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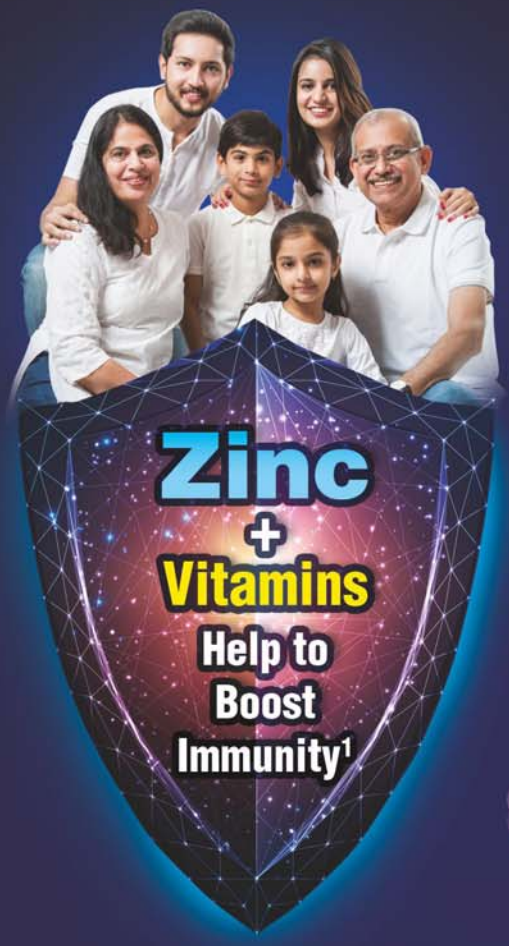

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Indian Pediatrics

March 2023 Volume 60 Number 3

CONTENTS

FROM THE EDITOR'S DESK

***Indian Pediatrics'* Policy Regarding Artificial Intelligence (AI) – Enabled Large Language Models**–DEVENDRA MISHRA 171

PRESIDENT'S PAGE

60th National Conference of Indian Academy of Pediatrics (PEDICON) 20-22 February, 2023, Gandhinagar, Gujarat–UPENDRA KINJAWADEKAR 172

INVITED COMMENTARY

Mother and Child Protection Card as a Development Screening Tool –MKC NAIR, ARPITA GUPTA 175

Does Every Child With Autism Need Investigations for Inborn Errors of Metabolism?–PRAJNYA RANGANATH, ASHWIN DALAL 177

Maternal-Fetal Iron Kinetics–MAMTA MANGLANI, RATNA SHARMA 179

The Burden of Vitamin D Deficiency in Indian Children: The Time is Right for Vitamin D Food Fortification–TOM D THACHER 181

PERSPECTIVE

Severe Acute Hepatitis: An Emerging Grave Illness in Children –RAGHVENDRA SINGH, AKSHAY KAPOOR 183

RESEARCH PAPERS

Diagnostic Accuracy of the Government of India Mother and Child Protection Card for Developmental Screening of Indian Children Aged 2-36 Months: A Hospital-based Mixed Method Study–SHARMILA B MUKHERJEE, S MEGHANA, ARUN KUMAR SINGH, SUVASINI SHARMA, DIPTI KAPOOR 187

Inborn Errors of Metabolism Associated With Autism Among Children: A Multicenter Study from Iran–HOSSEIN MORAVEJ, SOROOR INALOO, SAMAN NAHID, SHOKROLLAH MAZLOUMI, HAMID NEMATI, TOKTAM MOOSAVIAN, JAFAR NASIRI, FARIBA GHASEMI, MOHAMMAD REZA ALAEI, SETILA DALILI, MAJID AMINZADEH, PEGAH KATIBEH, ANIS AMIRHAKIMI, NEGAR YAZDANI, HOMA ILKHANIPOOR, ZHILA AFSHAR, FATEMEH HADIPOUR, ZAHRA HADIPOUR 193

Iron Profile in Term Small for Gestational Age Infants at 10 Weeks of Age and Correlation With Maternal Iron Profile : *A Prospective Cohort Study* –KRITIKA SHARMA, PRERNA BATRA, POOJA DEWAN, PRIYANKA GOGOI, BINDIYA GUPTA 197

Prevalence and Correlates of Vitamin D Deficiency Among Children and Adolescents From a Nationally Representative Survey in India–GARIMA RANA, RANSI ANN ABRAHAM, HARSHPAL SINGH SACHDEV, K MADHAVAN NAIR, GEETA TRILOK KUMAR, PRAWEEN KUMAR AGARWAL, ROBERT JOHNSTON, ARJAN DE WAGT, AVINA SARNA, RAJIB ACHARYA, AKASH PORWAL, NIZAMUDDIN KHAN, SOWMYA RAMESH, RAHUL BHARTI, KALAIVANI M, LAKSHMY RAMAKRISHNAN **202**

Neutrophil-Lymphocyte Ratio for Predicting Coronary Artery Lesions in Children With Kawasaki Disease–AAKASH CHANDRAN CHIDAMBARAM, JAIKUMAR GOVINDASWAMY RAMAMOORTHY, AVINASH ANANTHARAJ **207**

Evaluation of Diaphragmatic Thickness and Dysfunction by Ultrasonography in Mechanically Ventilated Children for Assessment of Extubation Success–SARANYA VADIVELU, DAISY KHERA, BHARAT CHOUDHARY, NISHA TOTEJA, BINIT SUREKA, KULDEEP SINGH, SURJIT SINGH **212**

Serum Amyloid A Levels and Severity of COVID-19 in Children–SELIN TASAR, EDA KARADAG-ONCEL, AYSEGUL ELVAN-TUZ, ASLIHAN SAHIN, GULNIHAN USTUNDAG, YILDIZ EKEMEN-KELES, SELIN ONUR, BANU ISBILEN-BASOK, AHU KARA-AKSAY, DILEK YILMAZ-CIFTDOGAN **217**

Composition of Common Junk Food Items and Their Contribution to the Dietary Requirement of Children and Adolescents–PRIYA MEHAR, RAJARSHI BERA, SWARNIM SWARNIM, DEVENDRA MISHRA **221**

REVIEW ARTICLE

mHealth Apps Delivering Early Intervention to Support Parents of Children With Autism Spectrum Disorder: *A Scoping Review*–RAHUL BHARAT, UZAINA TRIBHUVANESH YADAV, SANJAY NIRANJAN, PALOMI KURADE **224**

REMINISCENCES FROM INDIAN PEDIATRICS: A TALE OF 50 YEARS

Splenomegaly in Children- Significance Lies in the Cause!–KANIKA KAPOOR, ABHIJEET SAHA **231**

RESEARCH LETTER

Disposal Methods of Used Pressurized Metered Dose Inhalers and Spacers by Families of Children With Asthma–NISHA NARENDRAN, M DIVYA, RAJANY JOSE **233**

Genetic Expression of *CYP2B6* Gene in Phenobarbitone Responder and Non- responder Neonates–KRITIKA YADAV, PRERNA BATRA, VIKRAM BHASKAR, ANJU AGGARWAL, BASU DEV BANERJEE, HARENDAR KUMAR, TUSHA SHARMA **235**

CLINICAL CASE LETTER

Interstitial Lung Disease in an Adolescent Associated With a Novel *STAT5B* Mutation–SAMRITI GUPTA, ANITA MANDAL, KANA RAM JAT **237**

CORRESPONDENCE **239**

NEWS IN BRIEF **241**

CLIPPINGS **242**

IMAGES **243**

EVENT **182**

ADVERTISEMENTS **166-68,192,196,244-48**

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Indian Pediatrics' Policy Regarding Artificial Intelligence (AI) – Enabled Large Language Models

The effect of AI-enabled large language models (LLMs), most visibly ChatGPT (*https://openai.com/blog/chatgpt/*), on the scientific and educational world has been truly disruptive. Leading publishing groups like the Nature group [1] and JAMA group of journals [2] have come out with statements on journal policies regarding their use. The World Association of Medical Editors has also come out with its recommendations on chatbots in relation to scholarly publications [3]. The recent news of ChatGPT performing at or near the passing threshold for all three exams of the United States Medical Licensing Examination (USMLE) will probably create another outcry [4]. With LLMs demonstrating their ability all the way from the entrance to the medical profession (entrance exams) to the near peak of the academic achievement i.e., publishing in a respectable journal, academic clinicians may start to feel threatened.

Newer LLMs will continue to be developed [5], and will continue to improve with more people using them, and that is a welcome development. The progress of science and technology can neither be curtailed, nor should it be. Experts have already pointed out multiple short-comings of such programs [2], and other AI tools to delete their clandestine use are also on the horizon (*https://gptzero.me/*), which is likely to lead to a more ethical and regulated use of such technology.

The editors of *Indian Pediatrics* have discussed this issue. We feel that using ChatGPT or other LLMs for writing parts of a biomedical paper should not be prohibited, indeed it may even be advantageous to researchers from many low and middle-income countries, for whom English is a second language, and who wish to submit papers to English language journals. The Journal advises authors using such LLMs to clearly mention their use either in the 'Methods' section, or as an 'Acknowledgement,' inclu-ding the name of the LLM and the version used.

Additional guidance will be developed and communicated, as this field expands. The day is not far when it becomes the norm for journals to check for LLMs use for all manuscripts, as it is done for plagiarism/text-similarity nowadays.

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PRESIDENTIAL ADDRESS

60th National Conference of Indian Academy of Pediatrics (PEDICON) 20-22 February, 2023, Gandhinagar, Gujarat

UPENDRA KINJAWADEKAR
National President, Indian Academy of Pediatrics 2023
president@iapindia.org

Hon Chief guest, Guest of Honor, dignitaries on the dais, distinguished guest speakers from India and abroad, my teachers, friends, ladies and gentlemen, I stand before you with a deep sense of gratitude and humility for electing me as the President of the Indian Academy of Pediatrics for 2023 – the diamond jubilee year of our academy.

I congratulate immediate past President Dr. Remesh Kumar and the team of 2022 for the admirable work undertaken during the last year. As with every successive IAP president, Dr. Remesh has set the bar high for me, and I hope to continue the legacy in this prestigious office.

It was Confucius, the Chinese Philosopher, who proclaimed, "*Study the past if you would define the future*." The past is very important; it teaches us many a lesson. The most recent past for all of us has been that of a pandemic! It is now time to confront the intractable problems in public health; taking the lessons learned from these pandemic years and our collective trauma to protect the health of the next generation of children. Someone has rightly said that children are not little adults. Abraham Jacobi, the founding father of pediatrics in the US said "*Pediatrics does not deal with miniature men and women, with reduced doses and the same classes of diseases in smaller bodies, but …. it has its own independent range and horizon… Let alone adults, even in pediatrics, there is scarcely a tissue or an organ which behaves exactly alike in the different periods of life – from the neonatal to the adolescent stage*." For this reason, my Action plan for 2023 aims to cover every sphere of pediatrics – from the neonatal to the adolescent stage. For the duration of my term, I have identified five crucial pillars (5 Ps) of IAP that I would like to act upon with zest viz., Postgraduate students (PG), Pediatricians, Partnerships, Parents, and Publications.

For the benefit of our young PG students, we will introduce a platform to showcase New Research and Recommendations in Child Health (n-RICH). The aim of n-RICH is to synthesize and curate a collection of abstracts representing breakthrough developments in pediatrics, carefully selected from reputed journals published worldwide. These selections will be accompanied by the thoughtful and erudite commentary of Indian experts from various subspecialties, who will give an insight on how to read and analyze these articles. Secondly, with the aim to impart our best practices to students of pediatrics, we will be initiating 'PG Reach' every Tuesday through Friday. I am grateful to respected Dr. YK Amdekar, Dr. Baldev Prajapati and Dr. S Balasubramanian along with many other teachers who came together to make this outreach a reality.

IAP is now an association of over 40,000 pediatricians, and my core mandate is to serve this community. Through the pandemic years, we started conducting a series of weekly webinars consisting of lectures, panels and discussions. Considering the overwhelming positive response we received for this series, we will be continuing these 'Academic Pearls' webinars this year as well. Additionally, we will be launching several modules on pressing issues that, in my opinion, are the need of the hour. While newborn survival rates have improved everywhere (neonates born with low birth weight are routinely sent home), their risk stratification, assessment, clinical monitoring and early stimulation are all equally important for their optimal growth and development. The Risk stratification Assessment, Clinical monitoring and Early stimulation in high-risk neonates (RACE) module shall focus on the same. Through 'Rhythm,' a training module on Rheumatology, and 'Care to Cure,' a module on Hematology, we want to provide specialized insights into these emerging branches of medicine that are pertinent for pediatrics. The module on comprehensive nutrition (CNM) will have updated guidelines on child nutrition. The module on 'Hitting the Bulls Eye' will pivot around arriving at the accurate diagnosis using clinical cues. Infectious Diseases (ID) Conundrum is a case-study based interactive module wherein attendees will discuss a partially worked up case and present it to the experts in the panel. Under the ID ULTRA (Understanding Lab Test Rationale)

module, the focus is on the use of serology, microbiology and molecular testing, in day-to-day practice. In recent times, genetic testing and related laboratories have grown globally, yet the awareness around the appropriate time to take these tests and interpret results continues to be sub-optimal. The 'Genetics for Generalists' module will deal with the same. It is not just shocking, but appalling to find that in spite of the common knowledge that babies are most likely to survive, grow and develop to their full potential when exclusively breast fed, one in three babies in low- and middle-income countries are given some other fluids in the first three days of life, delaying early initiation of breastfeeding. Through the action plan program B4E i.e., 'Breastfeeding: Early initiation, Exclusive, Every baby and Every time,' I hope to come one step closer to our goal of universal early and exclusive breastfeeding. Hopefully, we can aim to reach at least a figure of 80% for early initiation as well as excusive breast feeding for six months. A final note on pediatricians – I am increasingly witnessing signs of fatigue and burnout around me, even amongst young colleagues. Doctors are the first line of defense that the population turns to. Lately; however, lifestyle disorders are on the rise among doctors themselves. These disturbing trends are urging us to look inward and make an effort to protect our mental health and happiness, so that we can serve our communities to the best of our abilities. I want to encourage all of us to have a life beyond pediatrics, where we consciously invest time in the little things that give us joy – our hobbies, passions, and interests outside of medicine.

For the year 2023-24, IAP will introduce a flagship program, '*Sankalp: Sampoorna Swasthya*' – a drive towards comprehensive preventive healthcare for school going children. The program is led by the belief that healthy habits, cultivated early in life, are more likely to be carried into adulthood. Childhood and adolescence are critical life stages that are primed to absorb and process new information. Hence, huge public health gains can be realized–including improvements in the health of current cohorts of children and adolescents, their future adult health trajectories, as well as the health of the next generation of children–by leveraging this crucial window of opportunity. Under the program, we are going to target seven environmental and social drivers (nutrition, physical activity, screen time, sleep, substance abuse, mental health and air pollution) that can have long-term repercussions on the health of the child. IAP has already signed a MOU with the Government of Maharashtra, thanks to chief minister Sh. Eknath Shinde, and very soon should be able to have similar support from the Government of Goa. Hence, our members will be allowed to conduct these programs in all the schools across these states. We have a force of 40,000 IAPians totally committed for the cause.

To understand the gravity of the situation and need of such a program, let me give you a couple of examples. Hypertension in adults has its origin in childhood, where it is often asymptomatic. Hypertension in childhood is more significantly associated with cardiovascular complications and target organ damage (TOD) like renal, retinal and cardiovascular. Given its correlation with obesity and physical inactivity, we can understand how important it is to prevent it. Secondly, 1.5 core children between 10-17 years of age in the country are addicted to either alcohol or smoking etc. which is so disturbing to know. About increased consumption of junk food and overexposure to screen, I need not quote any figures as each one of us is already aware of the reality around us. Friends, in the past 60 years of its existence, IAP has done tremendous work for the benefit of the children and adolescents of India, by continuously upgrading the knowledge and skills of pediatricians, advising governments on critical issues and advocacy on several fronts. Now, in its Diamond Jubilee year, it is only apt that through SSS, IAP is poised to take a big leap forward, by taking the benefits of the best of science, directly to the community and doorstep of all school going children of India, in an unprecedented manner.

While I have the vision, ambition and enthusiasm of all the equally motivated colleagues to undertake these programs and initiatives, I believe that *successful and sustainable* implementation depends on the strength of collaborative effort of not just IAP, but the entire public health academic community. I am also eager to build strong partnerships with other professional bodies of doctors, academics, civil society and private organizations that also work to improve child health outcomes in India. For instance, we are working with FOGSI towards the goal of zero thalassemia and evidence-based consensus statements on preconception care. A consultative meet followed by the publication of a white paper on developmental origin of health and disease (DOHaD) and the role of Epigenetics is also planned in the year. We have a vast collective knowledge on the rational use of antibiotics. Through collaborations with NIMA and IMA, I believe we can disseminate this knowledge to a wider audience. Reaching parents to guide and update them in various child health related issues has always been IAPs priority. Innovative partnerships with some popular forums is leading us into newer domains to broadcast curated pediatric content, that will benefit parents. Most importantly, we want to continue the trust and goodwill of our governmental partners, who have also committed to the cause of NC-ECD, NTEP etc.

Lastly, I am eager to initiate collaboration with our own diverse yet crucial sub-specialty chapters of IAP, and I anticipate a glorious working association that can culminate into seminal white papers and guidelines for the pediatric community at large. Through these partnerships, let us provide greater support also to academic publications on key issues such as pediatric obesity guidelines, thalassemia care and transition of care in chronic diseases, Grand rounds in Pediatrics etc. Keeping up with the times, I want to initiate digital aids in child care. One idea being explored is an app-based system to ensure the proper follow up of high-risk newborns. The objective of the app will be to provide a one stop, comprehensive, user-friendly way to provide structured care by pediatrician in office practice for neonatal intensive care graduates (NICU) graduates. It will cover screening for growth, development, sensory, behavior domains targeted for preterm/term infants up to two years of age. Red flags for different domains, tracking growth velocity, record keeping and screening charts are the highlights. Good nebulization practices, good oxygenation practices, good cannula care practices, good communication practice and many more such topics would be covered under it.

I would like to conclude with some personal expression of gratitude. In life, finding good mentors is so important. They watch us learn and grow over the span of decades, and are always present with words of affirmation or advice. I have had the good fortune to have several such extraordinary mentors–people who selflessly guided me over many years and who I continue to consult with and admire. They include Dr. MR Lokeshwar, Dr. YK Amdekar, Dr. Mamta Manglani, Dr. Nitin Shah, Dr. Bharat Agrawal, Dr. Bakul Parekh, Dr. Rohit Agrawal and my dear friend and inspiration, Dr. Vijay Yewale. I must also thank my wife Dr, Sucheta, for being my sounding board; my children Dr. Chinmay and Mugdha, my sisters Meena and Manju, my colleagues in IAP Navi Mumbai and of course my beloved *Aai* for being an incredible source of strength and support. I miss my Baba so much in this moment. He spent his life in selfless service to society, and he continues to be my idol and inspiration, especially as I step into this office as the President of IAP.

JF Kennedy once said that leadership and learning are indispensable to each other. In the year ahead, I foresee a brilliant journey of learning from the collective wisdom of this organization, while striving to give back in equal measure, serving you all to the best of my abilities, and to take our beloved IAP to scale newer heights. Friends, with strong solid and dependable friend Dr. GV Basavaraja besides me as president-elect and amazing task master Dr. Vineet Saxena as HSG, me and my team 2023 look forward to an exhilarating year with all of you. Indeed, I am quite sure that jointly with the help of each one of you together, we can achieve our objective of providing better and optimal healthcare to all the children of our land.

Jai Hind! Jai IAP!

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INVITED COMMENTARY

Mother and Child Protection Card as a Development Screening Tool

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Approximately, 10% of Indian children less than two years of age exhibit developmental delay in one or more developmental domains, needing early identification and timely intervention [1]. Developmental surveillance and developmental screening, using validated tools at specific age groups help identify deviation from normalcy. Despite the presence of a myriad of screening tests developed in LMICs [2], like Baroda Development Screening Test; Comprehensive Developmental Inventory for Infants and Toddlers; Developmental Assessment Tool for Anganwadi workers; Lucknow Development Screen; Parents' Evaluation of Developmental Status; Rapid Neurodevelopmental Assessment Tool; Ten Questions Screen; Trivandrum Developmental Screening Chart, very few fulfills all the criteria of an ideal community-based developmental screening tool. Al-though, there is no universally accepted criteria for assessing a community-based child developmental screening tool, generally the characteristics selected are; access, cost, time, reliability, usefulness to guide action, training, acceptance by caregivers, and most importantly validity. Additional considerations would be; cultural relevance, good ethnic and linguistic sensitivity; requiring minimum expertise of screeners; and pragmatic applicability [3].

Mother and Child Protection (MCP) card developed by Government of India (GoI) is a widely used simple community-based tool, which endorses an integrated approach to ensure proper maternal and child-care leading to survival and growth through family-focused, community-based intervention. MCP card has recently been revised to include development component for children between the age groups of 2 to 36 months [4]. The development component, divided across seven age groups namely 2-3, 4-6, 7-9, 10-12, 18, 24 and, 36 months, comprises of three-color zones (green, red and blue) containing age specific textual and pictorial information. It intends to act as a guide for developmental monitoring, identify presence of warning signs for timely referrals and give parenting tips for stimulation. The card has been designed to ensure family participation, motivate the community and encourage responsive caregiving by parents. The pictorial information ensures its standardization across all the states in India with different languages spoken. The Indian Academy of Pediatrics (IAP) recommends developmental screening at 9-12, 18-24, and 36 months, apart from the regular surveillance till two years of age [5] and endorses MCP card under IAP-NURTURE program in public as well as private sector [6]. Though, it appears to be a compre-hensive and acceptable tool with all required features, there is a dearth of the information about its psychometric properties.

Previous studies have explored the knowledge of community health workers on appropriate usage of MCP card for monitoring, awareness of beneficiaries in maternal and childcare, hitherto, none has investigated the psychometric properties, feasibility and acceptability of developmental component of this tool [7,8]. Hence, there is an ongoing need for studies that not only describe the psychometric properties of MCP card as a screening tool but also give information about its pragmatic relevance in our community.

In this issue of *Indian Pediatrics*, work by Mukherjee, et al. [9] investigates the role of MCP card for development screening of children aged 2 to 36 months. They provide much needed evidence in their mixed-method study (prospective and qualitative) regarding the diagnostic accuracy and feasibility of use of development component of MCP card in Indian children. In this hospital based study, 213 normally developing children across the seven age groups have been included and their age specific items marked on green and red zone of the MCP card are compared with the reference tool i.e., comprehensive clinical assessment (CCA). CCA is a self-designed tool which is an amalgamation of clinical assessment of hearing, vision and neurological signs along with objective assessment of development and adaptive functioning using norm-referenced validated tools like Development Profile (DP-3) and Vineland

Adaptive Behaviour Scale (VABS) [10,11]. They have also performed a qualitative research by conducting an in-depth interview of mothers regarding the difficulties encountered while using MCP card for developmental monitoring and performing stimulation activities. Their investigation revealed a good sensitivity and specificity of MCP card with acceptable diagnostic accuracy in four age groups namely 2-3 months, 7-9 months, 13-18 months and 24-36 months, along with reasonable acceptability and understandability of developmental component of MCP card among the mothers who have received primary education [9].

Previous studies have shown that though majority of healthcare workers have a satisfactory knowledge regarding the appropriate use of MCP card, majority of the beneficiaries do not [7]. Being a community based tool with focus on family centred intervention, MCP card requires active participation of the families, especially mothers in developmental monitoring and performing stimulation activities. This requires a basic level of education which may not be possible in our country where still approximately 30% of the total population is illiterate [12]. Thus, the authors have rightly questioned the generalizability and practical application of MCP card as a screening tool among the general population with varying levels of education and suggested need for further research at community level [9].

The present study [9] reveals reasonable psychometric properties of MCP card as a developmental screening tool and provides a good evidence for using it for our children. However, before recommending it at a large scale for developmental screening, training of mothers/ caregivers for using MCP card needs to be considered to ensure better results. Also, more research on practical usability and psychometric properties of MCP card against gold standard developmental assess-ment tools is required to recommend it as a comprehensive developmental screening tool for Indian children.

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INVITED COMMENTARY

Does Every Child With Autism Need Investigations for Inborn Errors of Metabolism?

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Autism spectrum disorder (ASD) is a complex neurodevelopmental disorder that affects communication and social interaction and is often also associated with repetitive behavior. ASD may occur in isolation when it is said to be non-syndromic, or it may be a component of a syndrome, where it may be associated with neurological abnormalities including seizures and neurological deficits, and/or multisystem involvement. Definite genetic causes such as chromosomal anomalies and monogenic disorders are more often found in patients with syndromic ASD. Majority of patients with non-syndromic ASD are believed to have a multifactorial etiology where there may be a complex interplay of multiple contributory genetic variations and environmental factors. Specific genetic causes are identified in only around 10-20% patients of non-syndromic ASD [1].

Fragile X syndrome, Rett syndrome and tuberous sclerosis are some of the common monogenic causes of ASD [2]. Disorders of inborn errors of metabolism (IEMs) caused by mutations in genes coding for enzymes and other important components of various metabolic pathways, are known to be associated with ASD. The build-up of toxic metabolites and deficiency of essential nutrients that occur in IEMs can affect the brain leading to various manifestations such as neurological deficits, seizures, microcephaly, and neurodevelopmental abnormalities including intellectual disability and ASD. A number of IEMs have been reported to be associated with ASD including organic acidurias, phenylketonuria, urea cycle disorders, neurotransmitter disorders, disorders of cholesterol biosynthesis, disorders of cerebral creatine metabolism, homocystinuria, disorders of purine and pyrimidine metabolism, mitochondrial disorders, and mucopolysaccharidosis (MPS) type III [3]. However, most of these IEMs have associated neurological and/or multisystem manifestations and isolated non-syndromic ASD is an uncommon clinical presentation [4]. In one of the largest studies on the utility of metabolic screening of patients with non-syndromic ASD, urine samples of 406 patients were screened for metabolites of cerebral creatine deficiency syndromes, purine and pyrimidine disorders, amino acid metabolism defects, mucopolysaccharidoses, and organic acidurias, and none of them were found to have IEMs. In the same study, a further retrospective analysis of 464 patients who had been diagnosed to have IEMs revealed that only one of them (who had urea cycle disorder) had presented with non-syndromic ASD [5]. In another large study, the results of the metabolic workup (urinary mucopolysaccharides, urinary purines and pyrimidines, urinary creatine and guanidinoacetate, urinary organic acids, and plasma and urinary amino acids) routinely performed in 274 non-syndromic ASD children were retrospectively analyzed and were found to be abnormal for only two patients; one with non-specific elevation of urinary creatine excretion and the other with persistent 3-methylglutaconic aciduria [4]. In another cohort of 32 patients with non-syndromic ASD who underwent metabolic screening for urinary mucopolysaccharides and organic acids, along with serum lactate, amino acids, ammonia, and acyl carnitine profiles, none of the patients were found to have abnormal results [2].

Occasionally; however, isolated ASD may be the initial early presentation of a few IEMs such as phenylketonuria, homocystinuria, mucopolysaccharidosis (MPS) III, and some urea cycle disorders, especially ornithine transcarbamylase deficiency [6-9]. The possibility of detecting such patients with IEMs manifesting with isolated ASD may be higher in countries where routine tandem mass spectrometry (TMS)-based newborn metabolic screening is not practised, and there is higher prevalence of consanguinity and endogamy with consequently increased prevalence of autosomal recessive disorders including IEMs [4,10,11]. A study of 277 patients with ASD from China screened using TMS and subsequently confirmed by molecular genetic testing, showed around 5% diagnostic yield of TMS-based metabolic evaluation

[12]. In the metacentric study from Iran published in this issue of *Indian Pediatrics* [13], 105 children and adolescents with ASD were screened for IEMs and a total of 13 patients (12.4%) were found to have various IEMs including five patients with cerebral creatine metabolism disorders and four with arginino-succinic aciduria. Four of these 13 patients had asso-ciated seizures and seven had global developmental delay.

Treatment for inborn errors of metabolism typically involves a special diet that is low in the nutrients that the body is unable to process properly, as well as supple-ments of essential nutrients and use of pharmacological agents to reduce the toxic metabolites. Early diagnosis and treatment can significantly improve the long-term outcomes for individuals with these disorders. Thus, even though IEMs account for a very small proportion of patients with autism, especially non-syndromic autism, it may be important to perform basic metabolic screening involving at least TMS, and blood lactate and ammonia assay, to ensure that treatable causes are not missed, especially for patients for whom routine newborn meta-bolic screening has not been done. More extensive meta-bolic tests and molecular genetic testing for IEMs can be reserved for patients with relevant associated clinical findings. It is important to screen for and identify IEMs in order to initiate therapeutic intervention before irrever-sible neurological injury sets in and to optimize the outcome.

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INVITED COMMENTARY

Maternal-Fetal Iron Kinetics

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Iron is not like gold that glitters or silver that sparkles, but it outshines both in its biologic importance. Iron is required for various biological processes like oxygen transport, ATP production, DNA biosynthesis, and cell proliferation [1]. Iron requirements increase 10-fold during pregnancy to support placental and fetal growth, increase in maternal RBC mass and blood loss during delivery [2]. This cannot be compensated by dietary iron intake or mobilizing iron stores, thus leading to iron deficient state in the mother, if not supplemented.

Iron deficiency (ID) and ID anemia (IDA) during pregnancy have been associated with adverse outcomes for the mother and the child, including increased risk of maternal morbidity and mortality, premature birth, low birth weight, and neurodevelopmental impairment in infants [3]. Hence, iron supplementation has been nearly universally recommended during pregnancy [4].

Transfer of iron from mother to fetus happens at the level of syncitiotrophoblasts in the placenta based on signals coming from fetus, mother or the placenta itself. This is a tightly regulated mechanism, though still lacking complete understanding. Under normal circumstances, maternal iron status and placental iron transportation are regulated in favour of fetal iron requirements, however, in severe iron deficiency, placenta prioritizes its own metabolic need over fetus [4].

The maternal body requirement for iron increases to approximately 1000 mg on an average during pregnancy. Of this, 350 mg is associated with fetal and placental growth, 500 mg with expansion in the red cell mass and 250 mg with blood loss at delivery. The increased requirement needs to be supported by higher maternal iron intake. However, iron requirements are not uniform throughout the three trimesters of pregnancy. In the first trimester, the requirements are lower than before pregnancy because menstruation stops. The estimated trend of requirement is around 6 mg/d in the 1st trimester, to 19 mg/day in the 2nd trimester to 22 mg/day in the 3rd trimester of pregnancy [5]. To meet these iron requirements, both dietary iron absorption and mobilization of iron from stores increase, a mechanism that is in large part dependent on the iron-regulatory hormone, hepcidin [2]. Absorption of both heme and nonheme iron increases as gestation progresses and iron stores are efficiently mobilized during pregnancy.

Hepcidin is an iron-regulatory hormone, produced by the liver and controls plasma iron concentrations and tissue iron distribution. The functions of hepcidin in the human body include maintaining stable iron stores, providing enough substrates for erythropoiesis, and preventing availability of iron to microorganisms. Thus, the factors that influence hepcidin production include iron (both circulating and stored iron increase hepcidin), erythropoietic activity (suppresses hepcidin), and inflammation (increases hepcidin) [6]. Hepcidin acts by inhibiting the following major iron flows into plasma: intestinal iron absorption, release from macrophages that recycle iron from old RBCs, and mobilization of stored iron from the liver. Hepcidin binds to iron-loaded ferro-portin (receptor for iron export) and triggers its degradation, resulting in iron sequestration in target cells and decreased iron flow into plasma.

Maternal hepcidin plays an important role in the regulation of iron availability during pregnancy. The concentrations of hepcidin decrease in the second and third trimesters resulting in an increased supply of iron into the circulation both from the enhanced absorption of dietary iron and the enhanced release of iron from stores. Lowering of maternal hepcidin during pregnancy increases iron bioavailability for placental transfer. Immediately after delivery, serum hepcidin concentrations increase, presumably because of dramatic physiologic changes that are associated with labor.

Fetal hepcidin may control placental ferroportin and the transfer of iron into fetal circulation. Maternal hepcidin regulates the amount of iron that is presented to the placenta for uptake, whereas fetal hepcidin regulates the export of iron from the placenta into the fetal circulation. Ferroportin is expressed on the basolateral side of the placental syncytiotrophoblast, facing fetal circulation, accessible only by fetal hepcidin. Whether endogenous fetal hepcidin

contributes to the regulation of placental transfer of iron still remains to be evaluated. In humans, only hepcidin from cord blood has been evaluated and the concentrations were higher than maternal hepcidin concentrations [7]. In animal studies very low concentrations of fetal hepcidin were observed during normal gestation [8].

Pregnancies associated with intense inflammation result in marked increase in hepcidin. This causes compromised iron availability during pregnancy. Resulting elevated hepcidin also causes impaired absorption of iron from supplements routinely prescribed to pregnant women and impairs the efficacy of intravenous iron therapy by trapping iron in macrophages. Obesity and pre-eclampsia are associated with mildly elevated serum hepcidin concentrations [6]. Mild elevation of hepcidin as observed with obesity in pregnant women and pre-eclamptic women did not have an obvious negative impact on hematologic or iron variables in the mother or neonate with conflicting reports suggesting otherwise [9].

Increased fetal iron demand or maternal iron insufficiency results in an increase in the expression of placental transferrin receptors on the syncytiotrophoblast and an increase in the expression of the ferritin receptor in the placental microvilli membrane. Expression of the endosomal membrane iron transporter, divalent metal ion transporter (DMT-1), has also been shown to be involved in the transfer of iron from the syncytiotrophoblastic endosome into the cytoplasm [10]. Moreover, placental iron regulatory protein 1 activity has been directly related to transferrin receptor messenger RNA concentrations in human placenta, and expression of this protein has been found to be related to the iron content of the placenta [11]. The role of various other hepcidin-independent factors in pregnancy, including apical iron transporter in duodenal enterocytes, associated ferrireductase duodenal cytochrome B and transcription factor hypoxiainducible factor (HIF)-2a in the duodenum, remains to be determined [2].

At the other end of spectrum are pregnant women who have iron replete rather than iron deficient status and, one needs to consider the potential risks of indiscriminate iron supplementation in them [12]. Iron overload and the resultant oxidative stress due to labile iron, leads to activation of programmed cell death pathways through ferroptosis. The intracellular accumulated iron causes lipid peroxidation of the cell membrane causing autophagy. Disorders during pregnancy like pre-eclampsia and gestational diabetes have been linked to ferroptosis [1] and high maternal hemoglobin, which is associated with failure of the plasma volume to expand. Recent studies have raised the possibility that giving too much iron to non-anemic women (50-60 mg/d) can result in hemo-concentration and associated poor outcomes [13]. Increased levels of the iron storage protein ferritin also are associated with preterm delivery likely via infection and inflammation. Thus, both extremes of the maternal hemoglobin distribution are associated with adverse pregnancy outcomes with low hemoglobin reflecting a mix of true and physiologic anemia and high hemoglobin reflecting failure of the plasma volume to expand.

In summary, iron deficient as well as iron replete states during pregnancy are known to adversely affect the mother and fetus. Iron deficient pregnant women should receive iron supplementation along with dietary counselling. How much iron should be supplemented and whether iron replete pregnant women need supplementation needs to be answered with more research in future.

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INVITED COMMENTARY

The Burden of Vitamin D Deficiency in Indian Children: The Time is Right for Vitamin D Food Fortification

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Vitamin D deficiency in children can lead to life-threatening hypocalcemia, seizures, cardiomyopathy, and cardiac arrest. Nutritional rickets and osteomalacia are the paradigm bone diseases resulting from vitamin D deficiency and inadequate bone mineralization. Vitamin D is produced in the skin by exposure to ultraviolet light from the sun, and apart from supplementation with vitamin D, sunlight exposure is the major source of vitamin D in children. Few foods naturally contain vitamin D, so in many countries, commonly consumed foods or milk are fortified with vitamin D to ensure adequate vitamin D status in children. Breast milk is generally low in vitamin D, but breast milk can be enriched with vitamin D if a nursing mother takes high doses of vitamin D [1]. Vitamin D is ultimately metabolized to $1,25(OH)_2D$, which promotes intestinal calcium absorption. Serum 25(OH)D is the intermediate metabolite used to assess vitamin D status, because of its long half-life and stability.

Nutritional rickets, despite being entirely preventable, is prevalent in India [2-4]. The first step toward implementing a program to prevent nutritional rickets is to understand the burden of vitamin D deficiency and its risk factors in a representative sample of children [5]. The Indian Ministry of Health and Family Welfare should be applauded for completing the largest ever Comprehensive National Nutrition Survey for 2016-2018 [6], filling the evidence gap related to vitamin D deficiency in children and adolescents 0-19 years of age.

In this issue of *Indian Pediatrics*, Rana and colleagues summarized the findings of the Comprehensive National Nutrition Survey and described the large burden of vitamin D deficiency in Indian children [7]. Vitamin D deficiency, defined as a serum 25(OH)D concentration below 12 ng/mL (30 nmol/L), was found in 14%, 18%, and 24% of children ages 1-4 years, 5-9 years, and 10-19 years, respectively. Vitamin D deficiency was associated with urban residence, greater household income, school attendance, greater BMI, winter sampling, and a vegetarian diet. Among adolescents, a greater proportion of females (34%) than males (14%) had vitamin D deficiency, and less than 3 hours of physical activity per week was associated with vitamin D deficiency [7]. In the original report of the Comprehensive National Nutrition Survey [6], Sikh children had the greatest frequency of vitamin D deficiency, ranging from 50-72% depending on age group. Geographic variation between states was notable, which did not strictly follow a north-south gradient. Many of these risk factors for vitamin D deficiency correspond to less exposure to sunshine in affected groups and were consistent with risk factors reported in other populations.

Dietary calcium intake is important to consider in the context of vitamin D deficiency, because of the interaction between calcium and vitamin D [8,9], and the role of dietary calcium deficiency in nutritional rickets [3,10]. Milk and dairy products are typically the major source of calcium for children when they are no longer fed breast milk or infant formula. In the Comprehensive National Nutrition Survey [6], 40% of school-aged children did not consume milk or curd at least once per week. Only 6% of children ages 6-23 months were fed a minimum acceptable diet, defined as either breastfeeding or receiving at least two milk feedings and the minimum dietary diversity and meal frequency. There was a large geographic variation in dairy product consumption between states, and dairy intake was positively correlated with the mother's education and wealth index.

It should be noted that 25(OH)D was measured with an antibody competitive immunoassay [6], rather than with the gold standard of LC-MS/MS, which may limit comparison of vitamin D status in this study with other countries. However, the laboratory participated in a validated external quality assurance scheme. Further research is warranted to explore the interaction of dietary calcium intake and vitamin D status on the risk of nutritional rickets and clinical manifestations of vitamin D deficiency in children [8].

The prevalence of vitamin D deficiency among children in India is a call to action. Fortification of commonly consumed staple foods with vitamin D is the most effective way to address the need for vitamin D at a population level [5,9]. For vitamin D fortification to succeed, children need to regularly consume the foods that are fortified. Government-mandated food fortification provides better coverage of the population than voluntary fortification [5]. In the absence of food fortification, vitamin D supplementation (400 IU/d in infants and 600 IU/d in older children and adolescents) [10], particularly targeting children unlikely to receive adequate sun exposure, will rapidly correct and prevent vitamin D deficiency. Alternatively, large bolus doses of vitamin D can be given to children during immunization visits to facilitate adherence [5,10]. Outdoor physical activity and exercise has many health benefits in addition to improving vitamin D status. If implemented successfully, these public efforts can eradicate vitamin D deficiency and its consequences in children.

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PERSPECTIVE

Severe Acute Hepatitis: An Emerging Grave Illness in Children

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Acute hepatitis of unknown origin in children has been recently described in the literature, and a case definition has also been proposed for this condition. The exact etiology is unknown and exclusion of infectious, metabolic, autoimmune and toxin mediated injuries is essential. Management for this condition is supportive, but some may require liver transplantation. Infection prevention and control practices are important as the etiology remains unidentified.

Keywords: *Adenovirus, Pediatric acute liver failure, Liver transplantation.*

The Lancet child and adolescent health recently published an editorial on severe acute hepatitis in children [1]. World Health Organization (WHO) was first notified of 10 cases of severe acute hepatitis of unknown origin (AHUO) by International Health Regulations (IHR) of the United Kingdom in previously healthy young children on 5 April, 2022 [2]. Laboratory testing excluded Hepatotropic A, B, C, D and E cases, while SARS-CoV-2 and/or adenovirus (HAdVs) were detected in several cases. The proposed case definition of AHUO by the WHO is summarized in **Box I.** As of 8 July, 2022, five WHO regions comprising 35 countries had reported 1010 cases fulfilling the WHO case definition with 22 deaths [3]. The proposed case definition of AHUO by WHO is summarized in Table I.

Delta testing is not required, as it is only undertaken in persons who are HBsAg positive to establish the presence of coinfection.

Acute non-A-E hepatitis is a well-known entity for years and has been described earlier from Europe and India accounting for 5-10% of all acute hepatitis in children [4,5]. A retrospective German study over 30 years observed a rising incidence of non-A-E in their cohort from 2019 onwards with stable numbers for Hepatitis A-E [6]. However, the recent reports of increase in the number of severe acute hepatitis reported by some European countries and WHO was not substantiated by a question-naire based survey across several European countries conducted in April and May, 2022 [7,8]

A Mixed Basket

HAdVs are non-enveloped double-stranded DNA viruses with a worldwide distribution and usually cause self-limited infections in the healthy population. However, severe or disseminated HAdV infections may occur in some immunocompromised individuals. The transmission mode consists of fecal-oral spread, conjunctival inoculation or inhalation of aerosolized droplets. The polymerase chain reaction from respiratory material, stool, blood or urine samples is the most common method to establish the diagnosis. Symptoms often reported are related to respiratory tract infections (e.g., pharyngitis, coryza or pneumonia), keratoconjunctivitis, gastro-intestinal symptoms (diarrhea, abdominal pain and vomiting, notably with serotype 40 and 41, but possible as concomitant symptoms for all serotypes, particularly in young children) or genitourinary tract infections [9]. Approximately 60% of United Kingdom (UK) cases and 45% of tested samples in the United States were positive for HAdVs. The precise role of HAdV induced acute severe hepatitis in immunocompetent individuals remains elusive. HAdV41 has tissue tropism to invade the gastrointestinal tract with clinical symptoms of diarrhea, vomiting, and

Box I WHO Working Group Proposed Case Definition of Severe Acute Hepatitis of Unknown Origin

Confirmed: Not available at present.

Probable: A person presenting with acute hepatitis (non-hepatitis A-E)[a] with serum transaminase >500 IU/L (AST or ALT), who is 16 years and younger, since 1 October, 2021.

Epi-linked: A person presenting with acute hepatitis (non-hepatitis A-E)[a] of any age who has been in close contact with a probable case since 1 October, 2021.

[a]*If hepatitis A-E serology results are awaited, but other criteria are met, these can be reported and classified as "pending classification." Cases with other explanations for their clinical*

fever [10]. However, HAdV41 is not known to cause acute liver failure in immunocompetent children. Enhanced HAdV surveillance in pediatric hepatitis cases as well as in animals and environments combined with specific laboratory and clinical investigations are urgently required to improve our knowledge of the impact and the spread of this new human threat [11]

CLINICAL PRESENTATION

Acute Hepatitis of Unknown Origin

Kelgeri, et al. [12] described the Birmingham liver transplantation (LT) unit, United Kingdom, experience in 44 children with acute hepatitis of unknown cause. The confirmed case definition for AHUO by the UK Health Security Agency (UKHSA) was utilized, i.e., acute hepatitis that is not due to hepatitis A through E viruses or a metabolic, inherited or genetic, congenital, or mechanical cause, with a serum aminotransferase level >500 IU per liter in a child who is ≤10 years of age presenting after 1 January, 2022 [13]. Most children were healthy before the illness, and only 3 had unrelated chronic health conditions. Jaundice in 93% was the most common condition, followed by vomiting in 54%, diarrhea in 32%, abdominal pain in 27%, and lethargy in 23%. The peak ALT levels ranged from 603-6279U/L (Normal 0-41U/L). The liver biopsy was performed in 9 children, but none had viral inclusions and immunohistochemical tests positivity for human adenovirus. Of these 44 children, 38 survived with the native liver; however, 6 (14%) children required liver transplantation [12].

Acute Hepatitis With Human Adenovirus Viremia

Gutierrez, et al. [14], in 2022, reported a case series of acute hepatitis in 15 children from United States, of whom 9 (60%) had no identifiable cause. Of these 9 non-identified cases, 8 were positive for HAdV infection. All nine children had been previously healthy. The symptoms began days to weeks before admission. The symptoms at admission in decreasing order were emesis, diarrhea, fever, fatigue, upper respiratory symptoms, poor appetite, and dark urine. The physical findings in 9 children included scleral icterus in 8, hepatomegaly in 7, hepatic encephalopathy, and splenomegaly in one each. The clinical symptomatology was similar to the above mentioned UK study. ALTs and ASTs ranged from 602-4696U/L and 447-4000U/L, respectively. The liver biopsy in six children revealed degrees of chronic and acute portal and lobular hepatitis characterized by mixed inflammation, consisting of lymphocytes, histiocytes, and neutrophils with interface activity in the majority of cases. Inflammation in the lobules was associated with extensive hepatocyte damage and foci of apoptosis. 3 of these 6 samples were positive for HAdVs by PCR, but none tested positive for immunohistochemical tests and viral inclusions for HAdVs. The INR ranged from 1.0-7.3, and three children met the criterion for pediatric acute liver failure (PALF). Two children with PALF required liver transplantation, and one recovered spontaneously. The remaining six children recovered with supportive treatment.

SARS-CoV-2 and Hepatic Involvement

Gastrointestinal tract involvement in severe acute respiratory syndrome coronavirus (SARS-CoV-2) infection has been described in adults and children. However, children with chronic liver disease, including autoimmune liver disease and liver transplant, do not fare poorly in the course of SARS-CoV-2 infection. A case series described severe hepatitis as a primary manifestation of SARS-CoV-2 infection, with two children out of four fulfilling the clinical definition for PALF [15]. The WHO also mentioned 78 cumulative SARS-CoV-2 positivity by PCR in severe hepatitis cases, with many having HAdV coinfection [2].

POSSIBLE HYPOTHESES

Several working putative mechanisms have been suggested for this enigmatic entity. The first multipronged hypotheses were suggested by the UKHSA [13] and endorsed by the ESPGHAN working group [16]. The working mechanisms proposed are a typical HAdV infection with abnormal susceptibility or host response which allows adenovirus infection to progress more frequently to hepatitis, a considerable number of typical adenovirus infections, uncovering a very rare or under-recognized complication, abnormal susceptibility or host response to adenovirus due to priming by prior infection or coinfection with SARS-CoV-2 (including Omicron restricted) or another infection or abnormal host response to a toxin, drug or an environmental agent. Other hypo-theses suggested by the same bodies include a novel variant adenovirus, with or without a contribution from a cofactor, a post-infectious SARS-CoV-2 syndrome (inclu-ding an Omicron restricted effect), drug, toxin or environ-mental exposure, a novel pathogen either acting alone or as a coinfection and new variant of SARS-CoV-2. Toxico-logic investigations have not revealed any specific toxico-logical or other environmental factors as the cause of the severe acute hepatitis in children detected in multiple countries, but public health investigations are ongoing.

Another interesting proposed hypothesis is that the SARS-CoV-2 infection can lead to a viral reservoir in the gastrointestinal tract and the repeated release of viral proteins can lead to immune activation. Immune activation might, in turn, be initiated by a superantigen motif within the SARS-CoV-2 spike protein that bears a resemblance to

staphylococcal enterotoxin B, triggering broad and non-specific T-cell activation. Adenovirus-induced type-1 immune skewing, which, upon subsequent staphylococcal enterotoxin B administration, led to excessive IFN-γ production and IFN-γ-mediated apoptosis of hepatocytes in mouse models [17]. Fortunately, no link with COVID-19 vaccination has been found as most children were unvaccinated.

MANAGEMENT

As the etiology of this condition is still not understood fully, the mainstay of therapy for severe acute hepatitis of unknown etiology in children is supportive. Patient consciousness, volume status, urine volume, blood electrolytes, liver function, and coagulation function should be closely monitored during the entire treatment period, as should maintenance of water, electrolyte and acid-base balance [18]. Approximately 6-10% of children require liver transplantation and liver transplantation is necessary for children with ALF who fail to improve with supportive measures. There are no well-defined and universally consistent indications for liver transplantation in ALF children. Treatment with cidofovir in HAdV infection has also been described in [19]. The role of steroids in treating this condition needs to be studied by clinical trials in the future [20].

Prevention

WHO advises general infection prevention and control (IPC) practices as the etiology is unknown. These include hand hygiene, physical distancing, good ventilation when indoors, use of masks, adopting safe and hygienic food measures and home isolation when unwell. Health facilities should adopt droplet precautions for suspected and probable cases [3].

RESEARCH AGENDA FOR SEVERE ACUTE HEPATITIS

The ESPGHAN Hepatology Committee has set a research agenda for this condition [16]. It stresses the need for running prospective, multicenter studies to collect data on the incidence of this cluster of acute hepatitis and acute hepatitis in general. It also recommends advanced virological, including metagenomic (from blood, serum, urine, stool, respiratory and liver samples) and toxicology (including environmental and food toxicity) testing should be undertaken. Ideally, DNA, blood samples, nasal and throat swabs and fecal samples should be stored for future centralized testing. It suggests a battery of investigations for the work up for infectious diseases of children with acute hepatitis as summarized in **Table I**.

Concurrently, excluding non-infectious etiologies, including autoimmune, metabolic and drug-related causes of severe acute hepatitis, is also essential. Histopathology results from a larger patient cohort would provide additional insights into the possible viral origin. Finally, there is a need to investigate the underlying susceptibility of a dysregulated immune response. The high cost of the detailed workup is the main limiting factor especially in lower-middle income countries (LMICs).

Table I Infectious Disease Investigations Suggested for Children With Acute Hepatitis

Sample type	*Test*	*Pathogen*
Blood	PCR	Adenovirus, enterovirus, CMV, EBV, HSV, hepatitis A, hepatitis C, hepatitis E, HHV6 and 7
Blood	Serology	Hepatitis A, B, C, E, CMV, EBV, SARS-CoV-2 anti-S, SARS-CoV-2 anti-N (only if locally available)
Blood	Culture	Standard culture for bacteria/fungi
Nasal/throat swab	PCR	Respiratory virus panel (including adenovirus/enterovirus/influenza, SARS-CoV-2)
Stool	PCR, stool culture	Adenovirus, sapovirus, norovirus, enterovirus

PCR: polymerase chain reaction, CMV: cytomegalovirus, EBV: Epstein-Barr virus, HSV: herpes simplex virus, HHV: human herpes virus.

Whether this entity is a new disease condition or a rediscovery of an already existing condition due to heightened surveillance and diagnostic measure is not known at present. Non-Hep-A-E were described in Indian children in earlier hospital based case series and its proportion ranged from 3.5-22 % [5,21-23]. The overall mortality ranged from 39-64% in two of these earlier series with fulminant hepatic failure but the unidentified causes constituted a smaller proportion in these series [22,23]. In a recently published study on 125 children with PALF, no cause could be identified in 23% of cases and mortality/LT was required in 41.4% of these indeterminate cases. However, the mortality/LT rates in indeterminate and infectious causes were comparable [24].

CONCLUSIONS

Severe acute hepatitis of unknown origin is an emerging entity with high risks to children afflicted with this disease. Human adenovirus infection has been found in high proportions of children, but this agent's direct cytopathic effect seems less likely. COVID-19 infection with immune dysregulation coupled with antigenic stimulation by HAdV or another infectious agent is another possibility. The majority of children recover with conservative measures but a liver transplant is required in the rest. Globally

coordinated multi-centric research with extensive tests for infectious agents and immunological mechanisms is the need of the hour.

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RESEARCH PAPER

Diagnostic Accuracy of the Government of India Mother and Child Protection Card for Developmental Screening of Indian Children Aged 2-36 Months: A Hospital-based Mixed Method Study

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Background: Universal developmental screening is recommended at 9, 18, 24 and 36 months. The Government of India Mother and Child Protection (MCP) card is an immunization record that is used to monitor child development, and identify children requiring further evaluation.

Objectives: To determine the diagnostic accuracy of the MCP card for developmental screening, and perform its item analysis.

Study design: Mixed-method study (prospective study of diagnostic accuracy and qualitative study).

Participants: Mother-child dyads of children between 2-36 months of age were recruited from the outpatient department or wards of a tertiary level children's hospital from November, 2019 to October, 2021. Children with confirmed neurodevelopmental disorders/disability, and mothers with less than 6th standard education were excluded.

Intervention: Each mother was given a MCP card, and taught how to mark the items. This was followed by the researcher's evaluation (index tool). The reference tool was a comprehensive clinical assessment (CCA) by the researcher and an expert. The CCA included clinical examination of hearing, vision, and neurodevelopment; and psychometric assessment of development and adaptive function. Each mother underwent an in-depth interview. Overall and group wise psychometric properties of diagnostic accuracy were computed. The interview transcripts were analyzed thematically.

Outcomes: The proportion of children with 'fail' and 'delay' by the evaluation of the researcher with the MCP card and the expert by the CCA, respectively.

Results: The study population included 213 children (40.4% females). Fifty-two (24.4%) children were identified as 'Fail' by the MCP card and 43 (20.2%) as 'delay' by the expert's CCA. The overall sensitivity and specificity was 83.7% (95% CI 69.3-93.2) and 90.6% (95% CI 85.2-94.5), respectively. Acceptable diagnostic accuracy was found in the age-group 7-9 months, 13-18 months, and 25-36 months.

Conclusions: The MCP card may be used for developmental screening at 9, 18, and 36 months.

Keywords: *Developmental monitoring, Developmental surveillance, Immunization card, National health mission*.

The Government of India's Mother and Child Protection (MCP) card is used by community health workers (CHW) throughout India to monitor maternal and child health, educate family regarding positive health practices, and provide information regarding healthcare services provided by the government for women, and children under the age of 3 years [1]. The children's sections include neonatal care, immunization, infant and young child feeding, growth, development, etc. The MCP card is given to the family by CHWs during pregnancy or at birth, who also teach them how to use it.

The developmental component is meant for children between 2 to 36 months, across seven age groups. Each group has three colored zones containing age-specific textual and pictorial information. The developmental component is meant to be used by the CHW for developmental monitoring, giving appropriate counselling, and referring for further evaluation if there is identification of a developmental issue.

Invited Commentary: Pages 175-76.

Given the high prevalence of children with developmental disability (52.9 million children under 5 years) [2], developmental screening plays an important role in identifying children with atypical development amidst apparently typically developing children. The Indian Academy of Pediatrics (IAP) [3] and the American Academy of Pediatrics (AAP) [4] recommend that all children should undergo developmental screening at 9, 18, 24 and 36 months of age. AAP advocates the use of the Ages and Stages Questionnaire (ASQ) or Parents Evaluation of Developmental Status (PEDS) [4], but IAP does not

specify the tools [3]. However, the IAP-NURTURE program promotes developmental monitoring by the MCP card in its well child visits [5,6].

One of the reasons for lack of universal developmental screening in India is non-availability of tools with desirable attributes [7-11]. The MCP card could fill this gap, but the psychometric properties are unknown. This study was planned to determine the diagnostic accuracy of the MCP card for developmental screening of children under three years, and also included qualitative assessment.

METHODS

This hospital-based, mixed-method (study of diagnostic accuracy and qualitative) study was conducted over two years (November, 2019 to October, 2021) after obtaining approval from the institutional ethics committee. Mother-child dyads of children between 2 and 36 months of age were recruited from the pediatric department on the basis of convenience. Children attending the specialty clinics due to established clinical diagnosis of a neurodevelopmental disorder/disability, or those presenting with parental concerns of developmental delay, and mothers with an educational level of less than the sixth standard were excluded.

The estimated sample size was 229; wherein 30% was the proportion of Indian children without risk factors who failed ASQ-2 screening [13], confidence interval (CI) was 90%, CI width was 10%, and standard normal deviate for CI was 1.65. Age stratification was used based on the MCP card age groups.

After obtaining written informed consent, each eligible child underwent standard evaluation that included elicitation of relevant demographic, socioeconomic, perinatal, and clinical details known to influence development. The mother was given a MCP card (2018), explained its purpose, and taught how to use the developmental component [14]; ticking the boxes in the green zone (GZ) related to what the child could do in the current and preceding age brackets; and marking any applicable item in the preceding red zone (RZ).

The researcher also administered the developmental component using the same method, except for marking a cross against any item that the child had not attained in the preceding green zone. A child was considered 'Fail' by the MCP card if there was any mark in the preceding red zone and/or cross in the preceding green zone. Absence of any marks in the red zone, and ticks in all items in the preceding green zone was considered a 'Pass.'

This was followed by a comprehensive clinical assessment (CCA), the reference tool. The CCA was designed to be in contextual alignment with the developmental component of the MCP card, which comprises of items pertaining to developmental skills, adaptive skills (activities of daily living), vision, hearing, and neurological signs. The CCA included five strategies: *i*) Structured age-specific clinical examination of hearing, according to which a child was identified as with or without hearing impair-ment; *ii*) Structured age-specific clinical examination of vision, according to which the child was labelled as with or without vision impairment; *iii*) Psychometric assessment of developmental status using Developmental Profile, 3rd edition (DP-3), in which a General Developmental Score of <70 was considered 'developmental delay' (<-2 SD) [15]; *iv*) Psychometric assessment of adaptive function using Vineland Adaptive Behavior Scale, 2nd edition (VABS-II), in which the Adaptive Behavior Composite of <70 was considered 'low' (<2 SD) [16]; and *v*) Clinical neurodevelopmental assessment according to which a child was categorized as with or without neurodevelopmental impairment. The overall interpretation of the CCA was 'Delay' (if an abnormality was detected in any components) or 'No delay.' Children with delay were reviewed by a pediatric neurodevelopmental expert on the same day (as far as possible), or within three days. The latter reviewed the CCA and performed a clinical assessment, on the basis of which the child was categorized as 'Delay' or 'No delay'. The expert and the researcher were blinded to the results of the evaluation by the index and reference tool, respectively.

The researcher also conducted a brief in-depth interview (IDI) of the mother regarding her understanding of individual items. Her responses were recorded verbatim. Transcripts of the interview were analyzed thematically by domain experts. The domains identified were coded as related to maternal understanding (M), cultural practices (C), and item content; technical errors (TE), theoretical issues (Th), or errors in translation from English to Hindi (Tr).

The outcome variable measures were number of children with 'pass' or 'fail' as per the MCP card when administered by the researcher, and the number of children with 'no delay' or 'delay' as deemed by the researcher and expert, respectively. All children with delay as established by the expert were enrolled in the pediatric neurology clinic and referred for early intervention.

Statistical analysis: The collected data were coded, tabulated and statistically analyzed using SPSS program (Statistical package for social science) software version 28. Descriptive statistics was used. Overall and individual age group wise psychometric properties were calculated: sensitivity, specificity, positive predictive value, negative predictive value, positive likelihood ratio and negative

likelihood ratio. Cohen Kappa was used to measure the level of agreement between mothers and researcher's responses in all items of both zones.

RESULTS

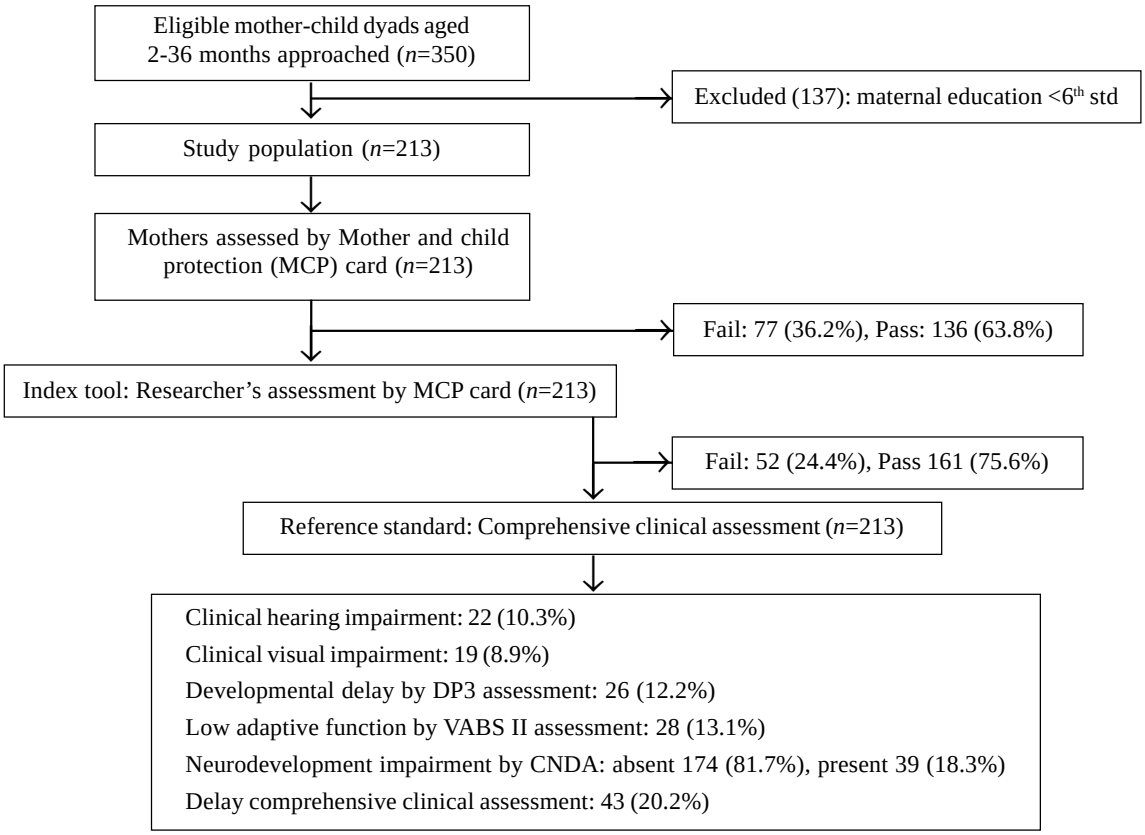
The study population comprised of 213 children; 28 (2-3 months), 30 (4-6 months), 29 (7-9 months), 23 (10-12 months), 35 (13-18 months), 33 (19-24 months) and 35 (25-36 months). The flow of study participants is depicted in **Fig. 1**. The mean age was 14.2 (9.5) months with male-female ratio of 1.5:1. The socioeconomic profile was comparable in all groups, predominantly the lower middle socioeconomic stratum (54.4%). The baseline characteristics of the study participants are given in **Table I**. Salient clinical findings included 64 (30%) children who were underweight, 43 (20.1%) who were wasted, 6 (2.8%) with dysmorphism, 17 (7.9%) with microcephaly, and 2 (0.9%) with macrocephaly.

The number of children identified as MCP card 'fail' by the researcher and CCA 'delay' by the expert were 52 (24.4%) and 43 (20.2%), respectively. Among the latter, 22 (10.3%) had hearing impairment, 19 (8.9%) vision impairment, 47 (22.06%) had delay on DP3, 28 (13.1%) had low adaptive function by VABS II, and 39 (18.3%) had neurodevelopmental impairment. The overall sensitivity and specificity was 83.7% (95% CI 69.3 -93.2) and 90.6% (95% CI 85.2 -94.5), respectively (**Table II**).

Table I Baseline Characteristics of the Study Population (*N*=213)

Characteristics	*No. (%)*
Low birth weight	13 (6.1)
Preterm birth	13 (6.1)
Small for gestational age	3 (1.41)
Perinatal asphyxia	10 (4.7)
Hypoglycemia	2 (0.09)
Hypocalcemia	1 (0.04)
Neonatal seizure	11 (5.2)
Neonatal infections	6 (2.8)
≥1 high risk factor	30 (14.1)

No participant was hospitalized for shock, hyperbilirubinemia requiring double volume exchange transfusion, or any major systemic illness.



CNDA: clinical neurodevelopmental assessment; DP3: development Profile (3rd ed); VABS II: vineland Adaptive Behaviour Score (2nd ed).

Fig.1 Flow of participants in the study.

Table II Psychometric Properties of the Developmental Component of the Government of India Mother and Child Protection Card (*N*=213)

Sge group (mo), n	*Sensitivity (%) (95% CI)*	*Specificity (%) (95% CI)*	*PPV (%) (95% CI)*	*NPV (%) (95% CI)*	*LR+ (95% CI)*	*LR- (95% CI)*
2-3, *n*=28	100 (66.4-100)	94.7(73.9-99.9)	90.0 (57.2-98.4)	100 (-)	19.0 (2.8-128)	0
4-6, *n*=30	66.7 (22.3-95.7)	100(85.8-100)	100	92.3 (79.5-97.4)	0	0.3 (0.11-1.0)
7-9, *n*=29	75.0 (19.4-99.4)	100(86.3-100)	100	96.2 (82.1-99.3)	0	0.3 (0.05-1.4)
10-12, *n*=23	66.7 (9.4-99.1)	95.0(75.1-99.9)	66.7 (20.1-94.1)	95.0 (79.3-98.9)	13.3 (1.7-105.8)	0.4 (0.1-1.7)
13-18, *n*=35	83.3 (35.9-99.6)	89.7(72.7-97.8)	62.5 (35.0-83.8)	96.3 (81.2-99.5)	8.1 (2.6-24.9]	0.2 (0-1.1)
19-24, *n*=33	100 (63-100)	60.0(38.7-78.9)	44.4 (33.1-56.4)	100	2.5 (1.6-4.0)	0
25-36, *n*=35	71.4 (29.0-96.3)	96.4(81.7-99.9)	83.3 (40.8-97.3)	93.1 (80.7-97.8)	20.0 (2.8-144.9)	0.3 (0.1-1.0)
Overall	83.7 (69.3-93.2)	90.6(85.2-94.5)	69.0 (58.1-78.5)	95.7 (91.8-97.8)	8.9 (5.5-14.4)	0.2 (0.1-0.4)

LR-: negative likelihood ratio; LR+: positive likelihood ratio; NPV: negative predictive value; PPV: positive predictive value.

Seventy-seven (36.2%) children were identified as MCP card 'fail' based on maternal assessment. **Web Table I** gives the level of agreement between mother and researcher for the GZ (33 items) and RZ (32 items). Details of the specific issues identified by qualitative assessment are listed in **Web Table II**. Difficulties in understanding were noted in 7 (25%) and 6 (21.4%) mothers for item 6 in the green zone (by 2-3 months) and item 6 in the red zone (at 3 months), respectively.

DISCUSSION

This study assessed the diagnostic accuracy of the developmental component of the MCP card (index tool) for developmental screening of children aged 2-36 months against assessment by an expert using the CCA (reference tool). It also included a qualitative dimension to add value to the quantitative parameters.

It is considered important that both the sensitivity and specificity of a screening tool be >70% [11], so that there is an acceptable balance between the number of false positive and false negative cases that are also identified with the true positives (delay present) and true negatives (delay absent). The overall diagnostic sensitivity of the MCP card was 83.7%, whereas the specificity was 90.6%. An exhaustive literature search could not identify other health cards that had been validated. We compared our results with screening tools recommended by AAP [4], and for LMICs [7,9], that have been validated in similarly aged Indian children. The Hindi translated and culturally adapted ASQ (second edition) had sensitivity and specificity of 83.3% and 75.4%, respectively [13]. The diagnostic accuracy of the Hindi version of PEDS revealed 33.3% sensitivity and 93.2% specificity [17]. The International Guide for Monitoring Child Development (IGMCD) had acceptable overall psychometric properties in children from India, Turkey, Argentina, and South Africa [18]. A screening tool popular in South America, 'Monitoring Child Development in Integrated Management of Childhood Illnesses Context' (MCDIC) that fulfils the criteria of tools suitable for LMICs, has a sensitivity of 88.0% and specificity of 85.7% [19].

On considering individual age groups, the MCP card had acceptable diagnostic accuracy in the 2-3, 7-9, 13-18, and 25-36 month brackets. Therefore, it can be used for developmental screening of all apparently asymptomatic children at 9, 18, and 36 months of age, but not at 24 months (since specificity was 60% for 19-24-month-olds). The probable reason for this is a likely theoretical error in a GZ item "*Name and identify common objects and their picture in the book*" due to which 54.5% children were MCP card 'fail,' despite displaying 'No delay' on the reference tool. MCDIC can be used for screening at 18 and 36 months, but not at 9 months (sensitivity sub-optimal in the 9-12 month bracket). The status at 24 months remains unclear as both parameters could not be computed for 24-30 month-old children [17]. Sub-group analyses are not available for ASQ-2 and IGMCD [13,18].

On the whole, the developmental component of the MCP card was found to be acceptable and easily understandable by most mothers. The thematic domains that emerged from qualitative analysis were due to maternal misinterpretation, socio-cultural taboos (children not being kept prone, and infants not being shown mirrors), technical errors (minor inaccuracies in placement of text, content of pictures, etc), errors due to incorrect translation, and theoretical inaccuracies. Correcting these would enhance overall and group wise diagnostic accuracy, if they are incorporated in the next revision. A few items in the red zone pertaining to atypical development (in his/her world) and/or abnormal neurological signs (persistently holds thumb inside the palm) were difficult for mothers

WHAT IS ALREADY KNOWN?

- Developmental screening is recommended for all children at 9, 18, 24, and 36 months of age.
- The diagnostic accuracy of a developmental screening tool is acceptable when both sensitivity and specificity are >70%, when validated in the intended population.

WHAT THIS STUDY ADDS?

- The overall sensitivity and specificity of the mother and child protection (MCP) card for developmental screening of children between 2-36 months of age was 83.7% and 90.6%, respectively.
- The sensitivity and specificity were acceptable (>70%) in the age groups of 7-9 month, 13-18 month and 25-36 month, but not in 10-12 month and 19-24 month age groups.

with typically developing children to comprehend, but easily recognized by those with children having neurodevelopmental issues. These should be retained as they serve their intended purpose.

The strengths of this study were that the reference tool used was a combination of psychometric tools assessing development and adaptive function, clinical neurodevelopment, and sensory (vision and hearing) evaluation so that it was in alignment with the content of the MCP card. In addition, we calculated the psychometric properties for each individual age group. There were a few limitations. We fell slightly short of the calculated sample size within the study period. The same person applied the index tool and the reference tool in cases of children with 'no delay,' but were different in the children identified with 'no delay'- due to logistic reasons, it was not possible for all the children (delay and no delay) to be confirmed by the expert. Although, this was a hospital-based study, we tried to simulate community settings by recruiting 'well' children from the immunization center, or children prior to being discharged from inpatient wards after recovery. In the field, the items ticked by the mother are cross checked by the CHW, who assesses the items that are left blank, and then decides upon the developmental status of the child. Since the index tool was the MCP card administered by the researcher, we determined the level of agreement between the mother and researcher, presuming that a CHW will perform at an intermediate level (superior to the mother, but inferior to the researcher). Hypothetically, our results may not be generalizable in caregivers with literacy levels below the sixth standard, since they were excluded from the study. However, since the cards are meant for the family, there is likelihood that most households will have one member with the requisite literacy level.

It is recommended that the issues identified in the developmental component be addressed in the next revision. Subsequently, a multi-centric, adequately powered study of diagnostic accuracy of the MCP card (administered by a CHW) should be conducted in the field with a similar reference tool and based on the responses of mothers, irrespective of literacy level. Till then, the developmental component of the MCP card can be used for developmental screening at 9, 18, and 36 months in Indian children, in addition to the existing practice of monitoring the developmental status at all visits, and providing developmental stimulation.

Ethics clearance: Institutional Ethics Committee, Lady Hardinge Medical College New Delhi; No. LHMC/IEC/Thesis/2019/95, dated Oct 28, 2019.
Contributors: SBM and AS conceptualized the study; SBM and SS planned the design of the study; SBM, SS, and DK were the neuro-developmental experts; SBM, MS, DK and SS were involved in collection and analysis of data; SBM and MS prepared the preliminary draft. All authors gave their intellectual inputs during critical revision, and approved the final manuscript.
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Web Table I Level of Agreement Between Mother's and Researchers' Assessment for Identification of Atypical Development in Children Aged 2.36 Months (*N*=213)

Age group	*Item 1*	*Item 2*	*Item 3*	*Item 4*	*Item 5*	*Item 6*
Green zone						
2-3 mo	0.9	0.6	0.7	0.2	0.7	0.9
4-6 mo	0.7	0.8	0.9	0.9	0.9	0.4
7-9 mo	NA	0.8	1	0.6	1	-
10-12 mo	1 Item	1	1	0.9	0.7	-
By 18 mo	0.9	0.9	0.9	0.8	-	-
By 24 mo	0.8	0.9	0.8	-	-	-
By 3 y	0.6	0.7	0.9	0.9	-	-
Red zone						
At 3 mo	0.8	0.8	1	0.9	0.9	0.9
At 6 mo	1	0.8	1	1	1	1
At 9 mo	1	1	1	1	0.6	-
At 12 mo	NA	1	1	1	1	-
At 18 mo	1	1	1	1	1	1
At 24 mo	0.8	1	1	1	0.8	1

Values are for Cohen Kappa.'-' means there was no item for that age group; NA: no children in which any item was crossed, hence k could not be computed, but there was 100% agreement between the researcher and mother in the assessment of all the ticked item. [a]As no child was aged 36 mo, items in RZ (at 3 y) were not administered to any child.

Web Table II Age-group Wise Thematic Qualitative Analysis of Items in the Green and Red Zones

Age	*Zone*	*Item No & text*	*Issue identified*	*k*	*Suggestion*
2- 3 m	GZ	2: Develop social smile	Non-social smiles were also incorrectly included [M]	0.6	Use a picture of mother and child smiling at each other
		4: Raise head at times when on tummy	Lots of babies are not placed in prone position [C]	0.2	Remove item. Add this activity to the blue zone.
		6: Keeps hand open and relaxed	Mothers understood open, but not 'relaxed' [M]	0.9	Remove relaxed as it is redundant
At 3 m	RZ	6: Persistently hold thumb inside the palm….	Mothers with typically developing infants were unable to understand [M]	0.9	Retain as mothers with atypically developing infants could identify this
4 – 6 m	GZ	1: Keeps head steady …and can sit with support	The corresponding picture is not appropriate for the 'sits with support' [TE]	0.7	Remove 'sit with support' and retain the image.
		4: Laugh aloud or make squealing…	Does not correlate with image in the box [TE]	0.9	Place this item separately from 'attempt to reach…'
		6: Likes to look at self in mirror	Many mothers do not show infants mirrors due to social taboos [C]	0.4	Substitute with a culturally acceptable item since this taboo is widely prevalent
At 6 m	RZ	6: Unable to raise head when on tummy	Lots of babies are not kept in the prone position [C]	1	Retain as mothers with atypically developing infants could identify this
7 – 9 m	GZ	2: Grasps toy by using all fingers	The corresponding inset pic is in the third box [TE]	0.8	Relocate the inset image to the first box.
		3: Turns head to visually follow…	Does not correspond to the picture in the box [TE]	1	Remove from the current box and place it separately
		5: Responds to name being called...	Does not correspond to the picture in the box [TE]	1	Remove from the current box and place it separately
At 9 m	RZ	2: Needs support to sit	Picture shows support of own and others hands [TE]	1	Specify that the support is someone holding the trunk
		4: Does not utter pa-pa, ma-ma…	Item does not match the image [TE]	1	Remove the image
		T5: Tilts head always to one side..	Item does not match the image [TE]	0.6	Change the image to a more appropriate one
10–12 m	GZ	2: Raises arm to be picked up	Place in a separate box as it does not match image [TE]	1	Or add an appropriate image depicting this item
		4: Uses one or two common words…	Place in a separate box as it does not match image [TE]	0.9	Or add an appropriate image with a speech bubble
At 12 m	RZ	1: Cannot pick small objects with finger and thumb…	Current image shows use of palmar grasp [TE]	NA	Replace with an image of a pincer grasp and a cross over it depicting inability
By 18 m	GZ	Age bracket name	This covers 13 – 18 m [TE]	-	Change to 13 – 18 months
		2: Uses a variety of gestures…	The corresponding image is placed elsewhere [TE]	0.9	Place the image in the proper location/ remove it
		4: Name and identify common objects and their pictures…	Naming and identifying are different skills. The number of words is age dependent [Th]	0.8	Retain only naming. Specify the age-appropriate number.
At 18 m	RZ	4: Does not respond to gestures… in his/her own world	Mothers were unable to understand 'in his/her own world' [M]	1	Not responding to gestures can be seen in both ASD and hearing impairment. Not responding to displays of affection would be more specific for ASD
		5: Does not use both hands…hand preference…	Any child would use the closest hand to pick up an item [TE]	1	Depict a child picking up an object with the opposite hand, crossing his/her trunk
		6: Does not say single words like…	Image does not convey the content of the item [TE]	1	Add a cross to the textbox conveying inability
By 24 m	GZ	Age bracket name	This covers 19 – 24 m	-	Change to 19 – 24 months
		2: Imitate household chores	The child's broom is not clearly visible [TE]	0.9	Make the broom larger or darker
		3: Correctly point out and name ≥ 1 body parts	Pointing out and naming are different skill sets. The number is age dependent [Th]	0.8	Use either pointing out or naming body parts, and specify the age-appropriate number

Contd....

At 24 m	RZ	1: Does not walk steadily while pulling a toy	The phrase 'while pulling a toy' implies the child walks steadily, otherwise [TE]	0.8	Remove the phrase 'pulling a toy' from the item, as it is irrelevant
		2: Cannot scribble	The Hindi word used for scribble is incorrect, and means 'writing' [Tr]	1	Correct the word. Use an image of a scribble and a cross over it.
		3: Does not use 2-word phrases…	Image does not convey the content of the item [TE]	1	Add a cross to the textbox conveying inability
		4: Does not make appropriate…	Image does not convey the content of the item [TE]	1	Add a cross to the textbox conveying inability
		5: Does not point to body parts	Image does not convey the content of the item [TE]	0.8	Add a cross to the textbox conveying inability
		6: Does not seem to understand & follow simple…	Image does not convey the content of the item [TE]	1	Add a cross to the textbox conveying inability
By 3 years	GZ	Age bracket name	This covers 25 – 36 m	-	Change to 25 – 36 months
		2: Name most familiar…identify colors, shapes, etc	Naming and identifying are different skills. Naming colors and shapes are age dependent. The Hindi phrase means naming and describing the color and shape of objects [Tr]	0.7	Use only one skill. Specify range of vocabulary or the age-appropriate number of colors. Correct the Hindi translation to convey the correct meaning.
		4: Climb up and down the stairs	It is unclear whether this skill is one foot at a time, or alternate feet per step [TE]	0.9	Specify one foot at a time which would be age-appropriate for 25-36 m.
At 3 years	RZ	1: Has trouble climbing up and down the stairs.	This skill is while erect, the image is of crawling, & the Hindi word is used for both ways [Th & Tr]	-	Use a picture for climbing stairs in the erect stance and add a cross to convey inability.

C Cultural issue; GZ green zone; k Cohen's kappa; m month; M Maternal issue; RZ red zone; TE Technical error; Th Theoretical issue; Tr Translation errors.

RESEARCH PAPER

Inborn Errors of Metabolism Associated With Autism Among Children: A Multicenter Study from Iran

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Objective: This study aimed to find the common inborn errors of metabolism in Iranian patients with autism spectrum disorder.

Methods: In this cross-sectional multicenter study, 105 children and adolescents with autism spectrum disorder from six centers in different cities of Iran were enrolled between August, 2019 and October, 2020. Metabolic screening, including measuring plasma levels of amino acids, acylcarnitines, creatine, and guanidinoacetate, and urinary levels of organic acids, purines, and pyrimidines was performed. Other data, including age, parental consanguinity, history of seizure, developmental mile-stones, and physical examination, were also recorded.

Results: An inborn error of metabolism was found in 13 (12.4%) patients. Five patients (4.8%) had cerebral creatine deficiency syndrome, 4 (3.8%) had arginine succinate aciduria, 2-methylbutyryl glycinuria, short-chain acyl-CoA dehydrogenase deficiency, and combined methylmalonic aciduria/malonic aciduria. There was a strong association between positive metabolic evaluation and parental consanguinity, history of seizures, microcephaly, and delayed development.

Conclusions: Our results suggest that metabolic screening should be performed in the cases of autism associated with parental consanguinity, developmental delay, and a history of seizures. The assays to be considered as a screening panel include plasma or blood amino acids, acylcarnitines, creatine and guanidinoacetate, and urinary levels of organic acids.

Keywords*: Cerebral creatine deficiency syndrome, Diagnosis, GAMT deficiency, Screening*.

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Autism spectrum disorders (ASD) are characterized by abnormalities in cognitive function and social and language communication problems. Although the etiology of ASD is unclear, genetic factos are known to be involved, and environmental factors may also play a significant role [1]. In this regard, impaired methylation and mutations of *MeCP2* have been associated with ASD. Genetic polymorphisms of cytochrome P450 enzymes have also been linked to autism, specifically CYP27B1, essential for proper vitamin D metabolism [2].

Invited Commentary: Pages 177-78.

Previous studies have demonstrated an association between inborn errors of metabolism (IEM) and ASD [3,4].

A wide range of IEMs has been found in patients with ASD, including aminoacidopathies, organic acidemias, urea cycle defects, cerebral creatine deficiencies, and abnormalities of purines and pyrimidines [5]. Early detection and timely treatment of these disorders may help improve some manifestations and prevent possible complications in patients with ASD [5]. Considering very high rates of consanguineous marriages in Iran, the association of IEM and ASD may be higher than expected. This study was conducted to document the occurrence of IEMs in patients with ASD in different centers in Iran.

METHODS

In this cross-sectional multicenter study, children and adolescents with ASD referred to either neurologists, psychiatrists, or endocrinologists in six centers in different cities of Iran were enrolled from August, 2019 to October, 2020. These centers were located in Shiraz, Isfahan, Tehran, Rasht, and Ahwaz cities. Patients with autism were referred to these specialists for different reasons such as neurologic evaluation, hormonal evaluation, or routine followups.

The protocol of the study was approved by a local ethics committee, and patients were enrolled after informed consent. We used Diagnostic and Statistical Manual of Mental Diseases, 5th edition (DSM-5) criteria to confirm the diagnosis of ASD [6]. All patients with a diagnosis of ASD were included. Patients with a history of hypoxic-ischemic encephalopathy were excluded. Information including age, history of seizures, history of other known medical conditions (such as autoimmune or inflammatory diseases) developmental assessment scales, and consanguinity of parents was collected. A detailed physical examination, including a neurologic examination, was performed.

Investigations for IEMs, including measurement of plasma amino acids, plasma acylcarnitines, urine purines and pyrimidines, plasma and urine level of guanidinoacetate and creatine were done by Liquid chromatography – mass spectrometry (LC-MS/MS) (AB-Sciex API 3000 with Agilent 1100 HPLC as front end), and measurement of urine organic acids and acyl glycines by Gas chromatography-mass spectrometry (GC-MS) (Agilent 5973 mass selective detector and 6890 GC system).

Statistical analysis: Data were analyzed using the Statistical package for the social sciences (SPSS) version 22. Continuous variables were checked for normal distribution using the Shapiro-Wilk test. The mean (SD), median (IQR), frequency, and percentages were used for descriptive analysis. The means of two independent variables were compared with the Student *t* test and the chi-square test was used for categorical variables. $P<0.05$ was considered statistically significant.

Table I Baseline Characteristics of the Study Population (*N*=105)

Variable	*Value*
Age, (y)[a]	5.8 (3.4)
Male gender	73 (69.5)
Head circumference	
Microcephaly	3 (2.9)
Macrocephaly	2 (1.9)
History of seizure	8 (7.6)
Development delay	26 (24.8)
Consanguinity	13 (12.4)

All values in no. (%) or [a]mean (SD).

RESULTS

In this study, six centers from different cities in Iran collaborated, and 105 eligible patients (69.5% boys) with ASD were enrolled (**Table I**), with a median (IQR) age of 5 (4-8) years. Thirteen patients (12.4%) were products of kinship marriages, 26 cases (24.8%) had some degree of developmental delay other than speech problems, three patients (2.9%) had microcephaly, and two (1.9%) had macrocephaly. A history of seizures was present in 7.6% (*n*=8) children.

Thirteen patients (12.4%) were diagnosed to have some form of IEM. Biochemical criteria for diagnosing these diseases, and the frequency and types of IEM found among these patients are listed in **Web Table I**. **Web Table II** shows the detailed characteristics of patients with ASD and IEM.

There was a statistically significant association between the presence of an IEM and parental consanguinity ($P<0.001$), history of seizure ($P=0.004$), and delayed development (other than speech) ($P=0.016$) (**Table II**). Among eight patients with a history of seizures, four had cerebral creatine deficiency syndrome (CCDS). There was a significant association between CCDS and history of seizure ($P=0.013$), consanguinity ($P=0.001$), and delayed development (cognitive and motor) ($P=0.04$). However,

Table II Results of Metabolic Assay in Iranian Children With Autism (*N*=105)

Variable	*Positive metabolic assay*	*P value*
Male gender, *n*=73	9 (12.3)	0.60
Microcephaly,*n*=3[a]	1 (33.3)	0.49
Seizure history, *n*=8	4 (50)	0.004
Development delay, *n*=26	7 (26.9)	0.016
Consanguinity, *n*=13	7 (53.8)	<0.001

Data in no. (%). [a]None of the two children with macrocephaly had positive metabolic assay.

WHAT THIS STUDY ADDS ?

- Cerebral creatine deficiency syndrome (CCDS) was a common type of inborn error of metabolism associated with autism among children in Iran.
- Screening is likely to be more important when autism is associated with a history of seizure, delayed development, or parental consanguinity in this population.

this association was not significant with head circumference (*P*=0.22).

DISCUSSION

Our multicenter study on 105 patients with ASD revealed a significant association between IEM and ASD in Iran. This association between IEM and ASD was found in other studies previously [7]. It may be promising to discover an IEM in a patient with ASD because, with IEM management, symptoms of autism probably ameliorate. However, this will need more extensive prospective studies from various settings [6,8,9].

Based on our results, the most prevalent type of IEM associated with ASD was CCDS. The prevalence of ASD is different in three types of CCDS including glycine amidinotransferase (AGAT) deficiency, guanidinoacetate methyltransferase (GAMT) deficiency, and *SLC6A8* deficiency or creatine transporter defect (CTD) [8]. According to previous studies,78-95% of patients with GAMT deficiency have symptoms of autism, whereas only 41% of patients with CTD have these symptoms [10,11]. It has been reported that management of GAMT deficiency can improve these patients' symptoms, such as seizures and movement disorders [3].

Phenylketonuria (PKU) and hyperphenylalaninemia are labeled as the most common types of IEM associated with autism [8,12]. However, this was not the case in our study; probably because of early detection and management through the neonatal screening program. The other metabolic disorders diagnosed by us have also been previously reported to be associated with ASD [3,12].

Although, previous authors proposed metabolic screening in only patients with ASD associated with other abnormalities [5,13,14], a recent study suggests IEM screening in all patients with ASD [12]. It is not documented whether early diagnosis and timely treatment of different metabolic disorders can improve symptoms of autism; nevertheless, some studies have reported a partial improvement in autism symptoms after starting treatment [8]. Although, CCDS has diverse signs and symptoms, including intellectual defect, neurodevelopmental delay, epilepsy, autism, and motor dysfunction, their pathophysiology is not well defined. However, the creatine-phosphocreatine system has an essential action for ATP production in the body [3] and creatine-phosphocreatine system malfunction may cause significant defects in neural signal production [15].

There were some limitations in our study. Although, the literature shows that different autism-related metabolic disorders and genetic factors are frequently associated with autism, and biotinidase deficiency and mucopolysaccharidosis are important metabolic causes, we could not evaluate for these disorders in our study due to financial constraints. Furthermore, due to the high cost of genetic testing and some limitations of accessibility of this in Iran, genetic confirmation was performed only for some patients.

Since metabolic screening in patients with ASD is not a routine practice in many countries, IEM diagnosis can be delayed, and irreversible neurologic damage can ensue. We underscore the need for further studies in different population groups regarding the yield of metabolic screening for patients with ASD, especially in communities with high rates of consanguineous marriages.

Ethics approval: Local Ethics Committee, Shiraz University of Medical Sciences; No. IR.SUMS.MED.REC. 1399.026, dated April 8, 2020.
Contributors: HM, SI, ShM: study concept and design, and preparation of the manuscript. NY: analysis and interpretation of data and the revision of the manuscript. All authors participated in data acquisition. All authors approved the final version of the manuscript and are accountable for all aspects related to the study.
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Web Table I Diagnostic Criteria for Diagnosis of Various Inborn Errors of Metabolism Found in Children with ASD (*N*=13)

Disorder	***n (%)***	***Diagnostic criteria***
Creatine transporter defect	5 (4.7)	Severely increased urinary ratio creatine to creatine and/or pathogenic variants in the *SLC6A8* gene
Guanidinoacetate methyltransferase deficiency	4 (3.8)	The urinary ratio of guanidine acetate to creatine severely increased and the urinary ratio of creatine to creatine: decreased and/or pathogenic variants in the *GAMT* gene
Argininosuccinate lyase deficiency	1 (0.95)	Increased plasma level of arginine succinic acid and citrulline+pathogenic mutation in *ASL* gene
2 methylbutyryl glycinuria	1 (0.95)	Very high levels of methylbutyryl carnitine in plasma and 2-methylbutyryylglycine and 2-hydroxyglutaric acid in urine
Combined methylmalonic aciduria/malonic aciduria	1 (0.95)	Increased urinary levels of methylmalonic acid and malonic acid
Short-chain acyl-CoA dehydrogenase deficiency	1 (0.95)	Very high levels of butyryl carnitine and isobutyl carnitine in plasma, and ethylmalonic acid and methyl succinic acid in urine

SLC2A8:Solute carrier family 2 members; ASL: Arginosuccinate lyase.

Web Table II Characteristics of Patients with Autism and Inborn Errors of Metabolism (*N*=13)

Disorder	***Age (y)***	***Seizure***	***Development***	***consanguinity***
CTD	4, M	N_O	Normal	No
CTD	5, M	N_O	Delayed	Yes
CTD	2, M	N_O	Normal	No
CTD	3, F	N_O	Normal	No
CTD	4, M	N_O	Delayed	Yes
GAMT deficiency	6, M	Yes	Delayed	Yes
GAMT deficiency[a]	2, M	N_O	Delayed	Yes
GAMT deficiency	3, M	Yes	Delayed	Yes
GAMT deficiency	5, M	Yes	Delayed	Yes
Combined MMA/MA	4, M	N_O	Normal	No
ASA	3, M	Yes	Delayed	Yes
2MBG	9, F	N_O	Normal	No
SCAD	11, F	N_O	Normal	No

ASD; Autism spectrum disorder, IEM; Inborn error of metabolism, CTD: Creatine transporter defect; GAMT: Guanidino acetate methyltransferase; MMA/MA: Methylmalonic aciduria/malonic aciduria; ASA: Argininosuccinate aciduria; 2MBG 2 Methylbutyryl glycinuria; SCAD: Short-chain acyl-CoA dehydrogenase deficiency. All patients had normal head circumference except [a]one with microcephaly.

RESEARCH PAPER

Iron Profile in Term Small for Gestational Age Infants at 10 Weeks of Age and Correlation With Maternal Iron Profile : *A Prospective Cohort Study*

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Background: Term small for gestational age (SGA) babies are at risk for developing iron deficiency anemia. The association between maternal and infant iron stores is not clear.

Objective: To assess proportion of term SGA neonates developing iron deficiency anemia by 10 weeks of age, and measure correlation between iron profile and hepcidin of babies at birth and at 10 weeks of age with maternal iron profile.

Design: Prospective cohort study conducted from November, 2018 to April, 2020.

Participants: 120 term SGA babies and their mothers.

Intervention: Hemogram, iron profile and serum hepcidin (every fourth case) estimated in mother, cord blood and baby at 10 weeks. Babies developing anemia at 6 weeks detected by hemogram and ferritin were started on iron supplementation and excluded from the study.

Outcome: Proportion of babies developing iron deficiency anemia at 10 weeks of age.

Results: 35 (29.2%) of 120 term SGA babies developed anemia (hemoglobin <9 g/dL) at 6 weeks. Proportion of infants who developed iron deficiency anemia (hemoglobin <9 g/dL and serum ferritin <40 µ/dL) at 6 and 10 weeks of age was 14.2% and 23.3%, respectively. No significant correlation was found bet-ween hemoglobin, iron and hepcidin of the baby in cord blood and at 10 weeks of age with that of mothers. Serum hepcidin in babies at birth (137.5 ng/mL) were higher than maternal values (128 ng/mL).

Conclusion: A significant proportion of term SGA infants developed anemia during early infancy, irrespective of maternal iron status.

Keywords: *Anemia, Ferritin, Hemoglobin, Hepcidin*

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Iron is an essential nutrient during all stages of human development. The requirement of iron increases during periods of rapid growth and differentiation such as pregnancy and infancy [1].

Transfer of iron from the mother to the fetus is an active process that occurs mainly in the third trimester. Most babies born to women with iron deficiency anemia (IDA) have serum iron levels comparable to those born to iron-replete women but with lower serum ferritin levels; thus, suggesting decreased iron stores [2]. Hepcidin operates as a negative feedback regulator of this iron homeostasis across the placenta and ensures appropriate physiological concentration of iron in the fetus. Maternal hepcidin levels gradually decrease in pregnancy from the first to the third trimester to meet the six-fold higher needs for iron absorption and facilitate its placental transfer. Lower hepcidin concentration in the mother increases duodenal iron absorption, allows release of iron from macrophages and hepatic stores to maternal circulation, thereby increasing iron availability to the fetus. On the other hand, higher hepcidin concentration in the placenta than in the mother avoids iron overload in the fetus [3].

Neonates born as preterm, very low birth weight (VLBW) and small for gestational age (SGA) are at risk of developing IDA due to poor iron stores. There are guidelines on iron supplementation to the preterm and VLBW babies but limited studies for term SGA neonates [4-6]. Prevalence of iron deficiency and timing of hemoglobin nadir in this group is not well documented. Existing guidelines do not recommend the time of initiation of iron supplementation in SGA babies. With India contributing to largest number of SGA births worldwide [7], an understanding of prevalence of IDA in SGA during early infancy and its association with maternal iron status will help develop focused preventive strategies. A decrease in hepcidin is considered an early marker of IDA with decreased transferrin saturation and ferritin levels.

Invited Commentary: Pages 179-80.

Thus, we planned this study to determine the correlation between iron profile of term SGA neonates and their mothers. The primary objective was to assess proportion of term SGA neonates who develop IDA at 10 weeks of age. The secondary objectives were to correlate serum iron,

total iron binding capacity (TIBC), percent transferrin saturation (TSat), ferritin and hepcidin of SGA babies at birth and at 10 weeks of age with maternal iron profile.

METHODS

This prospective cohort study was conducted in the neonatology unit of Departments of Pediatrics, Obstetrics and Gynecology and Pathology of a tertiary care teaching institute from November, 2018 to April, 2020 after obtaining ethical clearance from institutional ethical committee for human research. Verbal consent was taken from mothers at the time of screening for fetal growth restriction. Written informed consent was taken from the parent/guardian for participation in the study before enrolment.

Pregnant women admitted for delivery in the labor room were screened for suspected fetal growth restriction (FGR) by assessing symphysio-fundal height and abdominal circumference on available antenatal ultra-sound scans by obstetrician. FGR was defined as fetal weight or abdominal circumference less than 10th centile for that gestational age [8]. The neonates born at gestational age of 37- 42 weeks with birth weight less than 10th centile for that gestational age as per the 21st Intergrowth charts and their mothers were then enrolled in the study [9]. Neonates requiring admission in neonatal intensive care unit (NICU) in view of any of the ailments such as sepsis, birth asphyxia, respiratory distress at birth (defined as Downe score of more than 2), seizures or known Rh or ABO incompatibility were excluded from the study. Babies born to mothers who were known cases of hemolytic anemias including thalassemia and sickle cell anemia, chronic kidney disease, chronic liver disease, hemochromatosis, chorioamnionitis, infection with human immunodeficiency virus, hepatitis B, syphilis, toxo-plasmosis, rubella, cytomegalovirus, malaria and obstetric complications like antepartum and postpartum hemorrhage were also excluded from the study. Delayed cord clamping (DCC) was followed at the time of all deliveries as per the standard protocol. Mothers of all the infants were counseled regarding exclusive breastfeeding. Enrolments were done as per the work-shifts of the principal investigator.

Baseline demographic profile of both the mother and the baby with anthropometric parameters (including weight, length, head circumference and ponderal index) of the baby were noted at birth [10]. Three mL each of cord blood and maternal venous blood (within 2 hours of delivery) was collected for hemogram with indices, iron profile, ferritin and transferrin saturation at birth. Babies were discharged within 48 to 72 hours of birth on exclusive breast feeding.

The enrolled babies were followed up at 6 weeks (±1 week) and 10 weeks (±1 week) of age and their growth was monitored. Reinforcement of exclusive breast feeding was done at each visit. All the infants were immunized at each visit as per the National Immunization Schedule [11]. Hemogram with indices and serum ferritin of the baby was done at 6 weeks. Iron deficiency anemia was defined as fall in hemoglobin below 9 g/dL accompanied with serum ferritin <40 µg/L at 6-10 weeks of age as per the European Society for Paediatric Gastroenterology Hepatology and Nutrition (ESPGHAN) committee guidelines [12]. Those who developed anemia were assessed clinically for any features of sepsis, hepatosplenomegaly or blood loss. They were started on iron supplementation (4-6 mg/kg/day) after investigations and were excluded from the analysis [13]. Hemogram with indices, iron profile and serum ferritin were repeated for the rest of the babies at 10 weeks of age.

Samples for serum hepcidin assessment was taken from cord blood, mother (within 2 hours of delivery) and infant at 10 weeks (±1 week) of follow up in every fourth mother-infant pair and were stored at -20 ^{0}C for subsequent analysis.

Hemogram with indices was assessed using auto analyzer (Backmann culter LH 500). Venous sample was collected in iron free tubes for iron studies. Transferrin saturation (TSat), serum iron concentration and total iron binding capacity (TIBC) were done at the department of pathology, as recommended by the Iron Panel of the International Council for Standardisation in Hematology (ICSH) [14]. Serum samples for ferritin and hepcidin levels were stored at -20 ^{0}C until assayed using commercial ELISA kits (Diametra Ferritin ELISA Kit-96 wells Cat No. DKO039 and Human Hepc25 ELISA Kit E-EL-H5497).

In a study by Mukhopadhyaya, et al. [2], proportion of term SGA infants with serum ferritin <40 µg/L at birth was observed to be 34%. Considering absolute precision of 10% with confidence interval of 95% and power of 80%, sample size was calculated to be 87. Assuming that approximately 30% of babies might become anemic by 10 weeks of age and may need iron supplementation before final analysis, we decided to enroll 120 mother-infant pairs at birth.

Statistical analysis: Data were analyzed using SPSS software version 20.0. Proportions were expressed as numbers and percentages. Paired normally distributed variables (hemogram and iron profile) were compared using paired *t* test. Non-normally distributed variables (serum ferritin and serum hepcidin) were compared using Wilcoxon signed rank test/Friedman test. Linear correlation was explored using Pearson correlation (for normally distributed parameters) and Spearman correlation (for non-normally distributed parameters). *P* value <0.05 was considered significant.

RESULTS

The flow of study participants is shown in **Fig. 1**. Thirty-five (29.2%) enrolled babies developed anemia (hemoglobin <9 g/dL) at 6 weeks of age and were started on iron supplementation. Of the remaining 85 infants, another 24 infants (total 49.2%) became anemic at 10 weeks of age (**Fig. 1**). Proportion of term SGA infants who developed iron deficiency anemia (hemoglobin <9 g/dL and serum ferritin <40 µg/dL) at 6 and 10 weeks of age was 14.2% and 23.3%, respectively**.**

Anthropometric parameters of enrolled infants at birth, 6 weeks and 10 weeks are shown in **Table I**. The mean (SD) gestation age was 39 (1.13) weeks (67 boys), and 107 (89.2%) were exclusively breastfed till 10 weeks of age.

The maternal and infant profile of hematological parameters is shown in **Table II**. A significant fall was noted in mean serum iron, percentage transferrin saturation and median serum ferritin levels of infants at 10 weeks of age as compared to that in cord blood. It was also observed that serum iron levels and %transferrin saturation levels of infants in cord blood were more than that of mothers at the time of delivery.

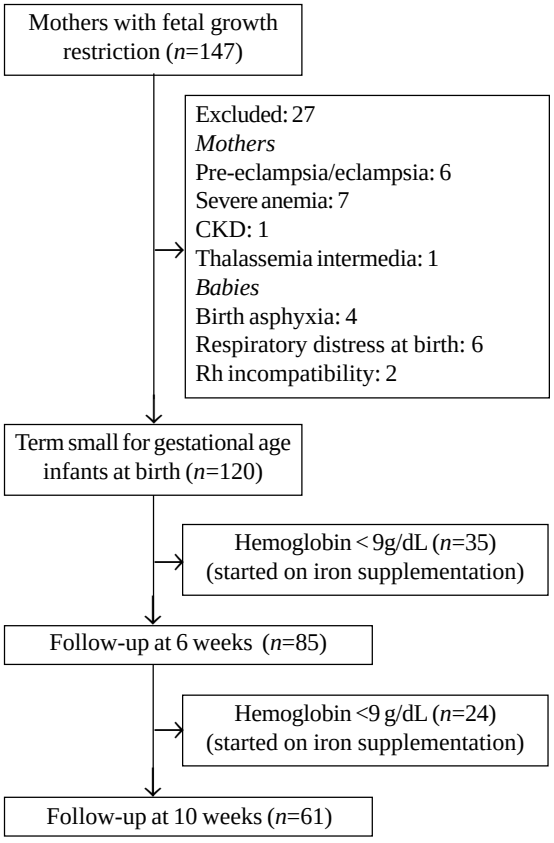

Fig. 1 Flow of participants in the study.

Table I Anthropometric Parameters of Term Small for Gestational Age Infants Enrolled in the Study (*N*=120)

Variables	*At birth, n=120*	*At 6 wk age, n=120*	*At 10 wk age, n=85*
Weight (g)	2431.5 (234.07)	3529.6 (278.10)	4307.8 (310.03)
Length (cm)	48.8 (0.76)	53.1 (2.44)	57.7 (1.30)
HC (cm)	34.5 (0.48)	36.5 (2.16)	38 (0.54)

Data expressed as mean (SD). HC: head circumference.

No significant correlation was found between hemoglobin, iron profile and hepcidin of the baby in cord blood and at 10 weeks of age with maternal profile iron (P>0.05), except a weak positive correlation between maternal serum iron and serum iron of the infant in cord blood (r=0.19, P=0.03). Serum hepcidin in cord blood showed a negative but significant correlation with maternal serum ferritin (r = – 0.39, P=0.033).

DISCUSSION

In our study, it was observed that nearly one-fourth of term SGA infants developed IDA by 10 weeks of age. There was no significant correlation between maternal iron profile with that of babies at birth and at 10 weeks of age. Serum hepcidin of babies at birth negatively correlated with maternal serum ferritin levels.

A higher risk of IDA in term SGA infants could be explained by their poor iron stores at birth with chronic utero-placental insufficiency, higher requirements for catch up growth and higher red cell mass [6]. A previous study [2] lower cord serum ferritin levels in SGA as compared to AGA infants. Cord serum iron levels were also lower in SGA infants than AGA infants suggesting poorer iron stores at the time of birth [15,16]. None of the babies in this study had anemia at birth; though, a significant proportion of these babies developed anemia by 10 weeks of age, the levels below the cutoff of 10 g/dL for physiological anemia of infancy at 8-10 weeks of age [17].

The transfer of iron from mother to fetus is an active process occurring against the concentration gradient via placenta. However, it is uncertain whether the transfer of iron across the placenta is proportional to iron available in the mother or whether it is transferred preferentially as per the requirements of the fetus. The iron status of cord blood in infants correlated weakly with maternal iron status, similar to as seen in earlier studies [18]. A weak positive correlation was reported between maternal iron and cord blood ferritin [19,20], unlike this study where no correlation between maternal hemoglobin and iron status of infants were seen [21,22].

Table II Hematological Parameters and Iron Profile of Mothers and Term Small for Gestational Age Infants in Cord Blood, and at 6 Week and 10 Week of Age

Variable	*Mothers (n=120)*	*Infants*			
		Cord blood (n=120)	*6 week (n=120)*	*10 week (n=85)*	*P value*[b]
Hemoglobin (g/dL)	9.9 (1.53)	15.5 (1.39)	9.8 (1.13)	9.3 (0.77)	<0.001
RBC count ($X10^9$/L)	3.7 (0.60)	4.5 (0.55)	3.47 (0.40)	3.51 (0.40)	<0.001
Hematocrit (%)	31.6 (5.61)	47.8 (6.04)	30.9 (3.67)	29.1 (2.62)	<0.001
Mean corpuscular volume (fL)	86.2 (12.86)	107.1 (7.21)	89.1 (4.83)	83.4 (6.04)	<0.001
MCH (pg/cell)	27.2 (4.10)	34.9 (3.13)	28.4 (2.47)	26.7 (3.19)	<0.001
MCHC (g/dL)	32.4 (9.85)	32.6 (2.82)	31.9 (2.03)	31.9 (2.53)	0.096
Total leukocyte count ($x10^9$/L)	12.1 (4.61)	11.3 (3.71)	8.7 (1.80)	8.9 (1.86)	<0.001
Platelet count ($x10^9$/L)	224 (0.79)	188 (72)	324 (77)	315 (62)	0.019
Serum iron (µg/dL)	88.21 (32.18)	153.23 (46.59)	-	75.16 (30.80)	<0.0001
TIBC (µg/dL)	375.75 (87.83)	249.15 (65.31)	-	242.27 (63.42)	0.901
Transferrin saturation (%)	32.02 (10.81)	63.71 (20.05)	-	25.21 (13.45)	<0.001
Serum ferritin (µg/L)[a]	18.5 (8.00-35.00)	106 (52-153.5)	143 (74.5-11.5)	98 (64-151)	0.032
Serum hepcidin (ng/mL)[a] (*n*=30)	128.0 (112.25-132.75)	137.5 (134.0-141.75)	-	139.5 (134.5-142.0)	0.802

Values expressed as mean (SD) *or* [a]*median (IQR).* [b]*P value for statistical significance between cord blood vs 10 weeks. TIBC: total iron binding capacity; MCH: mean corpuscular hemoglobin; MCHC: mean corpuscular hemoglobin concentration.*

Maternal serum hepcidin values were lower than the cord blood hepcidin values, without any correlation between the two. A few studies reported a low positive correlation between the two [23-25]. Lower hepcidin concentration in the mother increases duodenal iron absorption, allows release of iron from macrophages and hepatic stores to increase iron availability to the fetus. On the other hand, higher hepcidin concentration in the placenta than in the mother avoids iron overload in the fetus. A negative correlation between serum hepcidin in cord blood and maternal serum ferritin suggests that hepcidin at birth might be regulated by maternal iron stores.

Being a prospective cohort study, the infants who were anemic at 6 weeks of age were started on iron supplementation and excluded from the study. The improvement in their iron status, was not studied, thus amounting to a limitation of this study. We did not look at the maternal dietary and supplemental iron intake. Moreover, serum hepcidin values were assessed only in 30 infant-mother pairs due to budgetary constraints.

The study concludes that a significant proportion of term SGA neonates develop IDA and need iron supplemen- tation. There are no existing guidelines for prophylactic administration of iron in term SGA neonates. We recommend that iron status of SGA neonates should be evaluated in larger population-based studies. Further, multicentric interventional studies to determine time and dose of iron supplementation should be conducted to formulate guidelines regarding prophylactic iron supplementation to term SGA infants.

Ethics clearance: Institutional Ethics Committee–Human Research, UCMS; No. IEC-HR/2018/36/109, dated Oct 15, 2018.
Contributors: PB, PD: conceived the idea; KS, PB: conceptualized the study and devised its design; BG,PG: provided critical inputs; KS: collected the data; PB,PD,PG,BG: supervised data collection and helped in conduct of study; KS: drafted the manuscript and all authors have critically approved final version of study as submitted, and are willing to be accountable for all aspects of the study.
Funding: None; *Competing interests*: None stated.

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WHAT IS ALREADY KNOWN?

- Preterm and very low birth babies are at risk of iron deficiency anemia and need iron supplementation.

WHAT THIS STUDY ADDS?

- Significant proportion of term small for gestational age neonates developed iron deficiency anemia by 10 weeks of age.
- Iron stores of babies at birth were found to be independent of maternal stores.

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RESEARCH PAPER

Prevalence and Correlates of Vitamin D Deficiency Among Children and Adolescents From a Nationally Representative Survey in India

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Objective: To evaluate the prevalence of vitamin D deficiency (VDD) and its correlates among apparently healthy children and adolescents. **Methods:** We carried out a secondary analysis of data of Comprehensive National Nutrition Survey 2016-18 to analyze the pre-valence and predictors of VDD among Indian children and adolescents. **Results**: The over-all prevalence of VDD in preschool children (1-4 years), school age (5-9 years) children, and adolescents (10-19 years) was 13.7%, 18.2%, and 23.9%, respectively. Age, living in urban area, and winter season were significantly associated with VDD. Vegetarian diet and high-income households were the main risk factors observed in 5-19 years age category. Female sex and less than three hour of physical activity/week were independent risk factors among adolescents. **Conclusion**: The prevalence and determinants of VDD across different age-groups are reported, and these should be interpreted and addressed to decrease the burden of VDD in India.

Keywords: *Diet, Physical activity, Predictors, Rickets.*

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Vitamin D is essential in early period of growth and its deficiency causes adverse health consequences in later life [1]. Epidemiological studies in children have reported vitamin D deficiency (VDD) prevalence ranging from 50% to 94%, suggesting a high unmet vitamin D requirement [2,3]. However, these studies have methodological limitations such as small sample size, restricted to urban areas, improper sampling techniques, and different estimation methods and diagnostic criteria [4]. The Indian guidelines for addressing the burden of VDD were also released recently [5]. Environmental factors (air pollution, cloud cover), lifestyle factors (physical activity, sunscreen application), vegetarian dietary pattern, socio-demography and ethnicity influence the vitamin D levels.

This study evaluated the prevalence of VDD and its correlates among apparently healthy children and adolescents of India, using nationally representative data from Comprehensive National Nutrition Survey (CNNS) [6].

METHODS

The CNNS, a cross-sectional survey, was conducted in 30 states of India, between 2016 and 2018 [6]. The survey design, sampling methodology, sociodemographic characteristics and ethical approvals of the CNNS are published elsewhere [6-8]. Data were collected through household interviews and standardized protocols. Almost 50% of all the children who completed anthropometry were contacted through systematic random sampling for blood collection.

Invited Commentary: Pages 181-82.

Blood sample collection procedure and biomarker analysis methods are detailed elsewhere [6]. Briefly, trained phlebotomists collected 8 mL blood from children aged 1-4 years and 10 mL from 5-19 years for estimating micronutrient concentrations, and biomarkers for non-communicable diseases (5-19 years only). Biochemical analyses were done in a commercial laboratory (SRL Labs,

Mumbai, Gurugram, and Kolkata). Serum vitamin 25(OH) D was measured by the direct competitive chemiluminescent immunoassay that detects 25-OH vitamins D2 and D3 (Advia Centaur XP, Siemens). The quality control samples were estimated with each batch on all days and the inter-assay (values obtained in different runs) and intra-assay (within the same run) coefficient of variation (CV) was calculated, which ranged between <11.9% and <5.3%, respectively. Rigorous control and monitoring systems were included in the standard operating procedures for quality assurance of biomarker data [6].

Serum 25(OH) D level <12 ng/mL was considered as an indicator of VDD [9]. Body mass index (BMI) was categorized according to the WHO Child Growth Standards, 2006 for age group 0-4 years [10], and WHO Growth Reference Data for 5-19 years [11]. Vegetarian diet was defined as consumption of plant-based food items and abstinence from meat, game, poultry, egg and fish in food intake. Sports activity (for age group 10-19 years) was defined as engagement in sports for 45 min/day at least 3 days per week. It included one or more than one activity such as aerobics, basketball/volleyball, cricket, dancing, football, hockey, martial arts, rugby/kabbadi, running/ jogging, swimming lessons, swimming for fun, and tennis/ badminton/ squash/other racquet sport.

For analysis of wealth groups, we categorized households into three groups based on wealth quintile i.e., low income household group comprising poor and poorest wealth quintile, middle income household group comprising middle wealth quintile, and high-income household group comprising rich and richest wealth quintile [12]. Seasonal variation of vitamin D status was studied under three seasons i.e., summer (March-June), autumn (July-October) and winter (November-February) [13].

Statistical analysis: Participants were categorized into different sub-groups to analyze the predictors for vitamin D status. Sampling weights were used to ensure appropriate representativeness of all the estimates. Categorical variables were represented as percentages and continuous variables were represented as mean levels with 95% confidence intervals (CI). The association between categorical variables were computed by chi-square test and between continuous variables were computed by linear regression analysis. Further, unadjusted and adjusted odds ratios (aOR) with 95% CI were calculated by multivariable logistic regression analysis. Variables with *P* value <0.20 in univariate analysis were included in multivariable model [14]. All the analyses was conducted using Stata 15.0 (Stata Corp LLC). *P* value <0.05 (two sided) was considered statistically significant for all the tests.

RESULTS

The flow chart detailing the samples analyzed is given in **Fig. 1**. The prevalence of VDD in the age groups 1-4 years,

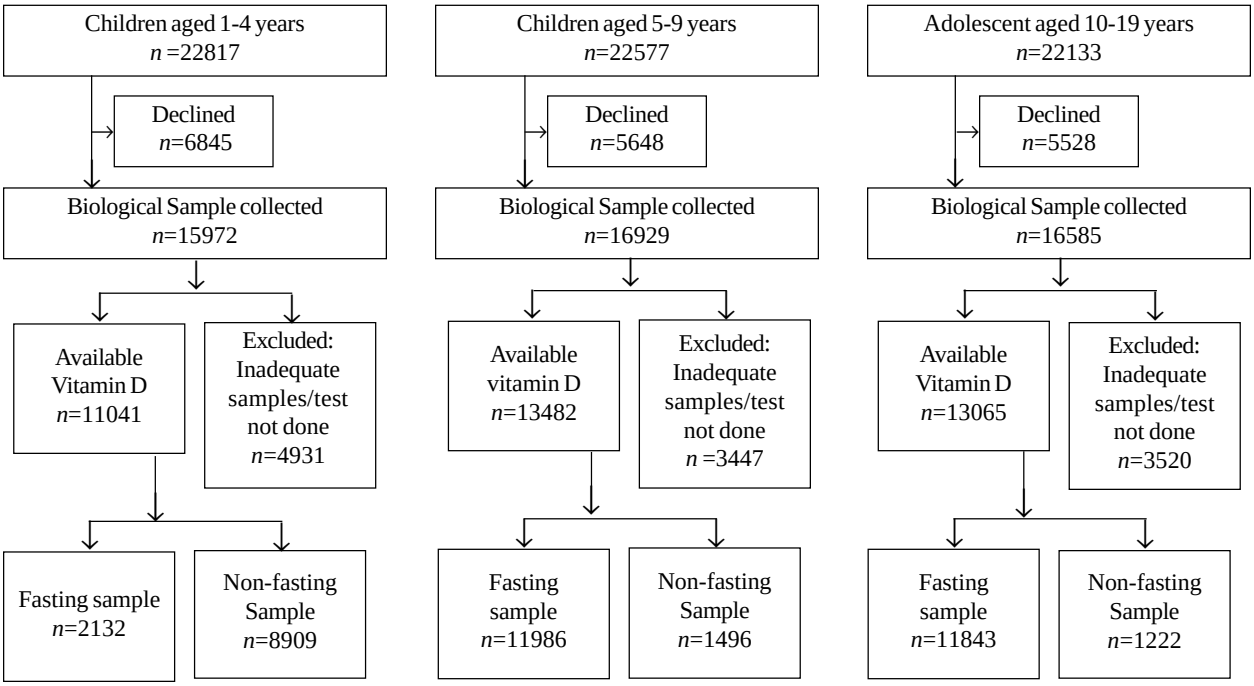


Fig. 1 Flow chart showing details of sample analysis as per different age-groups.

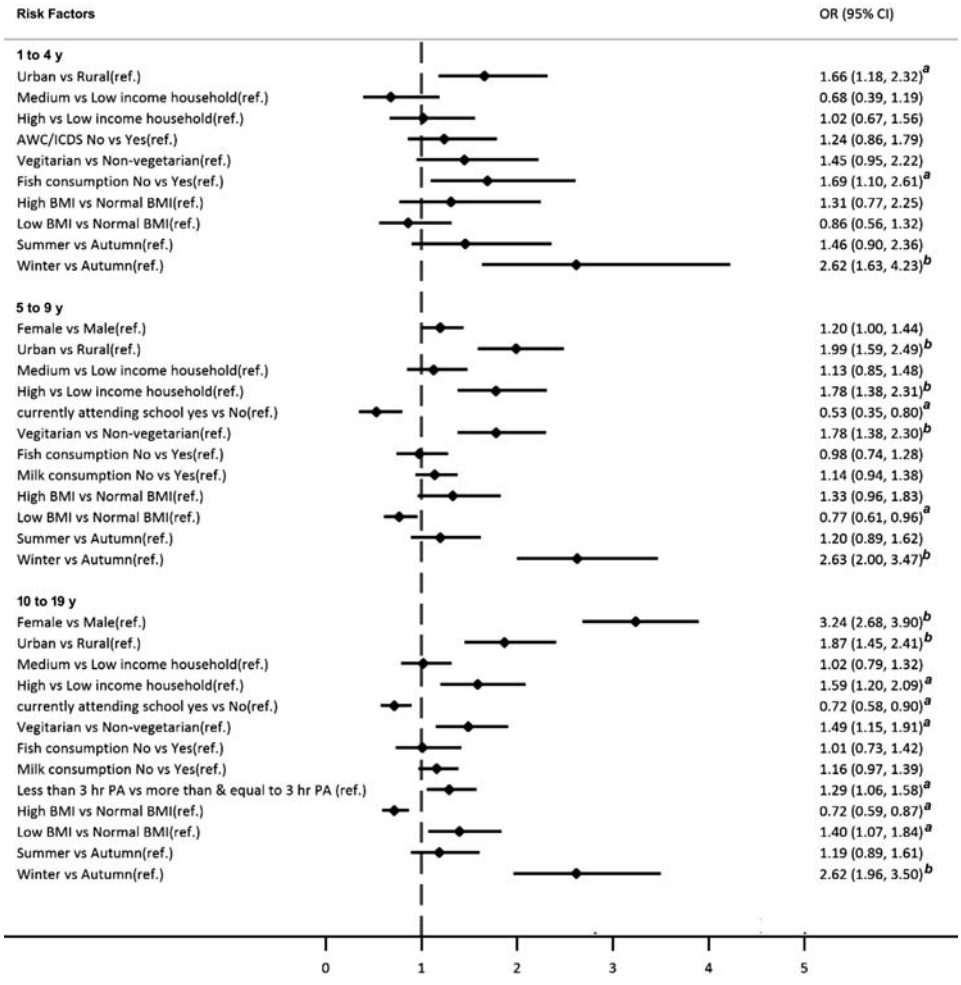
5-9 years, 10-19 years was 13.7%, 18.2% and 23.9%, respectively. Prevalence of VDD was higher in girls of age 10-19 years as compared to boys (34.3% vs 13.8%). The aOR for gender wise comparison was 1.20 in 5-9 years and 3.24 in 10-19 years. School aged children (aOR=0.6) and adolescents (aOR=0.7) who were not attending the school had lower risk for VDD than those attending the school. With respect to diet, VDD was higher among children and adolescent taking vegetarian diet than non-vegetarian diet in 1-4 years (17.5% vs 9.7%), 5-9 years (21.2% vs 14.6%) and 10-19 years (26.9% vs 20.6%) age groups, respectively. High income household (aOR=1.78 and aOR=1.59) and vegetarian diet (aOR=1.78 and aOR=1.49) were significant risk factors for VDD among children aged 5-9 year and adolescents aged 10-19 year.

The prevalence of VDD was lowest among individuals with low BMI [9.8% (aOR=0.86), 14.4% (aOR=0.77) and 17.6% (aOR=1.40)] followed by normal BMI (14.3%, 19.2% and 25.5%) and high BMI [17.2% (aOR=1.31), 27.3% (aOR=1.33) and 33.6% (aOR=0.72)] across ages 1-4, 5-9, and 10-19 years, respectively.

The association between the VDD and its correlates using multivariable regression analysis is shown in **Fig. 2**. Across all three age groups, subjects living in urban area and those who were sampled in winter season showed significantly higher odds of VDD.

DISCUSSION

The results of the present study showed a significant prevalence of VDD among the three age groups surveyed, and a disproportionate burden (1.1%-76.1%) of VDD across different states of India. Further, urban residence, non-consumption of fish, and winter season in children 1-4 years; urban residence, high income household group,



[a]P value <0.05; [b]P value <0.001.

Fig. 2 Association of vitamin D deficiency and its correlates across three age-groups. The dot shows the odds ratio and the bar shows the 95% CI.

WHAT THIS STUDY ADDS

- The age-specific and sex-specific prevalence of vitamin D deficiency from a nationally representative sample of children and adolescents aged 1–19 years in India is reported with its correlates.

attending school, high BMI, and vegetarianism among school-going children; female gender, urban residence, high income household, vegetarianism, less than three hours of physical activity/week, low BMI, and winter season among adolescents were significantly associated with increased risk of VDD. A similar Indian study using dry blood spot also reported high prevalence of VDD in children and adolescents aged 5-18 years with factors such as younger age, female gender, overweight and urban residence as risk factors [15]. Another study from Kerala also reported lower levels of vitamin D among girls than boys in children aged 5-13 years [16]. We also observed higher proportion of VDD among girls than boys in all age categories, and those residing in urban areas compared to those residing in rural areas. Similar trends of low vitamin D levels in urban population than rural have been reported earlier [17].

Higher VDD among vegetarians in the present study could be due to the limited intake of vitamin D rich products, most of which are animal source like fish and egg yolk [18]. Fortification of food with vitamin D in India is in a nascent stage, thereby making it difficult for a vege-tarian to meet the daily requirement of vitamin D.

An increased prevalence of VDD in winter season followed by summer and autumn was observed in all the age groups consistent with earlier studies [13,19]. Adolescent girls were less active physically as compared to boys in the present study. Physical activity, irrespective of indoor and outdoor in nature, was positively associated with vitamin D levels among children [20]. A reduced risk for VDD among school-aged children and adolescents who were not attending school was observed in this study, possibly linked to physical activity.

In this study, vitamin D was estimated using the chemiluminescence method rather than LC-UV or LC-MS/MS, which are the preferred methods. The factors related to sun exposure such as duration, ultraviolet B levels, air pollution levels, skin pigmentation, body coverage, sunscreen usage and vitamin D supplementation were not looked into, in the present survey. The strength of the study was that it examined the large scale population based data on vitamin D status of children and adolescents.

In conclusion, this study summarizes age- and sex-specific prevalence of VDD among children and adolescents in India. Children and adolescents should be encouraged to adopt healthy lifestyle with outdoor physical activity during sunshine and consumption of vitamin D fortified foods, specifically among girls and adolescents.

Ethics clearance: Postgraduate Institute for Medical Education and Research, Chandigarh; and the Institutional Review Board of the Population Council.
Contributors: LR, RAA, GR: conceptualized the manuscript; AS, RA, AP, SR, NK, PKA: survey data analysis; KM,GR,RB: statistical analyses for manuscript; HSS, MN, GTK, RJ, ADW, PKA: provided expert advice for the manuscript; GR: led the writing of the manuscript with inputs from LR, RAA. All authors have reviewed and approved the final version of manuscript, and are accountable for all aspects related to the study.
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Competing interests: HSS designed the draft protocol of the CNNS with consultancy support from the UNICEF, India and was member of the Technical Advisory Committee of the CNNS, constituted by the Ministry of Health and Family Welfare (MoHFW) of the Government of India (GoI), to oversee its conduct and analysis. He is a member of the World Health Organization Nutrition Guidance Expert Advisory Subgroup on Diet and Health and Guideline Development Group on 'Use and interpretation of hemoglobin concentrations for assessing anaemia status in individuals and populations', and member of the National Technical Board on Nutrition of NITI Aayog, and Expert Groups of the MoHFW; GoI on Nutrition and Child Health. GTK designed the draft protocol of the CNNS with consultancy support from the UNICEF, India and was member of the Technical Advisory Committee of the CNNS, constituted by the MoHFW, GoI, to oversee its conduct and analysis. She is also chair of a task force on Balanced and healthy diets of MoHFW, GoI, and member of other governmental expert groups and committees. MN and LR were members of the Technical Advisory Committee of the CNNS, constituted by the MoHFW, GoI, to oversee its conduct and analysis. None of the other authors listed on this manuscript report any competing interests.

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RESEARCH PAPER

Neutrophil-Lymphocyte Ratio for Predicting Coronary Artery Lesions in Children With Kawasaki Disease

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Background: Coronary artery lesions (CAL) are a specific feature of Kawasaki disease (KD), and develop during the second week of illness. This study was conducted to determine whether Neutrophil: Lymphocyte Ratio (NLR), assessed between the fourth and sixth day of fever onset in children with KD, can predict coronary artery lesion (CAL) development. **Methods:** In this review of hospital records, data of patients with KD admitted at our center between January, 2016 and January, 2020 was retrieved. The patients were divided into two groups based on the presence of CAL, and clinical characteristics of patients were compared between the two groups. **Results:** Out of the 79 patients enrolled, CAL was found in 40 (50.6%) patients and intravenous immunoglobulin (IVIg) resistance was seen in 13 (16.5%) patients. Multivariate logistic regression revealed NLR as an independent predictor of CAL [OR (95% CI) 2.0 (1.2-3.1); *P*<0.001], and erythrocyte sedimentation rate (ESR) [OR (95% CI) 1.03 (1.001-1.1) *P*=0.04], as an independent predictor of IVIg resistance. NLR ≥2.08 was 82% sensitive and 80% specific in predicting CAL. ESR ≥88 mm/h was 85% sensitive and 64% specific in predicting IVIg resistance. **Conclusions:** NLR is an independent predictor of CAL in KD. NLR ≥2.08 done between the fourth and sixth day of fever onset may identify children with KD at risk of CAL.

Keywords: *Aneurysm, Erythrocyte sedimentation rate, Intravenous immunoglobulin resistance, Prognosis.*

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Kawasaki disease (KD) is a systemic vasculitis resulting in inflammation of medium-sized vessels, predominantly the coronary arteries [1], with an incidence of 4.54 cases per 1,00,000 children below 15 years [2]. The most dreaded complication of Kawasaki disease is coronary artery aneurysm, which occurs in around 15-25% of patients [3].

Prognostic scores/biomarkers predicting the development of CAL will be of significant clinical utility to initiate early, aggressive treatment and follow up strategy, especially in resource-limited settings. The existing scoring systems viz., Kobayashi, Egami and Sano scores were designed to predict IVIg resistance rather than CAL [4]. The Harada score, which was designed to predict CAL, identified CAL with 90% sensitivity in US population, and with 83% sensitivity and 47% specificity in Turkish population [5]. Other novel biomarkers like NT-proBNP, thrombospondin-1, IL-12, IL-17, tenascin C have not been reliably validated in predicting CAL [6].

This study was conducted to study the role of neutrophil-lymphocyte ratio (NLR) and other markers in predicting the development of coronary artery abnormalities and intravenous immunoglobulin resistance in children with KD.

METHODS

We extracted hospital data of all admitted children diagnosed as KD between January, 2016 and January, 2020 at a tertiary referral hospital. Those with incomplete laboratory or echocardiographic details were excluded. As per hospital protocol, all patients suspected to have KD underwent 2D-echocardiography at admission or before administering intravenous immunoglobulin (IVIg) and at least 24 hours after IVIg administration. The coronary artery *z*-scores were calculated using the Cardio Z application, Version 3.0, as per reference values by Dallaire, et al. [7]. All patients were treated according to the American Heart Association (AHA) guidelines [8]. A repeat dose of IVIg (2 g/kg) was administered no earlier than 36 hours after the first dose completion for patients with IVIg resistance.

The standard definitions included classical KD, incomplete KD, atypical KD, CAL and IVIg resistance were defined according to AHA, 2017 guidelines [8]. NLR was calculated by dividing the absolute neutrophil count by the absolute lymphocyte count in the first complete blood count (CBC) sample between the fourth and sixth days of onset of fever. Platelet-lymphocyte ratio (PLR) was calculated by dividing the platelet count by the

absolute lymphocyte count in the first CBC sample between the Day 4-6 of onset of fever. Time to occurrence of CAL was the total number of days since the onset of fever till the day the echocardiography demonstrated CAL for the first time.

The patients were divided into two groups based on the presence or absence of CAL, and the clinical characteristics of the patients were compared across the two groups.

Statistical analysis: Normality was assessed using the Kolmogorov-Smirnov test. Continuous data were expressed as median (IQR), and nominal data were expressed as proportions. The predictive factors for CAL and IVIg resistance were compared and analyzed using the chi-square test for nominal variables and the Mann-Whitney *U* test for continuous variables. $P<0.05$ was considered statistically significant. Those variables with $P<0.05$ were analyzed for their predictive capacity using multivariate logistic regression for categorical variables, and linear regression for continuous variables. The receiver operating characteristic (ROC) curve was plotted for the variables found significant in multivariate analysis, and cut off value with the maximum Youden index was estimated. Survival analysis was undertaken with time to occurrence of CAL as the time to event. Kaplan-Meier survival analysis was plotted for the time to occurrence of CAL and the risk factors and compared using the log-rank test. The Cox-proportional hazard model was used to determine the significant predictors of time to occurrence of CAL. Data were evaluated using SPSS version 25.0.

RESULTS

Of the 103 records of eligible children identified, data of 79 patients was eligible for inclusion; 49 of them being diagnosed with incomplete disease, and 11 with atypical disease. CAL was found in 40 (50.6%) patients. Thirty-two (40.5%) patients had CAA, and 8 (10.1%) had CAD. IVIg resistance was seen in 13 (16.5%) patients.

Table I depicts the comparison of demographic and clinical characteristics between patients with and without CAL, and IVIg resistance, respectively. While children without CAL had significantly higher prevalence of conjunctivitis, children with CAL had higher total leukocyte

Table I Baseline Clinical and Demographic Characteristics of Children With Kawasaki Disease Enrolled in the Study (*N*=79)

	Without CAL (n=39)	*With CAL (n=40)*	*IVIg responsive (n=66)*	*IVIg resistant (n=13)*
Male[a]	27 (69.2)	29 (72.5)	48 (72.7)	8 (61.5)
Conjunctivitis[a]	27 (69.2)	22 (55)	43 (65.2)	6 (46.2)
Rash[a]	25 (64.1)	25 (62.5)	40 (60.6)	10 (76.9)
IVIg resistance[a]	2 (5.1)	11 (27.5)[b]	-	-
CAL[a]	-	-	33 (50)	11 (84.6)[b]
Age (years)	3 (1.17, 8.5)	2 (0.75, 5)	3 (0.9, 7.2)	2 (0.5, 4)
Hemoglobin (g/dL)	9.9 (8.6, 10.9)	9.4 (8.4, 10.4)	9.7 (8.4, 10.5)	9.6 (7.8, 10.3)
Total leukocyte count (x10^9 cells/L)	12.19 (9.2, 15.7)	17 (10.4, 23.2)[b]	13.5 (8.9, 19.1)	22.0 (16.1, 27.6)
Platelet count (x10^9 cells/L)	3.46 (1.5, 5.1)	4.0 (2.3, 5.4)	3.4 (1.4, 5.1)	4.6 (3.6, 7.0)
Neutrophil-lymphocyte ratio	1.6 (1.2, 2.7)	3.5 (2.1, 4.5)	2.44 (1.76, 4.11)	3.06 (1.92, 4.83)
Platlet-lymphocyte ratio	85.5 (51.7, 90.6)	110.8 (71.5, 104.8)[b]	86.9 (54.7, 160.3)	110.8 (73.5, 135.9)
Aspartate aminotransferase (U/dL)	38 (24.5, 49)	30 (22.5, 40.5)	33 (22, 51)	28.5 (23, 41.5)
Alanine aminotransferase (U/dL)	28 (16.5, 73)	27 (17, 33.5)	26.5 (18, 49.7)	21 (13, 28.8)
Albumin (g/dL)	3.2 (2.9, 3.5)	3.2 (2.9, 3.6)	3.2 (2.7, 3.6)	3.2 (2.9, 3.4)
C-reactive protein (mg/dL)	6.1 (3.4, 12.5)	6.4 (4.1, 16.2)	6.2 (3.1, 17.3)	6.4 (3.6, 8.3)
Erythrocyte sedimentation rate (mm/h)	79 (58, 97.5)	92 (58.8, 113.5)	80 (58.8, 104.3)	110 (93.8, 128.8)[b]
Sodium (mEq/L)	133 (131, 134)	132 (131, 134.5)	132 (130, 134)	132 (132, 133)
Duration of fever at presentation (d)	5 (3, 5)	5 (3,6)	5 (4, 7.2)	7 (5, 9.5)
Time to detection of CAL (d)	-	7 (6, 10.2)	-	-
Time to IVIg administration (d)	7 (5.5, 9)	7 (6, 10.2)	6 (5, 10)	8 (7, 11)
Time to defervescence (d)	8 (6, 10)	8 (7, 12.5)	7 (6, 11)	13 (10.3, 22.8)[b]

Values are in median (IQR) or [a]no.(%). [b]P<0.05. CAL:coronary artery lesion, IVIg:intravenous immunoglobulin.

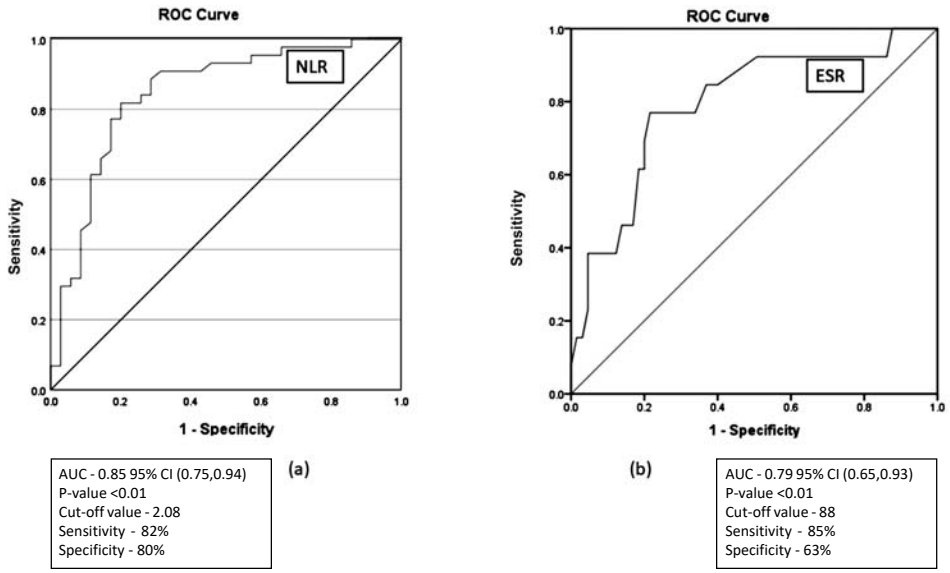

Fig. 1 Receiver-operating characteristic curve showing *a*) neutrophil-lymphocyte ratio (NLR) in predicting coronary artery lesions, and *b*) erythrocyte sedimentation rate (ESR) in predicting IVIg resistance.

count (TLC), NLR and PLR. Children with CAL were more likely to be IVIg resistant than those without. Further, children with IVIg resistance had higher TLC, ESR and were more likely to have had CAL than those without IVIg resistance.

On multivariate regression analysis (**Table II**), NLR was found to be an independent predictor for CAL [OR (95% CI) 2.0 (1.2-3.1); *P*<0.001]. Also, ESR was an independent predictor for IVIg resistance [OR (95% CI) 1.03 (1.0-1.1); *P*=0.04].

ROC curves for NLR and ESR were constructed to determine the appropriate cutoff values that predicted CAL and IVIg resistance, respectively (**Fig. 1**). An NLR value of 2.08 and above was 82% sensitive and 80% specific in predicting CAL [AUC (95% CI) 0.85CI (0.75, 0.94); *P*<0.001)]. ESR of ≥88 was 85% sensitive and 64% specific in predicting IVIg resistance [AUC (95% CI) 0.79 (0.65, 0.93); *P*=0.002]. The time to development of coronary artery lesion in patients with NLR ≥2.08 was 7.7 days as compared to 25.6 days in those with NLR<2.08 [hazard ratio (95%CI) 1.08 (1.03-1.13); *P*<0.001] (**Fig. 2**).

Table II Multivariate Analysis of the Risk Factors for Coronary Artery Lesion and IVIg Resistance

Risk factors	OR (95% CI)	*P value*
Coronary artery lesion		
Conjunctivitis	0.3 (0.1-1.1)	0.06
IVIg resistance	2.6 (0.4-17.4)	0.32
TLC	1.0 (1.0-1.0)	0.15
NLR	2.0 (1.2-3.1)	<0.001
PLR	1.0 (1.0-1.0)	0.51
IVIg resistance		
CAL	1.80 (0.3-12.8)	0.56
TLC	1.00 (1.0-1.0)	0.51
Platelet count	1.10 (0.8-1.6)	0.61
NLR	1.02 (0.9-1.2)	0.81
ESR	1. 03 (1.0-1.1)	0.04

IVIg: intravenous immunoglobulin; CAL: coronary artery lesion; TLC: total leukocyte count; NLR: neutrophil-lymphocyte ratio; PLR: platelet lymphocyte ratio.

DISCUSSION

In our study, the prevalence of CAL was 50.6%, which was higher than previous studies. This could partly be attributed to referral bias, retrospective nature of the study, exclusion of cases due to incomplete data, and partly to the fact that some of the incomplete KD masquerade viral exanthem and hence could be misdiagnosed unless a CAL is found. This relatively higher frequency of CAL needs to be confirmed in prospective studies.

In the early phase of KD, activated neutrophil-mediated endothelial injury is the likely pathogenesis of KD vasculitis. Therefore, NLR is higher in the initial phase, which gradually decreases with time. In our study, NLR and PLR, before administration of IVIg, were found to be significant predictors for CAL in univariate analysis. After multivariate regression analysis, an NLR cut-off score of 2.08 reliably predicted CAL, with moderately good sensitivity and specificity. Seven out of 36 patients with NLR <2.08 had CAL, while 33 out of 43 patients with NLR >2.08 had CAL in our study. The usefulness of NLR in predicting CAL was demonstrated only in two studies previously

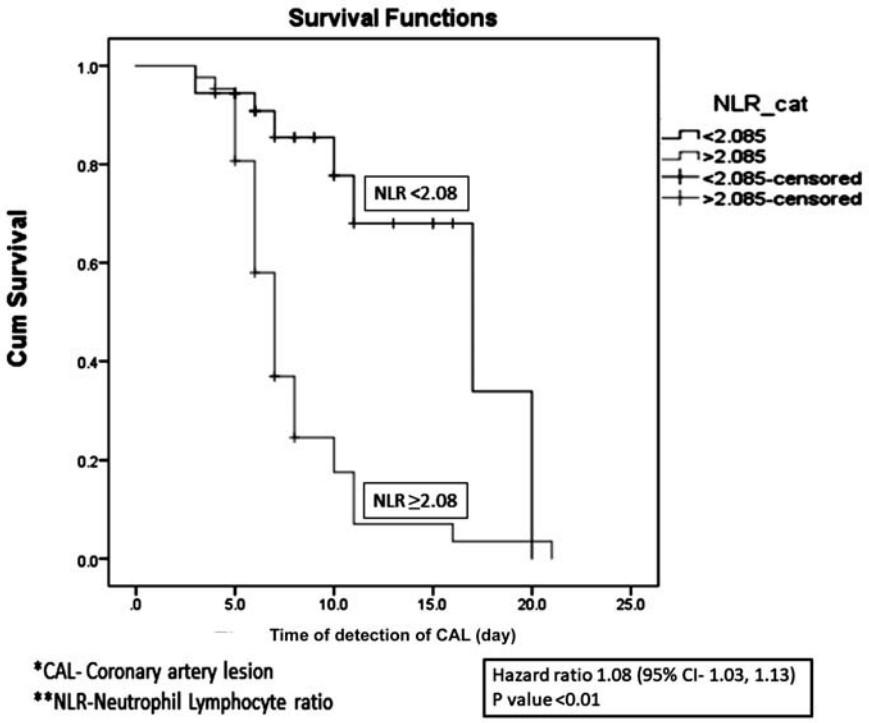

Fig. 2 Kaplan-Meier analysis curve showing time to detection of coronary artery lesions (CAL) in patients with neutrophil-lymphocyte ratio (NLR) of ≥2.08 and <2.08.

[9,10], where one was less sensitive, and the other had poor specificity. Ha, et al. [9] concluded that an NLR cutoff of 5.49 during the febrile phase predicted IVIg resistance with 39% sensitivity and 86% specificity. A cut off of 4.86 predicted coronary aneurysms with 60% sensitivity and 72% specificity. In another study from Turkey [10], NLR>1.32 predicted CAL with 92.3% sensitivity but was only 38.8% specific. A retrospective study from Japan [11] introduced a scoring system constituting NLR and PLR and concluded that the combination of NLR ≥3.83 and PLR ≥1.5 predicted IVIg resistance with high sensitivity and specificity. Further studies have shown the role of NLR and/or PLR in predicting IVIg resistance, but neither predicted the occurrence of CAL [12,13]. The meta-analysis [14] focusing on the role of NLR in predicting IVIg-resistant KD had an overall pooled sensitivity of 66% and specificity of 71%, with an area under the summary receiver operating curve (AUSROC) being 0.795. The subgroup analysis revealed that NLR detection after initial IVIg administration had larger AUSROC than pre IVIg NLR [pooled sensitivity-58%; pooled specificity-77%; AUSROC-0.844]. In our study, NLR alone or in combination with PLR failed to predict IVIg resistance. An ESR cut-off of 88 predicted IVIg resistance with 85% sensitivity and 64% specificity, though the number of patients with IVIg resistance was low.

The median time to detect CAL in our study was seven days, and the median time to presentation to the hospital was five days. This gives a lead time of 2 days and NLR application on the day of admission could help predict CAL and administer IVIg upfront, reducing CAL incidence.

There are certain limitations in our study. Being a retrospective study, it was subject to bias. Patients who did not have documented blood investigations before the diagnosis of KD could not be enrolled. The NLR after administration of IVIg was not available and hence could not be computed. Moreover, only the presence of CAL was evaluated, while neither the morphological characteristics nor its severity was assessed owing to the study's retrospective nature.

To conclude, NLR could offer direction whenever a clinician faces a diagnostic dilemma of tropical infection in a child with clinical features compatible with incomplete KD. A high NLR value (≥2.08) between days 4 and 6 of fever onset, before administration of IVIg, reliably predicted CAL but did not predict IVIg resistance. An ESR value of ≥88mm/h predicted IVIg resistance. Further multi-center, prospective studies with a larger sample size are needed to validate the results.

WHAT THIS STUDY ADDS

- Neutrophil-lymphocyte ratio ≥2.08, between the fourth and sixth days of onset of fever, can provide two days lead time in diagnosing coronary artery lesions in Kawasaki disease.

Ethic clearance: IEC, JIPMER; No. IEC/2021/102, dated July 7, 2021.
Contributions: ACC: participated in study protocol preparations, recruited patients, participated in data analysis and drafted the first version of the manuscript; JGR: conceptualized the study design, supervised the data collection, interpreted the data and critically revised the manuscript; AA: assisted in recruitment of the patients, data analysis and drafting the manuscript. All authors contributed to protocol preparation, drafting of the manuscript, and approved the final version of the manuscript. JGR: shall act as the guarantor of the paper.
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RESEARCH PAPER

Evaluation of Diaphragmatic Thickness and Dysfunction by Ultrasonography in Mechanically Ventilated Children for Assessment of Extubation Success

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Objectives: This study aimed to evaluate diaphragm thickness (DT) and diaphragmatic thickening fraction (DTF) in mechanically ventilated children, and study the association of these measurements with extubation success. **Methods:** Consecutive children aged one month to 18 years, who required mechanical ventilation (MV) for more than 24 hours at our institution, were enrolled between April, 2019 to October, 2020. Ultrasonographic measurements of DT were documented, and DTF was calculated from baseline (within 24 hours of MV) until 14 days of MV, and up to three days post-extubation. **Results:** Of the 54 children-enrolled, 40 underwent planned extubation trial, of which 9 (22.5%) had extubation failure. Pre-extubation and post-extubation DTF between children in extubation-success and extubation-failure groups were comparable (*P*=0.074). There was no significant difference in the diaphragm atrophy rate between the two groups (*P*=0.819). Binary logistic regression showed significantly decreased probability of successful extubation with total ventilation duration (*P*=0.012) and mean DTF% before extubation (*P*=0.033). **Conclusion**: Despite evidence of diaphragmatic atrophy in critically ill children receiving mechanical ventilation, there was no significant difference in DTF between extubation success and failure groups.

Keywords: *Diaphragm atrophy, Diaphragmatic thickening fraction, Mechanical ventilation*.

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Critically ill children requiring ventilation support account for one-third of patients admitted to pediatric intensive care units (PICUs) [1]. In mechanically ventilated patients, the diaphragm muscle undergoes disuse atrophy, also known as ventilator induced diaphragmatic dysfunction (VIDD). It is known to occur in nearly 44% of children within 24 hours to 4 days after the initiation of mechanical ventilation (MV) [1,2]. VIDD leads to extubation failure, defined as the failure to withstand spontaneous breathing, requiring re-intubation within 48 hours of extubation [3,4].

Diaphragmatic ultrasonography has recently been used in children to quantify the changes in diaphragmatic parameters and correlate them with the weaning outcomes. Limited literature is available in children on mechanical ventilation admitted to PICU [1,2,5]. Some diaphragmatic parameters used to assess VIDD include static measurements such as diaphragm thickness (DT) and dynamic measurements such as diaphragm thickening fraction (DTF).

This study aimed to evaluate the use of DT and DTF in predicting extubation failure in mechanically ventilated children.

METHODS

This prospective observational single-center study was conducted in the PICU of our tertiary care public hospital in Western Rajasthan between April, 2019 to October, 2020, after approval from the institutional ethics committee. All children aged one month to 18 years, requiring mechanical ventilation for more than 24 hours, were enrolled after written informed consent from parents. Children who required less than 24 hours of mechanical ventilation, those with neuromuscular disease, cerebral palsy, diaphragm paralysis, congenital lung or pleural malformation, any fluid or air collection between pleural spaces, and chronic respiratory failure were excluded from the study. The baseline demographics and clinical data were obtained for all enrolled patients.

The primary outcome was the assessment of DT and

DTF. The secondary outcome was to compare the number of patients who underwent reintubation, tracheostomy, prolonged ventilation (≥14 days), and death in successful-extubation and extubation-failure groups. The first ultrasound assessment (baseline) was done within 24 hours of mechanical ventilation. The study pediatrician (trained in diaphragm ultrasound for 4 weeks) and radiologist performed independent ultrasound assessments of diaphragmatic thickness parameters daily to measure DT and DTF until 14 days of mechanical ventilation, and up to three days post-extubation. The inter-rater reliability for sonography done by pediatrician and radiologist was assessed by intraclass correlation coefficient (ICC), which showed excellent absolute agreement (ICC varied from 0.939 (0.831-0.978) to 0.987 (0.968 -0.995); P <0.001).

Ultrasound was performed using a portable ultrasound machine (Sonosite M Turbo, Fujifilm Sonosite) with a 6-13 MHz linear probe. The horizontal view of the diaphragm was obtained by placing a linear probe perpendicular to the right chest wall and below the costal margin in the intercostal space between the eighth and tenth ribs (between anterior axillary and mid-axillary lines) in the zone of apposition of the muscle. Using the M mode image, the DT was measured from the middle of the pleural line to the center of the peritoneal line at the end of inspiration (DTi) and expiration (DTe). DTF was calculated using the formula: (DTi- DTe) /DTe x 100. In recent studies, this index has served as an accurate parameter for evaluating diaphragmatic function in ventilated and non-ventilated patients [6].

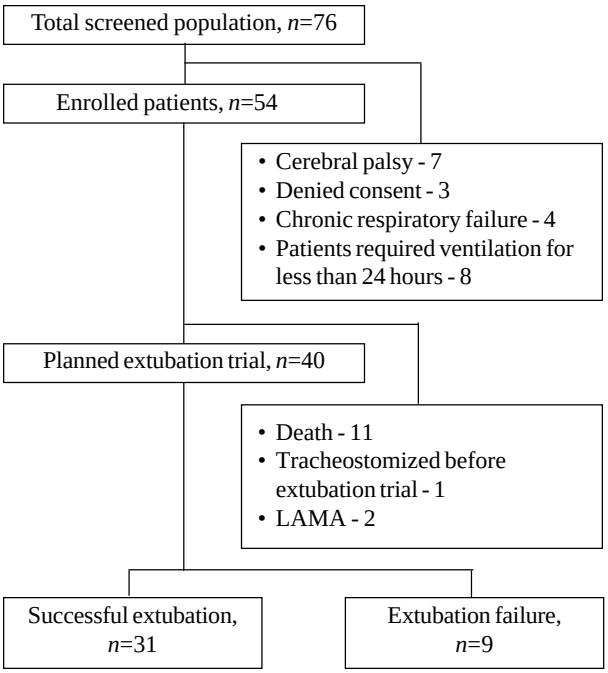

Fig. 1 Study flow chart.

The sample size calculation was derived from the study done by Lee, et al. [1]. Considering the success of extubation in study group to be 70% and precision of 85% with significance level of 0.05, a sample size of 36 patients was calculated. With a dropout rate of about 10%, 40 children were planned to be included in study.

Statistical analysis: Binary logistic regression for probability of successful extubation with independent clinical parameters was performed. All statistical analyses were performed using SPSS version 23, and a P-value of <0.05 was considered statistically significant.

RESULTS

During the study period, 76 patients admitted to the PICU were screened and 54 mechanically ventilated patients of age one month to 18 years were found eligible (**Fig. 1**). Eleven patients (20.3%) died before extubation. The baseline characteristics of the study population are detailed in **Table I.** A planned extubation trial was done on 40 patients, of which 31 patients were successfully extubated, and nine patients had extubation-failure requiring re-intubation

Table I Baseline Characteristics of Mechanically Ventilated Children Enrolled in the Study (N=54)

Characterstics	*Value*
Infants (<1 y)	21 (39)
Children (1-14 y)	30 (56)
Adolescents (15-18 y)	3 (5)
Age (y)[a]	3.5 (0.5, 7)
Male gender	33 (61.1)
Weight (kg)[a]	12 (5.1, 16)
Length (cm)[a]	65.3 (52.6, 116.8)
Weight for age z-score[a]	-1.57 (-2.21, -1.04)
Length for age z-score[a]	-1.74 (-2.06, -0.84)
Weight for length z-score[a]	-1.48 (-2.15, -0.97)
PIM-3 predicted mortality (%)[a]	6.75 (2.9, 14.42)
Patients survived	41 (75.9)
Deaths	11 (20.4)
Primary diagnosis	
Respiratory	14 (26)
Sepsis	14 (26)
Cardiovascular	8 (15)
Central nervous system	6 (11)
Renal	5 (9)
Gastrointestinal	2 (4)
Others	5 (9)

Data represented as no. (%) or [a]median (IQR). PIM: pediatric index of mortality.

Table II Profile of Mechanically Ventilated Children With Extubation Success and Extubation Failure (*N*=40)

Characteristics	*Extubation success (n=31)*	*Extubation failure (n=9)*	*P value*
Age (y)	5 (1, 8.5)	0.33 (0.25, 1)	0.031
Males[a]	18 (58.1)	7 (77.8)	0.440
Weight (kg)	14.5 (5.7, 19.7)	5.7 (4, 6.8)	0.021
Length (cm)	74.2 (48.5, 97.1)	52 (34.2, 64)	0.038
Weight for age *z*-score	-1.54 (-2.19, -1.05)	-1.79 (-2.38, -0.59)	0.185
Length for age *z*-score	-1.76 (-2.27, -0.86)	-1.35 (-2.6, -1.02)	0.670
Weight for length *z*-score	-1.35 (-2.05, -0.94)	-1.81 (-2.43, -1.53)	0.580
Total duration of PICU stay (d)	14 (9, 17.5)	26 (22, 37)	<0.001
Total duration of ventilation (d)	6 (5, 8)	17 (8, 30)	<0.001
PIM-3 predicted mortality (%)	9.2 (4, 18.1)	0.8 (0.7, 4.9)	0.094
Prolonged ventilation (≥14 d)[a]	16 (51.6)	8 (88.9)	0.046
Number tracheostomized[a]	0	2 (22.2)	0.006
Steroid use[a]	1 (3.2)	3 (33.3)	0.007
Duration (d)	3.2 (0, 5)	14 (4, 22)	0.041
Average Penn State sedation scale	3.5 (3.2, 3.8)	3.7 (3.6, 4)	0.489
Neuromuscular blocker use[a]	0	2 (22.2)	0.006
Duration (d)	0	5.5 (3, 9)	0.032
Maximum PEEP (cm of H_2O)	5 (5, 5)	5 (4.7, 5.3)	0.452
Maximum PIP (cm of H_2O)	15.2 (14.2, 16.7)	14 (13.5, 16.1)	0.434
Maximum FiO2 (%)	0.35 (0.3, 0.47)	0.3 (0.25, 0.35)	0.374
DTF before extubation (%)	30 (27.58, 35.55)	34.07 (30.85, 37.5)	0.074
DTF after extubation (%)	31 (25.83, 33.35)	34.33 (30.42, 35.5)	0.702

Data presented as median (IQR) or [a]no. (%). DTF:diaphragmatic thickening fraction, FiO2:fraction of inspired oxygen, NMB:neuromuscular blocker, PEEP:peak end expiratory pressure, PICU:pediatric intensive care unit, PIM 3:pediatric index mortality score, PIP:peak inspiratory pressure.

within 48 hours. The baseline characteristics and clinical profile of extubation success and extubation failure groups are detailed in **Table II**.

There was a significant reduction in daily DTe and DTi compared to baseline values. DTF decreased to a maximum of 6% in the first 24 hours following baseline measurement. The median (IQR) DTF before extubation was 30% (27.58%, 35.55%) and 34.1% (30.85%, 37.5%) (*P*=0.074) and after extubation was 31% (25.83%, 33.35%) and 34.3% (30.42%, 35.5%) (*P*=0.702) in the extubation-success and extubation-failure groups, respectively. There was no significant difference in the diaphragmatic indices between the two groups. The median (IQR) diaphragm atrophy (decrease in DTe from its baseline) was 11.4% (7.8%, 19.6%). The percentage decrease in DTe each day was calculated as the diaphragm atrophy rate, and the median (IQR) diaphragm atrophy rate was 1.43% (0.9, 1.98) per day. There was no significant difference in diaphragm atrophy rate between successfully extubated and extubation-failure groups [3.1% (1.22%, 3.97%) vs 3.4% (1.8%, 4.07%); *P*=0.819].

Only age had a moderate correlation with baseline DTe (r=0.677, *P*<0.001). Among the factors affecting diaphragm atrophy rate, high PEEP had moderate correlation (r=0.471, *P*=0.002). There was no correlation of diaphragm atrophy rate with age, sex, weight for age, length for age and weight for length; duration of MV; duration of PICU stay and neuromuscular blocking agent use.

Binary logistic regression showed significantly decreased probability of successful extubation with increase in total ventilation duration [aOR (95% CI) 0.76 (0.62, 0.94); *P*=0.012] and mean DTF% before extubation [aOR (95% CI) 0.79 (0.64, 0.98); *P*=0.033]. Receiver operating characteristic (ROC) curve showed poor diag-nostic accuracy of DTF% before [AUC (95% CI) 0.686 (0.504,0.869); *P*=0.092] and after extubation [AUC (95% CI) 0.586 (0.383, 0.749); *P*=0.549] for successful extubation (**Fig. 2**).

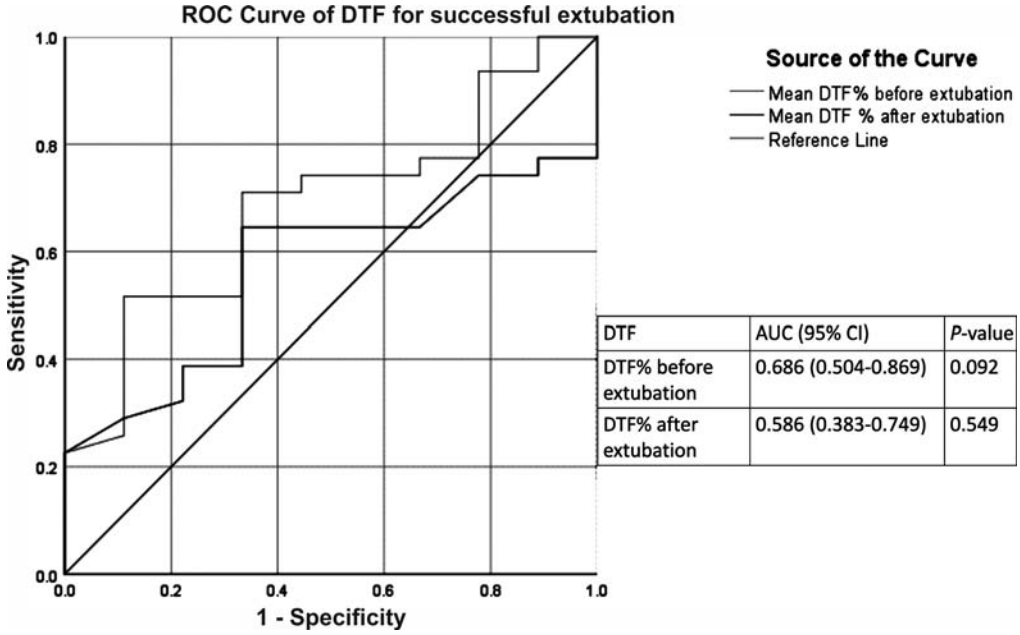

Fig. 2 Receiver operating characteristics curve of diaphragmatic thickening fraction (DTF) for successful extubation.

DISCUSSION

This study evaluated the utility of diaphragmatic thickness parameters (DTe, DTi, DTF) in mechanically ventilated children and their association with successful extubation. We found a significant and progressive decrease in DT during MV with the onset of diaphragmatic atrophy and reduction of DTF as early as the second day of MV. There was a decrease in DT starting within the first 48 hours and decreasing steadily thereafter. There was no significant difference in DTF between extubation-success and extubation-failure groups.

In contrast to our findings, previous authors [1,4,7] were able to demonstrate a significant difference in DTF between successful-extubation and extubation-failure groups. The increased levels of inspiratory effort reflecting load-induced muscle injury may explain the higher value of pre-extubation DTF in the extubation-failure group [8]. Similar results were also published by Goligher, et al. [9], in which 24% of the study population had increased DT predicting prolonged ventilation. The reasons for these differences could be different characteristics of the study cohorts. Similar to our findings, a reduction in three serial readings of DTe in the first week of mechanical ventilation has been documented by Glau, et al. [5]. In the post-extubation period, diaphragm tends to move cranially due to loss of PEEP, this leads to lengthening and thinning of diaphragmatic fibers resulting in smaller DTe and higher DTF [10]. This was noted in our cohort, and also by other authors [1].

Previous studies had defined cut-off values of DTF >36%, 20%, and 30%, respectively as a favorable predictor of successful extubation [4,11,12]. A study by Rahman, et al. [7] in children has shown that DTF at a cutoff value of >23.17% had sensitivity and specificity of 100% and 76.2%, respectively, for predicting weaning failure.

The median (IQR) diaphragmatic atrophy and atrophy rate over the first week of ventilation were 11.4% (7.8,19.6) and 1.43% (0.9, 1.98) per day, respectively. Glau, et al. [5] reported a diaphragmatic atrophy rate of 3.4% per day with a median age of 16 months in their study participants. Mistri, et al. [13] reported a diaphragmatic atrophy rate of 2% per day, with a median age of 6 years. The latter shares similar age characteristics as our study, with a median age of 5.8 years. In our study, age had moderate correlation with baseline DTe. This could be explained by the fact that infants have thinner diaphragm and with age diaphragm thickness increases [14]. Moreover, in our study, high PEEP had moderate correlation with diaphragm atrophy rate. This could be explained by the fact that children with higher PEEP were sedated to a higher degree and hence developed more diaphragm atrophy. Like previous studies, we did not find any correlation between diaphragm atrophy rate and nutritional status of children or duration of mechanical ventilation [13].

Diaphragm ultrasound can be easily done bedside in intensive care settings. The role of diaphragm ultrasound should be explored further in mechanically ventilated children for assessment of extubation success.

WHAT THIS STUDY ADDS?

- Ultrasonography measurement of diaphragm thickness and diaphragmatic thickening fraction did not predict extubation success in mechanically ventilated children.

To conclude, the use of DTF in predicting extubation outcomes cannot be concluded from this study. Hence, more extensive, well-designed studies are needed to assimilate evidence about the role of diaphragmatic indices in mechanically ventilated children.

Ethics clearance: EIC, AIIMS, Jodhpur; No. AIIMS/IEC/2018/779, dated Dec 24, 2018.
Contributors: SV: involved in developing the protocol, data collection, data analysis and manuscript writing; DK: involved in supervision of protocol development, data collection, data analysis and manuscript writing; BC: supervision of protocol development, data collection and manuscript writing; NT: involved in supervision of manuscript writing; BS: involved in training and supervision of diaphragmatic ultrasonography and supervision of manuscript writing; KS: involved in supervision of protocol development and manuscript writing; SS: involved in statistical analysis of the data and supervision of manuscript writing.
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RESEARCH PAPER

Serum Amyloid A Levels and Severity of COVID-19 in Children

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Objective: The aim of this study was to determine the availability of serum amyloid A (SAA) in the diagnosis of coronavirus disease 2019 (COVID-19), to asses disease severity and to predict hospitalization status. **Methods**: Between March, 2020 and March, 2021, a total of 80 children (40 cases with COVID-19 and 40 cases in healthy group) were included in this study. Patients were divided into two groups (mild and moderate/severe) to evaluate SAA levels in terms of clinical severity and also hospitalization status. **Results**: Comparisons between the two groups revealed that median SAA values were significantly higher in children with COVID-19 than in their healthy peers (21.45vs3.05 mg/L, *P*=0.002). There was no significant difference in the median serum SAA levels between mild and moderate/severe clinical disease (*P*=0.837). The SAA difference between the two groups with regards to hospitalization was not statistically significant (*P*=0.098). **Conclusions**: Although SAA level was found to be higher in children with COVID 19 compared to healthy controls, the sensitivity of SAA for the disease was found to be low. In addition, there was no difference between the groups in terms of clinical severity.

Keywords: *Cytokines, Diagnosis, Management, Outcome.*

Many biochemical markers have been investigated in the diagnosis and clinical severity in patients with coronavirus disease 2019 (COVID-19), including C-reactive protein (CRP), procalcitonin, lymphocyte count, and others [1-4]. Serum amyloid A (SAA) is an acute-phase protein mainly produced by the liver in response to proinflammatory cytokines secreted by the activated monocytes. This protein serves an essential role in inflammation and relates to the severity of inflammation. Currently, there are few reports about the relationship between SAA and COVID-19 [1]. Previous studies indicated that SAA plays essential roles in inflammatory diseases [5,6]. This study aimed to investigate the role of SAA in the diagnosis and clinical severity of COVID-19 in children.

METHODS

This study was carried out in our hospital after getting approval from the ethics committee of the pediatric infectious diseases department. Data of children diagnosed with laboratory-confirmed COVID-19 between March, 2020 and March, 2021 was retrieved. Patients with multisystem inflammatory syndrome in children (MIS-C) who tested positive for COVID-19, and those with missing details were excluded from the study. Serum samples were collected from COVID-19 positive patients attending outpatient clinics. The control group consisted of age- and gender-matched healthy individuals. Although reference values were available, comparisons were made with age- and gender-matched-healthy controls admitted in the same period.

Combined nasopharyngeal and or pharyngeal swab samples were taken from children with suspected COVID-19 and sent to medical microbiology laboratory of our hospital. Severe acute respirtory syndrome coronvirus 2 (SARS-CoV-2) was detected using reverse transcriptase-polymerase chain reaction (RT-PCR) (Bio-Speedy SARS-CoV-2 double gene RT-qPCR kit). Blood samples were collected between 8 AM and 12 PM. Serum samples were stored at –80 ºC after portioning. After all samples were collected, analysis was performed in batches. Serum amyloid A (SAA) levels were studied by nephelometric method in a BN II brand nephelometry device (Siemens Helathineers).

Demographic details, clinical symptoms, underlying diseases were also recorded, along with the laboratory reports. The guidelines of the CDC (Centers for Disease Control and Prevention) were used for classifications of COVID-19 severity into five categories according to the severity of the disease [7]. Asymptomatic patients were not included in our study. Disease severity was evaluated in two groups as mild and moderate severe.

Statistical analysis: Statistical analyses were performed by using SPSS software version 24.0 (IBM Corp). Descriptive statistics were used to summarize the patients' baseline characteristics, depending on the normality of distribution. Comparisons between groups for categorical variables were made by using the Chi square test. For two group comparisons of independent variables, the Student *t* test was used as a parametric test, whereas the Mann–Whitney *U* test was used as a non-parametric test. Receiver operating characteristic (ROC) curve analyses were conducted to determine diagnostic cut-off values and their sensitivity, specificity, and area under curve (AUC) values. Statistical significance was defined as *P* values less than 0.05.

RESULTS

A total of 80 children (40 COVID-19 positive; 40 healthy controls) were evaluated. Fever (57.5%), and cough (27.5%) were the most common presenting symptoms of the COVID-19 infected children. The demographic and clinical findings of children with COVID-19 is shown in **Table I**. Hospitalization rate was 42.5% (*n*=17), and 35.2% (*n*=6) of hospitalized cases were categorized into moderate/severe group, six (15%) patients had comorbidities. None were in the critical illness category. Three (50%) of the patients with underlying diseases were hospitalized and followed up in the moderate/severe group. The median (IQR) SAA values in COVID-19 group were significantly higher than that in healthy individuals [21.45 (5.94-105.4) vs 3.05 (1.6- 6.52) mg/L; *P*=0.002]. When the groups were compared according to disease severity, a significant difference was not found between mild disease and moderate/severe disease in terms of SAA levels (*P*=0.844). Comparison of the clinical and laboratory findings between mild disease and moderate/severe disease groups is shown in **Table II**. The serum SAA levels were similar across all categories of clinical severity. While neutrophil - lymphocyte ratio (NLR) was significantly higher in the moderate/severe group than mild group, all other inflammatory markers were similar in both categories of severity.

Comparison of the clinical and laboratory findings according to hospitalization status in patients with COVID-19 (**Web Table I)**, showed that there was no significant difference in SAA levels between the two groups. Other markers of inflammation were significantly higher in hospitalized patients.

The calculated cutoff value for SAA to identify COVID-19 patients was 5.82 mg/L. ROC curve for SAA values in COVID-19 patients vs healthy controls is shown in **Fig. 1** [AUC (95 % CI) 0.80 (0.70 -0.90); *P*<0.001].

DISCUSSION

Several biochemical parameters have been assessed for early diagnosis of disease, confirming and classifying disease severity, decision for hospitalization, and ICU admission [8,9]. SAA is one of the investigated parameters. The exact mechanism of increased SAA concentration in patients with COVID-19 is unknown. The differences in SAA levels between patients with COVID-19 and healthy participants observed in our study suggest that this biomarker may be particularly useful for identifying patients with COVID-19. However, SAA is not specific to COVID-19 and may be elevated in other respiratory diseases. In a similar study including adult patients in the literature, SAA was found to be higher than the healthy group [10]. In the results of our study, 80% sensitivity and 70% specificity are low for the diagnosing test. The reason for the low

Table I Demographic and Clinical Characteritics of Children With COVID-19 Enrolled in the Study (*N*=40)

Characteristics	*Value*
Male	18 (45)
Age (mo)[a]	156 (60-180)
Underlying medical conditions	6 (15)
Hospitalization	17 (42.5)
Symptoms and signs	
Fever	23 (57.5)
Cough	11 (27.5)
Runny nose	10 (25)
Weakness	8 (20)
Sore throat	7 (17.5)
Headache	6 (15)
Vomiting/nausea	6 (15)
Diarrhea	6 (15)
Abdominal pain	5 (12.5)
Laboratory tests	
WBC (x10³/uL)[a]	5700 (4300-10025)
ANC (x10³/uL)[a]	2750 (1750-5775)
ALC (x10³/uL)[a]	1850 (1300-2575)
N/L[a]	1.74 (1.06-2.84)
Platelets (x10³/uL)[b]	252800 (195750-291750)
Hemoglobin (g/dL)[a]	13.1 (12.12-14.05)
D-dimer (µg/L FEU)[b]	589.17 (300-632)
CRP (mg/L)[a]	4.35 (1.32-33)
Procalcitonin (µg/L)[a]	0.04 (0.012-0.08)
SAA (mg/L)[a]	21.45 (5.94-105.4)
Clinical severity	
Mild	34 (85)
Moderate and severe	6 (15)

Values in no. (%),[a]median (IQR) or [b]mean (SD). WBC:white blood cell; ANC:absolute neutrophil count; ALC:absolute lymphocyte count; N/L:neutrophil lymphocyte range; CRP:C-reactive protein; SAA:serum amyloid A.

Table II Clinical and Laboratory Findings in Children With Varying Severity of COVID-19 (*N*=40)

Characteristics	*Mild (n=34)*	*Moderate and severe (n=6)*
Clinical charateristics		
Male	16 (47.0)	2 (33.3)
Age (mo)[a]	156 (60-180)	109 (25-180)
Underlying medical conditions	6 (17.6)	0
Hospitalization	11 (32.3)	6 (100)
Fever	19 (55.9)	4 (66.6)
Cough	6 (7.6)	5 (83.3)
Runny nose/sore throat	9 (26.5)	1 (16.6)
Weakness	8 (23.5)	0
Laboratory characteristics		
WBC ($x10^9$/L)[a]	5.7 (4.3-9.2)	7.65 (4.1-10.5)
ANC ($x10^9$/L)[a]	2.65 (1.7-5.4)	5.15 (2.1-8.2)
ALC ($x10^9$/L)[a]	1.9 (1.3-2.6)	1.4 (1.1-2.4)
N/L range[a]	1.39 (1.02-2.64)	4.18 (1.9-6.3)
CRP (mg/L)[a]	4.25 (0.1-157.5)	18.2 (2.1-35.2)
Procalcitonin (µg/L)[a]	0.04 (0.01-0.08)	0.07 (0.04-0.09)
SAA (mg/L)[a]	18.4 (5.94-97.6)	55.9 (18.5-117)

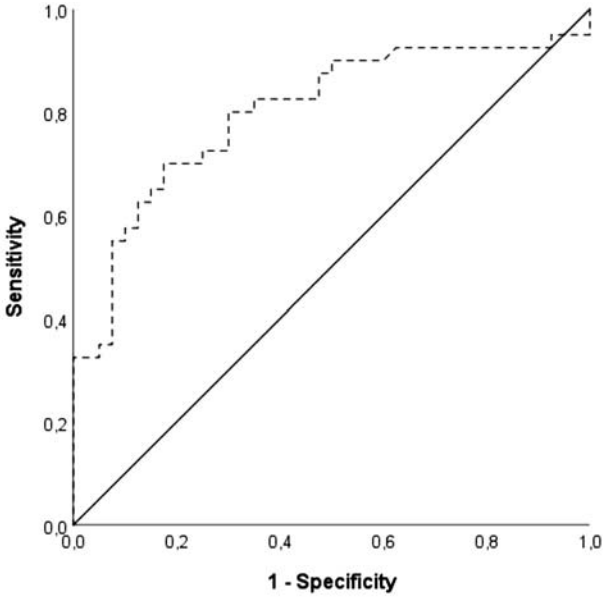
Values in no. (%) or [a]median (IQR). WBC:white blood cell; ANC: absolute neutrophil count; ALC:absolute lymphocyte count; N/L: neutrophil lymphocyte range; CRP:C-reactive protein; SAA: serum amyloid A.

sensitivity and specificity may be attributed to the small number of cases in our study. Investigation of the diagnostic value of SAA in the diagnosis of COVID 19 in children with studies involving more participants may reveal more clearer results.

In the literature, it has been reported that SAA can be one of the biomarkers for monitoring and it has a definite value in predicting the prognosis [11]. Previously, based on the data from 132 COVID-19 patients, Li, et al. [2] have reported that SAA/lymphocyte count, CRP, SAA, and lymphocyte count were valuable to evaluate the disease severity. In a meta-analysis examining adult patient studies, it was reported that NLR and SAA values were associated with disease severity [12]. A meta-analysis data shows that; while SAA was significant in showing the severity of the disease according to CRP, it was not sufficient to differentiate it from non-infected cases [13]. In a study that included only 13 severe patients, the variability of SAA according to disease severity was emphasized [14]. However, in our study, SAA was similar in groups in terms of clinical severity and hospitalization. Since our study is one of the limited number of publications on pediatric patients, studies involving larger participants are needed. Although SAA could not be helpful in terms of prognosis in our study, its higher detection rate in hospitalized patients may be an indirect guide about the severity of the disease.

Elevated levels of CRP, procalcitonin, D-dimer and low lymphocyte count have been associated with severe forms of COVID-19 as well as high NLR [10]. Our results indicated that WBC, ANC, absolutely lymphocyte count (ALC), CRP, platelets, and procalcitonin were not significantly different between the moderate/severe group and mild group. NLR was significantly higher in the moderate /severe group. Lymphocyte counts of the two groups were lower than the normal range and but not markedly different. A systemic review report compared the ALC of the severe and mild group and it concluded that lymphocytopenia is associated with in the risk of the COVID-19 severity [15].Our findings are consistent with other studies.

This study has several limitations. First, it was performed in a specific setting and only a relatively small number of children who were admitted to our center were enrolled. This prevents further generalization of the findings. This may also pose a bias in selection of the patients with COVID-19. Secondly, serum sampling of the children with COVID-19 was not based on the timing of different phases of the course of the SARS-CoV-2 infec-



AUC	Sensitivity	Specifity	PPV	NPV
0.800	80	70	72.7	77.7

SAA:serum amyloid A; AUC:area under the curve; PPV:positive predictive value; NPV:negative predictive value.

Fig. 1 ROC curve for serum amyloid A value in children with COVID-19 and healthy contols for a cutoff value of 5.82 mg/L.

WHAT THIS STUDY ADDS?

- Serum amyloid A levels did not differentiate between severity categories of coronavirus disease 2019 (COVID-19) in children.

tion. We enrolled healthy children as a control group, but it would have been more appropriate to choose patients with other respiratory tract infections. The low incidence of other respiratory tract infections and the increase in the frequency of admission findings other than respiratory tract infections led us to prefer healthy controls.

Larger studies of SAA in pediatric COVID-19 patients are needed, as most of the previous work was from adult patients. However, since the number of patients with moderate/severe clinical severity is low, this relationship can be revealed more clearly with studies with more extensive participation.

Ethics clearance: Provide name of IEC. Institutional Ethics Committee; Permission No. Provide, dated March 29, 2021.
Contributors credit: ST: conceptualizing, writing, and critically reviewing the article; EKO: statistical analysis, article writing; AET: data collection. AS: data collection. GÜ: data collection; YEK: data collection; SO: hormonal measurements; BIB: hormonal measurements; AKA: conceptualizing, writing and critically reviewing the manuscript; DYÇ: conceptualization, writing and critical review of the manuscript. All authors approved the final version of manuscript, and are accountable for all aspects related to the study.
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Web Table I Clinical and Laboratory Findings According to Hospitalization Status in Children With COVID-19 (*N*=40)

	Inpatients (n=17)	*Outpatients (n=23)*	*P*
Age (mo)	60 (17.5-168)	168 (96-192)	0.009
WBC (10^9/L)	10.5 (5.2-12.6)	4.8 (4.1-6.1)	0.001
ANC (10^9/L)	6.4 (2.65-9.5)	2.2 (1.6-3)	<0.001
NLR	2.45 (1.35-6.15)	1.3 (0.83-2.45)	0.015
Hemoglobin (g/dL)[a]	11.84 (1.73)	13.85 (1.57)	0.001
AMC (10^9/L)	0.9 (5.5-9)	0.5 (4-8)	0.020
CRP(mg/L)	33.4 (3.05-80.1)	3.4 (1-8.2)	0.005
Procalcitonin (µg/L)	0.08 (0.04-0.14)	0.03 (0.01-0.06)	0.004
SAA (mg/L)	28.2 (9.63-569.5)	15.1 (5.03-49)	0.098
D-dimer (µg/L FEU)[b]	770.67 (430-1030)	459.52 (215-530)	0.016
Albumin (g/dL)	4 (4-4.13)	4.7 (4.6-4.9)	<0.001

Values in mean (SD) or [a]median (IQR). WBC - White blood cell; NLR – neutrophil-lymphocyte ratio; AMC - absolute monocyte count; CRP - C-reactive protein; SAA - serum amyloid A.

RESEARCH PAPER

Composition of Common Junk Food Items and Their Contribution to the Dietary Requirement of Children and Adolescents

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Objective: To estimate the carbohydrate, energy, fat, protein, and sodium content of commonly consumed junk food items and to compare these to the Recommended Dietary Allowance (RDA) and Estimated Average Requirements (EAR) of children. **Methods:** A list of eight common junk food categories was made, and the median nutritional content of carbohydrate, energy, fat, protein and sodium was determined from the commonly consumed brands in these categories. It was compared to the RDA and EAR for two different age groups viz., age 4-6 year, and male adolescents aged 13-15 years. **Results**: The junk food groups with the highest carbo-hydrate were packaged potato chips and cakes, the group with the highest fat content was packaged potato chips, and the groups with the highest salt content were burgers and packaged potato chips. The %EAR of one packet of some items was 80-90% of daily fat requirement, and more than 60% of daily sodium requirement. **Conclusions:** Junk foods contribute substantially to the daily intake of carbohydrates, free sugars, total fats, saturated fats, and sodium of children.

Keywords: *Diet, Fast foods, HFSS foods, Obesity*.

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Junk food items are defined as foods with low nutritive value that are high in added sugars, saturated fats or sodium [1]. These are harmful for both adults and children, but nevertheless, due to their palatability and easy availability, the consumption of such items is quite high [1]. The most consumed junk food items are bakery products, beverages, burgers, caffeinated drinks, chips, noodles, pizza and sugar-sweetened drinks [1]. In a pan-India survey of children aged 9-14 years, it was found that 93% consumed packaged food and 68% consumed packaged sweetened beverages more than once per week, and 53% at least once in a day [2]. The major adverse effects related to intake of junk food items are obesity and its associated complications [3]. As per the National Family Health Survey (NFHS)-5 (2019-21) [4], the number of people with obesity has increased significantly in adults, with similar trend seen in children. Around 3.4% children below five years of age were overweight in 2019-21, as compared to 2.1% in NFHS-4 (2015-16) data [4].

In 2019, a National consultative group of the Nutrition Chapter of the Indian Academy of Pediatrics (IAP) recommended avoiding consumption of caffeinated energy drinks and limiting the consumption of junk foods to not more than one serving per week in children and adolescents [5]. These items now form a substantial part of the daily intake of the children and adolescents, but their nutritional value is not provided in any of the available food composition tables [6]. Food labelling is also not done for many of these items, especially those produced locally in the unorganized sector. We planned this study to collate the dietary composition and caloric values of the various junk food items, which are consumed by children in this region, and to compare it to the Recommended Dietary Allowance (RDA) for Indian children [6].

METHODS

This study was a survey of market items. A structured, verbal pilot survey was conducted of apparently well children attending the outpatient department for minor illnesses, vaccination, or accompanying relatives, at our public sector tertiary care hospital in Delhi. For this pilot, a total of 16 children were approached on one day [8, 4-6-year-old (5 boys) and 8, 13-15-year-old (7 boys)], and snack items commonly consumed by them were listed. These were arranged in a list of eight categories of junk food items (sweetened beverages, burgers, pizza, ice cream, doughnuts, cakes, and packaged chips and nachos). We selected three to four popular brands for each of these items and collected one packet of each from the market. Some additional items, which were commonly consumed by children but could not be categorized in the above categories, were classified as miscellaneous items.

Statistical analysis: We used the medium-sized packet of each brand, and the median (IQR) nutritional content was determined for each of the categories of carbohydrate, energy, fat, protein, sodium and caffeine. The values were

then compared to the RDA and Estimated Average Requirements (EAR) [6] for the two different age groups viz., child aged 4-6 year and male adolescent aged 13-15 years (since 7 out of 8 adolescents in the pilot were males).

RESULTS

We studied the packets of these 32 different junk foods. The dietary composition of these items is given in **Table I**. There was not much variation between brands with respect to the nutrient composition.

The junk food groups with the highest carbohydrate were chips and cakes, that with the highest fat content was packaged chips, and those with highest salt content were burgers and potato chips. The comparison with RDA and EAR is shown in **Table II**. The % EAR of some items were reaching 80-90% of fat EAR, and more than 60% of RDA for sodium.

DISCUSSION

In this survey of 32 popular junk food items, we found high carbohydrate, fat and sodium content in most junk food items, with a single serving of many items meeting about 2/3rd of daily permitted allowance of harmful fats and sodium. This agrees with the findings of studies depicting an increase in the contribution of ultra-processed foods to total energy intake, the dietary contents of carbohydrates, free sugars, total fats, saturated fats, and sodium [7,8]. Also, an inverse relationship was seen between the dietary contribution of ultra-processed foods and the dietary content of protein, fibers, potassium, minerals and vitamins [7,8]. In a study from the United States, it was found that more than 70% of calories and over 90% of total sugars intake were derived from junk foods, indicating unacceptably high intakes in children and adolescents [9]. Thus, children consuming junk food items are consuming high amounts of carbohydrates, fat and sodium, increasing the chances of obesity and cardiometabolic risk [10].

As the consumption of junk food items and ultra-processed foods increase, with children and adolescents being the most vulnerable victims, it is the need of the hour to find measures to control the problem. Food labelling enlisting all the ingredients should be available for all such items. The "front of the pack" labelling; although, an option, was found to be ineffective as only 24.6% of children always looked at the content label and

Table II Contribution of Junk Foods to the RDA and EAR of Average Indian Child (4-6 Years) and Average Indian Adolescent Male (13-15 Years)

Item	*% of RDA for carbohydrates*	*% of RDA for proteins*	*% of EAR for fats*	*% of RDA for sodium*
Average Indian male adolescent (aged13-15 years)				
Burger (200 g)	40	27.3	30	39
Pizza (~100 g)	12.7	12.9	11.5	16.5
Soft drinks (100 mL)	26.9	-	-	2
Chips (100 g)	42	14.5	40	41
Ice cream (100 mL)	22.3	7.09	22	1.9
Cakes (100 g)	40	10.63	47	15.7
Packaged noodles (100 g)	48.8	23.04	31	61.6
Average Indian child (aged 4-6 years)				
Burger (~200 g)	40	77	60	39
Pizza (~100 g)	12.7	36.4	23	16.5
Soft drinks (100 mL)	26.9	-	-	2
Chips (100 g)	42	41	80	41
Ice cream (100 mL)	22.3	20	44	1.9
Cakes (100 g)	40	30	94	15.7
Packaged noodles (100g)	48.8	65	62	61.6

RDA:recommended daily allowance; EAR: estimated average requirement.

Table I Composition of the Various Junk Food Items Studied

Item	*Carbohydrate (g)*	*Fat (g)*	*Sodium (mg)*	*Protein (g)*
Soft drinks (per 100 mL), *n*=4[a]	10.95 (10.88,11.25)	0	12.75 (8.5,19.3)	0
Veg burger (medium size~200 g), *n*=4	52.5 (51.5, 54.25)	15.7 (13, 19.3)	785 (735, 841.25)	12.3 (8.45, 16.75)
Pizza (1 slice, approx~100g), *n*=3	17 (15.5,18)	6 (5.3,6.5)	330 (320,330)	7 (5.3,7)
Ice cream (per 100 mL), *n*=3	29 (20.45,31.5)	11 (8.45,12.35)	37.1 (29.15, 39.55	3(2.6,3.4)
Donuts (approx~75g), *n*=3	27 (25,35)	14 (13.5,16.5)	270 (265,310)	3 (3,4)
Cakes (egg) per 100 g, *n*=3	53 (51.5,55)	15 (11.5,16.5)	315 (292.5, 317.5)	5 (4,7.5)
Potato chips (per 100g), *n*=4	54.85 (52.3,58.3)	35.25 (32.3,35.6)	854.95 (500.9,1155)	6.65(6.65,6.275)
Nachos (per100 g), *n*=2	63.05	26.65	803.5	6.55
Packaged noodles (100 g), *n*=1	63.5	15.7	1232.2	10.4
Chowmein (100g), *n*=1	67	18	847	11
Miscellaneous packaged snacks (per 100 g), *n*=4[b]	54.3 (54.1,61)	27 (19.5,34.6)	850(717,892)	6.3(6.1,6.6)

[a]*Two cola brands had caffeine content of 10 and 8.7 mg/100 mL;* [b]*consisted of popular brands of corn puffs and cheesy puffs.*

WHAT THIS STUDY ADDS?

- Composition of junk foods is equal to a substantial proportion of EAR of harmful carbohydrates, fats and sodium for children.

28.8% never checked the label [11]. A study by National Institute of Nutrition [12] revealed that even though the food labelling regulation in India is at par with the developed world, it is not looked at the point of purchase, therefore there is a need to evolve and experiment with different options like front of the pack labelling, and symbol-based labelling of foods in India. Another option is to control its advertising, marketing and availability to the children and adolescents. Prohibition of the sale of junk foods in and around the vicinity of schools can be effective in reducing its consumption [11]. IAP recommended legal ban on screen/print/digital advertisements of all junk foods for channels/magazines/websites/social media catering to children and adolescents through legislative measures. They also suggested providing tax discounts on healthy foods and beverages and regulation of discounts on large portions and multiple purchases of the junk food items [5]. The World Health Organization has provided a policy brief in 2022 for protecting children from the harmful impact of food marketing [12]. This includes a series of recommendations to restrict marketing of unhealthy junk food, and ways to reduce children's exposure to such marketing.

This study is an effort in pointing towards this often-neglected component of diet, which is frequently missed during routine dietary assessment. This study has several limitations including non-inclusion of roadside local snacks (*samosa, pakoda, vada,* chowmein, etc), type of oil used, and using a small number of children to decide on the items to be included in the study. More detailed studies in future may address these. Future studies are required to further explore the pattern of junk food consumption in children, and the various ways to curb them.

Junk foods contribute substantially to the daily intake of carbohydrates, free sugars, total fats, saturated fats, and sodium in children. Our data may guide clinicians while taking dietary history, to acknowledge and include junk food's contribution to the daily intake, at least till more standardized information is available from National bodies.

Ethics clearance: Ethics clearance is not required for survey of market items. Authors declare that the study procedures conform to the principles laid down in the Declaration of Helsinki.

Contributors: PM, SS: conducted the study, drafted the manuscript, collected the data, compiled, and analysed it. RB, SS: analyzed the data and drafted the manuscript. DM: Concept, study plan, contributed to data analysis and manuscript writing. All authors approved the final manuscript and agree to be accountable for all aspects of the study.

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REVIEW ARTICLE

mHealth Apps Delivering Early Intervention to Support Parents of Children With Autism Spectrum Disorder: *A Scoping Review*

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Context: Early intervention, and parent-mediated intervention are effective in achieving early childhood development goals for children with autism spectrum disorder. There is a surge in mHealth technologies delivering such interventions. This review aims to explore the concept, context and methodology of implementation of such mHealth apps. **Evidence Acquisition:** A search was conducted using NICE (National Institute of Clinical Excellence) healthcare database, including keyword 'early intervention,' 'mHealth,' 'parent support,' 'apps,' and 'autism.' The quantitative, qualitative, mixed-methods, case reports, grey literature, systematic reviews, clinical trials, and feasibility studies of children between 2 to 6 years with ASD were included from inception of database to December, 2021. Web/ Internet-based or computer-dependent programs were excluded. The initial search yielded 3786 studies; 17 were finally included based on the inclusion and exclusion criteria. **Result:** Studies on a total of mhealth apps were reviewed. Nine apps, apart from TOBY (Therapy outcome by you), lacked a holistic approach and instead targeted a specific difficulty in autism. The provision of support to parents using apps was equally beneficial as in-person support, reduced costs, and improved outcomes in children. **Conclusion:** The review revealed limited evidence-based mHealth apps available currently in a community setting. This also underscores an opportunity for clinicians to re-direct parents towards evidence-based information and interventions.

Keywords: *Early digital intervention, Digital technology, Mobile application-based support, eHealth, Parental support*.

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Autism spectrum disorder (ASD) is characterized by persistent deficits in the ability to initiate and sustain reciprocal social interaction and social communication with a range of restricted, repetitive, and inflexible patterns of behavior, interests or activities that are atypical or excessive for the individual's age and socio-cultural context [1]. Appropriate early interventions improve a child's development and behavior and reduce symptoms [2-4]. Early intervention has been defined as "services and supports available to babies and young children with developmental delays and disabilities, who are at risk of poor outcome and their families" [5]. The primary purpose of early intervention is to help in the acquisition and generalization of critical developmental skills, and to achieve independent functioning across environments [6]. Early intervention programs are more effective, if provided early [7]. The definition of early varies according to the age and developmental status of the child; areas where problems have been identified; the availability of professionals, the community and other resources at a local level; and individual choices made by parents [8,9].

Parents are key participants in the early intervention process. Information from parents, or carers can contribute to a better understanding of the complex interactions between neurodisability and its consequences. It helps in the planning of early intervention and providing support to parents through various implementation and theoretical models [10]. Early intervention through parents or caregivers, based on an integrated framework in a community setting, is helpful [11]. The limiting factors at the community, schools, parent group-based and home-based levels were lack of trained professionals, lack of such services in remote areas, time factor, parents' adherence and cost-effectiveness [12-14]. An early intervention must continue beyond the formal hours of contact to transfer skills into everyday activities [15]. To achieve this, there has been a rapid increase in the conversion of evidence-based in-person early inter-vention programs into remote and telehealth programs [16,17].

The emergence of mobile health (mHealth) has opened a new frontier for the delivery of early intervention [18]. The term mHealth was coined to describe a subset of eHealth that uses mobile technologies, including advancements in innovative applications to address health priorities [19]. It has great potential as a scalable and cost-effective delivery mechanism. This scoping review aims to present a review of currently available mHealth apps, which are used to deliver the early intervention to support parents of children with ASD between 2 to 6 years of age.

METHODS

A scoping review design based on Joanna Briggs Institute (JBI) methodology [20] for the conduction, and PRISMA extension (PRISMA-ScR) checklist and flow diagram [21] were utilized to guide procedures for identification, screening, eligibility, and inclusion of articles for review.

Search strategy and selection: A search was conducted from the inception of the database until December, 2021. Keywords for mHealth application, technology, early intervention, autism spectrum disorder, parent support and training, and digital program, were used (**Web Table I**, Priori protocol [10]). The following databases were used: NICE Healthcare Databases Advanced Search, Cochrane Library, EbscoHost, Sabinet, SAGE Journals, Directory of Open Access Journals (DOAJ), BioMed Central, Scopus, and ScienceDirect. Furthermore, grey literature was searched through Google Scholar, ShodhGanga, Journal Storage (JSTOR), CORE, and Bielefeld Academic Search Engine (BASE).

We defined autism apps as apps developed to support parents of children with autism aged 2-6 years. We defined a parent support app as any app to be used by parents, which helps them during the diagnosis pathway, during diagnosis, and after diagnosis during early intervention, in a community-based, hospital, clinic or research setting. We also aimed to understand the nature of the outcome measurements, their concept, context of implementation, methodological framework, and evidence quality.

Classification of mHealth applications was done using the NICE Evidence standards framework for digital health technologies [22]. TiDieR checklist reported the type of intervention, context, and outcome [23]. The quality and level of evidence was reported using the Evidence-Based Practice (EBP) tool developed by the Center for Evidence-Based Practice [24].

Eligible studies were uploaded to the Google Data extraction sheet (DES) (**Web Table II**). DES was screened for duplicates. In the second step, all the titles and abstracts were screened using the eligibility criteria, and those not matching were excluded. The final papers meeting the eligibility criteria were included for full data extraction using the DES.

Population/participants: Studies that included parents and carer of children with ASD between the ages of 2 to 6 years were included to review the apps supporting parents in the early years. Parents were defined as a biological parent, birth parents, carer or foster parents of children with ASD.

mHealth applications (apps) were defined as applications developed for use on mobiles or smartphones, tablets, or iPad that can be easily downloaded from the Play Store or App Store. Web/internet-based programs, or computer-dependent programs were excluded. The studies conducted or implemented for parents of children with ASD to provide support during the early intervention in community settings, school settings, special schools, clinics, hospitals, at home and child development centers were included. The inclusion was independent of region, gender, socio-cultural, or language factors.

Data extraction and analysis: Data extraction was done in two steps. The DES (**Web Table II**) included authors' names, year of publication, the purpose of the study, population and sample size, context, concept, outcomes, key findings, strengths, limitations, parental feedback, and theoretical framework. In the second step, the final papers meeting the eligibility criteria were analyzed using the Summary of the findings table (SOFT; **Web Table III**) developed based on the TiDier checklist [23], by two authors. The quality and level of evidence and classification, for the included apps, are shown in **Table I**.

RESULTS

The process of screening is shown in **Fig. 1**. The papers were excluded after being screened are mentioned in (**Web Table IV**). Seventeen papers were screened in full using TiDieR checklist and web-based software called Scholarcy (**Web Table III**).

All 17 papers were published between years, 2013 and 2021, with an increase from year 2017 onwards (15 of 17 papers) in the number of publications on mHealth. All papers were from high-income countries, most commonly from United States of America (*n*=6) and Australia (*n*=6, 35.3% in each). The sample size of parents and children in the studies ranged from 3 to 62 (415 mothers and 13 fathers), and 2 to 1514 (2735) children. All the papers included children within the age range of 1-12 years. In the final analysis, the findings for the age range 2-6 were included.

Table I Quality and Level of Evidence, and Classification as per the NICE Evidence Standards Framework for Digital Health Technologies

Research title	*App name*	*NICE*	*Quality*	*Level of evidence*
A pilot investigation of an iOS-based app for toilet training children with an autism spectrum disorder.	Toilet training app	Tier C: Interventions	B: Good-quality	Level I (RCT)
A randomized controlled trial of an iPad-based application to complement early behavioural intervention in Autism Spectrum Disorder	Therapy outcome by you (TOBY)	Tier C: Interventions	B: Good-quality	Level I
A randomised controlled trial of an information communication technology delivered intervention for children with autism spectrum disorder living in regional Australia.	Therapy outcome by you (TOBY)	Tier C: Interventions	B: Good-quality	Level I
A twelve-month follow-up of an information communication technology delivered intervention for children with autism spectrum disorder living in regional Australia.	Therapy outcome by you (TOBY)	Tier C: Interventions Methods)	B: Good-quality	Level III (Mixed
TOBY play-pad application to teach children with ASD – A pilot trial	Therapy outcome by you (TOBY)	Tier C: Interventions	C: Low-quality	Level III (Pilot Trial, with no control group, or randomization)
Appropriateness of the TOBY application, an iPad intervention for children with autism spectrum disorder: A thematic approach	Therapy outcome by you (TOBY)	Tier C: Interventions	B: Good-quality	Level III: Qualitative
Parental experiences using the Therapy Outcomes by You (TOBY) application to deliver early intervention to their child with autism.	Therapy outcome by you (TOBY)	Tier C: Interventions	B: Good-quality	Level III: Qualitative
A trial of an iPad™ intervention targeting social communication skills in children with autism.	Findme	Tier C: Interventions	B: Good-quality	Level I: RCT
Mobile technology to support parents in reducing stereotypy.	iStim Interventions	Tier C:	C: Low-quality	Level II: Quasi-Experimental
The use of behaviour modelling training in a mobile app parent training program to improve functional communication of young children with autism spectrum disorder.	Map4speech	Tier C: Interventions	C: Low-quality	Level 2
The effectiveness of a psychoeducation intervention delivered via WhatsApp for mothers of children with autism spectrum disorder in the Kingdom of Saudi Arabia: A randomized controlled trial.	Intervention delivered via whatsapp	Tier B: understanding and communicating	B: Good-quality	Level I
A comparison of PECS and iPad to teach requesting to pre-schoolers with autistic spectrum disorders	Sounding Bird	Tier C: Interventions	C: Low-quality	Level II
Evaluating the effectiveness of a tablet application to increase eye contact in children diagnosed with autism	Look in my eyes steam train	Tier C: Interventions	C: Low-quality	Level II

Contd....

table contd. from pre-page

Research title	*App name*	*NICE*	*Quality*	*Level of evidence*
An evaluation of a mobile application designed to teach receptive language skills to children with autism spectrum disorder	Camp Discovery	Tier C: Interventions	B: Good-quality	Level I randomized controlled trial (RCT)
Tablet-based cognitive exercises as an early parent-administered intervention tool for toddlers with autism - evidence from a field study	Mental Imagery Therapy for Autism (MITA)	Tier C: Interventions	A: High-quality	Level III
Mental imagery therapy for autism (MITA) - an early intervention computerized language training program for children with ASD	Mental Imagery Therapy for Autism (MITA)	Tier C: Interventions	B: Good-quality	Level III: non-experimental, descriptive study
Children with autism appear to benefit from parent-administered computerized cognitive and language exercises independent of the child's age or autism severity	Mental Imagery Therapy for Autism (MITA)	Tier C: Interventions	B: Good-quality	Level II

Level I: studies include randomized control trials (RCTs) or experimental studies; Level II: studies have some degree of investigator control and some manipulation of an independent variable but lack random assignment to groups and may not have a control group; Level III: studies lack manipulation of an independent variable; can be descriptive, comparative, or correlational; and often use secondary data. Quantitative Studies: A High quality: Consistent, generalizable results; sufficient sample size for the study design; adequate control; definitive conclusions; consistent recommendations based on a comprehensive literature review that includes thorough reference to scientific evidence. B Good quality: Reasonably consistent results; sufficient sample size for the study design; some control; fairly definitive conclusions; reasonably consistent recommendations based on a fairly comprehensive literature review that includes some reference to scientific evidence.C Low quality: Little evidence with inconsistent results; insufficient sample size for the study design; conclusions cannot be drawn.Qualitative Studies: A High quality: Contains high-quality quantitative and qualitative study components; highly relevant study design; relevant integration of data or results; and careful consideration of the limitations of the chosen approach.B Good quality: Contains good-quality quantitative and qualitative study components; relevant study design; moderately relevant integration of data or results; and some discussion of integration limitations.C Low quality: Contains low quality quantitative and qualitative study components; study design not relevant to research questions or objectives; poorly integrated data or results; and no consideration of limits of integration.

Ten apps were included in the final review; nine apps were available only on iPhone operating system (iOS) [25-35,37], and three apps were available on both iOS and android platforms [35,38-41] (**Web Table III**). Nine apps [25-34,36-41] were classified as Tier C: Intervention apps (**Table I**). All the apps differed in terms of interventions viz., toilet training [25], developmental abilities [28-31], social communication skills [32], reducing stereotypy [33], naturalistic language intervention [34], psycho-education intervention [35], picture exchange communication system [36], increasing eye contact [37], teaching receptive language skills [38], and cognitive exercises through a tablet [39-41]. The method of determining a participant's diagnosis also varied across all the studies, three studies did not mention the use of any diagnostic or screening tools used, two mentioned diagnosis was self-reported by parents, and four mentioned the use of the Autism Diagnostic Observation Schedule 2 (ADOS-2) [42], two mentioned diagnosis being done by the multi-professional team but did not mention any specific tool, one study reported use of Childhood Autism Rating Scale (CARS2) [43], and two used the criteria mentioned in Diagnosis and Statistical Manual of Mental Disorders (DSM-5) [44]. The context of delivery was home [28], home along with treatment as usual [28-35], remote at home [28-30], community- an school-based [32,34], intervention room/research setting [36,37], home and treatment center [38], and clinics [39-41].

Out of the seventeen papers included; one was rated high quality, eleven were rated as good quality and five were rated as or low quality. And using the EBP tool for the level of evidence rating, six papers were rated as level I, six were rated as level II and five were rated as level III (**Table I**).

The apps reported a variety of outcomes- a greater rate of skill acquisition in the intervention group [25]; no group difference on the primary outcome, significant improvements at the 6-month follow-up on three secondary outcomes [28]; no significant difference between baseline and post-intervention on other variables apart from expressive language [29]; improvements in receptive language, social skills, pragmatic language and playfulness [28]; positive feedback from parents on Therapy

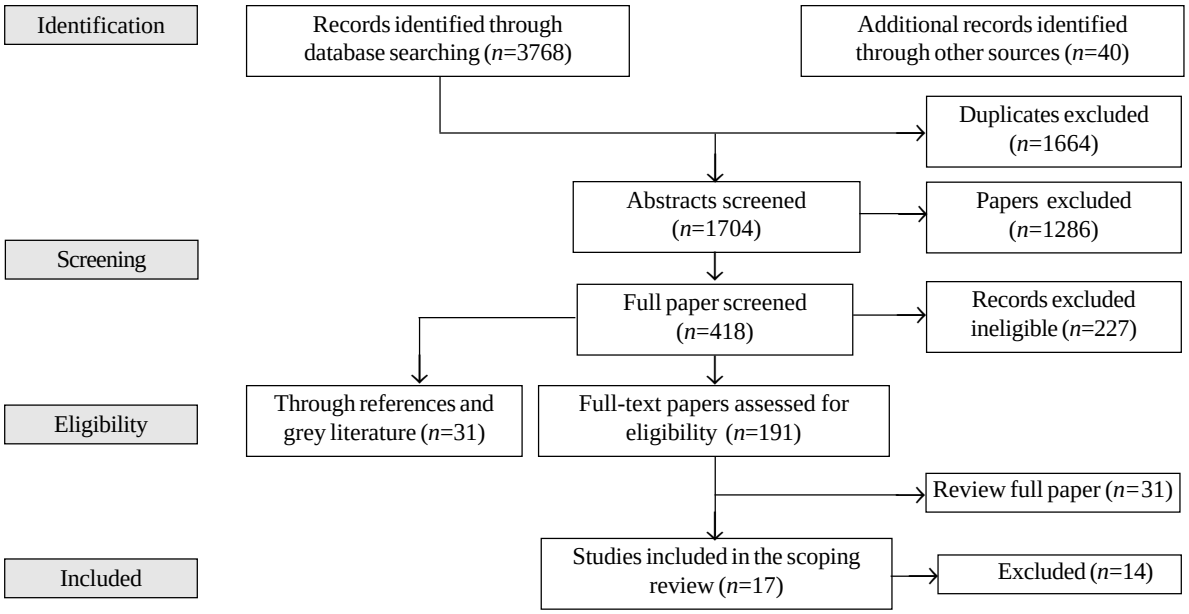

Fig.1 PRISMA flow diagram of the study.

outcome by you (TOBY) app [29-31]; no impact on real-world social communication skills [32]; reductions in stereotypy [33], improvements in parent's NLI skills and children's functional communication [34]; improvements in material happiness, and reducing maternal stress, depression, parent-reported ASD symptoms, child behavior problems [35]; and successful in teaching requesting skills [36].

The various theoretical framework which the included papers used were psychological models of stress and coping, and the double ABCX model (*n*=1**)**, applied behavioral analysis (*n*=7, 41.2%), behavioral inter-ventions (*n*=7, 41.2%), theory of mind cognitive model (*n*=1), and two papers did not mention the theoretical framework used.

Out of the included 11 mHealth interventions, only two did feasibility and usability testing. For implementation, clinical trials (*n*=1), randomized control trials (*n*=5, 29.4%), pilot investigations (*n*=2, 11.8%), and original research (*n*=7, 41.2%) were done.

DISCUSSION

This scoping review summarizes the evidence on current mHealth apps delivering early intervention in children with ASD. These mHealth apps were equally beneficial as in-person support, reduced cost, and improved outcomes in children, which were maintained till after 12 months. This review identified a gap in such technology support for parents in lower- and middle- income countries, as all publications were from high-income countries. The mHealth apps were similar in terms of the target population including parents (mostly mothers), and children aged between 2 years to 6 years, training parents, improving the child's skills, and treatment fidelity. Most of the studies used standardized measures of diagnosis; although, they varied in the type of assessment used. Few reported no method of diagnosis or did not use one, thus raising a question on the sampling process.

There was a significant difference in primary and secondary outcomes across all studies, that did not meet the defined goal of early intervention i.e., to assist in the acquisition and generalization of developmental skills. Instead, most mHealth apps focused on one aspect of daily life activities (DLA) or focused on improving autism-related behavior like eye contact, social communication, reducing stereotypy behavior, and teaching to request. This highlights the lack of interventions focusing on overall development, which might help in achieving the goal of early intensive evidence-based intervention for children with ASD.

All included mHealth apps were rated as Tier C: intervention level as per the NICE framework, but none were scrutinized for clinical safety, using Organization for the Review of Case and Health Apps (ORCHA) rating or WHO criteria for mHealth apps, apart from Mental Imagery Therapy for Autism (MITA). This suggests a clear lack of scrutiny of such apps on aspects like data security, clinical safety and clinical efficacy. They lack scientific evidence of being conducive to improve the outcome in affected children. The review suggests a clear

need for a mandatory real-world trial, patient and public involvement, and Digital Technology Assessment Criteria (DTAC) and ORCHA rating of all mHealth apps before making them available for parents and children with special needs. We also found a lack of any regu-lations or guidelines for such apps for app providers and publishers like the android play store.

All the mHealth apps have been grounded in a strong theoretical foundation, mostly based on behavior analysis, but the findings suggest most of them lack usability, feasibility, and efficacy before being used with a vulnerable population. Before being utilized by parents in general, only two apps, TOBY and MITA, underwent a pilot, RCT, feasibility, and usability test. However, TOBY lacks a sufficient sample size to generalize the findings.

The review revealed an opportunity for mHealth technology to aid early intervention in ASD. Although, there is an abundance of technology available, we found a lack of scientific rigor in the current mHealth apps. There is a need to create clear guidelines for the mHealth app stores to screen the apps for scientific evidence, and quality before making them freely available for parents of children with ASD. We suggest the need for a community-based mHealth app deliver early intervention to support parents of children with ASD based on the child's needs and at home, along with regular intervention. There is a need for a large-scale population trial of such apps, to generalize the finding to a larger population.

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

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Web Table I Result from search done on 21st December 2021 using National Institute for Health and Care Excellence (NICE) Healthcare Databases Advanced Search (HDAS)

Search	The search strategy used in MEDLINE (NICE Healthcare Databases Advanced Search)	**Records retrieved**
#1	Digit* OR exp TECHNOLOGY/ OR exp ELECTRONICS/ OR exp "DIGITAL TECHNOLOGY"/ OR exp "ANALOG-DIGITAL CONVERSION"/ OR digitisa* OR digitiza* OR onlin* OR technolog* OR computeriz* OR digitaliz* OR cell phone* OR mhealth* OR mobile technology* OR smartphone* OR mHealth apps* OR digital health intervention* OR digital health technology* OR e-health* OR tele-health* OR telemedicine*	2,263,054
#2	paren* OR exp PERSONS/ OR paren*or guardi* OR famil* OR Parent training* OR psychoeduca-tion* OR parent education* OR Parent Education Programmes* OR Parent Education Groups* OR Parent Psychoeducation* OR Parent Education Training*	178,797
#3	"Autism spectrum disorder"/ or "autistic disorder"/ or "pdd"/ or "asperger"/ or "neurodis"/ or "autism spectrum conditions"/ or "autistic spectrum conditions"/ or "autism"/ or "complex autistic spectrum"/ or "social communication disorders"/ or "neurodisability"/ or "autistic disorder"/ or "autistic condi-tion"/ or "asperger syndrome"/ or "spectrum"	461,50
#4	Child health services"/ or "preventive health services"/ or "early intervention, educational"	88604
MEDLINE	COMBINED- (1 AND 2 AND 3 AND 4)	500
Pubmed		546
PsycINFO®		904
EMBASE		765
HMIC		53
Note: The search keywords shown, are the one used in MEDLINE Abbreviations: MEDLINE - Medical Literature Analysis and Retrieval System Online EMBASE- Excerpta Medica database HMIC - Health Management Information Consortium		

Web Table II Data extraction sheet

Origin	Population and sample size	Methodology	Classification as per the NICE	Key findings that relate to the scoping review question	Major strengths and limitations	Parental feedbacks
United States of America	74 potential participants were screened, and 33 (45%) were randomized to either WMP or SBT.	pilot randomized controlled trial	Tier C: interventions	Parents used the app and related technology with few difficulties or malfunctions.	strength: RCT limitations: larger number of participants and longer intervention period (e.g., 6months) may have yielded different results; sessions, it is unknown how these interventions would fare under guidance from less academically trained providers or with fewer opportunities for consultation during intervention.	- parent satisfaction was high for both groups, parents saw benefit in participation. - most parents found the technology reliable and easy to use - minor challenges: replacing a dead battery, activating device, connectivity between transmitter and iPod, troubleshooting by interventionist and broad research team
Australia	80 children, randomised to either the 'TOBY group' (n = 41,80.6%male) or the 'Therapy as Usual' (TAU; n = 39,76.9%male) group.	This was a multicentre, stratified (site, socioeconomic status and developmental quotient, with 1:1 randomisation), parallel group RCT		Mhealth based support led to small improvements in developmental skills, and provided evidence that well-designed therapeutic apps can provide assistance to caregivers in delivering therapy, and increased exposure to therapy at relatively low cost.	Type of study, Limitation-lower level of use of App during the second 3-6 month, no significant improvement in autistic severities and drop outs.	Like' statements Helpful curriculum and therapy planning tool; lots of ideas for therapy and activities Easy to use Positive learning experience with off-iPad and social activities Front end of app attractive; presentation, structure and layout; colourful Relevant and reinforcing reward system Enjoyable experience for child and parent Enhanced understanding about child profile; increased awareness about therapy 'Dislike' statements Off iPad activities time consuming to prepare Curriculum too challenging Tasks were too repetitive; lacked variety Curriculum difficult to implement Curriculum too easy; below child's cognitive level and general abilities Child not interested Old interface
Australia	59 children and their families - 2-6 years	Exploratory Study		For hypothesis one, the expressive language subscale of the MSEL was the only statistically significant difference between the intervention and waitlisted groups between baseline and post intervention. For hypothesis two and three, when all the participants' scores were pooled and measured over time, statistically significant improvements were shown in receptive and pragmatic language and social skills and these gains were maintained, thus suggesting skill acquisition. These findings indicate limited effectiveness of the TOBY app for families living in regional areas. However, this was an exploratory study with a lower intervention dosage and fidelity than prescribed and a high partici-	strengths: RCT, significant gains were observed in the areas of receptive and pragmatic language and social skills from the intervention, suggesting skill acquisition. limitations: participant drop out, poor dosage and intervention fidelity, individual differences, location	

				pant drop-out rate.		
Australia	15 parents and their children with ASD (2-6 years)	12-month follow up of RCT		Findings demonstrate the receptive language, social skills, pragmatic language and playfulness of children with autism spectrum disorder improved during the three-month intervention period and were maintained at least 12 months after ceasing the Therapeutic Outcomes by You app intervention.	limitations - non-randomised sample, ceiling effect due to long term follow up, decrease in sensitivity of measures to detect change	- Twelve out of 15 parents reported their child had maintained at least one skill at 12 months post-intervention in one of the areas of receptive language, social communication or daily living skills, despite them no longer using the TOBY app. - Further, responses from the parents indicated 13 out of the 15 children were no longer using the TOBY app after twelve months, citing a lack of time and a loss of interest from their child as common reasons. - Parents reported the activities on the TOBY became too easy for their child and did not match their child's changing preferences since the initial intervention period.
Australia	33 families	Pilot trial study	Tier C: interventions	The TOBY play pad is an early intervention application through parent-mediation. This pilot trial sought out to collect information on the usage of the application rather than it's feasibility.	limitation: no independent pre or post intervention measures, nor was there a control group for comparisons; no independent data on indicators of functioning	positive - majority of families used toby to some extent during the trial, even without therapist support and in the absence of any kind of encouragement, parents were able to utilize this tool. Negative - accessibility issues, high levels of parenting stress,
Australia	24 parents of children with ASD	3-month trial		- Findings from this study partially support the appropriateness of the TOBY app for children with ASD and their parents who live in regional Australia. - Thematic analysis of interviews of parents who used the TOBY app as part of an effectiveness study identified the core theme that the TOBY app is not a panacea for the challenges associated with ASD. - Collectively, parents reported that that the TOBY app was appropriate for some children and not others, and should be used to complement other therapies and not in isolation. - Ongoing support from therapists, increased customisation through more choice and control, and a focus on user-experience was highlighted by parents as strategies that may improve the overall appropriateness of the TOBY app. - toby app provided variable benefits and experiences for parents and children. - the app did not accommodate the individuality of families by providing enough choice and control.	- Limitations: selection bias, generalisation to other sees, bias due to conflict of interest. - Strengths: first study to investigate the experience of using ICT based intervention for families of children with asd in regional areas,	- Most parents stated the TOBY app was straightforward to use, with clear instructions and easy navigation. - parents reported some issues with the TOBY app that tainted their experience: (1) it was challenging to get their children to engage with the app for 20 min per day; (2) a limited ability to choose and control the activities completed on the app; (3) the manifestation of problem behaviours in their children associated with using the TOBY app; and (4) the need for ongoing support from therapists, which they did not receive as part of this research project. - Parents in the study acknowledged the relevance of the TOBY app, with all participants expressing their desire to help their children overcome their developmental challenges. -Additionally, all parents interviewed would recommend the TOBY app to a friend, even if they felt it was not beneficial for their children, indicating they believe its utility and relevance for helping children with ASD.
Australia	17 parents of children with ASD	Semi-structured interviews and thematic analysis of parents' experiences using the		- positive experience with application use. -With sufficient support and guidance, parents reported that they were able to use TOBY effec-		- positive experience - One of the most significant barriers reported by parents was the time required in the organization of materials and equipment

		application.		tively in their home to provide successful intervention to their child with ASD. - The primary barriers identified by parents were occasional information overload, difficulty solving behavioural challenges, and a lack of sufficient support using the app. - Parents reported greater knowledge of the ASD condition and appreciated the guidance and structure by which TOBY provided so as to best support their child. -		to run the lesson plan, before using the TOBY app with their children. - Some parents found it challenging to orient themselves to the application's layout and structure. - Many parents reported feeling too time-poor and overwhelmed with the volume of information contained within the TOBY introduction. - Parents felt that training empowered them to deliver activities to their fullest potential, alleviated stress, and reduced time spent learning how to navigate the app itself. Parents also reported that this support also helped with understanding their contribution in delivery of the interventions and the importance of the NET for generalization of learning.
United Kingdom	54 children	Randomized Controlled Trial	Tier C: Interventions	There were no significant group differences in parent-report measures postintervention, nor in a measure of parent–child play at follow-up. Therefore, this intervention did not have an observable impact on real-world social communication skills and caution is recommended about the potential usefulness of iPad™ apps for amelioration of difficulties in interaction. However, positive attitudes among participants, lack of harms and the potential of apps to deliver therapeutic content at low economic cost suggest this approach is worth pursuing further.	strength: study design, limitation: usage of new, invalidated measure, lack of intervention effects no immediate effect on behaviours targeted by app.	- app was highly rated by parents - There were no problems with access of inappropriate content via the iPad and no parent reported concerns about the child becoming obsessive. - Parent perceptions of the intervention were largely positive and it is probable that one of the main advantages of this kind of approach is in the potential beneficial impact on family life and reduction of burden. - Some parents reported that their child was able to play and concentrate for longer using the FindMe app than with other toys, even at the end of the 2-month access period. - Other parents enjoyed the iPad as a way to sit with their child while mutually focussing on a rewarding activity.
Canada	12 children with ASD and one of their parents - 5 parents withdrew - 7 parents total	quasi-experimental AB design	Tier C: Interventions	1. a mobile application used by parents can teach them how to implement behavioural interventions to effectively reduce stereotypy. 2. more families could have access to effective services to reduce stereotypy in their child with ASD. 3. cost-effective since it does not require a professional being present during multiple sessions. 4. parents may need to use the iSTIM for longer periods of time in order to produce meaningful changes in functional engagement.	limitations: study design, no participation in functional analysis, participant withdrawal from study, didn't study placebo effect strengths: replication of intervention effects, a greater number of observation sessions to reduce participant bias.	
Singapore	three young children with asd and their mothers	multiple-baseline single-case experimental design	Tier C: Interventions	- mobile technology promising platform to deliver intervention for children with ASD. - effectiveness of specially developed PT program	limitations: low sample (3), response bias among participants, only included mothers. strengths: Findings from this study suggest that mobile apps may improve	

					access to those who want to develop their skills and desire more flexibility and convenience to access the training. Training apps may also be a valuable adjunctive tool for face-to-face training programs.	
United Kingdom/KSA	62 months of children with asd	randomized controlled trial	Tier B: understanding and communicating	- significant reduction in maternal depression post intervention - decrease in child behavioural problems - parental mean total stress significantly reduced	Strengths: This study has many strong points. This study used a randomized controlled trial (RCT) design to evaluate the efficacy of the intervention, which is considered to be the golden design for programme evaluation. The CONSORT statement guidelines for reporting RCTs were used in this study, which ensured a clear and comprehensive reporting of RCTs. Moreover, a follow-up data collection was used in this study to test the stability of findings overtime. limitations: lack of objective measures of maternal wellbeing and child behavioural problems, limited sample size, no info on health economics, low alpha value in some measures	
United Kingdom	3	A multiple baseline design (MBD) across participants was combined with an adapted alternating treatment design	Tier C: Interventions			
United States of America	3 Children with ASD	multiple baselines across participants design	Tier C: Interventions	- no significant result after usage of application, however after differential reinforcement was implemented, all three participants showed sig. increase in eye contact.	limitations: assessments run by researcher and not parent, inconsistent eye contact across different assessments, small sample size.	not given
United States of America	28 children	randomized, controlled design	Tier C: Interventions	the current findings show that participants demonstrated relatively high rates of learning over a short duration (i.e., 12 h in 1 month). Finally, CBI may increase the efficiency and accessibility of treatment for ASD.	limitations: small sample size, did not control for differing levees of asd severity, wide range in participant age, lack of treatment fidelity data.	not given
United States of America	823 children and their families	feasibility study	Tier C: Interventions	App based cognitive exercise for children with autism.	Study of tablet-based cognitive exercises administered to two-year-old toddlers with autism.	not given
United States of America	59 parents and 14 children	App development	Tier C: Interventions	Hypothesis children who begin training at an early age, and who make consistent progress over the course of training, will see drastic improvements in their language function.	The findings are from a feasibility study.	not given
United States of America	1514 children and their parents	feasibility study	Tier C: Interventions	MITA worked as designed, and parents were able to implement it an engage their children as young as 2 years with the app.	Major Strength: sample size, age range, Limitation: ATEC done by parents, and not professionals	not given

Web Table III Summary of finding table based on Template for Intervention Description and Replication (TIDieR) checklist

Author & Year	Brief Name [1]	Why [2]	What		Who/ trainer [5]	How [6]	Where [7]	When and How [8]	Tailoring [9]	Modifications [10]	How well	
			Materials [3]	Procedures [4]							Planned [11]	Actual [12]
Mruzek et al., 2019	Parent mediated toilet training intervention app	An innovative toilet training intervention that consists of WMP; an iOS-based app with transmitter/disposable sensor; and a corresponding manualized training program for use by parents in the home.	WMP and SBT intervention manuals, moisture paging device, connected to iPod.	At start of intervention, parents received a 1.5-h center-based training. During this initial training, all key aspects of the WMP or SBT intervention were reviewed, with a special emphasis on completing an individualized training program. For the WMP group, this initial review included development of an individualized training program based on the content of all six modules of the WMP intervention manual. Parents were then expected to carry out the intervention for 12 weeks in their home and participate in four 1-h, center-based 1:1 study visit (i.e., "booster sessions") at weeks 2, 4, 6, and 9 to troubleshoot and collect data on adherence and efficacy, and a closeout visit at week 12. Also, during these sessions, interventionists encouraged parents to continue with their training efforts and complete the data logs on the 3 days prior to the next booster session. Parents in both groups received a brief telephone call immediately prior to the onset of the 3-day data collection interval to remind them to complete the data logs. At 3 months following the close of intervention, parents were reminded by telephone to complete the data logs for 3 consecutive days and return them to the study team in a self-addressed stamped envelope.	Trained study interventionist (each with a master's or postdoctoral level of training in psychology) at each study visit using only the manual for their randomly assigned intervention.	delivered through manuals.	Home	Parents were then expected to carry out the intervention for 12 weeks in their home and participate in four 1-h, center-based 1:1 study visit (i.e., "booster sessions") at weeks 2, 4, 6, and 9 to troubleshoot and collect data on adherence and efficacy, and a closeout visit at week 12.	None	None	Yes, by measuring the number of sessions attended by participants of both group	At all visits, both study groups achieved mean treatment fidelity percentage scores greater than 80 (SBT mean = 94%, WMP mean = 90%) with median scores of 100.
Moore, et. al., (2015)	Therapy Outcomes By You (TOBY)	Pilot trail of TOBY app providing a comprehensive system for facilitating the delivery of intensive early intervention by parents in the home and as part of daily routines.	An iPad for each participating child loaded with the TOBY app and connected to the internet.	All participant responses were uploaded, automatically in the case of Solo and Partner activities and manually, by the parents, for NET activities, as an integrated part of TOBY use. Dependent variables generated by TOBY algorithms were (i) participant use patterns including total time engaged in Solo, Partner and NET activities, number of sessions and of completed learn units (stimulus, response, feedback – sequences) and (ii) indicators of child progress: correct/ incorrect response patterns differentiated across the four curriculum areas.	parents	iPad	Home using iPad	20 minutes per day	None	None	None	None
Whitehouse, et. al.,	TOBY	Use of the TOBY app as an addition to	The TOBY app installed on iPad	The length of the intervention period was 6 months, with follow-up	Trained Therapist	iPad	At home along	at least 20 min/day of TOBY based thera-	None	None	Yes	Yes, there was

(2017)		community-based therapy would help support home-based therapy, and thus facilitate greater improvement in a range of ASD-specific and broader developmental skills.		assessments taking place at the mid-point (3 months postbaseline) and conclusion (6 months postbaseline) of this period. Treatment group: TOBY intervention. Caregivers of children randomised to the TOBY intervention group received an initial 2-hr training session during which they were familiarised with the TOBY curriculum, and the entry point in the curriculum was determined for each child in consultation with caregivers. Caregivers were contacted by the study team every fortnight during the trial period to encourage use of the TOBY app, to provide an opportunity for caregivers to ask any questions, and for the research team to enquire about perceived barriers to use of the TOBY app.			with TAU.	py for the next 6 month				no change in n the number of fortnightly telephone calls made to families, however, there was a large drop in usage of TOBY in the second 3 months.
Parsons, et al., (2019)	TOBY	To evaluate the appropriateness of the ICT intervention and toby app and to examine the barriers and facilitators identified by parents who used TOBY app living in regional Australia.	The TOBY app installed on iPad	Twenty-four mothers of a child with ASD from a pool of 59 families from the RCT participated in a three-month RCT using the TOBY app were included in this study. Participants were ranked for use on three measures: (1) time spent using the app on the device; (2) items attempted; and (3) items completed. Phone interviews - semi structured interview: experience using the app; (3) if parents perceived the TOBY app to be effective for their child; (4) if parents perceived the TOBY app to be effective for themselves; (5) the ease of use, including the planning needed to implement the suggested dosage; (6) the level of support required to use the app effectively; (7) their intended future use of the app; and (8) suggested improvements to the app. Interviews lasted between 16 and 45 min in duration, and digital voice recorder was used to record the interviews, which were subsequently transcribed verbatim by a professional transcription service.	Occupational therapist and qualitative researcher	The TOBY app is a tablet (iOS©) along with face-to-face therapy.	Australia, remote intervention - phone interviews.	App use was measured using backend server data that is automatically gathered from the tablet device. Semi- structured interviews between 20 and 45 min in duration were conducted to explore the experience of the TOBY app	None	None	None	None
Parsons et. al., (2019)	TOBY	Comparative study of 3 months TOBY users and wait-list control group improving visual motor, imitation, language and social skills of children with ASD.	The TOBY app installed on iPad	In addition to receiving therapy-as-usual, the intervention group were instructed to practise at least 20 min on the TOBY app daily for 3-months using an iPad. Participants were then re-assessed at 3 and 6 months after the baseline assessment to establish post-intervention and follow-up measurements, respectively. The waitlisted group received an iPad without the TOBY app in-	psychologists and occupational therapists	Toby app, I-Pad	home, remote	20 min once per day, follow up every 2 weeks for 3-6 months. The intervention has three methods for the delivery of therapy: solo, partner, and Natural Environment Training (NET) (Venkatesh et al. 2013). The syllabus includes a variety of activities that utilise	None	None	20 min/day using the TOBY app.	low fidelity after three months

				stalled and therapy-as-usual after the baseline assessment. After the waiting period of 3-months, the control group received the TOBY app for 3-months. The waitlisted group were then assessed at 6 and 9-months to establish the post-intervention and follow-up measurements.				different methods of delivery to address the four targeted skill areas. The TOBY app solo activities involved the child interacting directly with the iPad. Caregivers then inputted the result directly into the TOBY app to track their progress. The NET activities of the TOBY app aimed to generalise learning from the solo and partner activities into natural situations by educating, prompting, and logging the caregiver's translational intervention with their child.				
Rogerson et al., (2019)	TOBY	Parental experience of using the TOBY app designed to provide targeted training in imitation, language, sensory discrimination, and joint attention; all of which are typically delayed or disrupted in young children with ASD.	The semi-structured interview guide	Data were obtained through semi-structured interviews with parents of children with ASD that were later analysed by initially identifying content manifesting perceived barriers and facilitators to the use of TOBY. A thematic analysis followed in which meaning-bearing units related to facilitators, barriers, and to parents' experiences were identified and later analysed as described in the data analysis section.	Paediatric occupational therapy student	In person and telephone interviews.	home	Prior use of the application within 2 years of child's diagnosis.	None	None	None	None
Parsons et. al., (2020)	TOBY	Use of the TOBY app was anticipated to lead to improvements in the longer term for the skills of language, social communication and playfulness as the children developed.	The TOBY app installed on iPad	This study used a single-site cohort design, with data collected at baseline (T1), post-intervention (T2) and follow-up at 12 months post-intervention (T3). After the assessment, participants were asked a series of open-ended questions lasting between 5–15 minutes to provide further explanation regarding the continued use and maintenance of skills learnt while using the TOBY app. The TOBY app comprises the following three types of tasks: solo, partner, and natural environment tasks (NET). Children begin the intervention with activities at their current level of functioning and progress through the curriculum at their own rate of development and ability.	parents	Through TOBY app using iPad. The NET tasks are performed separately from the iPad with caregiver support and are integrated into daily life to encourage generalisation of skills learnt during solo and partner	at home	Parents in this RCT, in the intervention group were provided with an iPad which had TOBY app installed and were instructed to use the applications for 20 min per day at a time convenient to the family. One-hour training by the researchers (occupational therapists and psychologists) on how to navigate and use the intervention was provided.	None	None	None	None

						tasks. Responses to each task are inputted into TOBY app, and a syllabus of future tasks is tailored for the child.						
Fletcher-Watson et al., 2016	FindMe	FindMe aimed to enhance the real-world social communication skills of the children through motivating, daily rehearsal of very basic subskills.	FindMe app	The length of the experiment/intervention was 72 days on average, and parents were suggested to aim for game play of about five minutes per day, or ten minutes every other day. Parents and children were filmed for 10 min playing with a standard set of toys with no specific instructions given. Intervention group: 2 months of app access at the same time as all usual treatments. Waitlist group: only treatment as usual.	parents	iPads were sent out to each child's home, for the intervention group, before shutting down functions aside from the app. Brief instruction document which dealt with the basics of working and charging the iPad and offering advice on troubleshooting, and suggested that parents aimed for game play of about five minutes per day, or ten minutes every other day	one to one support in nursery or primary school, including specialist units and integrated mainstream classes.	Children in the intervention group had access to the FindMe app for a period of 72 days on average (95% CI = 70–75 days).	None	The trial design set a high bar for measuring benefit by selecting as the outcome measure a parent–child play-based observational measure taken at the Follow-Up appointment and not immediately following the intervention.	Children in the intervention group had access to the FindMe app for a period of 72 days on average	The mean number of days on which children actually played the app was 28. Median length of game play was 339 min (interquartile range = 206–1074) over the intervention period (minimum 15 min, maximum 3522 min). Only four children in the Intervention group failed to reach the most complex level of the game. Of the 23 children reach-

						day.						ing the high-est level, 22 car-ried on to repeat the game cycle after achiev ing that top level.
Dunn et al., 2017	iStim	This applica-tion offers parents a solu-tion and a method to reduce stereo-typy in chil-dren who do not have the ability to use a self-monitoring method.	The iSTIM is an iOS appli-cation, cur-rently availa-ble for re-search pur-pose only, with four parent train-ing and sup-port modules.	To assess the effects of the app, researchers conducted a series of AB quasi-experiments wherein each partici-pant served as their own control. Research assis-tant measured stereo-typy before and during the implementation of the intervention while the parent was using the iSTIM.	research assistants and Parents	iOS devic-es	home	Families partici-pated in sessions once or twice per week over a period of 8 to 16 weeks.	Non e	None	None	None
Trudel, L., La-novaz, M. J., & Préfon-taine, I. (2021)	Map4speec h	Map4speech uses the BMT framework to conduct natu-ralistic inter-vention with young children with ASD to improve their functional communica-tion.	Each parent was loaned an iPad con-taining the Map4speech mobile app.	There were five phases in this experiment: baseline, PT, post-training intervention (PTI), novel settings, and 1-month follow-up. Phases 1, 2, and 3 (i.e., baseline, PT, and PTI) used a concurrent de-sign and were conduct-ed in the children's homes with the same toy materials within each parent–child dyad. Phases 4 and 5 (i.e., novel settings and 1-month follow-up) used a non-concurrent design and were conducted to learn how parents gen-eralized their new skills in different contexts.	Psycholo-gists	i-pad, as-sess-ment face to face	com-munity play-ground s, fam-ily dining areas, skype call	Phase 1: The shortest baseline period lasted 1 day with 5 ses-sions, and the longest baseline period lasted 7 days with 14 sessions. Phase 2: For 5 days a week, they were asked to spend 15 min to practice the learned interven-tion skills with their child. They used the app to take two or three 2-min video clips of them-selves practicing the skills with their child and uploaded them to a secure serv-er via the app. Phase 3: The average post-training period was around 9 days across three parents. The average feed-back sessions in Phase 3 were two sessions across three parents. Phase 4: set-tings. The aver-age intervention period on the playground and the snack time setting were 8 and 7 days, re-spectively. The average feed-back sessions for two novel set-tings were 3.3 sessions across three parents.	Non e	None	Parents were required to attain 90%–100% of the in-tervention skills in two consecu-tive practice videos in order to advance to the next stage of the app where they learned new skills while continu-ing to use the previ-ously learned skills.	Par-ents were able to main-tain a high level of im-ple-men-tation fideli-ty. They achiev ed 88%92% fideli-ty for inter-ven-tion im-ple-men-tation during the PTI, 83%–88% during novel set-ting—play-groun d, 91%97% during novel setting at snack time, and 90%–93% during the 1-month fol-low-up.
Law, G.	Psy-	The transac-	The interven-	Mothers who consented	Three thera-	Whats	Whats	Sessions 2–5	Non	None	None	None

C., Neihart, M., & Dutt, A. (2018)	choeducation Intervention delivered via WhatsApp	tional model of stress and the Double ABCX Model contributed to the development of the intervention in three sessions. Session 1 to inform mothers about the aetiology of ASD; Session 2 to target stress in mothers and how can they approach different stressful situations; Session 3 to child behaviour problems to help the mothers cope with the initial stressor of having a child with ASD; and Session 5 to inform mothers about the available resources in KSA.	tion was developed as a guided self-help intervention in line with the main principles and recommendations of NHS Good Practice Guidance on the use of self-help materials within Increasing Access to Psychological Therapies IAPT services.	were randomly allocated to the trial arm, and study information packs, and Copies of the training manual were provided. Sessions 2–5 consisted of 30-min therapist support via WhatsApp. Mothers in the CAU group received advice about their child's educational and behavioural problems from the organizations.	pists and one certified clinical psychologist assisted in delivering the intervention.	App	App	consisted of 30-min therapist support via WhatsApp.	e			
Hemdi, A., & Daley, D. (2017)	Sounding Bird	Investigating the use of an iPad as a SGD and to compare the relative efficacy of the iPad with PECS for developing requesting and navigational skills with preschoolers with ASD.	Child's table and chairs, a computer, A standard PECS book, An iPad-4 with a BigGripsTM4, and SoundingBoardTM 5 app	Stimulus preference assessment, parent interview and children were observed during unstructured free play prior to baseline. Modality Preference Assessment was conducted at the beginning of each baseline, intervention, and post-intervention to determine if a participant had a preference for one of the two AAC options.	researcher	face to face	intervention room	Each participant received six sessions of intervention over a 4-week period in each condition. Each session was of 20-min duration and focused on one AAC condition, PECS or iPad.	Non e	none	Treatment integrity was measured.	Overall treatment integrity for the three researchers was 97%
Agius, M. M., & Vance, M. (2016)	Look in My Eyes Steam Train	This application allows children to practice eye contact by displaying a number in a person's eyes and having the child complete a match- to-sample.	An Apple iPad© was used to allow the child to use the Look in My Eyes Steam Train application.	1. Before beginning the study, to ensure the child has the prerequisite skills required to use application, they were asked to complete a match-to-sample test while in the individual therapy room. The child was presented with a grid numbered 1 to 9, and he had to replicate that grid on the iPad application. On completion of the sample task only he was eligible for the study. 2. During baseline, the child participated in a two manding session that typically took place during therapy sessions. One took place in the individual therapy room and the other took place in the natural environment. For each baseline session, the occurrence or non-occurrence of eye contact when the child made a request was recorded for the first 10 times. Once there was a stable pattern of eye contact in both locations, the child was then moved to the intervention phase. 3. After baseline data were obtained, the child	Therapist	face-to-face and mhealth app	Each session took place in either the individual therapy rooms or the natural environment training room. The individual therapy rooms were 3x3 m with three individual cubicles in each. A 1.8 m wall divided the work-	A session consisted of 10 trials	Non e	none	None	None

				was instructed to play with an iPad application designed to increase eye contact while in the individual therapy room. The child only had access to this application during training. 4. Immediately after the child used the application, eye contact was assessed in the individual therapy room. After this assessment there was a 5 min delay in which the child was brought to the natural environment training room and was required to complete demands not associated with this study. Once the delay was complete an immediate generalization assessment was conducted to see if the change in eye contact generalized to another setting. If the eye contact application showed an increase in eye contact to 80% in all of the assessments, the child would be finished with the study. However, if the eye contact application was unsuccessful, the child then moved on to a differential reinforcement phase. 5. This phase consisted of 10-min training sessions in the individual therapy room. If the child made eye contact with the therapist when requesting and item, the therapist immediately reinforced the child's behaviour by providing him with praise and the requested item. If the child did not make eye contact with the therapist when requesting an item, the therapist waited until the child made the request again while using eye contact before reinforcing eye contact by allowing the child to have the requested item. The same prompting procedure that was used in baseline was used if a mand was not made within 5 s. 6. Immediately after the child completed the differential reinforcement training, eye contact was assessed in the same manner it was assessed following the iPad intervention.			spaces and each cubicle had a table and chairs, program materials, and reinforcers that were chosen by the child. The natural environment training room was 6x4 m open room with multiple reinforcing items available for the child to engage with.				
Jeffries, T. (2013)	Camp Discovery	The application incorporates modified discrete-trial training (DTT) procedures and other behavioural principles of ABA to teach receptive language targets across different lessons.	iPad tablet with Camp Discovery open. Application settings were adjusted to ensure that the participant only worked on the targets identified as unknown	1. Pre- and Posttreatment Probes: Probes were conducted lesson by lesson, in a random order. During probe sessions, reinforcement, corrective feedback was not provided for incorrect responses. Reinforcement was only provided for maintaining attention and exhibiting appropriate behaviour. Reinforcers were	Practicing behavioural therapists with extensive ABA training for ASD and field work.	through mhealth app on iPad, individually.	participant's home or treatment center	Probes took anywhere between 1 and 3 h and were conducted across one or multiple sessions performed within a 1-week period. Treatment sessions occurred for 3 h per week (i.e., three 1-h sessions) for 4	None	Application settings were adjusted to ensure that the participant only	None	None

			during his or her initial probe.	identified via a preference assessment conducted by the research assistant. Each participant experienced three total probe sessions. The first probe was used to identify approximately 100 unknown targets that were covered within the mobile application's learning content. Subsequent probes assessed the targets identified as unknown during the initial probe. 2. Immediate-treatment IT group and delayed-treatment control DTC group: After an initial probe, the IT group began interacting with the mobile application, whereas the DTC group continued with treatment as usual with no manipulations. After 4 weeks, both groups received a second probe (i.e., post-treatment for the IT group; pre-treatment for the DTC group) to determine if learning took place in the presence or absence of the mobile application. Following the probe, the DTC group entered the treatment phase while the IT group discontinued use of the mobile application, receiving only treatment as usual. After 4 weeks, both groups were administered a final probe to determine if any learning occurred (i.e., DTC group), as well as to evaluate whether previously acquired skills were maintained (i.e., IT group) without access to the mobile application.				weeks, separate from ongoing ABA sessions.		worked on the targets identified as unknown during his or her initial probe.		
Dunn, R.S., & Vyshedskiy, A. (2015)	Mental Imagery Therapy for Autism (MITA)	This paper is on the development of MITA app based on Pivotal Response Treatment (PRT), which was developed to deliver evidence-based early-intervention therapies made especially for very young children with ASD.	MITA app	MITA's exercises follow a systematic approach for training the skill of multiple cues responding.	N/A	using MITA app	at home	None	The MITA program follows a similar systematic approach for developing a child's ability to respond to multiple cues, starting	None	None	None

									with very simple exercises that require attending to only one cue or characteristic, namely colour			
Dunn et al., (2017)	MITA	Data from feasibility study of parent- administered tablet-assisted therapy for 1514 children of different ages and varying ASD severities for twelve months.	MITA app, Supplementary material	MITA was made available for free to download, and sample was selected from the pool of registered users on the app based on the pre-defined criteria; i.e., a self-reported diagnosis of ASD, availability of two ATEC scores at least three months apart, and age must be 12 or below.	Parents	using MITA app on mobile	at home	MITA consists of nine different developmental activities. To be done on a daily basis for 10 minutes, for a period of twelve months, using mobile app	Each of the nine MITA activities consists of multiple levels, starting with easier levels that require attending to a single cue, moving on to intermediate levels that require attending to two cues and culminating in challenging levels that require attend-	None	Yes, by recommending to be used at least for 10 minutes per day.	Subjects across all age and severity groups adhered to the recommendation at least 64 ± 19% of the time

									ing to three or four cues at a time. Most activities have as many as 50 levels which range from easy to difficult in a gradual and systematic manner.			
Dunn et al., (2017)	MITA	This study describes data from the feasibility study of this therapeutic intervention, through MITA.	MITA app	The objective of this study was to determine whether children as young as two who have been diagnosed with ASD could engage on a daily basis and over an extended period of time with a therapeutic application, and whether their parents would be willing to administer such an application.	Parents	through MITA app	home	At home, using the app. For a period of three to ten months.	None	None	Yes, by recommending to be used at least for 10 minutes per day.	There was one subjects who worked with MITA every day for over six months, the actual median (IQR) number of days MITA was used per week was 1.6 (0.9-2.6), significantly less than the recommendation. Only 161 subjects (20%) worked with MITA more than 3 days per

												week; only 60 sub-jects (7%) worke d with MITA more than 4 days per week; and only 24 sub-jects (3%) worke d with MITA more than 5 days per week.

Notes: § 1: Provide the name or a phrase that describes the intervention. 2.Describe any rationale, theory, or goal of the elements essential to the intervention. 3.Materials: Describe any physical or informational materials used in the intervention, including those provided to participants or used in intervention delivery or in training of inter-vention providers. 4: Procedures: Describe each of the procedures, activities, and/or processes used in the intervention, including any enabling or support activities. 5. For each category of intervention provider (e.g. psychologist, nursing assistant), describe their expertise, background and any specific training given. 6. Describe the modes of delivery (e.g. face-to-face or by some other mechanism, such as internet or telephone) of the intervention and whether it was provided individually or in a group 7. Describe the type(s) of location(s) where the intervention occurred, including any necessary infrastructure or relevant features. 8. Describe the number of times the intervention was delivered and over what period of time including the number of sessions, their schedule, and their duration, intensity or dose. 9. If the intervention was planned to be per-sonalised, titrated or adapted, then describe what, why, when, and how. 10.‡ If the intervention was modified during the course of the study, describe the changes (what, why, when, and how). 11. Planned: If intervention adherence or fidelity was assessed, describe how and by whom, and if any strategies were used to maintain or improve fidelity, describe them.12. Actual: If intervention adherence or fidelity was assessed, describe the extent to which the intervention was delivered as planned.

Abbreviations: ASD: Autism Spectrum Disorder, ABA: Applied Behavioral Analysis, PRT: Pivotal Response Training, MITA: Mental Imagery Therapy for Autism, ATEC scores: Autism Treatment Evaluation Checklist.

Web Table IV: Studies ineligible following full-text review

	Research Paper	Reason
1.	Parent scaffolding of young children's use of touch screen tablets	Different concept
2.	Investigating the use of technology in communication exchanges and visual support for students with autism	ineligible age range
3.	Comparison of therapist implemented and iPad-assisted interventions for children with autism	Different context
4.	Effectiveness Of Proloquo2go ™ in Enhancing Communication in Children with Au-tism During Aba Therapy	Different concept
5.	A video parent-training program for families of children with autism spectrum disor-der in Albania	Different concept
6.	Virtual Reality Support for Joint Attention Using the Floreo Joint Attention Module: Usability and Feasibility Pilot Study.	Different concept
7.	Features of Mobile Apps for People with Autism in a Post COVID-19 Scenario: Cur-rent Status and Recommendations for Apps Using AI	Different concept
8.	Applications for Children with Autism in Preschool and Primary Education	Different concept
9.	Talking Picture Schedules: Embedding Video Models into Visual Activity Schedules to Increase Independence for Students with ASD	Different Concept
10.	Proloquo2Go Enhances Classroom Performance in Children with Autism Spectrum Disorder	Different concept
11	Using Apps to Develop Social Skills in Children with Autism Spectrum Disorder	Different concept
12	mHealth for Mental Health: Integrating Smartphone Technology in Behavioural Healthcare	Different concept
13	JAKE® Multimodal Data Capture System: Insights from an Observational Study of Autism Spectrum Disorder.	No use of mhealth, and dif-ferent concept
14	The utility of LENA as an indicator of developmental outcomes for young children with autism	Different concept

Splenomegaly in Children- Significance Lies in the Cause!

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The spleen is palpable in 5-10 % of all healthy children and in <30% of all healthy neonates. The spleen is enlarged to at least twice its normal size to be clinically palpable. As a general principle, the length of normal spleen from the age of toddler until puberty is calculated by the formula spleen length [cm]=6 cm+1/3 cm per year of age [1]. The list of causes of splenomegaly is exhaustive, and patients often need a battery of tests before a final diagnosis is assigned. As noted by William Osler in 1908, "*nearly all diseases of the spleen are of a secondary nature"* [2]. Splenomegaly is not a disease of its own but a noteworthy sign of many systemic disorders. Fifty years after the seminal work by Reddi, et al. [3] on the etiology of splenomegaly in infants and children, we discuss the change in epidemiology in context of causes of splenomegaly and tools that have become available in the diagnostic pathway.

THE PAST

In third issue of 1973, Reddi, et al. [3] published their observation of cause of splenomegaly in 100 infants and children admitted to the pediatric wards of Institute of Child Health, Niloufer hospital, Hyderabad during the year 1970-71. About half of the cases were of less than 3 years of age. The most common cause of splenomegaly observed in the series was congestive splenomegaly due to Indian childhood cirrhosis (ICC). Other common causes reported were acute and chronic infections, blood disorders and malignancy. There were no cases of metabolic and collagen diseases. However, cause of splenomegaly remained undiagnosed in six cases. Conventionally, splenic edge palpable >2 cm below the left costal margin was considered as an enlarged spleen. The splenomegaly in this study was graded as mild, moderate and severe if it was <5 cm, 5-10 cm, >10 cm palpable below left costal margin, respectively. Massive splenomegaly was defined if it crossed the midline and reached right iliac fossa. Most cases had mild splenomegaly (57%) [3]. The authors observed that mild enlargement was due to acute infections, moderate by chronic infections as tuberculosis and blood disorders, and severe enlargement was due to portal hypertension. Tuberculosis accounted for 8% of cases. Mild splenomegaly was also observed in some cases of iron deficiency anemia. Massive splenomegaly was seen in 5 cases of which two had chronic myeloid leukemia. They found four cases of portal hypertension who had moderate to severe splenome-galy without any features suggestive of intra- or extra-hepatic portal vein obstruction, suggested by normal liver histology and dilated tortuous portal vein by spleno-venogram study. Banti syndrome was reported in one patient. A review article published around that time on etiology of splenomegaly stated infections as most common cause of an enlarged spleen in children in the Western population [4].

THE PRESENT

A correct diagnosis of splenomegaly in children must take into account age dependent size variations. Conventionally, the size of liver and spleen is determined by standard bedside techniques of palpation and percussion but are inaccurate to detect mild enlargement. A precise assessment of splenic dimensions i.e., "cranio-caudal length" can be obtained easily by ultrasonography and is reliable. The interpretation of the data must be based on age and body proportion dependent normal values in

pediatric patients from corresponding geographical area. Over the last two decades, there have been several studies to determine these age dependent values [5-7]. It correlates well with the splenic volume.

The underlying cause of splenomegaly is classified as infectious, hematologic, infiltrative, vascular, and immunological diseases with resulting abnormalities of the lymphoid, reticuloendothelial, or vascular components of the spleen. Though, congestive splenomegaly was the most common etiology in past, recent studies found infections as the most common cause [8]. Indian childhood cirrhosis (ICC) was a familiar entity in the past but there has been a considerable decline in incidence over last three decades [9]. Patra, et al. [10] reported five cases of ICC over a period of 10 years from a single center in Andhra Pradesh. The reason for a smaller number of cases was incriminated to unavailability of liver biopsies in large proportion and also due to lack of general unawareness among clinicians of the total spectrum of clinical and pathologic manifestations in the disease. The unexplained portal hypertension without cirrhosis or Banti syndrome has been given several names over years such as non-cirrhotic portal fibrosis or idiopathic portal hypertension. It is now known as non-cirrhotic intrahepatic portal hypertension (NCIPH) [11]. NCIPH is a vascular disorder of the liver, a consequence of chronic microangiopathy of portal vein branches, leading to intrahepatic portal vein occlusion.

Hematopoietic disorders are often steered by genetic mutations and epigenetic alterations. New advanced technologies including next-generation sequencing, ultra-deep PCR and whole-genome and exome sequencing are proving efficient in detecting mutations and rapid diagnosis of several disorders [12]. Moreover, due to availability of functional enzyme assays, tests for detection of metabolites in blood and urine for metabolic diseases, and flow cytometry for malignant disorders such as leukemia and histiocytosis, we are becoming better placed to diagnose the previously undiagnosed cases.

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Disposal Methods of Used Pressurized Metered Dose Inhalers and Spacers by Families of Children With Asthma

The choice of disposal method of used pressurized metered dose inhalers and spacers by families of children with asthma were evaluated by a questionnaire. The disposal methods used for inhalers and spacers by the 120 children enrolled included giving to plastic/metal waste collectors (28.3% and 33.3%), burning (15.8% and 8.3%), general waste (9.2% and 6%), burial (5.8% and 2.4%), hospital waste (1.7% and 1.2%), rivers (0.8% and 1.2%) and open dumping (0.8% and 0%), respectively. Further, 37.5% of inhalers and 47.6% of spacers were awaiting disposal after use, and were stored at home.

Keywords: *Greenhouse gases, Pollution, Product recycling*.

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A pressurized metered dose inhaler (pMDI) with a spacer is the preferred mode of drug administration in the long-term management of asthma [1]. After use, pMDIs are disposed of in an unscientific manner, which is harmful to our environment [2]. This descriptive cross-sectional study was conducted over a period of one year (December, 2019 to November, 2020) in the pediatric asthma clinic at Government Medical College, Thrissur, after obtaining clearance from the Institutional Ethical Committee. The primary objective was to find ways of disposal of metered dose inhaler canisters by patients/parents of children with asthma. The sample size, based on a pilot study done initially, was calculated to be 115 subjects. Data on disposal of pressurized metered dose inhalers and spacers from 120 patients diagnosed to have asthma, using/used pMDI with spacer as a mode of inhaled medications, either attending pediatric out-patient or asthma clinic or treated as in-patients, was collected using a structured questionnaire and analyzed. Children using only dry powder inhalers (DPIs) were excluded. For data entry and analysis, MS excel software was utilized. The results were expressed in proportions and percentages.

The mean (SD) age of children was 10.6 (3.83) years; 74 (61.7%) were males. The bulk of the children (45%) were aged 6-10 years. The clinical severity of asthma at diagnosis was by NAEPP EPR 3, 2007 guidelines [3]. Mild persistent asthma was found to be the predominant category (53.3%). The mean (SD) duration for which pMDI was used was 39.5 (29.95) months; 106 families (88.3%) had used only one method for disposing of pMDIs, and 14 (11.7%) used two methods. Of the first method adopted by 120 subjects, the majority (n=45) of used pMDIs were stored at homes and awaiting disposal as people did not know what to do with them (37.5%), 34 disposed of by handing over to plastic and metal waste collectors (28.3%), 19 burnt (15.5%), 11 threw into general waste (9.2%), 7 buried (5.8%), 2 threw into hospital waste (1.7%), 1 dumped into open space nearby (0.8%), and one dumped in the river (0.8%). Another method of disposal was adopted by 14 families. These were either by handing over to plastic and metal waste collectors (28.6%, n=4), burning (21.4%, n=3), to general waste (7.1%, n=1), hospital waste (7.1%, n=1), or handing over to pharmacy (7.1%, n=1); four pMDIs were stored at home and awaiting disposal. No scheme for recycling of pMDIs was available for these patients. The plastic actuator and canister components were disposed of together. Disposal of each component was not considered separate in the study.

Of the 120 children, 110 had used spacers (91.7%). The mean (SD) duration of usage of spacers was 35.6 (24.8) months. Among those who had used spacers, 26 (23.6%) had not disposed of and continued to use their old spacers. Of the 84 (76.4%) who disposed of the same, 81(96.4%) were disposed of by one method, and three (3.6%) used two methods for disposal.

The primary method for disposing of spacers (n=84) were handing them over to plastic and metal waste collectors (33.3%, n=28), burning (8.3%, n=7), to general waste (6%, n=5), burial (2.4%, n=2), to hospital waste (1.2%, n=1), into river (1.2%, n=1); 47.6% (n=40) were stored at home awaiting disposal. The second method adopted for disposing of spacers by three subjects was either throwing into general waste (33.3%, n=1) or handing over to plastic and metal waste collectors. (66.7%, n=2)

pMDIs with spacers play a main role in the treatment of asthma in children as well as adults [1]. The spacers are to be disposed of after six months ideally, following the duration of usage as per the available pharmaceutical literature. Their use became a necessity during the COVID-19 pandemic as per the protocols, even in non-asthmatics. In 1995, hydrofluoroalkane 134a (HFA 134a) and 227ea were recognized propellants by the European Union for incorporation in pMDIs. US Food and Drug Administration (FDA) approved using HFA 134a in pMDIs in 1996 [4]. Hydrofluoroalkane is one of the greenhouse gases [5]. Thomas, et al. [2] showed that patients using MDI were not informed about the disposal method of used MDI and they were disposing of them in the

waste bins, general waste, burning, storing, and flushing them in the toilet. These findings also point to the generation of greenhouse gases by burning, and the dangers to aquatic life posed by disposing of them in rivers.

'Complete the cycle' scheme in the United Kingdom [6], an example of recycling and recovering the pMDIs and DPIs, is aimed to prevent inhaler devices from landing up in landfills, and minimize the environmental threat from greenhouse gases remaining in the discarded inhalers. The plastic and aluminum parts are recycled and used, whereas the non-recyclable inhaler waste was recovered by converting to electricity or heat energy through incineration [6].

Switching from pMDIs to DPI-based maintenance therapy lowered greenhouse gas emissions without compromising patient care, with reduced annual drug costs [7]. The British Thoracic Society guideline on the management of asthma stresses the importance of choosing inhalers with low global warming potential, and recycling used inhalers [8]. NICE (National Institute for Health and Care Excellence) makes patients aware that pMDIs need an environmentally safe way of disposal [9].

Pharmaceutical companies should provide information on the disposal of pMDIs and spacers. Norms and instructions, a nationwide protocol for proper disposal, and recycling of pressurized metered dose inhalers and spacers under monitoring are needed to prevent its contribution to environmental pollution and climate change. These measures have not yet been adopted by our country. Switching to alternative, comparatively more environmentally friendly devices (e.g., DPI) for drug delivery, whenever possible, has to be considered.

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Contributors: NN: concept and design of the study, definition of intellectual content, literature search, acquisition of data and analysis, manuscript preparation, editing and review; MD: Partaking in designing study, literature search, acquisition of data and analysis, manuscript preparation, editing and review; MD: will be the corresponding author; RJ: Key role in framing study design, review of statistical analysis and manuscript time to time. All authors approved the final version of manuscript, and are accountable for all aspects related to the study.
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Genetic Expression of *CYP2B6* Gene in Phenobarbitone Responder and Non-responder Neonates

Quantitative real-time polymerase chain reaction for identifying *CYP2B6* gene expression was done on blood samples of 30 phenobarbitone responder and 30 non-responder neonates with seizures. *CYP2B6* was observed to be significantly down regulated among phenobarbitone non-responders as compared to phenobarbitone responders (Mean (SD) ΔCt 17.97 (1.19) vs 15.40 (1.83); *P*<0.001).

Keywords: *Gene expression, Drug resistance.*

Seizures are the most frequent neurological manifestation in newborns, majority seen during the first week of life. Phenobarbitone is used as the first line anticonvulsant for neonatal seizures. Non-response to phenobarbitone in some neonates may be related to the severity of the underlying condition; however, a genetic association remains a possibility. Polymorphism in expression of genes may alter the metabolism of phenobarbitone in different individuals, and inter individual variability may exist for drug response [1]. Thus, detection of genetic polymorphism using appropriately designed real time–polymerase chain reaction (RT-PCR) assays might help in identifying its role in phenobarbitone non-responsiveness in neonatal seizures.

This observational study was conducted in a medical college-affiliated hospital in the intramural neonatal intensive care unit (NICU), at the pediatrics and biochemistry departments over a period of 18 months. The study was conducted on a subpopulation of patients as a part of a research project, in which adverse outcomes of patients with neonatal seizures were identified [2]. Ethical clearance was obtained from institute ethics committee, and written informed consent was taken from parents/ guardians of the patients (including for the additional genetics tests). All the neonates with seizures recruited in the study were admitted and managed as per the NICU protocol. Phenobarbitone responders were defined as neonates who responded to one dose of 20 mg/kg of intravenous phenobarbitone by complete cessation of clinical seizures. Non-responders were those who did not have cessation of clinical seizures even after 40 mg/kg of phenobarbitone, and required a second-line anti-seizure medication. A convenience sample of thirty consecutive babies in each group, from the 220 babies enrolled in the original study [2], were enrolled. Neonates who succumbed to illness/ death before investigations were excluded.

Venous blood sample (3 mL) was collected after the first dose of phenobarbitone and immediately fixed with trizol and stored at -20^{0}C (to prevent RNA degradation). Gene expression (*CYP2B6*) analysis was done using RT-PCR. Clinical and biochemical data of enrolled patients was recorded in a predesigned case record form.

For statistical analysis, for normally distributed variables, differences in means were compared using *t* test for independent samples. For comparison of non-normally distributed parameters, differences in medians were compared using Mann-Whitney *U* test. *P* value <0.05 was considered significant.

It was observed that phenobarbitone non-responders had a significantly lower median (IQR) Apgar score at 5 minute [3.0 (1.66, 5.01) vs 9.0 (7.52, 9.10); *P*<0.001], lower mean (SD) maternal age [27 (5.49) vs 30.7 (8.5); *P*=0.047) and earlier time to mean (SD) onset of seizures [29.6 (28.73) vs 55.4 (33.88) hour; *P*=0.002] as compared to phenobarbitone responders. Mean (SD) of **Δ**Ct was significantly less in phenobarbitone responders as compared to non-responders [15.40 (1.83) vs 17.97 (1.19); *P*<0.001], which depicts down-regulation of the *CYP2B6* gene in phenobarbitone non-responders as compared to phenobarbitone responders.

Downregulation of *CYP2B6* gene in phenobarbitone non-responder neonates observed in our study might be a contributory factor responsible for phenobarbitone non-responsiveness in these neonates. *CYP2B6* is expressed primarily in liver and represents one of the fifteen CYP enzymes, predominantly responsible for xenobiotic metabolism. It has extremely polymorphic expression with high inter- and intra-individual variation in coding and non-coding regions of the gene. This highly variable coenzyme expression arises from multiple factors including genetic polymorphisms, non-genetic factors such as disease conditions, gender differences and transcriptional induction or suppression by xenobiotic and cytokines. Inflammation has also been recognized as an important factor for *CYP2B6* expression as depicted by down regulation in human hepatocytes in response to IL-6 and interferon gamma [3]. Goodwin, et al. [4], showed that constitutive androstane receptor (CAR, NR113) and the pregnane X receptor (PXR, NR112) are key modulators governing the inductive expression of *CYP2B6* [4]. Activation or inhibition of these receptors by known compounds including rifampicin, phenobarbitone, dexamethasone and phenytoin can have a significant impact on downstream expression of important drug metabolizing enzymes and drug transporters.

In human hepatocytes, induction of *CYP2B6* was reported for cyclophosphamide [5], artemisinine [6], carbamazepine, efavirenz and nevirapine [7], metamizole [8], and several statins. Genotyping for a variant of *CYP2B6*

gene has been proposed as a method to help in titrating efavirenz dosages for individual patients. This would help in recognizing patients who can be classified as poor metabolizers or ultra-rapid metabolizers of efavirenz, and may benefit from early therapeutic drug monitoring [9]. We could not find any human study on *CYP2B6* expression in babies receiving phenobarbitone. A case control study by Alves, et al. [10] concluded that the allele frequencies of an *ABCB1* gene polymorphism in intron 1 were different between phenobarbitone-resistant and phenobarbitone-responsive idiopathic epilepsy in dogs. Phenobarbitone-resistant dogs were more likely to have the variant G-allele at single nucleotide protein (SNP) [10].

A major limitation of this study is its small sample size, and defining response on the basis of only clinical seizures cessation. We also did not carry out genotyping of the *CYP2B6* gene, and only studied *mRNA* expression. Low APGAR score might be a significant confounder for phenobarbitone non-responsiveness. More extensive studies explaining role of genes in drug response can be done to find out the relationship between gene polymor-phism and seizure control in neonates.

Ethics clearance: Institutional ethics committee – human research, UCMS; No. IEC-HR/2017/32/102 dated Oct 17, 2017.
Contributors: KY: Data collection, writing first draft of manuscript; PB, AA: conceptualized the study, devised its design, and provide critical inputs into manuscript revision; BDB, VB: supervised data collection and conduct of study, and provided inputs into manuscript revision; TS,HK: supervised data collection and performed analysis.
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Interstitial Lung Disease in an Adolescent Associated With a Novel *STAT5B* Mutation

Signal transduction and activation of transcription 5B (*STAT5B*) protein is involved in signal transduction of growth hormone (GH) receptor and mediates actions of IL-2 and hence, plays an essential role in growth and immunity. Mutations in *STAT5B* gene are associated with growth failure, immunodeficiency, autoimmunity and diffuse lung disease (DLD) [1].

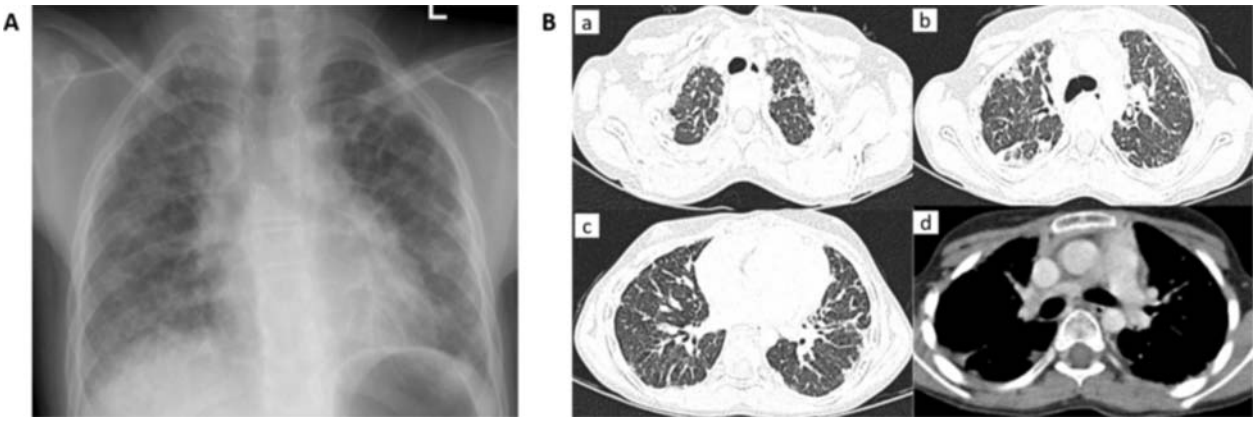
We report a 12.5-year-old girl who presented to us with poor growth since one year of age and fast breathing for last 4.5 years. The child was born out of non-consanguineous marriage with normal birth weight (3.2 kg) and length (51 cm). At 1 year of age, she presented with recurrent loose stools along with poor weight and height gain. At 5 years of age, she was diagnosed to have celiac disease with high anti-tTG IgA levels- 300 U/mL (normal <15 U/mL) and hypothyroidism [Thyroid stimulating hormone (TSH) 52.3 mIU/L]. She was started on gluten free diet and L-thyroxine, and had shown symptomatic improvement with some weight gain. At 8 years of age, she developed insidious onset fast breathing which gradually progressed from Medical Research Council (MRC) grade 1 to 4 over 3 years along with dry, nonproductive cough. She had minimal response to bronchodilators and inhaled steroids. There were no similar family history or any sibling death. There was history of exposure of child to pigeons. At presentation, she had tachypnea and chest retractions with normal oxygen saturation, grade 3 clubbing and growth failure [weight 19.3 kg (-4.23 *z*-score), height 122 cm (-4.55 *z*-score), body mass index (BMI) 12.96 (-3.28 *z*-score)]. Her weight and height from previous records were plotted on growth chart which revealed her height consistently below third percentile (less than -3 *z*-score using WHO child growth standards 2006 and growth reference 2007 tables). Respiratory system examination revealed bilateral reduced breath sounds with fine crackles, more evident on right side. She had loud second heart sound on cardiovascular system examination. The possibilities considered were childhood interstitial lung disease associated with autoimmune diseases, chronic hypersensitivity pneumonitis and NKX2.1 mutation.

On evaluation, complete hemogram showed lymphopenia (absolute lymphocyte count- 1.74×10^9/L) while metabolic panel was within normal limits. Chest *X*-ray revealed bilateral reticulonodular shadows in all lung fields (**Fig. 1A**). Computed tomography of chest showed organizing pneumonia in right upper lobe. Other lobes showed septal thickening and fibrotic changes (**Fig. 1B**). Spirometry was suggestive of severe restrictive disease. Autoimmune antibody panel and serum precipitins for avian antigen were negative. Bronchoalveolar lavage (BAL) CD4:CD8 ratio (0.79) was reduced. Lung biopsy was not done due to refusal of consent by parents. 2D-echocardiography revealed mild tricuspid regurgitation with systolic pressure gradient between right atrium and ventricle as 40 mm Hg, suggestive of mild to moderate pulmonary arterial hypertension.

Genetic testing in the form of whole exome sequencing revealed a novel homozygous missense variation of *STAT5B* gene involving exon 16 at chromosome 17 (40359647A>C; c.2006T>G) that results in the amino acid substitution of Glycine for Valine at codon 669 (p. Val669Gly) and reported as likely pathogenic. Further detailed investigations like chromosomal array and genetic testing of her parents and siblings could not be done due to financial constraints. However, the parents and sibling were phenotypically normal and had no family history of similar presentation.

Due to the reported associations of STAT5B mutation with immunodeficiency and growth hormone insensitivity (GHI), we evaluated the patient for the same. Her primary immunodeficiency evaluation suggested lymphopenia, CD8 cell deficiency (5.99%) and raised IgG (1501 mg/dL). On hormonal evaluation, normal baseline (3.32 ng/mL) and stimulated growth hormone (GH) levels (8.07 ng/mL) while reduced baseline and post IGF-1 generation test IGF-1 levels (59.6 ng/mL vs 68 ng/mL) were suggestive of GHI. Her thyroid function tests revealed hypothyroidism (total T3- 0.92 ng/mL, total T4-8.97 mcg/dL, TSH-5.58 mIU/L) with normal anti-TPO antibody levels (0.48 IU/mL). Repeat celiac serology (anti-tTG IgA) was negative.

The child was started on oral prednisolone (1 mg/kg/day) and hydroxychloroquine (10 mg/kg/day). On follow up after 8 months, there was improvement in breathlessness (MRC scale 4 to 2) and cough, as well as weight gain. There was also some improvement in lung function parameters (**Web Table I**). There were no exacerbations in this period. She was continued on low dose steroids (0.5



(*a*) (*b*)

Fig. 1 *a*) Chest *X*-ray image of the index case showing multiple reticulo-nodular shadows in all lung fields. High resolution computed tomography chest images of the index case showing septal thickening with fibrotic changes in all lobes (*a, b* and *c*) with normal mediastinal structures and pulmonary artery caliber (*d*).

mg/kg every alternate day) and hydro-xychloroquine. She was being followed up with pediatric endocrinologist for hypothyroidism and GHI and conti-nued on L-thyroxine (50 µg/day). Thereafter, she could not be further evaluated due to loss to follow up.

STAT5B mutation is an uncommon cause of interstitial lung disease. There are 12 case reports of *STAT5B* mutation or deficiency till now. Most of these cases had growth failure secondary to GHI, immunodeficiency due to defects in regulatory T (Treg) cells and autoimmune diseases [2-4]. Severe pulmonary disease in the form of recurrent pulmonary infections, LIP and progressive pulmonary fibrosis presenting in early childhood was characteristic feature of most of the cases except in one case [5]. The clinico-radiological profile in our case suggested fibrosing ILD, which could not be further characterized due to lack of lung biopsy. Pulmonary involvement in *STAT5B* mutation has been hypothesized due to decreased numbers of regulatory T cells (Treg) and attenuated effector T cell (Teff) function, underlying immunodeficiency or associated autoimmune phenomenon [1,2].

This case highlights STAT5B mutation as a novel cause of interstitial lung disease in children. This condition may be thought of in any child with unexplained pulmonary manifestations or childhood onset interstitial lung disease of unknown etiology associated with GHI, autoimmune conditions and immunodeficiency, particularly lymphopenia.

Acknowledgement: Professor SK Kabra, Head, Division of Pediatric Pulmonology and Intensive care, Department of Pediatrics, AIIMS, New Delhi for his guidance in the diagnosis and management of the patient as well as in writing the manuscript.

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Web Table I Spirometry Values of the Index Child Before and 8 Months After Treatment

Spirometry values	*Before treatment*		*After treatment*	
	Observed	*% predicted*	*Observed*	*% predicted*
FVC (L)	0.42	32%	0.53	51.4%
FEV1 (L)	0.42	35%	0.51	42%
FEV1/FVC	100	103%	96	98%

Premature Atherosclerosis in Children With Transfusion-Dependent Thalassemia

I read with interest the recently published paper on premature atherosclerosis in children with transfusion-dependent thalassemia by Kumaravel, et al. [1]. I have the following observations related to the study:

The authors state that "age and sex-matched healthy volunteers were recruited from the outpatient department and taken as control." It is surprising that 84% of children with thalassemia in this study were normally nourished, despite having evidence of poor chelation in the form of high ferritin levels. Several studies from India and other developing countries have reported high rates of malnutrition in children with thalassemia [2-4].

The authors report significant differences in the mean leucocyte count, serum glutamic oxaloacetic transaminase, serum glutamic pyruvic transaminase, and serum alkaline phosphatase levels between the two groups, and also the serum total cholesterol, high-density lipoprotein and low-density lipoprotein levels. However, results of statistical tests applied to reach this conclusion have not been provided .

There were two children with pre-hypertension amongst the cases. The authors have not defined if these measurements were evaluated with a 24-hour ambulatory blood pressure monitoring (ABPM). A study from Iran [5] found 16% of children with transfusion-dependent thalassemia having high blood pressure. Hypertension could be one reason why children with thalassemia may have changes in CIMT.

Authors should elaborate on the possible reasons why nutritional status and blood pressure readings in the children from their study were so deviant from other studies.

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AUTHORS' REPLY

We thank the reader for the interest shown in our publication. Many studies have demonstrated significant malnutrition in children with thalassemia in the past; but with the advent of specialized thalassemia care units like ours, where comprehensive management including growth monitoring and nutritional advice are regularly given, we are observing improvement in the nutritional status of these children [1]. The statistical analysis for the liver enzymes and lipid profile between cases and controls is given in the footnotes of table I in the paper [2]. Though, a study from Iran has observed a 16% incidence of hypertension in children with thalassemia [3], in the present study we did not observe any child with hypertension. On the contrary, a study by Veglio, et al. [4] has documented lower mean systolic, diastolic, and mean arterial pressures in children with thalassemia, due to myocardial dysfunction. A study by Karimi, et al. [5] considered thalassemia trait as a protective factor against hypertension in young adults. Hence, blood pressure measurement studies in children with thalassemia remain inconclusive. To the best of our knowledge, there is no published data regarding 24-hour ambulatory blood pressure measurement in Indian children with thalassemia, and it will be a good research question to answer for the thalassemia care units in India.

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Iron Overload in Rh-Isoimmunization

We read with interest the recent article on iron overload in a late-preterm infant with Rh-isoimmunization [1], and would like to share our experience and practices on this aspect.

Iron overload is a rule rather than an exception in Rh-isoimmunization, where the fetus receives intrauterine transfusion (IUT) [2]. The degree of iron overload is directly proportional to the number of IUTs received. Double-volume exchange transfusion in the immediate neonatal period further accentuates the problem, which becomes a grave concern if the infant gets top-up transfusions in the first month of life [2,3].

In an ongoing study, we enrolled 30 mother-infant dyads where IUTs were given. Among those 30, 28 (93%) had elevated serum ferritin (>200 ng/mL) at birth. We followed them until they were three months of age. At three months, 20 (66.7%) had anemia (16 received at least one packed red blood cell transfusion), and 79.3% (23/29) had elevated serum ferritin.

As described in the literature, iron overload is widespread in hemolytic disease of the fetus and new-born (HDFN); we avoid iron supplementation until three months and prescribe only folic acid (50-300/µg/day; we prefer the upper range) [4]. Anemia during the intervening period is treated with top-up transfusions, and iron is initiated if there is documented iron deficiency.

In our practice, we observed that despite prescribing folic acid drops, many parents continue to give pre-paration containing a combination of iron and folic acid; hence, inadvertently exposing them to excess iron. The common reasons identified for these errors were incorrect medication supply by the pharmacy (most drug formu-lations in the Indian market have a combination of iron and folic acid), and lack of knowledge among prescribers about not giving iron to these infants.

Folic acid supplementation is essential for regene-rating new red blood cells. In the index case report, there is no mention of folic acid prescription since birth. Currently, there are no evidence-based guidelines on chelation therapy in Rh-isoimmunized infants [5]. As a consensus, ferritin levels above 1000 ng/mL are consi-dered toxic for the liver, and chelation is advocated in most other conditions (including thalassemia).

Therefore, there is a need to emphasize the problem's magnitude, and increase awareness among pediatricians. A clear policy of not giving iron supplementation in infants with HDFN, unless a ferritin test shows a true deficiency, might avoid the iatrogenic iron overload to some extent.

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Regional Action Plan for Prevention and Control of Snakebite Envenomation

Snakebite is an emergency, if not managed properly it can cause serious physical and mental effects including death. According to the World Health Organization, globally 5.8 billion people are at the risk of snakebite resulting in approximately 80,000-140,000 deaths every year. Approximately 70% of snakebite deaths occur in the South-East Asian region, as there is large agrarian population, rapid urbanization causing shrinkage of natural habitat of snakes and high population density. Majority of these deaths and serious effects of snakebite can be prevented by use of antivenoms. Despite this, lack of knowledge, timely access to the healthcare facility and antivenoms are the some of the main reasons for high fatality in snakebite envenoming in the South-East Asia.

Working on the lines of resolution taken during 71st World Health Assembly for addressing the global burden of neglected tropical disease through a coordinated response, recently the "Regional Action Plan for prevention and control of snakebite envenoming in the South-East Asia 2022-2030" was launched. This action plan aim to reduce snakebite related morbidity and mortality by 50% by year 2030 in South-East Asian region. This document provides the framework for the coordinated res-ponse between various stake holders to achieve common objective of prevention of snakebite, providing effective first-aid, ensuring timely access to health facility and availability of life-saving, effective, affordable treatment like antivenom to all snakebite cases (*WHO.int 5 February, 2023*)

Proton Pump Inhibitors for Treatment of Tuberculosis

Globally each year approximately 10 million people get infected with tuberculosis. Despite the availability of an effective treatment, ~1.5 million die annually due to tuberculosis. For cure tuberculosis require continuous longer treatment, but poor compliance and high default rates has led to emergence of drug resistant forms which is a major public health threat. Rifampicin - the most effective anti-tubercular drug inhibits the mRNA synthesis by binding to the β-subunit of the RNA polymerase which is coded by *rpoB* gene. Mutations in *rpoB* gene are the major cause of rifampicin-resistant *M. tuberculosis.*

Short term treatment with multiple drugs have improved the overall compliance to the treatment and improved the cure rates. But this success has been threatened by the newer mechanisms developed by the *M. tuberculosis* to counter the drugs. Recently, studies have found that even the actively growing, drug sensitive variants of *M. tuberculosis* become drug tolerant on entering the macrophages. This phenomenon – macrophage induced drug tolerance - is mediated by the *M. tuberculosis* drug efflux pumps acting through proton gradient dependent mecha-nism. In a recent paper, the researchers have studied the effect of efflux pump inhibitors on the rifampicin efflux. Verapamil a known efflux pump inhibitor was tested and found to be effective in inhibiting macrophage induced rifampicin tolerance and *M. tuberculosis* rifampicin efflux. This effect was mediated through its human P-glycoprotein (PGP)- like inhibitory activity. Due to its calcium channel blocking property and cardiac effects verapamil analogs and other drugs with incidental PGP-inhibitory activity were tested. Among these proton pump inhibitors are found to be potential candidates which can inhibit the intramacrophage drug tolerance and mycobacterial growth by inhibiting the mycobacterial rifampicin efflux pumps. (*Proceedings of the National Academy of Sciences 10 February, 2023*)

Home Based Detection and Monitoring of Lupus Nephritis

Renal involvement in the cases of systemic lupus nephritis is of associated with poor outcome as approximately 10-30% progresses to end stage renal disease over a decade and half period. According to the American college of Rheumatology presence of persistent proteinuria, hematuria, cellular casts or raised serum creatinine levels indicates towards possibility of an underlying lupus nephritis. Patients with lupus nephritis need renal biopsy, which is a gold standard for diagnosis as well as for monitoring the response to the treatment. But it has been associated with its own limitations like invasive nature, inter-observer variation and procedure associated infection risk. A team from University of Houston recently has devised a new point of care testing method which estimates levels of a noninvasive biomarker (protein-coding gene ALCAM). In this method an easy, affordable and rapid technique – lateral flow assay (LFA) along with a smart phone app was utilized to determine Urinary ALCAM (uALCAM) levels. This test had 86% accuracy for distinguishing active lupus nephritis from all other lupus patients. Thus helping the lupus nephritis patients in early diagnosis and home based monitoring of the disease in without the need of renal biopsy. (*Frontiers in Immunology 09 December*, 2022)

Association between Meal Frequency and Body Weight in Children

With improvement in the standards of living and access to better healthcare there is significant reduction in the communicable diseases. On the other side there is progressive surge in the non-communicable diseases. According to recent World Health Organization estimates globally approximately 340 million children aged between 5-19 years and 39 million under-5 children were either overweight or obese. Lack of outdoor activity, increased screen time, poor eating habits in children are some of the major reasons for this epidemic. Studies have shown that adoption of healthy dietary habits, and intake of nutritious food containing fruits and vegetables is associated with healthy weight and reduced risk of lifestyle diseases. In a cohort study published from Spain, the researchers studied the association between the meal frequency, body weight and abdominal obesity in 1400 participating children with a follow up of 15 months.

Results revealed a significant reduction in the odds of developing abdominal obesity or excessive weight with an increase in the meal frequency during the follow-up after controlling for the same confounders. Thus, giving frequent healthy meals can help in maintaining a healthy weight in children. (*Nutrients 08 February, 2023*)

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Food Insecurity and Child Development (Int J Environ Res Public Health. 2021;18:8990)

The given review examined the last decade research to: (1) describe the impact of the severity and persistence of food insecurity on child development; (2) use a socio-ecological framework to examine significant proximal and distal factors which may interplay; and (3) outline directions for future research. The articles were included from the search if > 50% of participants were ≤ 12 years, >50% of participants were living in high income countries and if the study addressed any one of the key child development outcomes-behavior, cognitive or non-cognitive performance, academic achievement, psycho-social, emotional, developmental risk e.g. as assessed by PEDS and motor development. While one study found that children in food insecure households had lower scores on measures of both vocabulary and letter-word recognition, another study reported that after adjusting for immigrant protective and risk factors, there were no significant differences in reading or math scores according to food security status. In some of the studies, a significant negative effect of food insecurity persistence on academic/ cognitive outcomes was found. The effect of food insecurity on behavior (externalizing, internalizing, self-control, self-regulation, general conduct) showed positive associations between food insecurity and behavioural problems. The analysis of effect of food insecurity persistence on behavior showed significant negative associations of the phenomenon of self-control with food insecurity. Shorter and more transient forms of food insecurity were associated with increased externalizing behaviours, while more persistent food insecurity was associated with internalizing and self-control behavioural issues. The studies on food insecurity and developmental concerns reported that food insecurity was associated with increased developmental concerns reported by parents using the Parents' Evaluation of Developmental Status (PEDS). The review indicated that food security status, severity and persis-tence do adversely impact upon child development outcomes. The strongest evidence of an effect of food insecurity was found in academic/ cognitive outcomes and externalizing behaviours.

Minerals in Pregnancy and Their Impact on Child Growth and Development. (Molecules. 2020;25:5630).

This review aimed to assess the consequences of gestational deficiency of iodine, selenium, iron, zinc, calcium, and magnesium and their impact on child growth and development. Iodine deficiency during the first years of a child's life causes changes in brain development that can lead to reduced mental activity. In the case of children, the authors found that low iodine consumption was directly related to low parental edu-cation and low consumption of breastmilk throughout the first years of life. The concentration of iodine present in the colostrums could predict the child's motor development. In childhood, according to some studies there is an association between the deficiency of this mineral with autism and attention deficit and hyperactivity disorder in children. A systematic review on supplementation and mental development among children suggested that light to moderate iodine deficiency at infancy had adverse effects on cognitive and motor perfor-mance. Selenium deficiency in neonates, especially premature, was found to be associated with higher risk for developing diseases due to increased susceptibility to oxidative stress e.g. retinopathy, bronchopulmonary dysplasia and other lung disorders. Iron deficieny during pregnancy was observed to be responsible for the development of neuronal alterations in newborns, persisting until adulthood despite supplementation. Iron deficiency in early years of life was found to be associated with delayed development, reduced school performance, beha-vioural disorders, ADHD and risk of cerebrovascular accident (CVA) in healthy young children. Gestational Zinc deficiency is thought to have adverse effects on learning ability and reduced attention and memory in the offspring as per the studies on experimental animals. Magnesium deficiency in pregnancy was related to prematurity, hypoxic-ischemic encephalopathy in neonates, whereas deficiency in children was related to impairments in cognitive capacity and processing as well as lack of concentration apart from hypoparathyroidism, hypocal-cemia and impaired bone growth. Based on this review, it was concluded that, for a child to grow and develop properly, it is necessary to start planning pregnancy from conception. There are many problems associated with brain function under nutritional deficit, such as hyperactivity, attention deficit, autism, speech delay and memory problems which can pro-bably be prevented by optimum maternal nutrition during pregnancy.

Parenting interventions to promote early child development in the first three years of life (PLoS Med. 18: e1003602)

Responsive parent-child relationships and parental support for learning during the earliest years of life are crucial for promoting early child development (ECD). Parenting interventions have been underscored as a key strategy for improving early child development (ECD) outcomes. The present study was a global systematic review and meta-analysis to evaluate the effective-ness of parenting interventions on ECD and parenting out-comes. It included 102 randomized controlled trials of parenting interventions for children during the first 3 years of life that were implemented across a total of 33 countries. The study found that parenting interventions improved early child cognitive, language, motor, socioemotional development, and attachment and reduced behavior problems. Parenting inter-ventions additionally improved parenting knowledge, parenting practices, and parent-child interactions. However, they did not significantly reduce parental depressive symptoms. Parenting interventions had significantly greater effects on child cognitive, language, and motor development and parenting practices in low middle income countries than high income countries. Parenting interventions that included content on responsive caregiving had significantly greater effects on child cognitive development, parenting knowledge, parenting practices, and parent-child interactions than interventions that did not include content on responsive caregiving (e.g. effect on parenting practices was nearly 4 times greater for interventions with responsive care-giving content versus those without responsive care giving content).

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Heck's Disease

A 10-year-old boy presented with multiple, asymptomatic, sessile, confluent, verruciform papules in right buccal mucosa for 7 months (**Fig. 1**). Family history was negative and physical examination was normal. Routine hemogram revealed no abnormalities. Histopathological examination showed hyperkeratosis, acanthosis, papillomatosis, thickened and elongated rete ridges, koilocytes and mitosoid bodies (**Fig. 2**). A diagnosis of Heck's disease was made - Multiple asymptomatic, skin coloured, soft, sessile papules or nodules in buccal or labial mucosa presenting as exophytic lesions with histological findings of mitosoid bodies and koilocytosis (hyperchromatic nuclei with perinuclear cellular vacuolisation) are diagnostic clues to this condition.

Focal epithelial hyperplasia a benign condition caused by human papilloma virus 13 or 31, is frequently seen in childhood and adolescence with genetic factors, immunosuppression, poor oral hygiene, and malnutrition being associated factors. It is rare in Caucasians and Indians. Differential diagnosis include verruca, verruciform xanthoma, papilloma, and irritation fibromas. Verruca and papilloma presents as localized, small wart like growths with irregular surface. Verruciform xanthoma is usually single, pinkish lesion occurring in areas of irritation or trauma, whereas irritation fibromas are pale with increasing size at irritation sites, with proliferation of fibroblasts and collagen histologically. Apart from biopsy, HPV DNA can be detected by PCR of scraping or biopsy material. The disease undergoes spontaneous resolution in months to years. Topical imiquimod, intralesional interferons, surgical excision, diathermy, CO2 laser, diode laser and systemic retinoids are various treatment modalities available. Our patient was treated with 5% topical imiquimod cream, applied thrice weekly, with complete resolution achieved in three months without any adverse effects.

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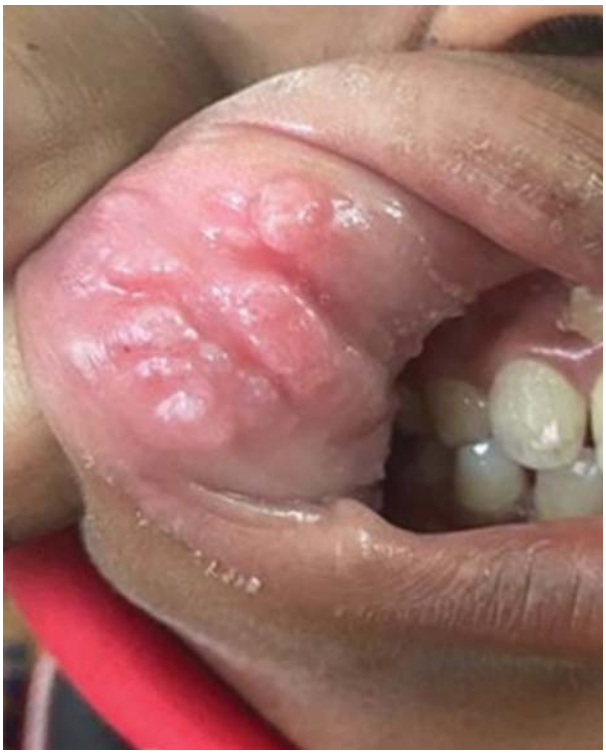

Fig. 1 Multiple sessile, confluent and verruciform papules in right buccal mucosa.

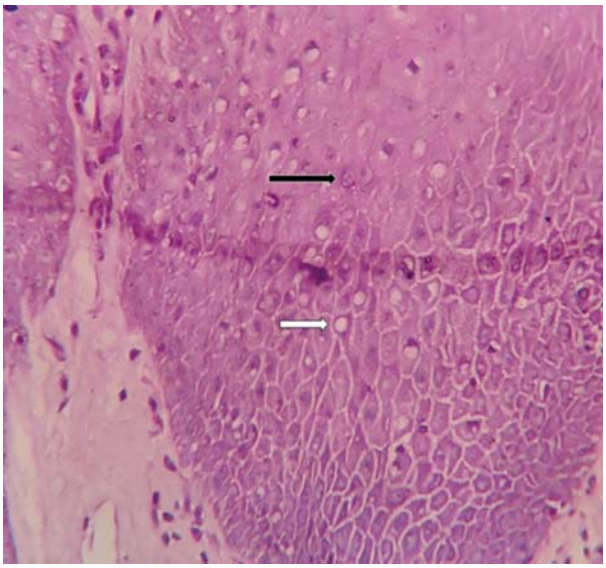

Fig. 2 – Histopathological image (40 x, H &E) – Koilocytes - Vacuolated Keratinocytes indicated by white arrow and Mitosoid bodies indicated by black arrow.

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