. The researchers enrolled 132 infants aged 7 months, one month after the last (4th) dose of recombinant DNA hepatitis B vaccine containing pentavalent vaccine. All infants were negative for hepatitis B surface antigen and hepatitis B core antibody. On the basis of birthweight, participants were divided into group 1: weighing between 2-4 kg (*n*=63), appropriate for gestational age, and group 2: weighing >4kg (*n*=69), large for gestational age. Anti-Hbs antibody titers ≥10 IU/L were taken as adequate. Mean birthweight of the groups was 2.98 (0.53) and 4.19 (0.19) kg, respectively. The mean (SD) anti-HBs antibody titer in group 1 and 2 was 13701.0 (11744.4) and 8997.1 (2827.2), respectively (95% CI of difference, -7607.4 to -1800.2; *P*=0.002), even though the titers were in protective range in both the groups.

RAJESH KUMAR MEENA
raj.mamc@gmail.com

Hyperinsulinemic Hypoglycemia in Neonates Due to Perinatal Stress: A Case Series

Hyperinsulinemia (HI) is a well-known cause of persistent hypoglycemia in neonates. Early diagnosis is crucial, as management needs to be aggressive because insulin blocks alternative fuels like ketones for cerebral metabolism resulting in long-term neurological sequelae. It can be persistent or transient. The persistent forms are inherited and are due to mutations in genes associated with insulin secretions regulation. The transient state; however, is non-genetic and also known as perinatal stress-induced hyperinsulinemia (PSHI). Various factors associated with perinatal stress are intrauterine growth restriction, birth asphyxia, cesarean delivery, and maternal toxemia that can lead to HI [1]. Collins and Leonard in 1984 reported cases of small for gestational age and birth asphyxia, who responded well to medical therapy, followed by spontaneous resolution [2].

We retrospectively analyzed the medical records of newborns who presented with hypoglycemia (lethargy, poor suck, or poor feeding) and biochemical evidence of hyperinsulinemia in the neonatal intensive care unit. Hyperinsulinemic hypoglycemia was defined as hypoglycemia (<50 mg/dL) with inappropriately elevated plasma insulin (>3 mIU/mL) and/or evidence of an excessive insulin effect, such as an increased glucose consumption rate (>8 mg/kg/min) and inappropriately suppressed plasma β-hydroxybutyrate (<2 mmol/L) [3]. Out of 111 babies with hypoglycemia, 14 (12.6%) babies were diagnosed to have hyperinsulinemia. All the babies were treated initially with intravenous glucose requiring a median glucose infusion rate (GIR) of 12 mg/kg/min. Diazoxide was started as soon as the diagnosis of hyperinsulinemia was made. To wean off the baby from intravenous glucose, oral diazoxide was initiated as first-line medication – 11 (78.6%) patients responded to it. Three (21.4%) babies did not respond even after maximum doses of diazoxide, following which a second-line drug, octreotide, was added. All three babies who required octreotide treatment were small for gestational age (SGA). The maximum dose of octreotide required was 14 mcg/kg/day along with diazoxide. Duration of therapy for diazoxide with octreotide ranged from 7 to 14 days. All three babies tolerated octreotide well with no adverse events.

Most of the babies were an early term with a male to female ratio of 1.3:1. Early term (71%), SGA (43%), cesarean section (71%), and fetal distress (28%) were found as risk factors for PSHI in this series. Each baby had one or more mentioned risk factors. Hoe, et al. [4] in their study of 26 neonates with prolonged HI, found it to be frequently associated with the male sex, low birth weight, perinatal stress, and cesarean deliveries; however, they could not find any risk factor in 19% of babies. Insulin levels in all our babies were elevated, with a median insulin level of 13.6 mU/L, along with hypoketonemia. However, there are reports of perinatal stress-induced hyperinsulinemia with normal insulin levels, specifically in SGA babies [4]. Response to diazoxide was consistent with previous findings [4]. In the study by Hoe, et al. [4] only 2 out of 26 babies were started on octreotide. All 3 of them were born by cesarean section and were SGA. In 2 babies, octreotide was added at a maximum diazoxide dose of 10 mcg/kg/day as they required some dextrose support to maintain their blood sugars. In one baby, octreotide was added at a maximum diazoxide dose of 15mcg/kg/day as he intermittently maintained blood sugars on feeds and diazoxide. Other authors have reported treatment time ranging from 18 to 402 days. In our study, 100% responded to medical treatment, whereas it was 80-95% in published literature [4,5]. Pan, et al. [6] reported their experience of octreotide therapy for HI in 7 cases of SGA neonates with treatment duration between 9 to 45 days, and with an excellent response to treatment in all patients. As all babies remained admitted till the treatment was completed and responded well to medications, we suspected it due to perinatal stress; therefore, genetic analysis was not considered.

To summarize, the requirement of a high glucose infusion rate in a neonate should raise suspicion for HI. Routine glucose screening of high-risk neonates can help in its early identification. Identification and appropriate treatment of a neonate with HI are essential to prevent long-term neurologic sequelae. Unlike congenital HI, neonates with perinatal stress-induced hyperinsulinism should recover with medical management within a few days to few weeks. However, larger series are required to draw firmer conclusions.

APARNA PRASAD, KUMAR ANKUR* AND SANJEEV CHETRY
Department of Neonatology,
BLK Superspeciality Hospital, Pusa Road, New Delhi.
**sahankur@gmail.com*

REFERENCES

1. Vora S, Chandran S, Rajadurai VS, et al. Hyperinsulinemic hypoglycemia in infancy: Current concepts in diagnosis and management. Indian Pediatr. 2015;52:1051-9.
2. Collins JE, Leonard JV. Hyperinsulinism in asphyxiated and small-for-dates infants with hypoglycaemia. Lancet. 1984;2:311-3.
3. Stanley CA. Hyperinsulinism in infants and children. Pediatr Clin North Am. 1997;44:363-74.
4. Hoe FM, Thornton PS, Wanner LA, et al. Clinical features and insulin regulation in infants with a syndrome of prolonged neonatal hyperinsulinism. J Pediatr. 2006;148:207-12.
5. Agladioglu SY, Savaþ Erdeve S, Cetinkaya S, et al. Hyperinsulinemic hypoglycemia: experience in a series of 17 cases. J Clin Res Pediatr Endocrinol. 2013;5:150-5.
6. Pan S, Zhang M, Li Y. Experience of octreotide therapy for hyperinsulinemic hypoglycemia in neonates born small for gestational age: A case series. Horm Res Paediatr. 2015;84:383-7.

CORRESPONDENCE

Severe Headache in Emergency Room: Migraine or Digital Eye Strain

Among primary headaches in children, tension type headache and migraine form the most common causes of headache. With the increasing use of digital devices globally, digital eye strain (DES) or computer vision syndrome (CVS) has been increasing, with the 2016 digital eye strain report documenting a self-reported prevalence of nearly 65% [1]. Headache has been considered to be one of the five most common symptoms associated with DES according to American Optometric Association [2].

A 14-year-old girl presented to the emergency room with the complaints of severe bitemporal headache with heaviness in eyes, vomiting and undocumented fever for past 15 days. The headache was severe enough to hinder studies, and she had to quit her online examinations due to the headache. Vitals of the patient were within normal ranges and she was afebrile during hospital stay. No signs of meningeal irritation were present. Fundus evaluation was normal. Lumbar puncture and magnetic resonance imaging of brain were done to rule out causes of secondary headache, and were found to be normal. A provisional diagnosis of migraine without aura was made but there was neither previous history of such attacks nor any positive family history. Since the girl had a history of watering of eyes while watching television (TV), an ophthalmic evaluation was performed that revealed dry eyes and a refractory error of -0.25D in both eyes. On further detailed history, it was found that the adolescent was having a screen time of 7 hours daily for past 10 months (4 hours of online classes on smartphone due to the pandemic and 3 hours of TV watching). A computer vision syndrome questionnaire (CVS-Q) [3] was used to rule out digital eye strain as the cause of headache, and the total score was found to be 18 indicating severe CVS. Initially the patient was given oral analgesics and was advised to have a reduced screen time for next 4 weeks. After one week, the analgesics were stopped. Presently the patient is asymptomatic.

Educational screen use, with appropriate precautions, was advised. The symptomatology of DES or CVS can be related to extraocular, ocular surface or accommodative mechanism leading to severe headache [4]. So objective visual assessment of such patients should not be limited to the assessment of refractory error alone but should also include an orthoptic vision screening for detecting errors of accommodation including unilateral and alternate cover and uncover tests at near vision [5]. Even small aberrations in these tests can lead to symptoms, and may continue progressing uncorrected.

SANDHYA CHAUHAN* AND HIMANSHU GARG
Department of Pediatrics,
Sri Ram Murti Smarak Institute of Medical Sciences,
Bareilly, Uttar Pradesh.
**drsandhyapedia@gmail.com*

REFERENCES

1. Sheppard AL, Wolffsohn JS. Digital eye strain: Prevalence, measurement and amelioration. BMJ Open Ophthalmol. 2018; 3:e000146.
2. American Optometric Association. Computer vision syndrome 2017. Available from: *https://www.aoa.org/patients-and-public/caring-for-your-vision/protecting-your-vision/computer-vision-syndrome?sso=y*
3. Seguí MdM, Cabrero-García J, Crespo A, et al. A reliable and valid questionnaire was developed to measure computer vision syndrome at the workplace. J Clinical Epidemiol. 2015;68:662-73.
4. KY Loh, Redd SC. Understanding and preventing computer vision syndrome. Malays Fam Physician. 2008;3:128-30.
5. Wallace DK, Morse CL, Melia M, et al. Pediatric eye evaluations preferred practice pattern: I. Vision screening in the primary care and community setting; II. Comprehensive ophthalmic examination. Ophthalmology. 2018 ;125:184-227.

Non-availability of Parenteral Preparations of Vitamin A: Is a Silent Surge of Bronchopulmonary Dysplasia Happening in India?

Bronchopulmonary dysplasia (BPD) continues to be one of the most important challenges in the care of the preterm infants, affecting approximately one-quarter of very low birth weight infants [1]. With better availability and improved quality of care of neonatal intensive care units (NICUs) in India, more and more such babies are surviving. As vitamin A is accumu-lated mainly in the third trimester, preterm infants may have low vitamin A levels at birth, which may contribute to an increased risk of developing BPD. With the large number of preterm babies surviving, there is possibility of an increase in number of BPD cases.

Most of randomized trials to study efficacy of vitamin A supplementation to prevent BPD used parenteral preparations. Globally, trials testing efficacy of oral vitamin A supplemen-tation in preventing BPD has not shown its role [2].

During the last few years there is increasing difficulty in getting intramuscular preparations of vitamin A. Thus currently

virtually no vitamin A injections are available in Indian market, making it one of latest addition in orphan drugs. India still lacks appropriate policy framework for orphan drugs, making a country-specific Orphan Drugs Act (ODA), need of the hour [2]. Well-designed multicenter trials should be done in Indian setting to study role of oral vitamin A in preventing BPD. Until efficacy of oral vitamin A is proved, Indian Academy of Pediatrics should engage with the government to ensure easy availability of injection vitamin A throughout the country.

SHAHID AKHTAR SIDDIQUI* AND MANISHA MAURYA
Department of Pediatrics, SN Children Hospital, MLN Medical College, Allahabad, Uttar Pradesh.
**sha.akht@yahoo.com*

REFERENCES

1. Guimarães H, Guedes MB, Rocha G, et al. Vitamin A in prevention of bronchopulmonary dysplasia. Curr Pharm Des. 2012; 18: 3101-13.
2. Rakshasbhuvankar AA, Simmer K, Patole SK, et al. Enteral vitamin A for reducing severity of bronchopulmonary dysplasia: A randomized trial. Pediatrics. 2021;147:e2020009985.
3. Reddy DS, Pramodkumar TM, Reddy Y, et al. Orphan regulations for orphan drug development in India. Asian J Pharm. 2014;8: 130-2.

Dexmedetomidine vs Midazolam for Sedation in Mechanically Ventilated Children: Few Concerns

We read with interest the recently published research paper on dexmedetomidine vs midazolam for sedation in mechanically ventilated children [1]. We have the following concerns related to the study.

The recommended approach for noninferiority trials is to perform both intention to treat and per protocol analysis and to conclude noninferiority if both analysis produce the same result [2]. Although we could infer from the study flow chart that per protocol analysis was done, but there could be doubt in the minds of the readers if modified intention to treat or per protocol analysis was done. The estimated sample size in the methods section is written as 39 per group whereas in the discussion section the intended sample size is written as 36 in each group. Bradycardia in dexmedetomidine group is mentioned as 17.4% in the results section as well as in the fourth paragraph of discussion section while it is mentioned as 14.4% in the first paragraph of discussion section.

We understand your concern of not giving bolus of dexmedetomidine in your study to avoid bradycardia and hypotension as it has been reported in many studies. There have been few pediatric randomized control trials in which bolus dose of dexmedetomidine was given and there was no difference in the occurrence of bradycardia and hypotension and they found that the rate of adequate sedation was higher in the dexmedetomidine group with lower requirement of rescue drugs and shorter onset of sedation time [3]. We are of the opinion that not giving bolus dose of dexmeditomidine could have been a contributory factor in non-establishment of non-inferiority of dexmedetomidine as compared to midazolam in your study, and this point could have been discussed in the discussion section.

SATVIR SINGH AND DAISY KHERA*
Department of Pediatrics, AIIMS, Jodhpur, Rajasthan.
**daisykhera78@gmail.com*

REFERENCES

1. Gulla KM, Sankar J, Jat KR, et al. Dexmedetomidine vs midazolam for sedation in mechanically ventilated children: A randomized controlled trial. Indian Pediatr. 2021;58:117-22.
2. Hahn S. Understanding noninferiority trials. Korean J Pediatr. 2012;55:403-07.
3. Koroglu A, Demirbilek S, Teksan H, et al. Sedative, haemodynamic and respiratory effects of dexmedetomidine in children undergoing magnetic resonance imaging examination: preliminary results. Br J Anaesth. 2005;94:821-24.

AUTHORS' REPLY

We thank the readers for their interest in our study [1]. The analysis was a per-protocol analysis; the same is highlighted in the study flow chart.

The errors in discussion section in the values of adverse events in dexmedetomidine group as well as the sample size are typographical errors.

The authors have opined that not giving bolus dose of dexmedetomidine could have been a contributory factor in non-establishment of non-inferiority of dexmedetomidine as compared to midazolam in our study. The median (IQR) duration of dexmedetomidine infusion was 26 (14, 48) hours and even without bolus dose, the serum levels of the drug are likely to be in the therapeutic range to cause desired sedation. Moreover, the frequency of adverse events in the dexmedetomidine group argue against the lack of therapeutic levels. Hence, we feel that bolus dose of dexmedetomidine would not have changed the outcomes.

KRISHNA MOHAN GULLA AND RAKESH LODHA*
Division of Pediatric Pulmonology and Intensive Care, Department of Pediatrics AIIMS, New Delhi.
**rakesh_lodha@hotmail.com*

REFERENCES

1. Gulla KM, Sankar J, Jat KR, et al. Dexmedetomidine vs midazolam for sedation in mechanically ventilated children: A randomized controlled trial. Indian Pediatr. 2021;58:117-122.

Analysis of Young Infant Deaths Using Verbal Autopsies: Clarifications Needed

We compliment the authors on their work on verbal autopsies for infant deaths [1]. We seek the following clarifications:

i) The study mentions about a State verbal autopsy tool, but no details of it could be found either in the text or the references.

ii) We want to know source of individual death information, which was crucial for timely execution of verbal autopsy tool. What was number and qualification of the field workers? There is no mention of parental consent before collecting the information.

iii) Authors mention that clinical summaries of babies were collected from health posts near their residence. Were they public/private hospitals/clinics? What if they refused to provide confidential data to field workers? Even if they had access to individual case sheets of all babies who died, how did they interpret it in the absence of a pediatrician on site?

iv) The study mentions an infant mortality rate of 5.2 per 1000 live births, which is much below the national average. Kindly elaborate.

v) Discussion section mentions about the 108 neonatal ambulance service, but the number 108 is for general ambulance and 102 is the number for dedicated maternal and neonatal/infantile services.

SHAHID AKHTAR SIDDIQUI* AND MUKESH VIR SINGH
Department of Pediatrics, SN Children Hospital, MLN Medical College, Prayagraj, Uttar Pradesh.
**sha.akht@yahoo.com*

REFERENCE

1. Devi RU, Bharathi SM, Kumutha J. Analysis of young infant deaths using verbal autopsies and accuracy of verbal autopsy tool in Chennai, India. Indian Pediatr. 2021;58:363-66.

AUTHORS' REPLY

We thank the readers for their interest in our article [1], and provide the desired information:

i) The contents of verbal autopsy form are available at *https://nhm.gov.in/images/pdf/programmes/childhealth/guidelines/Operational_Guidelines_Child_Death_Review.pdf* and have been modified in the Tamil Nadu State verbal autopsy form to include health seeking behavior, each cause of death based on symptoms or problems in the child. The State verbal autopsy tool has maternal characteristics as well as neonatal characteristics. For neonatal deaths as well as post neonatal deaths, there are set of questions under each cause of deaths and depending upon the answers, one of the major causes is selected. Probable main cause of death (only one cause) is to be given at the end.

ii) All young infant deaths (< 2 months) between March, 2013 and March, 2015 were line-listed as per government orders. Investigation of the same was done using the State verbal autopsy forms for infant deaths. Line-listing of young infant deaths is routinely done by field workers assigned in each Chennai corporation zone, and it was not done solely for the purpose of the study. Investigation of these deaths are done using verbal autopsy forms after getting informed consent from the parents by the field workers (auxiliary nursing midwives, ANM), also called as urban health nurse in urban area. These health workers had finished the ANM course and had undergone a two-week training on verbal autopsy.

iii) Permission for procuring line listing as well as verbal autopsy questionnaires from all zones of Chennai Corporation for the purpose of our study was obtained from the Deputy Project coordinator, district family welfare, Chennai Corporation. Babies having residence within Chennai Corporation zones and who died within 2 months of age between April, 2013 and March, 2015 were included in the study.

Deaths that occurred at home and private hospitals were also line-listed and included. Case sheets of those collected and scrutinized before the district level verbal audit of infant deaths (government, private, home deaths) done bi-monthly and the physician who treated the baby also attends the audit. The case sheet details and clinical diagnoses of deaths in private setup were noted by us for the study. In case of home deaths, diagnosis as given at arrival to hospital or in the death certificate was taken.

iv) We have provided the "young" infant (<2 months) mortality rate in the article and not the infant mortality rate. Total live births among the residents of Chennai corporation zones were 164009, out of which there were 865 young infant deaths.

v) Regarding transport, 108 ambulance service is utilized for neonatal services as well in Tamil Nadu, with the calls requesting for neonatal services getting filtered and channelized separately.

MANGALA BHARATHI S,* USHA DEVI R
Department of Neonatology, Institute of Child Health and Hospital for Children, Madras Medical College, Chennai, Tamil Nadu.
**drmangalabharathi@gmail.com*

REFERENCE

1. Devi RU, Bharathi SM, Kumutha J. Analysis of young infant deaths using verbal autopsies and accuracy of verbal autopsy tool in Chennai, India. Indian Pediatr. 2021;58:363-66.

Parent-mediated Training for Children with Autism Spectrum Disorder in India

In India, prevalence of autism spectrum disorder (ASD) is estimated to be 1-1.4% (1 in 100 children) in children aged 2-9 years [1]. However, there continues to be a deficit of trained therapists to address these children [2]. Parent-implemented interventions with adequate guidance from trained therapists could be the best alternative for young ASD children [3]. We have developed an online intervention program (SCoPE-EDITT: Socio Communication Play and Educational Program-Educating Parents on Direct and Interactive Teaching Techniques) to involve and support parents in the early intervention of children with ASD (or at risk for ASD). Parent-implemented intervention also extends the benefits of intervention to the home environment thereby supporting in generalization.

The purpose of this pilot study was to find whether the short term parental training brings about changes in parental interactive behavior and child engagement skills and also to assess effectiveness of the SCoPE program. Participants were 22 young children (Mean age 27.3 months) with diagnosis of ASD/at risk for ASD. Before beginning the intervention, baseline data on children's language and cognitive skills was assessed using Mullen Scale of Early Learning (MSEL). Parents were asked to play and interact with their child in their usual way for 10 min in the centre and it was videotaped. Parents' baseline interactive behavior and children's engagement skills were observed and rated using Modified Maternal Behaviour Rating Scale (MBRS) and Modified Child Behaviors Rating Scale (CBRS) [7]. CBBS identiûes two domains (attention domain and initiation domain) of children's interactive engagement with their parents. In our study, CBRS scale was modified by excluding affect and persistence items.

Parents were trained over 4 weeks to learn and use interactive parenting techniques with structured EDITT program and suggested to use individualized SCoPE based activities during homework sessions. All children continued regular weekly (weekly twice for 45 minutes) language therapy sessions (with/without sensory integration sessions based on individual child requirement) with trained therapists. Parents and their children were followed up for next 9 months. After 1 month of EDITT parental training program, parents showed significant improvement in all the interactive behaviors, and decline in directive behaviour parameters of MBRS. At 3 months follow up, parents showed significant improvement in their interactive behavior and further decline in directive behaviors (Sensitivity d=4.87, Responsiveness d=3.78, Effectiveness d=3.37, Achievement d=3.78, Reinforcing behaviour d=3.08 and Directiveness d=-2.74) with maximum improvement in their sensitivity towards their children.

At 1 month and 3 months follow up, children showed significant improvement in their engaging skills in all the parameters of Modified CBRS, Attention d=4.16, Cooperation d=2.8, Involvement d=2.5, Initiating behaviors d=1.94). Children showed maximum improvement in their attention behavior and least improvement in their initiating behavior.

At 9 months follow up, children showed significant improvement (P<0.01) in language and cognitive domains of MSEL. On average, children showed 12.3 months improvement in receptive language, 10.7 months improvement in expressive language and 9.6 months improvement in cognitive domain over 9 months of SCoPE based intervention. 66% children showed improvement equivalent or more than 9 months in all domains.

As per existing scientific evidence, active involvement of caregivers with appropriate supervision and training as a form of co-therapy is desirable but there is not much evidence for the efficacy of parent-mediated approaches [4]. Our study adds evidence to efficacy of parent mediated approach. SCoPE-EDITT program not only empowered the parents to serve as co therapists but also facilitated joint attention, imitation skills, language and cognitive development in the children with ASD.

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KIRTHIKA RAJARAMAN,* NANDINI MUNDKUR
Center for Child Development and Disabilities, Bengaluru, Karnataka, India.
**rkirthi83@gmail.com*

REFERENCES

1. Arora NK, Nair MKC, Gulati S, et al. Neurodevelopmental disorders in children aged 2-9 years: Population-based burden estimates across five regions in India. PLoS Med. 2018; 15: e1002615.
2. Mahapatra P, Pati S, Sinha R, et al. Parental care-seeking pathway and challenges for autistic spectrum disorders children: A mixed method study from Bhubaneswar, Odisha. Indian J Psychiatry. 2019;61:37-44.
3. Zwaigenbaum L, Bauman ML, Choueiri R, et al. Early intervention for children with autism spectrum disorder under 3 years of age: Recommendations for practice and research. Pediatrics. 2015; 136:S60-81.
4. Mahoney G, Kim J, Lin C. Pivotal behavior model of developmental learning. Infants Young Child. 2007;20:311-25.
5. Oono IP, Honey EJ, McConachie H. Parent mediated early intervention for young children with autism spectrum disorders (ASD). Evid Based Child Health. 2013;8: 2380-479.

Messaging Applications: Implications for Healthcare

In the last two decades, there has been an increase in the ownership of mobile devices like tablets, mobile phones, and computers. The constantly improving networking service worldwide is transforming the healthcare sector with the introduction of eHealth or mHealth – the use of mobile devices for health research and delivery [1]. Some of these include Facebook messenger, iMessage, Koo, Signal, Telegram, WeChat and WhatsApp.

The coronavirus disease 2019 (COVID-19) has disrupted the conventional ways of practicing medicine and demanded socially distant healthcare practice. Recently, the Government of India issued a telemedicine guideline that said, "telemedicine includes all communication channels with the patient that leverage information technology platforms, including voice, audio, text and digital data exchange " [2]. In this situation, WhatsApp came in as a handy tool to consult not only for routine check-ups but also for consultation and monitoring of COVID-19 patients [3]. The World Health Organization (WHO) health alert service also uses WhatsApp to give people up-to-date COVID-19 information.

Various scenarios show that using messaging apps can accelerate communication, streamline workflows, enhance productivity, and improve patient healthcare [4]. They help improve hospital administration efficiency, secure hand-off, faster delivery of laboratory reports, create a discussion thread with seniors and juniors, and collaborate with those outside their workplace easily with seamless integration of health records, and reach healthcare workforce in the peripheral areas [4].

India is a developing nation, and doctors with minimum access to large funds refrain from using professional EMR platforms and use what is freely available and convenient. Platforms like Magpi, Google forms, Survey monkey can be used to collect text and numerical data but when it comes to collecting pictures and videos of the patients, messaging apps are the most convenient option, in addition to being used by researchers to send out survey forms to potential participants.

Despite the benefits, the use of telemedicine has always been concerning due to the lack of clear guidelines in India, unlike some countries like the USA where a healthcare professional can only use a HIPAA compliant platform for telemedicine. Third-party apps like iMessage do not have access to user info, it is not the case with WhatsApp business accounts anymore, making users migrate to other more privacy-oriented appli-cations. Even end to end encryption poses a problem as no data is being stored by WhatsApp (only saved locally on the users' device) which makes it nearly impossible to audit. With messaging applications, it might become very difficult to keep a record of patient data. If either user changes devices, it might lead to complete loss of data unless a full backup is done prior to the shift, which cannot be done remotely. Use of multiple messaging platforms by various professionals may not allow crosstalk between them. Also, app performance is in the control of the company, which may terminate or modify it, based on corporate priorities.

In the current situation, messaging apps can also be used for patient education services like by sharing links to reliable sources of information. The acceptance of messaging is better if the doctor and patients know each other. If it is the first consult, then having a preliminary round of introductions may help build trust. Having ground rules for communication such as when will the clinician respond and the charges for communication via this medium, should be conveyed early on.

Messaging applications can be used in the future for regular follow up of patients. With the development of patient support groups for chronic as well as rare disease using messaging groups would be of excellent use. As capabilities of messaging apps get enhanced, including artificial intelligence, there may be possibilities and threats, which are difficult to foresee presently.

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SOMASHEKHAR NIMBALKAR,* PRIYANKA PARIKH
Department of Neonatology,
Pramukhswami Medical College,
Bhaikaka University, Karamsad 388 325,
Gujarat.
**somu_somu@yahoo.com*

REFERENCES

1. Langford AT, Solid CA, Scott E, et al. Mobile phone ownership, health apps, and tablet use in US adults with a self-reported history of hypertension: Cross-sectional study. JMIR Mhealth Uhealth [Internet].2019;7:e12228.
2. National Medical Commission. Telemedicine Practice Guidelines. NMC, 2020. Accessed June 2, 2021. Available from: *https://www.mohfw.gov.in/pdf/Telemedicine.pdf*
3. Sabýrlý R, Karsli E, Canacik O, et al. Use of WhatsApp for polyclinic consultation of suspected patients with COVID-19: Retrospective case control study. JMIR Mhealth Uhealth [Internet]. 2020;8:e22874.
4. Opperman CJ, Vuuren MJ van. WhatsApp in a clinical setting: The good, the bad and the law. South African Journal of Bioethics and Law. 2015;11:102-103.
5. Parikh PA, Shah BV, Phatak AG, et al. COVID-19 pandemic: knowledge and perceptions of the public and healthcare professionals. Cureus [Internet].2020;12:e8144.

NEWS IN BRIEF

Heterologous Prime boost in COVID vaccines

Heterologous prime boost in vaccinology means using different vaccines for the primary and booster shots in an individual. Nowadays, there is a cafeteria style choice of various COVID vaccines. Both advantages and availability of each vaccine is variable. So it may be useful to see what happens when one vaccine is followed by a different kind of vaccine.

The mRNA vaccines induce T-cell mediated immune response which may have a long lasting immunity. The inactivated adeno-viral vector vaccines on the other hand have a more robust antibody response. When two doses of the inactivated viral-vector vaccine are used, it appears that the second dose may have lower responses as the body mounts a response against the vector.

In Europe there are several trials evaluating the effects of using various combinations of inactivated viral vector vaccine and the mRNA vaccine. The Com-Cov study from UK, has four study groups: those who receive both doses of the Astra-Zeneca vaccine, both doses of the Pfizer vaccine, first Astra-Zeneca vaccine followed by booster with the Pfizer vaccine, and the reverse. Preliminary safety data published shows there were more systemic effects like fever following heterologous vaccination 34-41% *vis-a-vis* 10-21% when both vaccines used were same. There were no major adverse effects with heterologous combination. Hematological and biochemical tests done were normal.

The order of vaccines may also make a difference. For example, in trials involving HIV vaccines, giving the DNA vaccine first followed by protein subunit vaccine had best responses. Another strategy is to inject both DNA in plasmid form with recombinant spike protein together in a single shot. This has only been tested in animals so far.

Overall, it appears that combining two different types of vaccine may confer better protection at the cost of mildly increased initial systemic effects like fever. Long term data are still awaited.
(Lancet 29 May 2021)

The Pfizer vaccine in adolescents

Safety, immunogenicity and efficacy data of the Pfizer vaccine in children aged 12-15 years was recently published. This was a multi-centric randomized controlled trial in 2260 adolescents. The main adverse effects were local pain (79-86%), fatigue (60-66%) and headache (55-65%). Higher levels of neutralizing antibody titers were seen in the 12-15 year age group as compared to those aged 16-25 year. No cases of COVID-19 were reported in those who received the vaccine while 16 cases were reported in the placebo group.

This is welcome news for pediatricians all over the globe.
(NEJM 27 May 2021)

Efficacy and safety of the Sputnik vaccine

This vaccine was developed in Moscow by the Gamaleya Research Institute of Epidemiology and Microbiology. It is an inactivated adeno-viral vector based vaccine. Its first dose has the recombinant Adeno virus 26 (rAd26). The second dose has a different adeno virus vector rAd5 and is given after 21 days. The E1 gene has been removed from the adeno virus to prevent replication. The use of two different adeno viral vectors is to pre-empt any existing immunity against adeno viruses in the community.

Initial Phase I/II data published in September, 2020 had shown adequate safety. They had also shown both neutralizing antibodies and robust T-cell responses suggesting long term immunity. Phase III data in 21970 adults published recently in the Lancet demonstrated an efficacy of 91.6%. Incidence of infections in vaccinated individuals plotted over time showed that by 18 days after the first dose, there was adequate immune response to prevent SARS-CoV-2 infection. Only minor adverse effects were observed. Four deaths noted in the study group were in individuals with severe co-morbidities and were deemed to be unrelated to the vaccine.

A single dose of the vaccine is being marketed as Sputnik light and is considered to have an efficacy of 79.4%. An Indian pharma company has already launched the vaccine in India, and another will soon commence manufacturing the vaccine.
(Lancet 20 February 2021; The Economic Times 27 May 2021)

The Milennnium technology prize

Shankar Balasubramanyan, an Indian born British chemist from Cambridge has won the Millennium technology prize for developing one of the fastest DNA sequencing technologies called the Solexa-Illumina platform. As a child he wanted to be a professional footballer but decided later it would be safer to be a scientist.

The idea for the technology was born while brainstorming with his colleagues over several pints of beer in a pub in Cambridge. They came up with an idea to dramatically increase the speed of DNA sequencing 100,000 fold while steeply cutting down the cost. Finally along with his colleague David Klenerman, they founded the company Solexa and the rest is history.

The method involves fragmenting the DNA and immobilizing it on a chip. The sequence is then decoded base-by-base using fluorescent labelled nucleotides. Sophisticated software is then applied to create the final sequence. Today the Solexa-Illumina technology is the most widely used platform for next generation DNA sequencing. This technology has helped in rapidly sequencing the SARS-CoV-2 and subsequently developing effective vaccines.
(The Economic Times 19 May 2021)

GOURI RAO PASSI
gouripassi@hotmail.com

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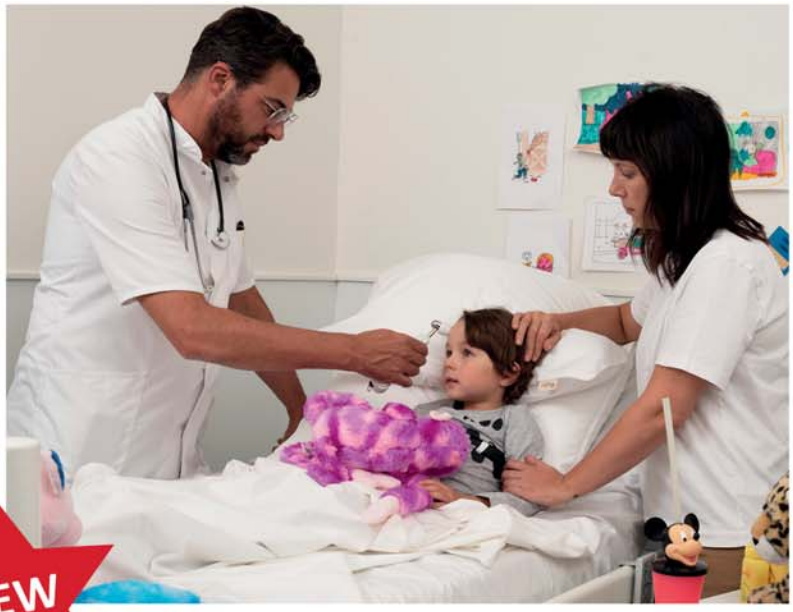

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