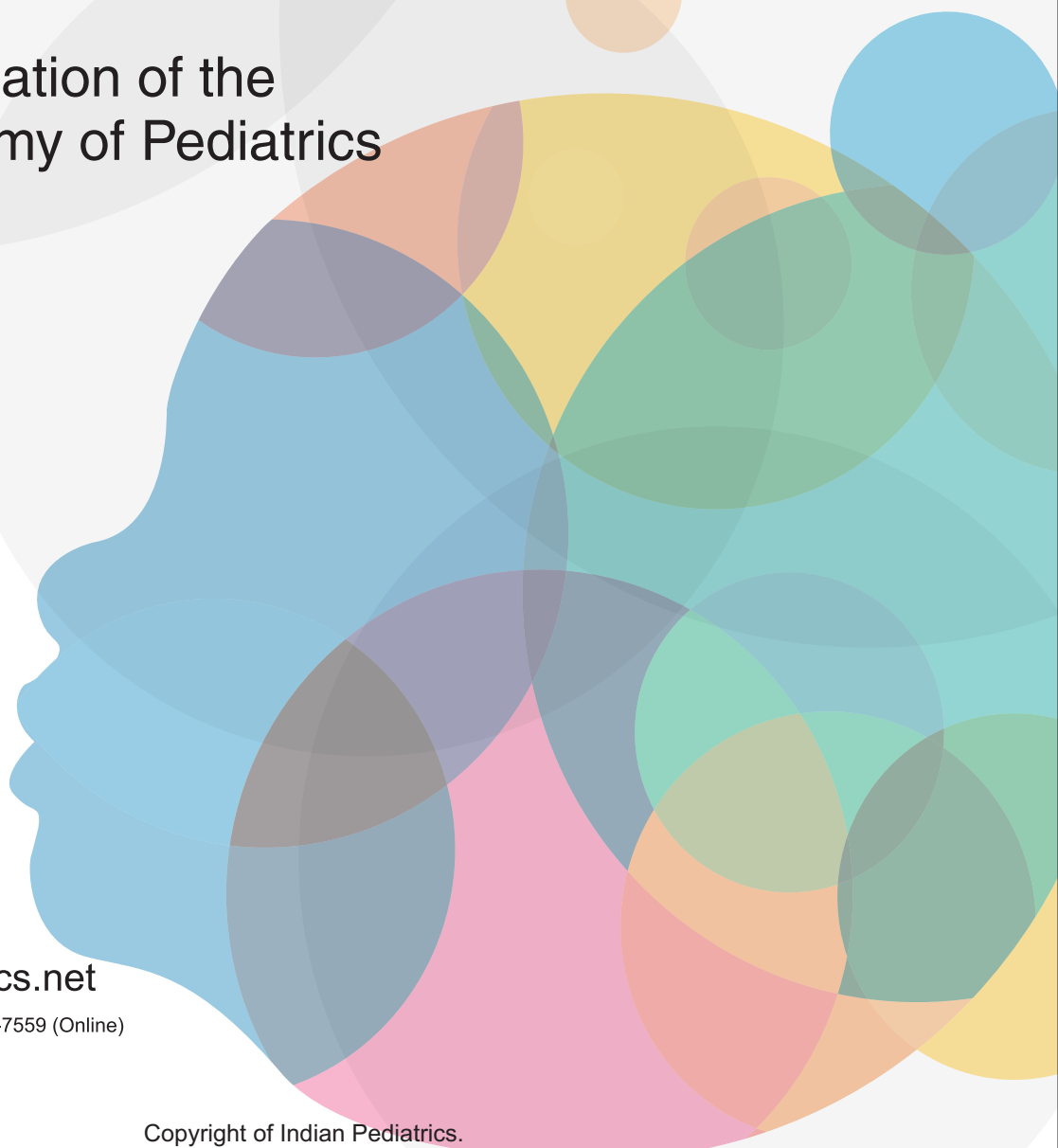
E-copy

Indian Pediatrics

Official Publication of the
Indian Academy of Pediatrics

VOLUME 59
NUMBER 5
May 2022



www.indianpediatrics.net
ISSN0019-6061 (Print) | ISSN0974-7559 (Online)

Copyright of Indian Pediatrics.
It is meant for personal use only, and not to be shared widely over social media platforms, blogs and mass e-mails.

IAP Guidelines for pediatric skincare

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

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

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

Johnson's® CHOOSE gentle™

CLINICALLY mildness PROVEN®

Evidence-based skincare regimen

Cleansing regimen

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

Helps maintain the skin barrier function[5]

Moisturizing regimen

Improves the skin barrier integrity[6,7]

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

*From birth to the first five years of life

References: 1. Madhu R, et al. Indian Academy of Pediatrics Guidelines for Pediatric Skin Care. Indian Pediatr. 2021;58(2):153-161. 2. Telofski LS, et al. The infant skin barrier: can we preserve, protect, and enhance the barrier? Dermatol Res Pract. 2012;2012:198789. 3. Data on file. 4. Lund C, et al. Baby's first bath: Changes in skin barrier function after bathing full-term newborns with water vs liquid baby cleanser. Pediatr Dermatol. 2020;37(1):115-119. 5. Garcia-Bartels N, et al. Use of baby wipes in the diaper area in newborns: A prospective, randomized clinical study on skin barrier. Arch Dis Child. 2008;93:ps222. 6. Johnson's clinical moisturizing report. Appendix 2. Claim table for F#1185-056. 7. Williams N, et al. Does evidence suggest that the use of barrier enhancing emollient is beneficial in the care of preterm neonates? Infant. 2012;8(4):120-25. 8. Patzelt A, et al. In vivo investigations on the penetration of various oils and their influence on the skin barrier. Skin Res Technol. 2012;18(3):364-9.

RA/IB/8Mar2021/05

EXERGEN
CORPORATION

New professional light thermometer: TAT-2000

NEW

The 7 advantages of the Temporal Artery Thermometer

1. Very accurate
2. User-friendly
3. Very fast
4. Effcient
5. Comfortable
6. No caps needed
7. Can be cleaned with alcohol cloth

Changing the Way the World Takes Temperature

Clinical accuracy of ± 0.1°F - Temperature range of 60-107.6°F - response time ~ 0.04 sec - FIVE YEARS warranty

More info on the Temporal Artery Thermometer

#1 preferred thermometer used by pediatricians in the USA
Supported by more than 100 clinical studies
Exergen also has a high-performance model: the TAT-5000

For more details you can contact:
Dr. Pushpa Goyal - Marketing Manager Exergen India | +91 98114 24550 | drpushpa@exergen.com

www.exergen.com


30TH IPA CONGRESS & 60TH PEDICON 2023
Quality Care for Every Child Everywhere
Gandhinagar, Gujarat, India | 19th-23rd Feb, 2023
30th International Pediatric Association Congress 2023 &
60th Annual Conference of The Indian Academy of Pediatrics
Organised by:
IPA International Pediatric Association
Every Child · Every Age · Everywhere
Hosted by:
REGISTER TODAY FOR 30TH IPA CONGRESS & 60TH (DIAMOND JUBILEE) PEDICON 2023
19th Feb - 23rd Feb, 2023
Venue: Mahatma Mandir Convention & Exhibition Center, Gandhinagar, Gujarat
To register, log on to www.ipa2023congress.org
CONFERENCE HIGHLIGHTS:
• Hybrid Congress
• Unique 24x7 Virtual Congress
• Networking opportunity
• Oral papers and ePosters
Scan the QR code to Register Today
c/o INDIAN ACADEMY OF PEDIATRICS (IAP)
Kamdhenu Business Bay, 5th Floor, Plot No. 51, Sector 1,
Near Juinagar Railway Station, Nerul, Navi Mumbai, Maharashtra - 400 706
Email: centraloffice@iapindia.org
Phone: +91-022-27710857 | 27715578 | 27716573 | 27710118
For more details log on to:
www.ipa2023congress.org
info@ipa2023congress.org
+91 93722 75650

Indian Pediatrics

May 2022 | Volume 59 | Number 5

Editor-in-Chief Devendra Mishra
Executive Editor AP Dubey
Managing Editor Rakesh Lodha
Associate Editors
Anup Mohta
Pooja Dewan
Joseph L Mathew
Aashima Dabas
Abhijit Saha
Executive Members
Sunita Bijarnia-Mahay
JS Kaushik
Ujjal Poddar
Kirtisudha Mishra
Somshekhar Nimbalkar
Ashish Jain
Kana Ram Jat
Sumaira Khalil
Romit Saxena
Amit Devgan
Nidhi Sugandhi
Rajesh Meena
International Advisory Board
Prashant Mahajan
Sanjay Mahant
PSN Menon
John M Pettifor
Sudhin Thayyil
Gaurishankar Shah
National Advisory Board
Central Zone Anil Kumar P
Mahesh Maheshwari
East Zone Mritunjay Pao
Santanu Deb
North Zone Anurag Tomar
Muzaffar Jan
South Zone Raghunath CN
Riaz I
West Zone Kavita Shrivastav
Samir R Shah
Chief Advisor Siddharth Ramji
Central IAP Advisors (*ex-officio*)
R Remesh Kumar
Upendra S Kinjawadekar
S Thangavelu
Vineet K Saxena
Biostatistics Amir M Khan
Rajeev K Malhotra
Electronic media Sanjeev Goel
Ethics Jagdish Chinappa
Office Matters AS Vasudev
Peeyush Jain
Social Media Arva Bhavnagarwala
Chandermohan Kumar
Website C Vidyashankar

CONTENTS

Impact Factor 2020 of *Indian Pediatrics* is 1.411

Indian Pediatrics, the official journal of the Indian Academy of Pediatrics, is a peer-reviewed journal with monthly circulation of print/e-copies to over 33,000 pediatrician members. The journal is indexed in Current Contents/Clinical Medicine, Science Citation Index Expanded, Medline, PubMed, Indian Science Abstracts, getCITED, POPLINE, CANCERLIT, TOXLINE, Psych Line, and Dermline. The journal gives priority to reports of outstanding clinical and experimental work, as well as important contributions related to common and topical problems related to children and adolescents. Information for Authors is available on the website of the journal. *Indian Pediatrics* is available free online at *www.indianpediatrics.net.* There are no charges for publishing in the journal.

All rights reserved. The views and opinions expressed in the articles are of the authors and not of the journal. *Indian Pediatrics* does not guarantee directly or indirectly the quality or efficacy of any product or service featured in the advertisements in the journal, which are purely commercial.

Address for ordinary letters: **The Editor-in-Chief, Indian Pediatrics, P. Box No. 3889, New Delhi-110 049, India.**
Address for registered/speed post letters: **Dr. Devendra Mishra, Editor-in-Chief, Indian Pediatrics, 115/4, Ground Floor, Gautam Nagar, New Delhi 110 049, India. Tel: (011) 46052593**
E-mail: *jiap@iapindia.org*; Website: *www.indianpediatrics.net*; LinkedIn: *indianpediatrics*
Facebook: *www.facebook.com/Indianpediatrics;* Twitter: *@EditorIndPed;* Instagram: *@indianpediatrics*

Healthy Air – The Right of Every Child

Remesh Kumar R
President, Indian Academy of Pediatrics 2022
drremesh2006@yahoo.com

Environmental pollution is one of the banes of modern civilization. Today our living spaces are bombarded with various types of pollutants, which directly impact our health, happiness and general well being. Of these, air pollution is definitely the worst as it pervades every aspect of our life and is almost unavoidable. Even here, children are the ones most affected by air pollution. WHO estimates that 93% of the world's children under the age of 15 years (1.8 billion children) breathe polluted air, placing their health and development at serious risk [1]. According to a report published in *The Indian Express* last year [2], India is one of the most polluted countries in the world with an estimated 16 lakh deaths attributed to air pollution in 2019. The report further states that nearly 1.5 lakh children below the age of five died of lower respiratory infections in the same year, with more than 90% of these being infants, less than a year old [2]. As Julianne Moore, the famous actress and Artist Ambassador for Save the Children Fund, emphatically put it, "*Air pollution is terrible for our children. Every single scientist, every single doctor will tell you the same thing: air pollution damages our children's brains, their hearts, and their lungs*."

WHY IS AIR POLLUTION MORE HARMFUL?

Air and water are the two major types of pollution which directly impact human health. Of these, water pollution may be considered to be less threatening to health as we have a greater degree of control over its use and consumption. The same is not true with air pollution as the very air that we breathe cannot be controlled on a continuous basis. While many of the air pollutants such as smoke are visible and avoidable, there are many more types of pollutants such as toxic gases, organic and inorganic particulates and biological molecules carried by air which are not visible. Air pollution can occur from various sources like industrial smoke, vehicular emission, dust from construction activity and household activity. We inhale these pollutants unconsciously, there by endangering our health. The significant risks associated with long term exposure to air pollution include frequent respiratory infections, aggravated asthma, heart disease and stroke. There is also growing evidence that exposure to air pollution may be associated with reduced IQ scores, impaired cognition, increased risk for psychiatric disorders such as depression and detrimental perinatal health.

WHY ARE CHILDREN MORE AT RISK?

It is a commonly accepted wisdom that children are the most affected by air pollution. Their exposure risk begins even before birth as the ill effects can be transmitted from the mother transplacentally and through breastfeeding. They inhale more pollutants per kilogram of body weight than do adults. Because their airways are narrower, irritation can result in proportionately greater airway obstruction.

Then there is the explorative behavior of children, which increases their chances of exposure. Children live closer to the ground and they breathe more rapidly than adults and so absorb more pollutants. Constant exposure to household smoke, such as from kitchen fire (wood and charcoal stoves), burning of incense sticks or mosquito coils, tobacco smoke combined with poor ventilation and congested living spaces can be problem areas typical to developing countries like India. Exposure to vehicular smoke during commute to school adds to these problems. All these forms of air pollution can emit harmful elements like carbon monoxide (CO), nitrogen dioxide (NO2), sulfur dioxide (SO2), nitrogenated compounds and particulate matter (PM), which can result in chronic problems impacting respiratory and cardiovascular health.

AIR POLLUTION AND CHILD RIGHTS

With air pollution, chemical exposure and other environmental risks leading to 13.7 million deaths – about 24% of mortalities worldwide-each year, the United Nations Human Rights Council found it imperative to declare access to clean, healthy, and sustainable environment as a human right. Following the Resolution, which was approved on October 8, 2021, the UNHRC called on UN member states to act aggressively and promptly towards implementing this decision. The UN Environment Program

(UNEP), UN Children's Fund (UNICEF), and UN Human Rights (OHCHR) subsequently jointly launched the Principles and Policy Guidance on Children's Rights to a Safe, Clean, Healthy and Sustainable Environment in the Association of Southeast Asian Nations (ASEAN) Region [3].

STEP UP OUR ADVOCACY FOR MITIGATING AIR POLLUTION

The issues associated with air pollution are far too deep and all encompassing to be dealt with in one stretch. There are many more aspects to it than we can enumerate here. Our primary focus should be on fostering a healthy home and school environment, as these are two places where children spent most of their time. The following are some of the simple measures that we can actively promote in schools to negotiate air pollution and its adverse impact on children:

- Scheduling outdoor activities at school during a time of the day when air pollution and sun exposure are the lowest**.**
- Prohibiting the entry of vehicles including school buses inside the school campus, which minimizes the exposure to diesel and petrol exhaust fumes.
- Prohibiting the use of polluting fuels inside homes and schools for cooking.
- Planting maximum trees around school grounds to create shade and prevent ultraviolet ray exposure.
- Eliminating the use of tobacco both at home and in or around schools.
- Schedule painting works, floor refinishing and renovation during seasons when windows can be kept open and when school is not in session.
- Forming a school health committee to ensure that class rooms and premises are safe for children.

As the professional guardians of child health, let us all work together to minimize children's exposure to air pollution. There is a lot that we can do, as individuals and through our local IAP units, to make a real difference and ensure a healthy upbringing for our children. As rightly mandated by global bodies, access to a clean environment is the right of every child and we have a bounden duty to fulfill it.

Funding: None; *Competing interests:* None stated.

REFERENCES

1. World Health Organization. More than 90% of the world's children breathe toxic air every day. Accessed April 30, 2022. Available from: *https://www.who.int/news/item/ 29-10-2018-more-than-90-of-the-worlds-children-breathe-toxic-air-every-day#:~:text =Air%20pollution %20also% 20impacts%20neurodevelopment,cardiovascular%20 disease%20later%20in%20life*
2. Let the children breathe easy. Indian Express Jan 25, 2021. Accessed April 30, 2022. Available from:*https://indian express.com/article/opinion/columns/who-health-pollution-children-breathing-7159999/*
3. United Nations Human Rights Council. Accessed April 30, 2022. Available from:*https://www.ohchr.org/en/hr-bodies/ hrc/regular-sessions/session48/res-dec-stat*

NOTICE

IAP RESEARCH GRANTS 2022

Under IAP Action Plan 2022, the Indian Academy of Pediatrics (IAP) has initiated the following IAP Research Grants, to foster a research environment amongst the undergraduate medical students, pediatric postgraduate students, young pediatric faculty, and private practitioners. The awards will be granted every year for carrying out a research project related to child health. Applications are invited from interested researchers for the following categories of grants:

1. Undergraduate Pediatric Research Grant: 10 grants of Rs. 10,000/each
   (*Who can apply*: MBBS Students)
2. Postgraduate pediatric Research Grant: 4 grants of Rs. 25,000/each
   (*Who can apply*: MD/DNB Pediatrics Students)
3. Young Researcher Grant: 4 grantsof Rs. 25,000/each
   (*Who can apply*: Young faculty/Residents/Tutors in Medical Colleges/Hospitals, within 5 years of completing postgraduation in Pediatrics)
4. Practitioner Research Grant: 8 grants of Rs. 25,000/each
   (*Who can apply*: Life members of IAP in office practice)

The facility for online submission of application form and other details of the grant scheme is available on ICP website (*icpindia.org.in*). Last date of receiving project proposal with completed application form is 15 June, 2022.

INVITED COMMENTARY

Are We Keeping Our Nebulizers Clean?

ANSHULA TAYAL, S K KABRA*
Department of Pediatrics, AIIMS, New Delhi, India.
**skkabra@hotmail.com*

Recurrent wheezing occurs in a large proportion of children, often with viral upper respiratory tract infections. Many of these children, particularly in developing countries like ours, are administered bronchodilators at home by parents by means of nebulizers. These medications and devices are easily available over-the-counter across the country. Nebulizers are at times the only viable drug delivery system for young children who cannot be taught effective use of spacers. Nebulizers are also indispensable for the care of children with certain chronic diseases like cystic fibrosis (CF) and other chronic suppurative lung diseases. As part of their airway clearance therapy, these children require daily nebulization with mucolytic agents like hypertonic saline that cannot be delivered via pressurized metered dose inhaler (MDI). These patients are also frequently prescribed nebulized antibiotics and anti-fungal agents for microbial eradication.

It is a cause of concern that the nebulizers used on domiciliary basis have been found to be contaminated by several microorganisms, which predisposes these patients to frequent pulmonary exacerbations. A study on CF patients demonstrated that 70.5% of the home nebulizers were contaminated, with *Pseudomonas aeruginosa* being the most commonly isolated organism [1]. They also found a significant increase in number of pulmonary exacerbations in these patients. Another study highlighted the risk of acquisition of colistin-resistant, gram-negative bacteria like *Burkholderia cepacia* and *Stenotrophomonas maltophilia* from contaminated home nebulizers [2]. They also found a similar proportion of nebulizers (69%) to be contaminated. Similar studies have also been conducted on asthmatic children who use home nebulizers. The nebulizers were shown to be contaminated in 66.7% children and filters of 78.3% nebulizers were found to be contaminated [3].

In this regard, the study by Ranjan, et al. [4] published in this issue of *Indian Pediatrics* is highly commendable. They studied the bacterial colonization of home nebulizers and assessed the robustness of cleaning practices using a physician-administered questionnaire. About 20% of the samples from nebulizers showed bacterial growth, predominantly drug-resistant gram-negative bacteria [4].

It would be logical to wonder if the nebulizers are the primary sources of these organisms or are contaminated secondarily from the patients' secretions. Hutchinson, et al. [2] showed that in six CF patients whose nebulizers yielded *B.cepacia* and *S. maltophilia*, the same organisms were not isolated from concurrent sputum samples, indicating that nebulizers were likely primarily contaminated. This is further supported by the finding that a similar rate of contamination of home nebulizers is seen in asthmatic children, whose airways are not likely to be chronically colonized with bacteria [3,4]. In addition to microbial colonization, home nebulizers can also get contaminated with common indoor allergens in homes with pet dogs and cats, and these may lead to adverse consequences in sensitized individuals [5].

There is a wide variation in the maintenance practices of home nebulizers. Frequency of cleaning the nebulizer parts varies from none to once a week to daily [2,6,7]. Cleaning and drying the reservoir after each use leads to significantly lesser contamination [3]. Technique of cleaning and drying the nebulizer parts is also highly variable. Lower rates of contamination have been seen on cleaning with soap and water than with tap water alone [3]. Nebulizers with visible moisture have been shown to yield the heaviest growths of bacteria, as Gram-negative bacteria survive better in a moist environment [2]. Infrequent changing of nebulizer circuits has also been frequently observed, although the duration of use has not been shown to correlate with the degree of contamination [2].

The ideal standards and methods for cleaning the nebulizers have not been established [8]. However, a study has shown similar efficacy with both tap water and sterile distilled water, and a better efficacy with soak-then-rinse method (soaking for 10 minutes, followed by rinsing for 30 seconds in tap water) [9].

There is a pressing need to enhance awareness about this potentially serious problem of infections caused by contaminated nebulization equipment. The study by Ranjan, et al. [4] showed that about one-fifth of the parents used nebulizers on advice of friends or family. This makes them prone to improper handling and suboptimal hygiene practices, owing to a lack of appro-priate advice from healthcare personnel. An even more alarming observation made was that even amongst those who were using nebulizers on the advice of a physician, only a third receiving instructions on cleaning the equipment [4]. Another study on asthmatic children showed that only a fifth of the parents received instruc-tions on maintenance of nebulizers from healthcare personnel.

All the stakeholders – the patients, caregivers and healthcare personnel, need to be educated about the need and techniques of proper cleaning and maintenance of these devices. These instructions need to be reinforced to the caregivers at every follow-up visit to ensure maximal compliance.

Funding: None; *Competing interests:* None stated.

REFERENCES

1. Tabatabaii SA, Khanbabaee G, Sadr S, et al. Microbial contamination of home nebulizers in children with cystic fibrosis and clinical implication on the number of pulmonary exacerbations. BMC Pulm Med. 2020;20:33.
2. Hutchinson GR, Parker S, Pryor JA, et al. Home-use nebulizers: a potential primary source of Burkholderia cepacia and other colistin-resistant, gram-negative bacteria in patients with cystic fibrosis. J Clin Microbiol. 1996;34:584-87.
3. Cohen HA, Kahan E, Cohen Z, et al. Microbial colonization of nebulizers used by asthmatic children. Pediatr Int. 2006;48: 454-8.
4. Ranjan N, Singla N, Guglani V, Randev S, Kumar P. Bacterial colonization of home nebulizers used by children with recurrent wheeze. Indian Pediatr. 2022;59:377-79.
5. Bollinger ME, Butz A, Mudd K, Hamilton RG. Contamination of nebulizers with environmental allergens. Ann Allergy. 2005;95: 4.
6. MacFarlane M, Carson L, Crossan A, Bell J, Moore JE, Millar BC. Nebuliser cleaning and disinfection practice in the home among patients with cystic fibrosis. J Infect Prev. 2020;21: 14-22.
7. Murray TS, O'Rourke TK, Feinn R, Drapeau G, Collins MS. Nebulizer cleaning and disinfection practices in families with cystic fibrosis: The relationship between attitudes, practice and microbe colonization. J Cystic Fibrosis. 2019;18:823-8.
8. Force CC of the T, Boe J, Dennis JH, O'Driscoll BR, Force M of T, Bauer TT, et al. European Respiratory Society Guidelines on the Use of Nebulizers: Guidelines Prepared by a European Respiratory Society Task Force on the Use of Nebulizers. Eur Respi J. 2001;18:228-42.
9. Tai CH, Lin NT, Peng TC, Lee RP. Cleaning small-volume nebulizers: The efficacy of different reagents and application methods. J Nursing Res. 2011;19:61-7.

ADVERTISEMENT

APPLE SARASWATI
Apple MULTISPECIALITY HOSPITAL

We are a 200 Bed, NABH Accredited Multispeciality Hospital from Kolhapur (MS). The NICU is 40 Bedded and Paediatric ward consists of 25 beds including 10 bed PICU. Centre is recognised for PG Teaching - NNF, CPS, MUHS (Nashik)

We are looking for

NEONATOLOGIST : DM/DNB(Neonatology), Post MD, DCH Fellowship in Neonatology

PAEDIATRIC INTENSIVIST : MD / Fellowship in Paediatric Intensive Care

Apple Saraswati Multispeciality Hospital,
Kolhapur, Maharashtra
Contact : 9823022623, 9370525204
Email : *appledocs@rediffmail.com*

Specific Learning Disabilities in India: Current Situation and the Path Ahead

Sunil Karande
From Seth Gordhandas Sunderdas Medical College and King Edward VII Memorial Hospital, Parel, Mumbai, Maharashtra. Correspondence to: Dr Sunil Karande, Professor of Pediatrics and Head of Department, In-Charge Learning Disability Clinic, Department of Pediatrics, Seth GS Medical College and KEM Hospital, Parel, Mumbai 400 012, Maharashtra. karandesunil@yahoo.com

Students with specific learning disabilities (SpLD) need timely remedial education and provisions to continue their education within the mainstream. The Government of India has enacted the Rights of Persons with Disabilities (RPwD) Act, 2016, and legitimized SpLD as a disability, nationally. This Act mandates screening of every school student for SpLD on completion of eight years of age, setting up of resource rooms for imparting remedial education in all schools, and provisions in examinations for all afflicted students. This Act authorizes that students with SpLD get benefit of reservations in higher education seats and government jobs. To ensure that this Act is implemented effectively, all stakeholders in the field of education and health will have to collaborate to set up sufficient number of assessment clinics, create sufficient number of special educators, and develop validated screening and assessment tools for diagnosing SpLD in all the regional languages of our country.

Keywords: *Dyslexia*, *Education mainstreaming, Psychosocial support systems.*

Published online: 19 February, 2022; ***PII:*** S097475591600407

Specific learning disabilities (SpLD) are a group of neurodevelopmental disorders characterized by severe and persistent difficulties in learning to efficiently read (dyslexia), write (dysgraphia) and/or perform mathematical calculations (dyscalculia); despite normal intelligence, conventional schooling, intact hearing and vision, adequate motivation and socio-cultural opportunity [1,2]. Students with SpLD present with one or more of academic problems like reading slowly and incorrectly, skipping lines while reading aloud, making repeated spelling mistakes, untidy/illegible handwriting with poor sequencing, and inability to perform even simple mathematics [2,3]. These afflicted students have poor school performance [3], anxiety [4], and social maladaptation [5]. If SpLD remains undiagnosed, they invariably fail to achieve school grades at a level that is matching with their intellectual abilities [3]. In our country, up to 5-15% school-going children (almost 35 million), who otherwise have no visible physical, intellectual, visual, or auditory impairment, have this invisible disability [3,6]. Dyslexia afflicts 80% of all school students identified as having SpLD [3]. Additionally, out of 35 lakh children who are not in the formal school system [7], quite a few may be having a SpLD; though, no data is available.

SpLD is believed to be a result of functional disruption in neural systems, rather than an anatomic problem, and is genetically inherited [2,3]. Ideally, SpLD should be diagnosed while the afflicted student is in primary school, so that there is adequate time to avail adequate remedial education for a few years to achieve academic competence [3].

Landmark Events

Let us recapitulate a brief history of the landmark events related to SpLD in our country so as to be able to understand its present status and plan for the future (**Table I**). The state government of Maharashtra was the first to recognize SpLD as a disability; and passed a Government Resolution in 1996 empowering school students with SpLD in standards IX and X the option of availing provisions (accommodations) during their schooling and even for the Secondary School Certificate (SSC) board examinations. These accommodations included: *i*) extra time of 30 minutes for all written tests with spelling mistakes being overlooked; *ii*) employing a writer for children with dysgraphia; *iii*) exemption of a second language (Hindi or Marathi in an English medium school) and substituting it with a work experience subject; and *iv*) exemption of standard X mathematics (algebra and geometry) and substituting it with lower grade of mathematics (standard VII) and another work experience subject [3].

Over the years, other state governments have also granted provisions to students with SpLD so that they could successfully complete their education in mainstream schools (inclusive education). The critically acclaimed Hindi movie, *Taare Zameen Par* (Stars on Earth), accurately depicted the academic and social problems faced by a young 8-year-old boy with undiagnosed

Table I Landmarks Events Related to Specific Learning Disabilities in India [3,10-12]

Event	*Year*
Provisions given to SpLD students in standards IX-X in Maharashtra	1996
ICSE and CBSE national educational boards allow SpLD students to avail provisions	1999
Provisions given to SpLD students from class standards I-XII in Maharashtra	2000
GoI launches Sarva Shiksha Abhiyan ('Education for All' movement)[a]	2001
Provisions given to SpLD students in college courses in Maharashtra state	2003
Reservations for SpLD college students in disability category in Maharashtra state	2003
Provisions given by other states (Karnataka, Tamil Nadu, Kerala, Gujarat, Goa, others)	2003 to 2015
Hon. Bombay High Court verdict: denying provisions make school/college authorities liable for prosecution	2006
GoI implements Right of Children to Free and Compulsory Education (RTE) Act, 2009	2010
GOI implements Rights of Persons with Disabilities (RPwD) Act, 2016	2016

SpLD: specific learning disabilities; ICSE: Indian Certificate of Secondary Education; CBSE: Central Board of Secondary Education; GoI: Government of India; [a]Incorporated into Samagra Shiksha (Holistic Education) in 2018.

dyslexia [8,9]. This movie played a vital role in increasing awareness about dyslexia in India [8,9]. The launch of the Sarva Shiksha Abhiyan (Education for All movement), and implementation of the Right of Children to Free and Compulsory Education (RTE) Act (2009) have mandated that all children in the 6 to 14 years age group, including children with disabilities, have access to free and compulsory education [10,11]. A recent landmark event is the Government of India's (GoI's) implementation of the Rights of Persons with Disabilities (RPwD) Act, 2016 [12] which, for the first time, has recognized SpLD as a disability at the national level. With the backing of this Act, Indian students with SpLD all over the country can look forward to a brighter future.

The RPwD Act, 2016 and SpLD

The RPwD Act, 2016 [12] empowers students with SpLD all over the country. The salient features of this path-breaking legislation mandate that these students: should not face any discrimination; should continue education in mainstream schools/colleges (inclusive education); get access to occupational therapy and remedial education; get reservations in higher educational seats and government jobs; and get access to vocational training for self-employment.

The RPwD Act, 2016 [12] has also stated that: *i*) SpLD should be detected at the earliest and regular 1-yearly screening carried out; *ii*) adequate number of resource centers to support school education at all levels be established; *iii*) adequate number of training institutions to create special educators or remedial teachers be established; *iv*) induct SpLD as a component for all education courses for schools, colleges and university teachers, doctors, para-medical personnel, social welfare and rural development officers, Accredited Social Health Activist (ASHA) and Anganwadi workers; and *v*) all universities should promote research in SpLD.

A subsequent GoI notification [13] has stated that teachers, in both public and private schools, should screen every student in standard III (8-year-olds) and refer those who test positive to a learning disability clinic for further evaluation [13]. This screening can be done for students studying in English-medium schools by using a validated Screening Check list for Specific Learning Disability [3], which has been devised by the Maharashtra State Council of Educational Research and Training's, Divisional Office and Institute of Vocational Guidance and Selection, Mumbai [3].

A study from Mumbai [5] has reported that newly-diagnosed students with SpLD [mean (SD) age 12.5 (2.2) years (range 8.0 -16.0 years)] perceive themselves as: *i*) being "socially excluded," viz., they feel different from their peers, are lonely, feel stigmatized by their teachers and peers, have problems concentrating at school and feel left out; *ii*) having developed "emotional reactions," viz., worries, concerns, anger, and problems; *iii*) being "physically limited" in performing physical activities, having a poor health status, and have difficulties with sleeping; *iv*) lacking "independence," viz., they are insecure about their future and unable to live an autonomous life; and *v*) lacking in qualities for "social inclusiveness," viz., they feel that their peers and friends do not enjoy their company or understand their problems or care about their condition, and they therefore find it difficult to develop social relationships. A large majority (75%) of mothers of school students with SpLD already develop mild anxiety levels by the time this disability is diagnosed in their child [mean (SD) age 12.0 (2.32) year (range 7-16 year)] [14].

Unique Disability Identity Card

The GoI has implemented the 'Unique ID for Persons with Disabilities (UDID)' project [15] in 2018 with the objectives of creating a national database for persons with disabilities. A school student with suspected SpLD has to apply online (*<https://www.swavlambancard.gov.in/pwd/application>*) to get an appointment from the nearest recognized center for assessment. On confirmation of the diagnosis of SpLD the student will receive the disability certificate and UDID card [15]. This card will mention the student's name, date of birth, unique ID number, percentage of the disability and enable the student to avail provisions in school and board examinations. It is important to remember that a primary school student (older than 8 years) who is diagnosed with SpLD will have to undergo revaluation first at the age of 14 years; and again, at the age of 18 years [13]. Only a UDID card issued after evaluation on completion of 18 years of age states that the disability is permanent and entitles the student to get reservation in higher educational seats and government jobs [15].

Diagnosis of SpLD in India

The RPwD Act, 2016 guidelines for diagnosing SpLD [12] follow the twenty-year old DSM-IV-R criteria [1] for diagnosing this disability in our country. This involves directly, without any prior remedial education, doing educational testing to document that the student has significant difficulties in reading (dyslexia), writing (dysgraphia) and/or performing mathematical calculations (dyscalculia) [1,16]. Lately, many Western countries have adopted the latest DSM-5 [17] criteria for diagnosing SpLD, which states that the educational testing to diagnose SpLD should be done only after the school student has been given the necessary provisions and after having attended regular 'one-hourly' remedial education sessions with a special educator, twice- or thrice-weekly, for a minimum period of six months [17]. The application of the DSM-5 criteria is not suitable in the Indian educational setting as there is a dearth of qualified special educators; and, most schools do not have infrastructure for providing remedial education [3].

The only test that has been recommended by the GoI to diagnose SpLD [18] is the National Institute of Mental Health and Neurosciences (NIMHANS) Battery [19], which is available in English, Hindi and Kannada. However, the NIMHANS battery can only be utilized to diagnose SpLD in school students who are 8 to 12 years of age [19]. Other validated tests which are available in India, but not mentioned in the GoI's protocol [18], include: *i*) the Grade Level Assessment Device (GLAD) [20] (available in English and Hindi), which can be used to diagnose SpLD in students up to class standard IV level; *ii*) the Dyslexia Assessment for Languages in India (DALI) [21] test (available in English, Hindi, Kannada and Marathi), which can be used to diagnose dyslexia in students up to standard V level; and *iii*) Sholapurwala RF's Curriculum Based Test for Educational Evaluation of Learning Disability [22] (available in English and Marathi), which can be used to diagnose SpLD in students up to standard X.

It is not possible to quantify the severity of SpLD by following the DSM-IV-R criteria for diagnosing SpLD [1]. As per GoI rules [12], unless the severity of a disability is ≥40 per cent (benchmark disability), an afflicted student cannot qualify to avail any benefits. To solve this dilemma, the GoI has stated that "any student who tests positive on NIMHANS Battery shall be considered as having SpLD disability of more than 40%" [18].

Existing Challenges in India

Currently there are significant challenges to overcome in order to ensure effective implementation of the RPwD Act, 2016 [3]. Validated screening tools to identify a student who needs to be referred for assessment; and validated assessment tools for diagnosing SpLD are not available in all the regional languages of our country [3]. There is a dearth of assessment clinics and special educators to diagnose SpLD and impart remedial education to afflicted students [3,23]. Also, a large majority of schools do not have resource rooms to impart remedial education [3].

The RTE Act, 2009 [11] has mandated that no student shall be held back until completion of schooling till standard VIII. One of the consequences of this is that students with suspected SpLD are getting referred for assessment at a very late age [4], when they are in standard IX (14 years of age). At this stage, only the Sholapurwala RF's test [22] or the Woodcock-Johnson Test of Achievement [24] (in English) can be used to diagnose SpLD. It is important to note that the Woodcock-Johnson Test of Achievement is not validated for use in the Indian population [24,25].

The Path Ahead

To make India an SpLD-friendly country, active collaboration between all stakeholders in the field of education and healthcare is needed. The action points to achieve this include: *i*) school trustees and principals should set up resource rooms within the school to ensure that students with SpLD get the remedial education at an affordable cost; *ii*) all students at the age of 8 years should be screened for SpLD and those who test positive should be referred to the nearest assessment clinic for evaluation; *iii)* school counselors should play a proactive role to

ensure that students with SpLD do not feel stigmatized; *iv)* government medical colleges and hospitals (including those at district levels) should set up assessment clinics; and, *v)* all universities related to the field of teacher education should start courses to create special educators, and conduct research to develop validated screening and assessment tools for SpLD in all the 22 regional languages of our country.

Over the years, SpLD in our country will get detected early, there would be adequate qualified special educators available, and the afflicted students will be able to avail timely remedial education to achieve academic competence [3]. The path ahead is arduous but is achievable in the next decade with determined efforts from all stakeholders.

Funding: The Learning Disability clinic at my institute is partially funded by a research grant by MPS Limited (*https://www.mpslimited.com*). *Competing interests*: None stated.

REFERENCES

1. American Psychiatric Association. Diagnostic and Statistical Manual of Mental Disorders. 4th ed. Revised. American Psychiatric Association;2000.
2. Peterson RL, Pennington BF. Developmental dyslexia. Annu Rev Clin Psychol. 2015;11:283-307.
3. Karande S, Sholapurwala R, Kulkarni M. Managing specific learning disability in schools in India. Indian Pediatr. 2011;48:515-20.
4. Thakkar AN, Karande S, Bala N, et al. Is anxiety more common in school students with newly diagnosed specific learning disabilities? A cross-sectional questionnaire based-study in Mumbai, Maharashtra, India. J Postgrad Med. 2016;62:12-9.
5. Karande S, Venkataraman R. Self-perceived health-related quality of life of Indian children with specific learning disability. J Postgrad Med. 2012;58:246-54.
6. Arora NK, Nair MKC, Gulati S, *et al*. Neurodevelopmental disorders in children aged 2-9 years: Population-based burden estimates across five regions in India. PLoS Med. 2018;15:e1002615.
7. Vishnoi A. Over 35 lakh children out of school, states tell Centre. The Economic Times, 2021 Oct 10; New Delhi. Accessed February 11, 2022. Available from: *https://economictimes.indiatimes.com/news/india/over-35-lakh-children-out-of-school-states-tell-centre/articleshow/86920024.cms*
8. Sathyanarayana Rao TS, Krishna VS. Wake up call from 'Stars on the ground.' Indian J Psychiatry. 2008;50:2-4.
9. Chakravarty A. Taare zameen par and dyslexic savants. Ann Indian Acad Neurol. 2009;12:99-103.
10. Department of School Education and Literacy, Ministry of Education, Government of India. *Samagra Shiksha* ("Holistic Education"). Accessed November 21, 2021. Available from: *https://samagra.education.gov.in/*
11. Legislative Department. Ministry of Law and Justice, Government of India. The Right of Children to Free and Compulsory Education Act, 2009. Accessed November 21, 2021. Available from: *https://legislative.gov.in/actsofparliament fromtheyear/right-children-free-and-compulsory-education-act-2009*
12. Legislative Department. Ministry of Law and Justice, Government of India. The Rights of Persons with Disabilities Act, 2016. Accessed November 21, 2021. Available from: *https://legislative.gov.in/actsofparliamentfromtheyear/rights-persons-disabilities-act-2016*
13. Department of Empowerment of Persons with Disabilities (*Divyangjan*). Ministry of Social Justice and Empowerment, Government of India. Notification on Guidelines for Assessment of Various Specified Disabilities. Accessed November 21, 2021. Available from: *https://disabilityaffairs.gov.in/content/page/guidelines.php*
14. Karande S, Kumbhare N, Kulkarni M, Shah N. Anxiety levels in mothers of children with specific learning disability. J Postgrad Med. 2009;55:165-70.
15. Department of Empowerment of Persons with Disabilities (*Divyangjan*). Ministry of Social Justice and Empowerment, Government of India. Unique Disability ID. Accessed November 21, 2021. Available from: *https://disabilityaffairs.gov.in/content/*page/unique-disability-id.php
16. Karande S, Kulkarni M. Specific learning disability: The invisible handicap. Indian Pediatr. 2005;42:315-9.
17. American Psychiatric Association. Diagnostic and Statistical Manual of Mental Disorders. 5th ed. American Psychiatric Association Publishing; 2013.
18. Department of Empowerment of Persons with Disabilities (*Divyangjan*). Ministry of Social Justice and Empowerment, Government of India. Assessment guidelines for disability has been amended with regard to specific learning disability on 9.12.2000. Accessed November 21, 2021. Available from: *https://disabilityaffairs.gov.in/content/page/guidelines.php*
19. Hirisave U, Oommen A, Kapur M. Psychological assessment of children in the clinical setting. NIMHANS index of specific learning disabilities. 2nd ed. Samudra Offset Printers; 2006.
20. Narayan J. Grade level assessment device for children with learning problems in schools. Secunderabad: National Institute for The Empowerment of Persons with Intellectual Disabilities (*Divyangjan*); 2003 reprint. Accessed November 21, 2021. Available from:*https://niepid.nic.in/list.php*
21. National Brain Research Centre. DALI - Dyslexia Assessment for Languages of India. National Brain Research Centre; 2015.
22. Sholapurwala RF. Curriculum Based Test for Educational Evaluation of Learning Disability. 2nd ed. Jenaz Printers; 2019.
23. Does India have enough special teachers – Supreme Court asks. India Today 2021 Oct 29; New Delhi. Accessed January 16, 2022. Available from: *https://www.indiatoday.in/education-today/news/story/does-india-have-enough-special-teachers-supreme-court-asks-1870970-2021-10-29*
24. Woodcock RW, McGrew KS, Mather N. Woodcock-Johnson Tests of Achievement. 3rd ed. 2001.
25. Abu-Hamour B, Al Hmouz H, Mattar J, Muhaidat M. The use of Woodcock-Johnson tests for identifying students with special needs - a comprehensive literature review. Procedia Soc Behav Sci. 2012; 47:665-73.

RESEARCH PAPER

Implementation of Nationwide Evidence- and Consensus-Based Guidelines to Harmonize Neonatal Care in The Netherlands

ELISE TALSMA,[1] ROGIER DE JONGE,[2] FLORIAN CASSEL,[3] MONIEK VAN DE LOO,[4] KAREN DE BIJL-MARCUS,[5] ESTELLE MULDER,[6] HENDRIK NIEMARKT,[7] SANDRA PRINS,[4] MAYKE VAN DER PUTTEN,[8] MARGRIET VAN STUIJVENBERG,[9] REMCO VISSER,[10] ANKE OERLEMANS,[11] MARIJE HOGEVEEN[1]

From [1]Division of Neonatology, Departments of Pediatrics, Amalia Children's Hospital, Radboudumc, Nijmegen; [3]Erasmus MC Sophia Children's Hospital, Rotterdam; [4]Amsterdam University Medical Centers, Amsterdam; [5]University Medical Center Utrecht, Utrecht; [6]Isala Women and Children's Hospital, Zwolle; [7]Maxima Medical Centre, Veldhoven; [8]Maastricht UMC+, Maastricht; [9]Beatrix Children's Hospital University Medical Center Groningen, University of Groningen, Groningen; [10]Leiden University Medical Center, Leiden; [2]Pediatric Intensive Care Unit, Department of Pediatrics and Pediatric Surgery, Erasmus MC Sophia Children's Hospital, Rotterdam; [11]IQ healthcare, Radboud Institute for Health Sciences, Radboudumc, Nijmegen; The Netherlands. Correspondence to: Dr Marije Hogeveen, Amalia Children's Hospital, Radboudumc, Geert Grooteplein Zuid 6525 GA Nijmegen, The Netherlands. marije.hogeveen@radboudumc.nl

Received: November 20, 2021; Initial Review: December 16, 2021; Accepted: February 23, 2022.

Background: A Dutch committee for National Guidelines in Neonatology developed nineteen evidence- and consensus-based guidelines to be used in all Dutch neonatal intensive care units (NICUs). The primary goal was to make clinical practices more uniform and consistent.

Objective: This study investigated to what extent the guidelines were implemented and which factors played a role in implementation.

Study design: A mixed method study design was used to investigate both the level and the process of implementation. A nationwide, multicenter, cross-sectional survey was performed using a validated instrument for measuring the level of implementation (Normalization MeAsure Development questionnaire, NoMAD). The number of implemented guidelines per NICU and the frequency and content of the amendments that NICUs made to the original consensus guidelines were analyzed. Through semi-structured interviews, perceived barriers and facilitators for implementation were explored.

Participants: Fellows and neonatologists working at all ten Dutch level 3-4 NICUs were eligible.

Results: On an average, NICUs implemented 12.6 (of 19) guidelines (range 6-17). The Normalization Process Scale was 54 (of 65). Main influencing factors impeding implementation were guideline-related (e.g., unpractical, lengthy guidelines) and personal (e.g., an active representative responsible for local implementation).

Conclusion: The implementation of our guidelines appears to be successful. Ways for improvement can be distinguished in personal, guideline-related and external factors. Empowerment of local representatives was considered most essential.

Keywords: *Guideline development, Neonatal intensive care unit, Quality improvement*.

Published online: March 10, 2022; ***PII:*** S097475591600410

In medical practice, evidence is often lacking, and local agreements are frequently made, potentially resulting in divergence from guidelines [1]. In newborn care (where evidence is particularly scarce), these divergences are extensive [2]. To create more consistency in newborn care, a consensus targeted approach is necessary.

In 2014, a Dutch committee called N3recommendations (N3R), part of Neonatology Network Netherlands (N3) was founded. Through the development of national evidence- and consensus-based guidelines, aim was to improve harmonization of neonatal care. The European Foundation for the Care of Newborn Infants (EFCNI) addresses disparities in the provision and quality of European neonatal care by developing reference standards as a source for the national development of guidelines and protocols [3,4]. In the Vermont Oxford Network (VON), an international collaboration in neonatology, neonatal intensive care units (NICUs) work together to formulate potentially better practices that are implemented locally [5].

The success of guidelines depends on content quality and on their actual implementation [6,7]. Successful implementation depends on the consideration of a variety of barriers and the use of adequate strategies to overcome them [8]. We studied the implementation level of our guidelines, and the factors influencing implementation.

METHODS

The Netherlands consists of 17.4 million inhabitants and has approximately 170,000 live births per year [9]. There are ten level 3-4 neonatal intensive care units (NICUs) with 108

neonatologists and 26 fellows. In total, there are 195 NICU beds, where approximately 5000 patients are admitted annually [10]. Every NICU has one representative on N3R.

The guidelines were developed based on a comparison of existing local protocols and comprehensive literature searches followed by Grading of Recommen-dations Assessment, Development and Evaluation (GRADE) processes. Where evidence was lacking or inconclusive, N3R followed Delphi structured processes to form consensus. After multiple feedback rounds available for all neonatologists and fellows, the final guidelines, based on input from all Dutch NICUs, available evidence and consensus, were approved (**Web Fig. 1**). The intention was for every NICU to upload the consensus guidelines into their local guideline system. The agreement was to allow NICUs to make logistic, but not substantive amendments.

To achieve both a breadth and depth of under-standing, this study follows a mixed-method approach, combining quantitative data from a questionnaire and qualitative data from semi-structured focus group interviews [11]. All parts of the study received clearance from the Radboudumc medical ethical committee. The Standards for Quality Improvement Reporting Excellence (SQUIRE) 2.0 were used as a framework [12]. A nationwide, multicenter, cross-sectional, digital survey was conducted.

One of the theories for understanding and measuring implementation is the normalization process theory (NPT) [13,14]. Finch, et al. [15,16] developed and validated an NPT-based questionnaire, the Normalization MeAsure Development questionnaire (NoMAD). A validated Dutch translation, customized to our particular situation, was used [17]. The original NoMAD contains twenty questions, of which thirteen were deemed appropriate for our survey. The NoMAD distinguishes four constructs playing a central role in generating implementation. A more practical approach, considering three groups of factors (personal, guideline-related and external factors) was used [8], and therefore, five questions were added.

The survey was pilot-tested by two neonatologists (non-N3R members), and modified based on their feedback. The final version contained three parts: Part A concerned demographic information; Part B collected three general normalization ratings about current and future use; and, Part C contained 13 items from the NOMAD instru-ment and five additional questions. Answer options were according to a five-point Likert scale. There was option B ("I don't know" or "not relevant") to ensure that non-applicable questions were skipped.

Survey invitations were sent via e-mail. Informed consent was obtained at the beginning. Data were collected over three weeks in April, 2019; reminders were sent after one and two weeks. The completed surveys were automatically collected (Castor Electronic Data Capture EDC 2019.1) and stored anonymously.

Local versions of the guidelines were retrieved and compared with the original documents. Amendments were recorded, distinguishing logistic (defined as necessary changes due to local logistic circumstances) and substantive (defined as changes in content) amendments. The COREQ (COnsolidated criteria for REporting Qualitative research) checklist was used to report items of importance [20].

The interview guide was created by three investigators (ET, MH and RdJ) and critically appraised (AO). The interview guide concerned two themes (perceived facilitators and barriers) each subdivided into three subthemes (personal, guideline-related and external factors [8]. Questions concerning the aim of N3R, the acceptance, and the development of the guidelines were added. Printed forms showing results from the survey and an overview of local amendments per NICU were used as background information.

To reach depth, group interviews were conducted or (in case of planning issues) dyadic interviews [21]. Neonatologists and fellows working at Dutch level 3-4 NICUs, except N3R members, were considered eligible. The interviews were conducted between May and June, 2019. A convenience group participated in the interviews from each NICU. The interviews were recorded (WS-806 voice recorder, Olympus), transcribed anonymously, and deleted after transcription.

Statistical analysis: While the data were considered ordinal, nonparametric analyses were performed. The individual normalization process scale (NPS) was calculated by summing 13 construct items per person [15]. Differences between fellows/neonatologists, N3R member or not, and gender were analyzed, and correlations between age and years of experience were calculated. The total factor score (TFS) was calculated by summing 17 factor questions, as was the score for each factor group. Differences between factor groups were analyzed with the Kruskal-Wallis test or a median test (depending on the difference in variance between factor groups, analyzed with Levene test for equality of variances). Option B answers were valued at zero points.

The construct scores were correlated to the three general normalization ratings to check the validity after our alterations [16,17]. Outcomes were interpreted following the categorization (0: no correlation, 0.1-0.29: poor, 0.3-0.59: fair, 0.6-0.79: moderate, 0.8-0.99: very strong, 1: perfect) [19]. IBM SPSS statistics, version 25 (IBM Corporation) was used.

For the qualitative part, a thematic template analysis based on the interview guide was used [22]. Transcripts were analyzed independently by two researchers (ET and MH). Discrepancies were discussed with a third researcher (RdJ) until consensus was reached. A qualitative analysis tool (ATLAS.ti Scientific Software Development GmbH Version 8.3.20) was used.

RESULTS

When the survey aired, all the 134 neonatologists and fellows working in the NICUs were approached; 63 (47%) completed the survey (**Table I**). Distribution of completed surveys among the NICUs is shown in **Web Fig. 2**.

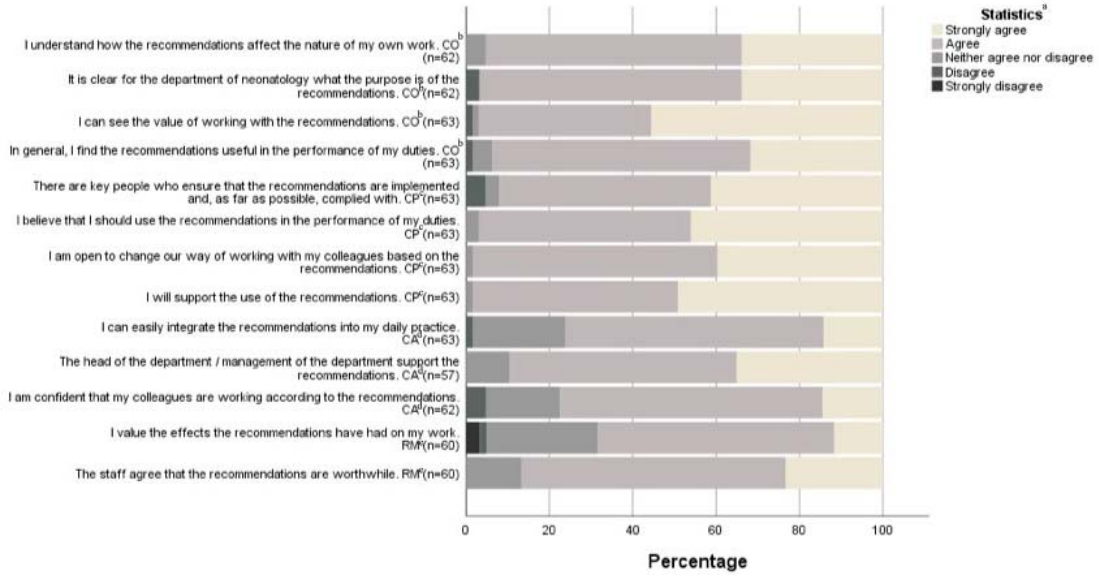
Distributional characteristics of the scale scores are shown in **Fig. 1**. With respect to the NPS, majority of the participants (strongly) agreed with the statements. The total NPS scores of all participants, neonatologists, fellows, and N3R and non-N3R members followed similar response patterns. There were no significant differences in total NPS score by role (neonatologists/fellows or gender). Age and years of experience were also not related to NPS results [age: r= 0.257 (P=0.042); years of experience: r=0.231 (P=0.069)].

Median (IQR) scores were 4 (4-5), 4 (3-4) and 4 (4-5) for personal, guideline-related and external factors, respectively. There was a significant difference between guideline-related factors and the other groups (P<0.001). Factor group explained 13.8% of the variability in rank scores. Correlations between construct scores and the three general normalization ratings were assessed to investigate the

Table I Demographic Background of Survey Participants and Total Research Population (N=63)

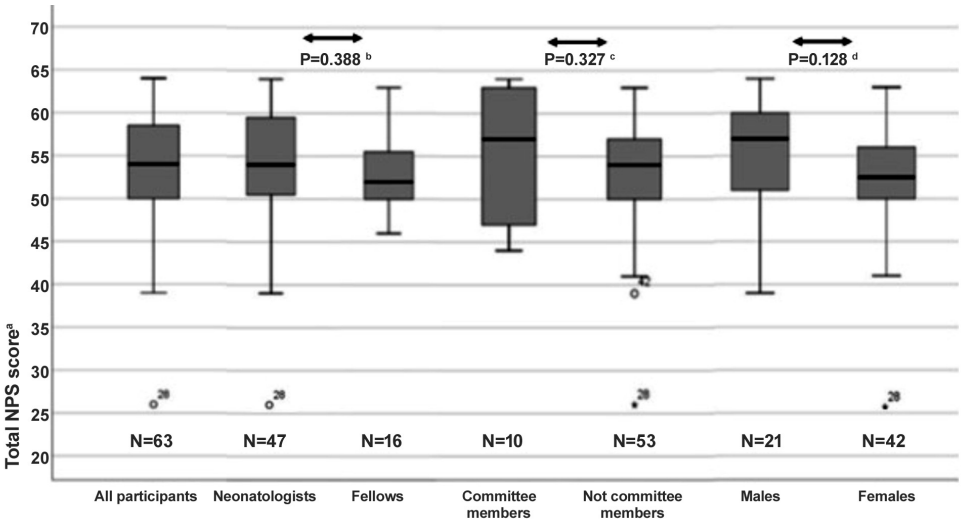
Characteristics	*No. (%)*
Role[a]	
Fellow, *n*=26	16 (61.5)
Neonatologist, *n*=108	47 (43.5)
Committee member[a]	
Yes, *n*=10	10 (100)
No, *n*=124	53 (42.7)
Male gender	21 (33.3)
Age (y)	
30-39	20 (31.7)
40-49	26 (41.3)
50-59	13 (20.6)
60-65	4 (6.3)
NICU experience (y)	
0-5	19 (30.2)
6-10	16 (25.3)
11-15	13 (20.6)
16-20	5 (7.9)
21+	10 (15.9)

Data in no. (%). [a]*Percentages are out of total with that role.*



[a]*Percentages of responses reporting strongly disagree, disagree, neutral, agree, strongly agree are shown.* [b]*coherence (CO);* [c]*cognitive participation (CP).*

Fig. 1 Frequency distribution of responses to questions that are part of the normalization process scale. For interpretation: maximum score is 65 points (five points per question, thirteen questions). More agreement with the statements indicates a more positive attitude towards the guidelines.



[a]Total normalization process scale (NPS) score. All boxplots are presented as medians and IQR. [b]Differences in total NPS score between neonatologists and fellows; [c]between committee/noncommittee members; and [d]between males and females were tested using Mann-Whitney U test.

Fig. 2 Boxplots of the total normalization process scale score per group.

validity of the survey. Similar to the out-comes in NoMAD validation studies, all correlation coeffi-cients can be interpreted as fair to moderately strong [19].

The amendments of the 19 guidelines for all NICUs were studied (n=190). In total, 126 (66.3%) guidelines were uploaded into local protocol systems. On an average, NICUs implemented 12.6 guidelines (range 6-17). Every NICU made logistic and substantive amendments; in total, 379 amendments were made, of which 69 were logistic.

Interviews were conducted with at least one fellow and one neonatologist per NICU. In total, 12 fellows and 14 neonatologists were interviewed. Interviews lasted between 28-54 minutes. The majority of the participants agreed with the aims of N3R. Positive aspects mentioned were the value of collective expertise, insight into the gaps in evidence-based medicine, and the need for more consistency towards patients, parents, and colleagues. Negative aspects were scarce and almost exclusively related to guidelines where it was harder to reach consensus. Fellows were in general very satisfied with the existence of the guidelines and felt more secure when using them (**Web Box I**).

Perceived factors with corresponding barriers and facilitators and illustrative quotations are demonstrated in **Web Table I**. An active representative for local implementation was considered to be the key by the most participants. Being part of the developmental process or feedback rounds was also considered a facilitator.

For guidance-related factors, participants were unanimous on the fact that guidelines should be concise with a clear step-by-step plan. Many were in favor of accompanying flowcharts. Few participants stated that information was not always substantiated. In contrast, others said that the underlying reason for action is clear and that information is sufficiently retraceable. For external factors, some guidelines were not applicable to a specific NICU and therefore not implemented (for example a surgical guideline). In other situations, the guideline was not compliant with the NICU (for example, the congenital diaphragmatic hernia guideline has been developed for NICUs without extra corporeal membrane oxygenation (ECMO) and therefore not implemented in NICUs with ECMO).

The availability of consulting specialists (for instance, pediatric cardiologists) was mentioned as both a facilitator and a barrier. When consulting specialists were familiar with the guidelines, participants would use the guidelines during consultation. Otherwise, participants stated that they would rather ask a consultant's opinion.

A local culture open to change was considered an important factor, whereby a clear aim supported by the whole team is considered the key.

DISCUSSION

The primary aim of the study was to evaluate the implementation of national evidence- and consensus- based NICU guidelines. Considering the NPS results, this implementation seems successful: most Dutch neonatologists and fellows have implemented (the majority of) the guidelines into their daily practice (total NPS 54/65).

WHAT IS ALREADY KNOWN?

- The success of guidelines depends on content quality and their implementation.

WHAT THIS STUDY ADDS?

- The structure of a national committee, comprised of local representatives from every practice involved, appears to be appropriate for the development of readily accepted evidence- and consensus-based guidelines.

However, there is room for improvement. Not all guidelines were implemented, and NICUs differed in the amount of local implementation and (logistic and substantial) amendments. Based on our results, focus should lie primarily on guideline-related factors and active representatives. Furthermore, many interview participants stated that just by performing this study, N3R increased awareness and therefore implementation of their guidelines. Repetitive evaluation of guideline implemen-tation is therefore recommended. A generally applicable advice regarding guideline implementation strategy is presented in **Web Table II**.

An important factor was having an active local representative. This finding is supported by a study of Lago, et al. [23]. Creating and maintaining a strong connection between representatives was considered beneficial, supporting the N3R structure with represen-tatives from every NICU. In consonance with our results on guideline-related factors, a study by Donnell, et al. [24] states that a guideline should have clear action steps. Some participants emphasized the importance of the scientific background of the guidelines, which has also been previously reported [24]. It is important to realize that as consistent high-level evidence is often lacking in medicine, consensus may be the only way to achieve guidelines.

In contrast to the study by Davis, et al. [25] suggesting that early-career physicians are more receptive to clinical practice guidelines, there was no indication for less implementation among more experienced doctors com-pared to early-career doctors [25].

Due to the mixed-method study design, broad and in-depth insight was gained at both the level and process of implementation. Neonatologists and fellows from every NICU in the Netherlands participated, which resulted in a representative sample. However, this study also had some limitations. In the absence of a gold standard, the level of implementation was measured with the second-best option: a validated tool. Even though slight alterations were made, a validation test shows correlations similar to those in the original study [16]. Furthermore, with this study design, presumed practice was investigated instead of actual practice. However, this does not make the answers less relevant. Future research could comprise repetitive audits investigating actual practice. The distribution of fellows/ neonatologist and N3R members/no N3R member in the survey is different from the distribution of practicing physicians; this may have led to response bias. In this study, no other stakeholders were investigated, such as NICU nurses.

Our process of development and implementation of national guidelines, combined with the lessons learned from this study, demonstrates a suitable approach for those in other nations or specialties with the desire to develop nationwide guidelines. Due to the coronavirus (COVID-19) pandemic, doctors have become more accustomed to meeting digitally, making our strategy applicable for larger geographical areas. Our strategy appears to be applicable for countries with identical numbers of NICUs, but this is unclear for countries with large numbers of NICUs. In future research, a follow-up study demonstrating the impact of the suggestions for improving implementation could be performed.

Strategies for improving implementation are multifactorial and can be distinguished in personal, guideline-related and external factors. Improving guideline-related factors seems a good starting point since they scored lowest and are probably easiest to change. Ways to empower representatives should be discussed among N3R members and their staffs, since they were considered essential.

Almost all participants in this study supported the aim of N3R and valued the guidelines. It turns out that even when experts' opinions seem far apart, forming a national consensus is desired by most.

Ethics clearance: Institutional Ethics Committee of the Radboudumc; No. IRB 2020-6274 dated March, 2020.
Note: Additional material related to this study is available with the online version at *www.indianpediatrics.net*
Contributors: EST, RCJdeJ, FC, MvdL, KAdeB-M, EEMM, HJN, SAP, MEvdP, RV: substantial contributions to the study, including: Concep-tualization, methodology, investigation, data curation and formal analysis, participating in writing (drafting the initial manuscript); MH,MvS,AJMC: substantial contributions to the study, including: Supervision, conceptualization, methodology, investigation, interpretation of data, data curation, formal analysis, resources, participating in writing (review and editing of the

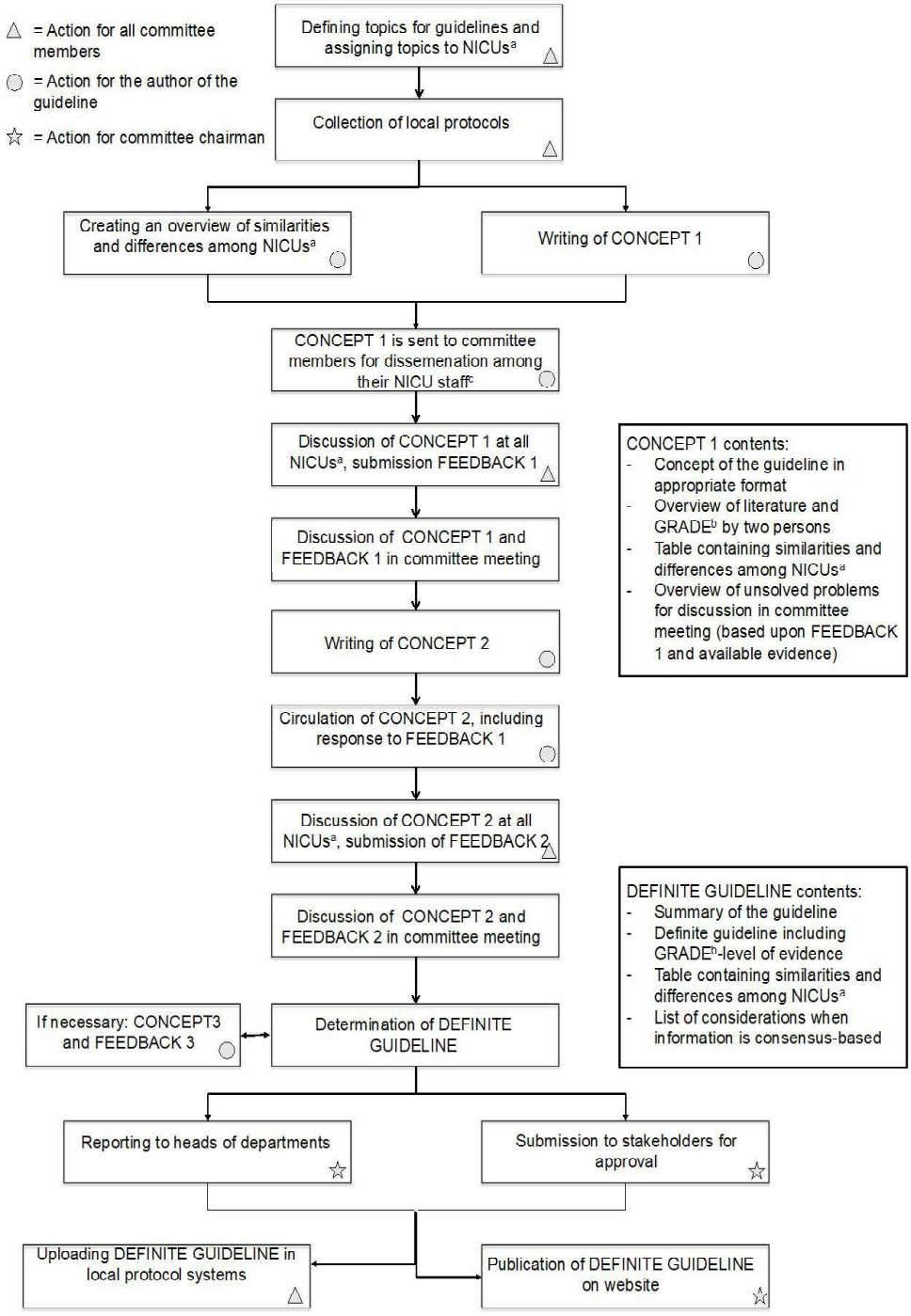
manuscript). All authors provided final approval to the version to be published and agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. *Funding*: None; *Competing interest*: None stated.

REFERENCES

1. McClellan MB, McGinnis JM, Nabel EG, Olsen LAM. Evidence-Based Medicine and the Changing Nature of Healthcare: Meeting Summary (IOM Roundtable on Evidence-Based Medicine). National Academy of Sciences;2008. p. 49-70.
2. Meyer S. Evidence-based medicine in neonatology: The need for multifaceted improvements. Neonatology. 2020;117:123-24.
3. European Standards of Care for Newborn Health EFCNI (2020) Standards Overview. Accessed July 15, 2020. Available from: *https://newborn-health-standards.org/standards/overview/*
4. European Standards of Care for Newborn Health EFCNI (2020) Project Methods. Standard development process – in detail. Accessed July 15, 2020. Available from: *https://newborn-health-standards.org/project/methods/*
5. Horbar JD. The Vermont Oxford Network: evidence-based quality improvement for neonatology. Pediatrics. 1999; 103:350-9.
6. Prior M, Guerin M, Grimmer-Somers K. The effectiveness of clinical guideline implementation strategies-a synthesis of systematic review findings. J Eval Clin Prac. 2008; 14:888-97.
7. Proctor E, Silmere H, Raghavan R, et al. Outcomes for implementation research: conceptual distinctions, measurement challenges, and research agenda. Admin Policy Ment Health. 2011;38:65-76.
8. Fischer F, Lange K, Klose K, et al. Barriers and strategies in guideline implementation-a scoping review. Healthcare. 2016;4:36.
9. Centraal Bureau Statistiek, CBS Bevolkingsteller. Accessed November 23, 2020. Available from: *https://www.cbs.nl/nl-nl/visualisaties/bevolkingsteller*
10. Perined, Perinatale zorg in Nederland anno 2018: landelijke perinatale cijfers en duiding. Published November 26, 2019. Accessed March 23, 2020. Available from: *URL: https://www.perined.nl/Perinatale zorg in Nederland anno 2019*
11. Palinkas LA, Aarons GA, Horwitz S, et al. Mixed method designs in implementation research. Adm Policy Ment Health. 2011;38:44-53.
12. Ogrinc G, Davies L, Goodman D, et al. SQUIRE 2.0 (Standards for QUality Improvement Reporting Excellence): Revised publication guidelines from a detailed consensus process. BMJ Qual Saf. 2016;25:986-92.
13. May CR, Mair F, Finch TL, et al. Development of a theory of implementation and integration: Normalization process theory. Implementation Science. 2009;4:29.
14. Rabin BA, Purcell P, Naveed S, et al. Advancing the application, quality and harmonization of implementation science measures. Implement Sci. 2012;7:119.
15. Rapley T, Girling M, Mair FS, et al. Improving the normalization of complex interventions: part 1 - development of the NoMAD instrument for assessing implementation work based on normalization process theory (NPT). BMC Med Res Methodol. 2018;18:133.
16. Finch TL, Girling M, May CR, et al. Improving the normalization of complex interventions: part 2 - validation of the NoMAD instrument for assessing implementation work based on normalization process theory (NPT). BMC Med Res Methodol. 2018;18:135.
17. Vis C, Ruwaard J, Finch TL, et al. Toward an objective assessment of implementation processes for innovations in health care: Psychometric evaluation of the normalization measure development (NoMAD) questionnaire among mental health care professionals. J Med Int Res. 2019;21:e12376.
18. Bishop PA, Herron RL. Use and misuse of the Likert item responses and other ordinal measures. Intern J Exercise Sci. 2015;8:297-302.
19. Akoglu H. User's guide to correlation coefficients. Turk J Emerg Med. 2018;18:91-3.
20. Tong ASP, Craig J. Consolidated criteria for reporting qualitative research (COREQ): a 32-item checklist for interviews and focus groups. Int J Qual Health Care. 2007;19:349-57.
21. Morgan DL, Ataie J, Carder P, Hoffman K. Introducing dyadic interviews as a method for collecting qualitative data. Qual Health Res. 2013;23:1276-84.
22. King N. Chapter 21: Using Templates in the Thematic Analysis of Text. *In*: Cassell C, Symon G. Essential Guide to Qualitative Methods in Organizational Research. SAGE Publications Ltd; 2004. p. 256-70.
23. Lago P, Garetti E, Boccuzzo G, *et al.* Procedural pain in neonates: the state of the art in the implementation of national guidelines in Italy. Paediatr Anaesthesia. 2013;23:407-14.
24. Donnell Z, Hoffman R, Myers G, Sarmiento K. Seeking to improve care for young patients: Development of tools to support the implementation of the CDC Pediatric mTBI Guideline. J Safety Res. 2018;67:203-9.
25. Davis DA, Taylor-Vaisey A. Translating guidelines into practice: a systematic review of theoretic concepts, practical experience, and research evidence in the adoption of clinical practice guidelines. CMAJ. 1997;157:408-16.

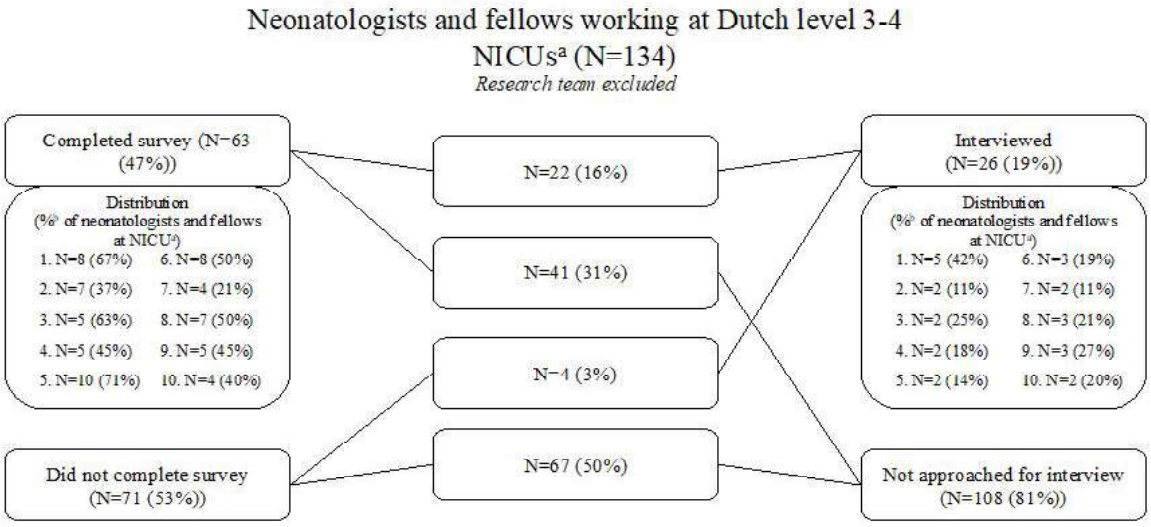
Web Box I Illustrative Quotes from Participants

Quotations from various neonatologists and fellows about making care more consistent
"I think it is indeed good to work in a more consensus-based and evidence-based manner in the Netherlands. Because it is quite special when you hear how in… well, the Netherlands is not that big at all, but when you hear how ten different NICUs sometimes follow ten different policies…".
"We need a lack of ambiguity that is also directed towards parents, so that everybody knows what can be expected."
"Make healthcare a bit more consistent. Not only inwards, but also outwards. I think that's pretty important."
"The collective expertise that such a group can bring is of course worth a lot."
"I always like to hear that other people do things completely different, while the results are almost the same. That puts things into perspective that we actually don't know yet. That you can do different things, but that you don't necessarily do it wrong if you do it slightly differently."
"If I compare it to when I was still in training, when those national protocols were not there yet, I think this is a huge improvement, that you also have the feeling that you have agreed nationally: that's how we do it. And so, you get fewer differences among the NICUs."
Quotations from fellows about the advantages of the guidelines
"It is very nice if you can achieve a basic level in a short time of what is good evidence-based care."
" 'And look! It is here in our national protocol!' Then you are in a bit stronger position."
"And if it's in the protocol, you think okay. This is the protocol, I have come that far. And then I can do that and then you can sometimes also give it your own twist. So, I think that you actually learn how to work independently. I like that about the protocols."
"They give a bit more completeness. After a whole shift, and you think "Oh, wait a minute", because it can be so hectic that you sometimes forget things and then you can walk through them, a sort of checklist or something."
"And they are usually beautiful protocols, I think, with a lot of background information. For me, when I started as a fellow, I was really happy with those protocols."



NICU –Neonatal intensive care unit; GRADE – the graing of guidelines assessment development and evaluation.
[a] all fellows and neonatalogist working at Dutch level 3-4 NICUs.

Web Fig. 1 Flow chart of the development process of the guidelines.



[a] Neonatal intensive care unit (NICU); [b]Percentages of total fellows and neonatologists working at that NICU.

Web Fig. 2 Distribution of participants in the survey and interviews.

Web Table I Perceived Facilitators and Barriers, and Illustrative Quotations During the Survey of Neonatologists in the Netherlands

Personal factors			
	Facilitator	*Barrier*	*Illustrative quotation*
Awareness	- Key person at NICU[a] who drives the local implementation - Guidelines are part of standard local training program for new employees - Integration of guidelines into local protocol system	- Difference in local and national guidelines is unclear - Guidelines aren't integrated into local protocol system	*"If you have a key person who is active and who plays an active role in the assessment... And helped again on time, guys, we still have to look at it. If that is not there, I think it will be much more difficult"*
Familiarity	- Regularly planned plenary sessions with the department - Mentioning of guidelines during rounds and morning report	- Not standard part of plenary sessions - Not mentioned during morning report - Guidelines aren't integrated in national fellow training days	*"During the training days, there is no word of the guidelines. So, I really have to take it from the field."*
Motivation	- Clear aim and agreement with the aim of the guidelines - Curiosity for the way others work	- Too many protocols - No agreement with aim of guidelines - Lack of agreement with the content	*"If you would issue new guidelines and we do something completely different here, I think it will take a little longer before we adhere to the guidelines."*
Self-efficacy	- Being part of development - Taking part in feedback round	- No response to given feedback	*"When you are more actively involved in developing a guideline, if only in refining it, then you can support it more easily."*
Guideline related factors			
	Facilitator	*Barrier*	*Illustrative quotation*
Access to guidelines	- App or website where guidelines can be found - Being integrated in local protocol system	- Not integrated in local protocol system - No app or site available	*"I think it would be very useful if there could be a sort of app for certain subjects, that you could find a flowchart or something on your phone, possibly offline. Imagine, I'm in an ambulance somewhere, and I want to know, what about this or that."*
Clear intervention goals	- Step-by-step plan - Clear parameters to watch and following actions - Useful background information	- Too ambiguous - Chaotic - Information has no practical value/consequences	*"You have an acute problem, and it clearly gives you a perspective on where the problem is now and what you can do. And it makes it easy for you to communicate with each other: 'I have taken these steps, and I can still do this.' So, these are very practical guidelines."*

Contd....

from pre-page

Personal factors			
	Facilitator	*Barrier*	*Illustrative quotation*
Simplicity	- Short guideline - Fast access of practical knowledge	- Too much (irrelevant) text	*"A protocol that is too long is not used."*
Lay-out	- Flowcharts, or entire guideline as flowchart - Bullet points, spaces - Logical structure	- Too much background information for acute problems - No logical structure	*"That protocol just is a flow diagram. And you can't do more than follow those things. That is of course delightful."*
Evidence	- Literature for information in guideline is retraceable - Underlying reason for action is clear - Level of evidence is clear	- Lack of (adequate) references - Quality of guideline is unclear - Guideline is not up to date with latest evidence	*"When you can check which literature they have used, you get an idea how up to date the literature is, who the authors are, if you once have read something from it. You have a much better image of…whether someone has seriously investigated this or whether you're missing some things."*
Subjects of guidelines	- Frequently occurring - Complex situations where consultations or paramedics are needed	- Subject has too many aspects and considerations	*"That is why the apnea guideline is so good. It is of course something we all have to deal with and struggle within the same way, and someone has now finally extensively determined…for what do we have proof and for what we don't, and then made a practical guideline out of it regarding when to intensify treatment or not. That is, to me, the gain."*
External factors			
	Facilitator	*Barrier*	*Illustrative quotation*
Organizational	- Guidelines are part of standard local training program for new employees - Familiarity with guidelines by subspecialists and paramedics - Guidelines are adjusted to logistic specifications of the NICU[a]	- Local research projects - Being a center of expertise on a topic - Adequate materials or specialists are not available	*"It works much easier when you say during consultation, 'We have done this. We looked at the N3recommendations protocol. In that context, I am now consulting with you, because we are now on this point.' That makes it easier for the consultation."*
Other recourses	- Other hospitals work with the same guideline	- Paramedic or subspecialists available	*"That also applies to the MRI and its follow-up. You also need to have the radiologist for that..."*
Existing protocol	- No existing protocol about the subject available - Guideline is of a higher quality then the local protocol	- Existing protocol of good quality	*"If they are concrete, practical and better than the protocol that we already have, or if we do not have a protocol, that would be a reason for us to use the national guideline."*
Consensus in team	- Final goal of guideline is clear for the whole group - The atmosphere of the NICU[a] is open for change	- Employees of the NICU[a] not open for change - A strong opinion that differs from the guideline	*"If its ultimate goal is clear, then it is easier as a group to go that way."*
[a] Neonatal intensive care unit (NICU)			

RESEARCH PAPER

Bacterial Colonization of Home Nebulizers Used by Children With Recurrent Wheeze

NAVKIRAN RANJAN,[1] NIDHI SINGLA,[2] VISHAL GUGLANI,[1] SHIVANI RANDEV,[1] PANKAJ KUMAR[1]
From Departments of [1]Pediatrics and [2]Microbiology, Government Medical College and Hospital, Chandigarh.

Correspondence to:
Dr Shivani Randev, Assistant Professor, Department of Pediatrics, Government Medical College and Hospital, Chandigarh.
Received: October 23, 2021;
Initial review: December 01, 2021;
Accepted: March 14, 2022.

Objective: To look for bacterial colonization of parts of home nebulizers used for children with recurrent wheeze and asthma. **Methods**: Children aged 1 mo-12 y, using home nebulizers for recurrent cough and wheeze were enrolled from May to October, 2019. Caregivers were administered a structured questionnaire by a single researcher, during their hospital visit, to elicit information on their nebulizer cleaning practices. Samples were taken from nebulizer medicine chamber and tubing for bacterial culture and sensitivity. **Results:** Bacterial growth was observed in 17 culture samples obtained from medicine chamber and/or tubing of nebulizers used by 12 (20.3%) out of the 59 enrolled children. The bacteria isolated were *Klebsiella pneumoniae, Pseudomonas aeruginosa and Staphylococcus aureus* (Methicillin resistant *S. aureus* and Coagulase negative staphylococci) and these were resistant to many of the commonly used antimicrobials. Almost 20% parents had never cleaned the nebulizers. Diluent re-use was significantly associated with bacterial colonization of nebulizer parts [AOR (95% CI) 20.6 (2.26-188.5); *P*=0.007]. **Conclusion**: Home nebulizers, if not cleaned properly as per set protocols, may get colonized with potentially harmful bacteria. There is a need to increase awareness about their proper use amongst parents of children with recurrent wheeze.

Keywords: *Asthma, Contamination, Domiciliary, Infection.*

Published online: March 28, 2022; ***PII***: S097475591600414

The home use of nebulizer has expanded in recent times from indications of chronic respiratory diseases like cystic fibrosis to more common conditions like asthma and recurrent wheezing in infants and toddlers [1]. However, not enough evidence is available for their safety for home use. Being medical devices, their use is covered under Infection Prevention and Control guidelines, which recommend that cleaning of these devices should involve removal of drug residues, dirt, and microbes [2]. Protocols have been formulated and published by various professional organizations for the proper use of these devices [2,3]. However, there are inconsistencies amongst the guidelines published. Another challenge is lack of awareness regarding the existence of these protocols amongst manufacturers, health personnel and users. Moreover, much of the home use in otherwise healthy children is based on over-the-counter purchase of these devices [4], so caregivers may not be receiving any instructions on proper usage and maintenance of these devices, setting the stage for colonization with potentially harmful microbes. It has been shown that in the hospital setting, bacterial contamination of nebulizers is clearly associated with nosocomial pneumonia [5-8]. Also, inhalation of aerosols contaminated with gram-negative bacteria generated from home use nebulizers in cystic fibrosis patients have been shown to act as primary route of bacterial colonization of lung [9-11]. Studies in adult COPD (chronic obstructive pulmonary disease) patients using domiciliary nebulizers have shown a high prevalence of bacterial contamination which was difficult to eradicate with the recommended washing methods, possibly due to formation of biofilms, and a higher probability of exacerbations in patients using contaminated nebulizers [12].

Invited Commentary: Pages 365-66.

In children, most of the research work on colonization of home nebulizers has been done in patients of cystic fibrosis; whereas, almost 50% of children under six years suffer from at least one episode of wheeze [13]. As use of domiciliary nebulizers in this group of children is common [4], we conducted this study to ascertain their safety so that remedial measures can be undertaken.

METHODS

This descriptive study was conducted in the outpatient department of a tertiary care hospital after approval by institutional ethics committee. Between May to October, 2019, caregivers of children aged one month to 12 years presenting with history of recurrent cough, wheeze and asthma were asked about use of home nebulization. Eligible

children were enrolled after taking parental consent. Patients diagnosed as cystic fibrosis, primary ciliary dyskinesia, immunodeficiency disorders and those who had been diagnosed to have pneumonia within last four weeks were excluded from the study. Caregivers of all enrolled children were asked to bring their devices along with tubing and medicine chamber at next visit. The equipment was examined for visible dirt and moisture; samples were collected from the nebulization chamber by rotating a swab moistened with sterile saline, and from tubing by flushing it with saline collected in sterile container. The collected samples were sent immediately to the microbiology laboratory. These were processed as per standard protocol on liquid medium, Brain heart infusion broth (BHIB) and solid medium, blood agar and MacConkey agar. All the cultures were incubated at 37^0C for 24 hours. BHIB was observed for turbidity next day and if turbid, sub-culturing was done on solid media. All the isolates obtained were identified as per micro-biological techniques. The antibiotic sensitivity was done as per CLSI (Clinical and Laboratory Standards Institute) guidelines [14]. The parents were administered a questionnaire on device cleaning and maintenance procedures being followed by them.

Considering the expected contamination rate as 65%, similar to a previous study [15], assuming 95% confidence interval and 13% deviation for absolute precision, sample size of 52 was determined.

Statistical analysis: Comparison of various parameters between devices colonized and those not colonized with bacteria was done with Chi-square or Fisher exact test for categorical variables. Significance was defined as *P* value <0.05. Analysis was done using Stata/MP 14.0 software.

RESULTS

A total of 59 patients (66% boys) were enrolled in this study, majority (72.8%) of whom were younger than 6 years [median (IQR) age, 80 (55-108) months] (**Table I**). All enrolled patients were using jet nebulizers, 38.9% for more than a year. At least one previous episode of fever and fast breathing with wheeze was reported by 21 (35.6%), of which 10 required hospitalizations, but it was unclear whether the episodes qualified as pneumonia or just a viral infection associated with wheeze. Eleven (19%) parents used nebulizers on advice of friends/family, while the rest were advised by a doctor; out of which only 15 (31.2%) received cleaning instructions from the advising physician.

Reuse of the same medicine bottle beyond one day was seen in 12 (20.3%) patients and never in 42 (71.3%). Out of these, five had re-used the bottle during the same episode of wheezy illness while the remaining had reused the bottle opened for the previous episode. Normal saline was used as diluent for medications by 25 patients, while one used tap water. Most of those who cleaned the nebulizer (*n*=12), used water alone or soap and water to wash the tubing and chamber.

Out of a total of 118 samples, 17 had positive bacterial culture from 12 (20.3%) nebulization sets (6 samples from medicine chamber, 11 from nebulizer tubing). We found a predominance of gram-negative bacteria on culture, out of which the most frequent isolates were *Klebsiella pneumoniae* (*n*=6/17) and *Pseudomonas aeruginosa* (*n*=5/17). Most isolates were resistant to third generation cephalosporins while being sensitive to amikacin, piperacillin-tazobactam and imipenem. Other isolates included *Staphylococcus* aureus, Methicillin resistant

Table I Characteristics of Children With Recurrent Wheeze Using Home Nebulizers (*N*=59)

Characteristic	*No. (%)*
Father's education (below 10th standard)	7 (12)
Mother's education (below 10th standard)	11 (19)
Child nebulized by	
Mother	35 (59)
Father	7 (12)
Both parents	10 (17)
Relative	7(12)
Nebulizer use advised by	
Doctor	48 (81)
Friends and relatives	11 (19)
Read the instruction manual	17 (28.8)
Re-use of diluent bottle, [a]*n*=25	
Never	8 (32)
Beyond 1 day	17 (68)
Re-use of syringe,[b]*n*=18	
Never	1 (5.6)
Within same day	1 (5.6)
Beyond 1 day	16 (88.9)
Cleaning of nebulizer	
After every use	32 (54.3)
Occasionally	15 (25.4)
Never	12 (20.3)
Part of nebulizer cleaned, *n*=47	
Only mask	21 (44.7)
Mask and medicine chamber	15 (31.9)
Mask, medicine chamber, tubing	11 (23.4)
Drying the tube after use	
After every use	39 (66)
Sometimes	13 (22.2)
Never	7 (11.8)
Handwashing before nebulization	
Before every use	46 (78)
Sometimes	12 (20.3)
Never	1 (1.7)

[a]*None reused diluent within the same day;* [b]*only 18 used syringes.*

S. aureus, Coagulase negative staphylococci and *Citrobacter koseri*.

The only significant associations of colonization of nebulizer device were found with 'past history of fever with fast breathing' and diluent re-use on univariate analysis, and only diluent re-use [AOR (95% CI) 20.6 (2.26-188.5); *P*=0.007] on multivariate analysis.

DISCUSSION

It was observed that a high proportion of nebulizer devices used for home nebulization in healthy children with recurrent wheeze were colonized with potentially pathogenic bacteria, most of which were resistant to several commonly used antimicrobials. The spectrum included organisms usually associated with nosocomial infections, despite only domiciliary use of these devices.

The current study is one of the first attempts to learn about the nebulizer use and cleaning practices in otherwise healthy children in our country. The limitation of the study is that we have only looked for bacterial colonization, whereas fungi may also colonize the damp parts of the device. Another limitation is that this study does not conclusively tell us whether colonization of the device resulted in colonization of the patients' respiratory passages or in increased risk of respiratory infections.

High rates of bacterial colonization of home nebulizer devices have been reported earlier. Barnes, et al. [16] observed bacterial contamination in 35% home nebulization units, while Cohen, et al. [15] reported a rate of over 65%, figures much higher than the 20% rate observed by us, but with a similar spectrum of microbes being isolated. Both studies had few differences which could have accounted for a higher colonization rate reported by them, like composition of patient population and sites of sampling for culture.

A nebulizer generates very small particles (1-3 microns) which reach the terminal bronchioles and alveoli and may cause delivery of microbes to distal airways, if the device is contaminated. In children, this may result in respiratory infections due to an immature immune system. Whether this risk is real, needs to be evaluated by well-designed studies on this aspect, but more important is the need to generate awareness about balanced decision regarding the appropriate home-use device, and proper use and care of the nebulizers used at home.

Ethics clearance: IEC, GMCH, Chandigarh; No. GMCH/IEC/2019/348, dated May 29, 2019.
Contributors: NR, SR: planned the study and wrote the draft. NR,SR,NS: collected data; NR,SR,NS,PK,VG: involved in data interpretation. All authors approved the final draft and are accountable for for all aspects related to the study.
Funding: Short Term Studentship (NR), Indian Council of Medical Research (2019-02055); *Competing interest*: None stated.

REFERENCES

1. Global Inhalation Therapy Nebulizer Market Analysis, Trends, Size, and Forecasts up to 2024 - ResearchAnd Markets.com. Business Wire. Accessed December 1, 2021.
2. Boe J, Dennis JH, O'Driscoll BR, et al. European Respiratory Society Guidelines on Use of Nebulizer. Euro Resp J. 2001;18:228-42.
3. Ghoshal AG, Salvi S, Dhar R, et al. Consensus Document on Home Nebulization for Maintenance Treatment of Obstructive Airway Diseases: A Joint Initiative by the National Allergy Asthma Bronchitis Institute (NAABI) and Chest Research Foundation (CRF). J Assoc Phys India. 2017;65:60-73.
4. Kahlon GK, Pooni PA, Bhat D, et al. Role of montelukast in multitrigger wheezers attending chest clinic in Punjab, India. Pediatr Pulmonology. 2021; 56: 2530- 2536.
5. Mertz J, Scharer L, McClement JH. Hospital outbreak of Klebsiella pneumonia from inhalation therapy with contaminated aerosol solution. Amer Rev Resp Dis. 1967;95: 454-60.
6. Grieble HG, Colton R, Bird TJ, et al. Fine particle humidifiers-Source of *Pseudomonas aerugenosa* infections in respiratory disease unit. N Eng J Med. 1970;282:531-5.
7. Arnow M, Chou T, Weil D, et al. Nosocomial Legion-naires' disease caused by aerosolized tap water from respiratory devices. J Infect Dis. 1982;146:460-7.
8. Roberts FJ, Cockcroft WH, Johnson HE, Fishwick T. The infection hazard of contaminated nebulizers. Can Med Assoc J. 1973;108:53-6.
9. Hutchinson GR, Parker S, Pryor JA, et al. Home use nebulizers: A potential primary source of *Berkholderia cepacia* and other colistin–resistant gram-negative bacteria in patients with cystic fibrosis. J Clin Microbiol. 1996;34: 584-7.
10. Vassal S, Taamma R, Marty N, et al. Microbiologic contamination study of nebulizers after aerosol therapy in patients with cystic fibrosis. Am J Infect Control. 2000; 28:347-51.
11. Blau H, Mussaffi H, Mei Zahav M, et al. Microbial contamination of nebulizers in the home treatment of cystic fibrosis. Child Care Health Dev. 2007;33:491-5.
12. Jarvis S, Ind PW, Thomas C, et al. Microbial contamination of domiciliary nebulizers and clinical implications in chronic obstructive pulmonary disease. BMJ Open Resp Res. 2014;1:e000018.
13. Martinez FD, Wright AL, Taussig LM, et al. Asthma and wheezing in first six years of life. N Eng J Med 1995;332: 133-8.
14. Clinical and Laboratory Standards Institute . Performance Standards for Antimicrobial Susceptibility Testing 2013. Clinical and Laboratory Standards Institute: 2013. Twenty third informational supplement M100-S23.
15. Cohen HA, Kahan E, Cohen Z, et al. Microbial colonization of nebulizers used by asthmatic children. Pediatr Int. 2006;48:454-58.
16. Barnes KL, Murphy D. Bacterial contamination of home nebuliser. Br Med J (Clin Res Ed). 1987:295:812.

RESEARCH PAPER

Mutational Spectrum of the *CFTR* Gene in the Kazakhstan Population

MUNIRA BULEGENOVA,[1] MILAN MACEK,[2] MALGORZATA LIBIK,[3] ANARA IMANGALIYEVA,[4] ANNA MAKHNEVA[1]

From the [1]Laboratory Department, and [4]Department of General Somatic, Scientific Center of Pediatrics and Children Surgery, Almaty, Kazakhstan; [2]National Center for Cystic Fibrosis, Prague,Czech Republic; [3]Department of Biology and Medical Genetics, Motol University Hospital, 2nd Faculty of Medicine, Charles University in Prague, Czech Republic.

Correspondence to:
Dr Munira Bulegenova,
Laboratory Department, Scientific Center of Pediatrics and Children Surgery, Almaty, Kazakhstan.
mbulegenova@yandex.kz.
Received: July 01, 2021;
Initial review: September 20, 2021;
Accepted: February 12, 2022.

Objective: To study the frequency and spectrum of *CFTR* gene variants in different ethnic groups of Kazakhstan. **Methods:** We reviewed the records of 58 patients with cystic fibrosis. All the patients underwent molecular genetic analysis to reveal genotype-phenotype correlations. **Results:** The median (IQR) age of the patients was 5.4 year (7 months, 18 year); 40% were diagnosed at the age of 5-10 year. The study identified 28 specific variants: p.Phe508del, the variant most common in the European population, was detected in 30 patients (51.7%). Variants other than p.Phe508del were revealed in 31% (21 patients). **Conclusions:** We found a number of specific variants characteristic of the Kazakhstani population. A pronounced regression of disease symptoms was detected in patients with mild mutations; whereas in patients with severe mutations, therapy produced very little effect.

Keywords*: p.Phe508del, Pancreatic elastase, Pseudomonas aeruginosa, Sweat test*.

Published online: March 10, 2021; ***PII***: S097475591600409

Cystic fibrosis (CF) is a monogenically inherited autosomal recessive disorder characterized by the presence of mutations in *Cystic fibrosis transmembrane conductance regulator (CFTR)* gene [1-3]. There are more than 2000 known variants of the CF gene- the following variants are most common in the European population: p.Phe 508del (52.8%), p.Ser18ArgfsX16 (6.3%), p.Glu92Lys (2.6%), p.Gln685Thrfs (2%), 3849+10kbC>T (1.6%), p.Ser670_Leu671insTer (1.6%), p.Gly542Ter (1.3%), p.Asn1303Lys (1.3%), p.Trp1282Ter (1.1%), p.Leu138dup (1.1%) [4]. Incidence of cystic fibrosis varies in different populations of the world; varying from 1:1800 newborns in Ireland, to 1:9000 in Africa, and 1:3,50,000 in Japan. These data indicate differences in the population gene pool of Europe, Asia and Africa [5]. There is minimal data on the frequency and spectrum of *CFTR* gene variants in different ethnic groups of Kazakhstan.

A study of the CF variants spectrum most specific to a population helps to identify groups at increased risk of cystic fibrosis, conduct timely measures to prevent secondary complications, optimize the therapeutic algorithms and, consequently, ensure a more favorable prognosis of the disease. Thus, the purpose of this study was to analyze the CF variants most specific to the Kazakhstani population.

METHODS

We reviewed the records of patients with cystic fibrosis, based on clinical symptoms, undergoing treatment at the Scientific Center of Pediatrics and Pediatric Surgery at Almaty, Kazakhstan between 2016-2019. The diagnosis was confirmed by sweat test (sweat chloride concentration >80 mmol/mL) and decreased levels of stool pancreatic elastase. To study genotype-phenotype correlations and the possible effect of identified variants on the aspects of the disease, all the patients underwent molecular genetic analysis at the Molecular Laboratory of the Department of Biology and Human Genetics of Motol University Hospital, Prague, Czech Republic. The patients went through a so-called 'cascading' approach in molecular genetics diagnostics, which involves identifying gene variants in several stages of testing – starting from the most common variants and gradually moving on to rarer ones. First, the most common variants of the *CFTR* gene were examined using the commercial Elucigene CF-EU2 kit (Longwood Diagnostics), which permits simultaneous detection of 50 mutations (supple-mentary material). Then, additional multiplex ligation-dependent probe amplification (MLPA) was performed for identifying extensive intragenic rearrangements i.e., deletions or deletions by duplication of one or more exons within the entire *CFTR* gene, which are not detectable by conventional PCR-based methods. Finally, MPS-based analysis of the entire *CFTR* coding region, adjacent splice site junctions and several introns was done using a locus-specific library preparation assay (CFTR NGS assay; Devyser) and MPS sequencing was performed on

MiSeqSystem (Illumina). Bioinformatic analysis was carried out using the SOPHiA platform for hereditary disorders (*www.sophiagenetics.com*). Positive cases were confirmed by targeted Sanger DNA sequencing on ABI 3130xl DNA Analyser (ThermoFisher). MLPA analysis of intra-*CFTR* rearrangements and copy number variation was performed using the SALSA MLPA P091 CFTR Assay followed by the analysis of raw data on the proprietary software Coffalyser.Net (MRC-Holland).

RESULTS

We studied the data of 58 children (52% boys) with CF with a median (IQR) age of 5.4 year (7 month, 18 year) (range 3 month – 18 year). Majority of the patients (46.6%) were between 1 year and 5 year of age. Of the remaining, 16 (27.6%) were infants and 13 (18.9%) were between 5-10 years of age. Majority (55%) of patients were ethnic Kazakhs or Russians (35%). Pulmonary form of the disease was seen in 15 patients, 10 patients had intestinal manifestations, and the rest of the children were diagnosed with a mixed form of the disease.

In 88% of cases (51 patients), variations associated with CF were identified, whereas in the remaining children (*n*=7, 12%) no variations were detected, and the diagnosis was based on high sweat chloride levels, pancreatic insufficiency, low levels of stool pancreatic elastase, and presence of *Pseudomonas aeruginosa* in culture.

We identified 28 variants specific to cystic fibrosis. p.Phe508del, the variant most common in the European population, was detected in 30 patients (51.7%) (9, homozygous state and 21, heterozygous state). Variants other than this were found in 21 (41.2%) patients (3 in homozygous state).

In patients with p.Phe508del variant (homozygous or compound) *P. aeruginosa* was found in culture more commonly than in patients with other variants (46.5% vs 21.6%; *P*=0.04). Moreover, 66.7% of patients homozygous for p.Phe508del and positive for *P. aeruginosa* were infants. Secondary complications in the form of bronchiectasis in patients with p.Phe508del (homozygous or composite) were detected more commonly (21,70%) than in patients with other mutation (14, 50%) (*P*=0.12). However, there was no difference in frequency of fibrotic changes in lung tissue between the two variants.

Of the identified variants, 21 (75%) were classified as severe mutations, whereas the remaining 25% (*n*=7) were ranked as mild (supplementary material). The following variants, not common to the European population, were identified in the study: p.Arg553X, c.2818_2819delAC, c.185G> T, p.Tyr515_Arg516delinsTer, p.Val392Gly, c.488A>C, p.Tyr1092His, p.Gln290Ter, c.4111_4113dupGAA (severe mutations) and, mild mutations (c.2491G>T, p.Ser1235Arg).

p.Tyr515_Arg516delinsTer variant was detected in four patients (1 homozygous). This variant had originated from the Caucasus geographic region [6], but despite its occurrence in many other regions of Russia, it is mainly found in Georgians, Megrels and Chechens [4,7,8]. Some of the patients in the study were ethnic Kazakhs, but since Kazakhstan is a mult-ethnic country with a wide prevalence of inter-ethnic marriages, the probability of transfer of this mutation to the Kazakh population is high. In patients with the heterozygous variant, the disease develops in a more severe form, most likely due to it often being a compound with one of the other severe variants. P.Trp1282Arg variant was identified in one case in the study – in an ethnically Russian child; although, this variant is reported to occur in Ashkenazi Jews [9].

Patients with mild variants, either in the homozygous or compound/heterozygous state, had complete absence of steatorrhea or moderate steatorrhea; moderate pancreatic elastase concentrations accompanying mild pancreatic dysfunction; no *P. aeruginosa* in culture, and significantly fewer gastrointestinal complications.

We identified Å92Ê (p. Glu92Lys, c.274G>A) and p.Gln290Ter variants, each of them in homozygous state, for the first time in the Kazakhstani population; of which, p.Glu92Lys is reported to be prevalent among the Turkish people.

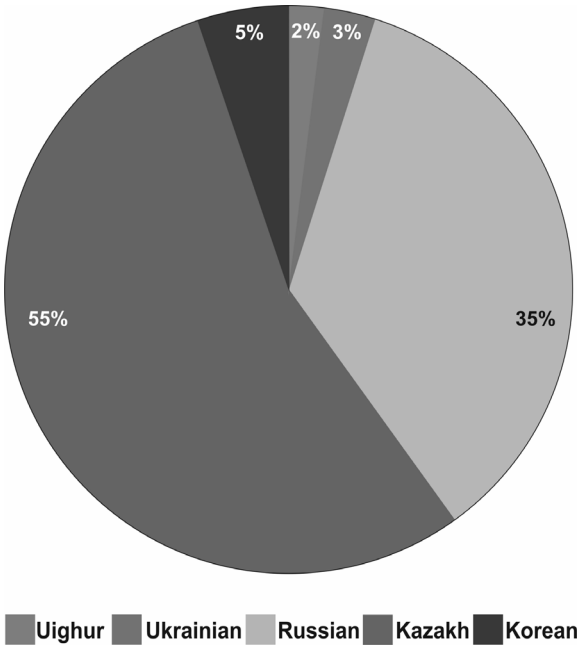


Fig.1 Distribution by ethnicity.

WHAT THIS STUDY ADDS?

- Mutational spectrum of *CFTR* gene in Kazakhstan population revealed a member of specific variants characteristic to this population and not common in the European population.

Analysis of different gastrointestinal complications showed that milder genetic variants were mostly associated with fewer complications like liver cirrhosis or diabetes. In seven (12%) patients with unidentified variants of the *CFTR* gene, the disease manifested itself mainly through pulmonary symptoms with only slow detrimental effect on other organs.

Data on bacterial infection showed that in infants, *Staphylococcus aureus* was seen in 51% and *H. influenzae* in 33%, while pneumococcus was less prevalent (16%). Thus, the pulmonary form prevailed among clinical manifestations despite the diagnosed mixed form. However, as the disease progressed, the frequency of *P. aeruginosa* colonization steadily increased in patients. Twenty five patients (43%) were carriers of *P. aeruginosa*, whereas three other patients (12%) with bronchiectasis had an intermittent form, which is more favorable due to its milder symptoms and less accumulation of sputum. The remaining 22 patients were found to be chronic carriers who did not respond to treatment (*P. aeruginosa* was constantly present in culture).

Residual wheezing in the lungs was mainly observed in patients with severe variants and with p.Phe508del (homo/heterozygous states), while in children with identified mild variants, residual wheezing was not observed. In children with unspecified variants, residual wheezing persisted in 43% of cases. Following therapy, steatorrhea was still present in most children with severe variants and with p.Phe508del in heterozygous state; whereas in patients with the homozygous variant, steatorrhea was found only in 55%. No steatorrhea was observed in children with mild variants of the gene. In the group of children with unidentified variants, steatorrhea persisted only in one patient.

DISCUSSION

In Kazakhs (32 patients), the p.Phe508del variant was identified in 40.6% (13 patients), while among the representatives of the Russian ethnic group (20 patients) it was seen in 70% of cases (14 patients). According to the available literature, this deletion in codon 508 is most common among Europeans and is much less frequent among Asians [10]. Alibakhshi, et al. [11] analyzed a cohort of Iranian patients, and detected p.Phe508del in 18/1% cases [11], while in the Turkish population, it was detected in 23% [12]. The frequency of p.Phe508del reaches about 60% in Pakistani CF patients, but is much lower in Indian (about 20%) and Japanese patients (about 10%) [13]. In 36 Asian CF patients in the United Kingdom (26 Southern Asians and 10 Central Asians), 26% were homozygous for p.Phe508del [5]. Unfortunately, there is limited information available from most Asian countries. Genetic analysis revealed no p.Phe508del variants in patients of Chinese nationality [6]. It may also be assumed that a fairly high percentage of p.Phe508del variants detected in the Kazakhstani population may be due to a large number of inter-ethnic marriages.

It may be inferred that the severe mutation causes early secondary complications of the respiratory system in children under the age of 1 year due to the poor immune system and poor resistance to external infectious agents. In contrast to the data from other countries [14,15], wherein the first manifestation of the disease in patients homozygous for p.Phe508del is in the second year of life and in the third year in heterozygotes, the present study did not reveal any significant age differences in the first manifestation of symptoms of cystic fibrosis.

We feel that this study of the CF-related variant spectrum most specific to the Kazakhstani population will help to identify groups at increased risk of cystic fibrosis and take timely measures to prevent secondary complications, optimize the therapeutic algorithms and, consequently, ensure a more favorable prognosis of the disease and improve the life expectancy of the patients.

Ethics clearance: Scientific center of Pediatrics and Pediatric Surgery; Protocol No. 2, dated May 05, 2021.
Contributors: MB,ML,AI: conception and design, acquisition of data, analysis and interpretation of data; MM: final approval of the version to be published; AM: drafting of the manuscript, critical revision of the manuscript for important intellectual content; MB: agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All authors approved the final version of manuscript, and are accountable for all aspects related to the study.
Funding: Vertex grand scheme- CG-2015-104643; *Competing interest*: None stated.
Note: Additional material related to the study is available with the online version at *www.indianpediatrics.net*

REFERENCES

1. ManamiIso, Suzuki M, Yanagiet K, et al. The CFTR gene variants in Japanese children with idiopathic pancreatitis. Human Genome Variation. 2019:6;17.
2. Golubtsova OI, Krasovskiy SA, Kozhevnikova SL, et al. Clinical characteristics of respiratory involvement in children with cystic fibrosis in the Chuvash republic. Issues of Modern Pediatrics. 2012:11;54-59.
3. Gembitskaya TE, Ivashchenko TE, Chermensky AG, et al. Phenotypic characteristic and genetic heterogeneity in patients with late-onset unclassical cystic fibrosis. Pulmo-nologya. 2014;1:66-70.
4. Mogayzel PJ, Naureckas ET, Robinson KA. Cystic fibrosis pulmonary guidelines. Am J Respir Crit Care Med. 2013: 187;680-89.
5. Callaghan BD, Carr S, Balfour-Lynn I. et al. Do asian cystic fibrosis patients have more severe disease than their caucasian peers in childhood? Pediat Pulm. 2002;24;329-29.
6. Cordovado SK, Hendrix M, Greene CN, et al. CFTR mutation analysis and haplotype associations in CF patients. Mol Genet Metab. 2012:105;249-54.
7. Dodge JA, Brock DJH, Widdicombe JH. Cystic fibrosis. Curr Topics. 2009: 370p.
8. Lahiri T, Hempstead SE, Brady C, et al. Clinical practice guidelines from the cystic fibrosis foundation for preschoo-lers with cystic fibrosis. Pediatrics. 2017:137;e20151784-e20151784.
9. The molecular genetic epidemiology of cystic fibrosis: report of a joint meeting of WHO/IECFTN/ICF(ýM)ýA/ECFS, Genoa, Italy, 19 June, 2002.
10. Alibakhshi R, Kianishirazi R, Cassiman JJ, et al. Analysis of the CFTR gene in Iranian cystic fibrosis patients: Identification of eight novel mutations. J Cyst Fibros. 2008; 7:102-9.
11. Bonyadi M, Omrani O, Rafeey M, et al. Spectrum of CFTR gene mutations in Iranian Azeri Turkish patients with cystic fibrosis. Genet Test Mol Biomarkers. 2011;15: 89-92.
12. Wang W, Okayama H, Shirato K. Genotypes of cystic fibrosis (CF) reported in the world and polymorphisms of cystic fibrosis transmembrane conductance regulator (CFTR) gene in Japanese. Nihon Rinsho (Japanese Journal of Clinical Medicine). 1996;54:525-32.
13. Zilfalil BA, Sarina S, Liza-Sharmini AT, et al. Detection of F508del mutation in cystic fibrosis transmembrane conductance regulator gene mutation among Malays. Singapore Med J. 2006;47:129-33.
14. P Farrell 1 , S Joffe, L Foley, G J Canny, P Mayne, M Rosenberg. Diagnosis of cystic fibrosis in the Republic of Ireland: epidemiology and costs. Ir Med J. 2007;100:557-60.
15. Isa HM, Al-Ali LF, Mohamed AM. Growth assessment and risk factors of malnutrition in children with cystic fibrosis. Saudi Med J. 2016;37:293-98.

ADVERTISEMENT

IAP NEONATOLOGY FELLOWSHIP

Department of Pediatrics
Goa Medical College, Bambolim, Goa

The neonatal unit of Dept of Pediatrics, Goa Medical College offers an opportunity to do IAP Neonatology Fellowship in the 40 bedded tertiary level Neonatal intensive care unit. This is a centre providing high risk perinatal care with 5000 deliveries per year and intensive care facilities like high frequency and conventional ventilation, CPAP, parenteral nutrition, etc with all invasive procedures being done during care of the newborns. We have trained faculty and excellent academics with support from other specialist units like Pediatric surgery, Radiology and Cardiology. We also run a high risk follow up clinic with full fledged neurodevelopment centre run by a developmental Pediatrician.

No of seats available : Two
(MD/DNB – 1 year, DCH – 1 1/2 year)
Last date of application : 15th June, 2022.
Entrance exam and interview : 9 AM to 5 PM, 17th June, 2022.
Selected candidates will start course : 1st July, 2022.
(Stipend and fees according to Institute policy)

Send your CV/Application to :

Dr. Kavita Sreekumar (MD), Course Coordinator
Associate Professor, Department of Pediatrics,
Goa Medical College, 403202
Mobile: 9422454850 Email: *kantakum@yahoo.com*

RESEARCH PAPER

Vitamin D Levels and Cardiopulmonary Status in Infants With Acute Bronchiolitis

ANA ESTALELLA-MENDOZA,[1,4] ANA CASTELLANO-MARTÍNEZ,[2,4] JOSE CARLOS FLORES-GONZÁLEZ,[1,4] LORENA ESTEPA-PEDREGOSA,[1,4] PATRICIA RODRÍGUEZ-CAMPOY,[1,4] MOISÉS RODRÍGUEZ-GONZÁLEZ[3,4]

From [1]Pediatric Intensive Care Unit, [2]Pediatric Nephrology Division, and [3]Pediatric Cardiology Division; and [4]Biomedical Research and Innovation Institute of Cádiz (INiBICA) Research Unit; Puerta del Mar University Hospital, Cádiz, Spain.

Correspondence to: Dr Ana Castellano-Martínez, Pediatric Nephrology Division, Puerta del Mar University Hospital, Cádiz, Spain. anacastellanomart@gmail.com
Received: July 23, 2021; Initial Review: August 26, 2021; Accepted: February 28, 2022.

Background: To assess association of vitamin D deficiency with cardiac and pulmonary status in infants with acute bronchiolitis. **Methods**: Infants hospitalized with acute bronchiolitis were enrolled and classified as those with serum 25-hydroxyvitamin D (25-OHD) below or equal and above 20 ng/mL. The primary outcomes were cardiopulmonary involvement defined by elevation of NT-ProBNP, alteration of echocardiographic parameters and respiratory support requirements. The secondary outcomes were the need for PICU admission and duration of hospitalization. **Results**: 92 (50 males) infants with median (IQR) age of 1 (0.5-3) month were included with median (IQR) serum 25-OHD level 27.4 (11.4-40.3) ng/mL. 43 (47%) patients had serum 25-OHD level below 20 ng/mL with left ventricle dysfunction (*P*=0.008), right ventricle dysfunction (*P*=0.008) and pulmonary hypertension (*P*=0.007) on echocardiography more commonly than those with serum 25-OHD ≥20 ng/mL. The median (IQR) serum NT-ProBNP levels were higher in those with low 25-OHD levels than normal 25-OHD levels [2232.2 (461.4-4313.3) and 830.4 (312.7-2579.5)], respectively *(P*=0.003). Low 25-OHD levels were associated with increased risk for PICU admission (OR 3.9 (95% CI 1.5-10.1); *P*=0.004), higher rates of non-invasive ventilation (*P*=0.048) and mechanical ventilation (*P*=0.005) and longer duration of hospitalization (*P*=0.015). **Conclusion:** Low serum vitamin D level was associated with clinical severity and impaired cardiac and pulmonary status in infants with acute bronchiolitis.

Keywords: *Heart failure, NT-Pro BNP, Troponin, Vitamin D deficiency; Ventricular dysfunction.*

Published online: March 10, 2021; ***PII***: S097475591600413

Acute bronchiolitis is a respiratory infection commonly caused by Respiratory syncytial virus (RSV). It is the most frequent cause of hospitalization in infants and treatment is mainly supportive [1].

Recent evidence suggests that vitamin D has immunomodulatory properties that attenuates the intensity of the inflammatory response in acute respiratory tract infections [2,3]. Hypovitaminosis D is associated with heart failure, ventricular dysfunction and pulmonary hypertension in chronic diseases such as diabetes, congenital heart disease and chronic kidney disease [4,5]. Recently, we observed that cardiac dysfunction and pulmonary hypertension could predict adverse outcomes in infants hospitalized with acute bronchiolitis [6,7]. Therefore, we hypothesize that a plausible relationship could exist between vitamin D deficiency and altered cardiovascular status.

The purpose of this study was to explore the association between serum 25-hydroxyvitamin D (25-OHD), pulmonary hypertension status and myocardial dysfunction, and worse clinical outcomes in infants with acute bronchiolitis.

METHODS

This observational study evaluated infants at the pediatric department of a tertiary university-affiliated hospital in Spain, who were admitted with acute bronc-hiolitis between October 1, 2018 and March 31, 2020.

The study was approved by the Institutional Review Board. Informed written parental consent was obtained for all cases before inclusion in the study. Eligible infants were diagnosed and treated as per standard guidelines [1]. Patients previously diagnosed with malnutrition, chronic heart disease, pulmonary, kidney, endocrine and metabolic diseases, a previous episode of acute bron-chiolitis, and with incomplete medical records were excluded.

All patients underwent clinical, laboratory, microbiologic and echocardiography evaluation within the first 24 hours of admission. Biochemical data including serum 25-

hydroxyvitamin D (25-OHD) calcium, phosphorus and N-terminal pro hormone B-type natriuretic peptide (NT-proBNP) levels were recorded. Serum 25-OHD levels of less than 20 ng/mL was defined as hypovitaminosis D.

Bronchiolitis score of Sant Joan de Déu hospital (BROSJOD) was used to assess the respiratory state severity at admission [8]. A BROSJOD score greater than 10 points was indicative of a severe clinical state. Polymerase chain reaction (PCR) analysis of naso-pharyngeal swabs for respiratory viruses was routinely analyzed in all patients. Pediatric intensive care unit (PICU) admission was defined by the need of advanced respiratory support or presence of apneas.

Standard techniques to obtain M-mode, two-dimensional and Doppler (colour, pulsed, continuous and TDI) echocardiograms were performed by a single experienced pediatric cardiologist, who was blinded to the patient's clinical profile. Images were obtained using a Phillips IE33 ultrasound scanner with an 8 or 12-MHz sectorial transducer. All echocardiographic measurements represent the average of three beats.

The primary outcome was the identification of cardiovascular involvement defined by elevated levels of NT-ProBNP and/or detection of myocardial dysfunction or pulmonary hypertension on echocardiography. Secondary outcomes were the need for PICU admission and the duration of hospitalization. Due to the skewed distribution of NT-ProBNP, we carried out a base 10 logarithmic transformation to achieve normal distribution, and this value was used in the statical analysis.

Statistical analysis: Statistical analyses were performed using Stata software (StataCorp. 2014. Stata Statistical Software: Release 14). Continuous data were presented as median (IQR) or mean (SD) after testing for normality with the Shapiro-Wilk test. Categorical data were presented as frequencies and percentage. The comparison of mean was performed using Student t-test or Wilcoxon Mann-Whitney test as appropriate. Proportions were compared using the Chi-square test or exact methods. Pearson or Spearman coefficients were used to assess correlations between continuous data. A P value <0.05 was considered statistically significant.

RESULTS

During the study period, 149 infants were assessed for

Table I Baseline Characteristics and Outcome of Infants With Bronchiolitis

Variable	*Serum Vitamin D level*		
	<20 ng/mL (n= 43)	*>20 ng/mL (n=49)*	*P value*
Age,[a] mo	1 (0.5-1)	2 (1-4)	< 0.001
Weight,[a] kg	4.5 (3.6-5.3)	5.5 (4-6.2)	0.006
Comorbidity	9 (21)	10 (21)	0.95
BROSJOD score[a]	8 (5-11)	7 (5-9)	0.18
Respiratory syncytial virus positive	33 (77)	37 (75)	0.97
Bacterial superinfection	3 (7)	4 (8)	0.8
Apneas	5 (12)	0 0.01	
SpO2[a]	97 (88-99)	95 (90-99)	0.83
pH	7.30 (0.06)	7.34 (0.05)	0.003
pCO2[a]	58 (48-63)	46 (41-54)	0.002
Nebulization[a]	20 (46)	33@ (67)	0.1
Antibiotics	3 (7)	4 (8)	0.81
Oxygen (nasal canula)	16 (37)	33 (67)	0.004
High flow nasal canulae	1 (2)	2 (4)	0.653
Pediatric intensive care unit admission	20 (47)	9 (18)	0.003
Pediatric intensive care unit stay,[a] d	6 (4-12)	5.5 (3.5-8)	0.44
Non-invasive ventilation	14 (33)	8 (16)	0.048
Mechanical ventilation	6 (14)	0 (0)	0.005
Duration of hospitalization,[a] d	7 (2-11)	4 (2-6)	0.01

**Data presented n (%) or [a]median (IQR). BROSJOD (Bronchiolitis score of Sant Joan of Deu hospital); LU (Lung ultrasound).*

eligibility of whom 57 were excluded as we had insufficient blood sample, resulting in a study sample of 92 (50 boys, 19 with prematurity). The median (IQR) age was 1 (0.5-3) months. The most prevalent (70%) causative agent was RSV. Twenty nine (32%) patients required PICU admission for a median (IQR) duration of 6 (4-9) days. Twenty two (25%) infants required non-invasive ventilation (NIV), and 6 (6.5%) required mechanical ventilation (MV). **Table I** compares the baseline characteristics as per serum 25-OHD levels.

The median (IQR) 25-OHD levels were 27.4 (11.4-40.3) ng/mL, with hypovitaminosis D seen in 43 (47%) infants. Hypovitaminosis D was associated with increased risk for PICU admission [OR 3.9, 95% CI 1.5-10.1; *P*=0.004]. The echocardiographic measurements and cardiac biomarkers are compared as per vitamin D status in **Table II**. A significant negative correlation was seen between log-NT-pro BNP and 25-OHD levels (r=-0.35, *P*=0.002).

DISCUSSION

In this study, hypovitaminosis D was associated with impairment of myocardial function, increased pulmonary pressures, higher risk of PICU admission for advanced respiratory support, and prolonged hospitalization.

Severe lung involvement can be accompanied by a significant impairment of the cardiovascular status in acute bronchiolitis [9]. Increased troponin I levels and echocardiographic measures indicative of pulmonary hypertension and right ventricular diastolic dysfunction have been observed earlier in patients with hypoxia and acidosis [10]. The wide-ranging functions of microRNAs in the cardiovascular system have provided new perspectives on disease diagnostics for a variety of cardiovascular disorders [10].

Cardiac biomarker NT-proBNP is secreted by myocytes in response to increased stress, and it is an accurate diagnostic and prognostic biomarker of heart failure and pulmonary hypertension [11,12]. Notably, we observed that infants with hypovitaminosis D had increased rates of abnormal echocardiographic parameters indicative of pulmonary hypertension and myocardial dysfunction, with increased values of NT-proBNP. Vitamin D exerts its action through the vitamin D receptor, which has also been localized in the cardiovascular system on vascular smooth muscle cells, endothelial cells, and cardiomyocytes. Vitamin D exerts cardioprotective actions and regulates cardiac function by modulating serum and calcium parathyroid hormone levels. Vitamin D also exerts an inhibitory action on the renin-angiotensin-aldosterone axis in vitro, such that hypovitaminosis D can increase the renin levels promoting arterial hypertension, myocardial hypertrophy and raised plasmatic levels of natriuretic peptides [13,14]. Vitamin D deficiency is associated with the development of dilated cardiomyopathy with severe hypocalcemia in infants with nutritional rickets and in the development of pulmonary hypertension [14,15].

It is possible that hypovitaminosis favors a baseline subclinical myocardial dysfunction in infants that could worsen in the acute respiratory setting leading to the development of a severe course of the disease. Nevertheless, the pathophysiology of hypovitaminosis D causing altered cardiovascular status might be different in acute respiratory disease and in chronic cardiovascular setting. The lack of immunomodulatory effect of vitamin D in deficiency states would lead to more severe airway inflammation and subsequent, hypoxemia, and acidosis, leading to raised pulmonary pressures and myocardial dysfunction [14].

Table II Echocardiographic Parameters and Cardiac Biomarkers in Infants With Bronchiolitis

Variable	*Serum vitamin <20 ng/mL (n= 43)*	*D level >20 ng/mL (n=49)*	*P value*
Left ventricle			
FS, %	43 (39-57)	41 (38-55)	0.702
S‘, cm/s	8.5 (7-11)	10 (8-12)	0.030
E´, cm/s	8 (7-10)	10 (9-11)	0.020
A´, cm/s	9 (7-11)	9 (8-11)	0.607
MPI, %	0.48 (0.40-0.63)	0.41 (0.36-0.56)	0.008
Right ventricle			
TAPSE	14 (12-16)	15 (12-16)	0.293
S‘, cm/s	10 (7-11)	11 (8-12)	0.039
E´, cm/s	10 (8-11)	10 (8-11)	0.823
A´, cm/s	9 (7-10)	9 (8-11)	0.100
MPI, %	0.49 (0.41-0.68)	0.40 (0.35-0.58)	0.008
Pulmonary hypertension			
TRJG, mmHg	21 (19-37)	24 (18-37)	0.725
ATET	0.32 (0.08)	0.36 (0.07)	0.007
LVEI	1.2 (1.06-1.41)	1.03 (1-1.27)	0.007
RVLVr	0.58 (0.48-0.68)	0.48 (0.40-0-6)	0.003
Cardiac biomarkers			
NT-proBNP, pg/*mL*[a]	2232.2 (461.4-4313.3)	830.4 (312.7-2579.5)	0.003
Troponin I, ng/L[b]	19.2 (10.1-41.3)	10.3 (10.1-17.6)	0.163

Data presented in median (IQR). FS (Fraction shortening); S' (Systolic wave); E´(Early diastolic wave); A´ (Active atrial contraction wave); MPI (Myocardial performance index); TAPSE (tricuspid annular plane systolic excursion); TRJG: (Tricuspid regurgitation jet gradient); ATET (Acceleration time/ejection time ratio); LVEI (Left ventricular ejection index); RV/LV (Right/left ventricular end-diastolic diameter ratio); [a]n=92 for NT-proBNP (N-terminal pro-brain natriuretic peptide). [b]n= 92 for troponin.

WHAT THIS STUDY ADDS?

- We found a high incidence of vitamin D deficiency among infants hospitalized with acute bronchiolitis.
- An association was found between low levels of vitamin D and clinical severity.

The present study had few limitations. Few of the included patients were only 15-days-old, and it is likely that their pulmonary pressures were still physiologically raised. As these patients with hypovitaminosis D were younger, age could act as a cofounding factor limiting our results. Besides, up to 20% of the included patients were born prematurely, who could have high pulmonary pressures and subclinical myocardial dysfunction due to a delayed drop of pulmonary vascular resistances and delayed myocardial function maturation. Parathyroid hormone levels were not measured in this study, and therefore, we could not assess the calcium and phosphate metabolism. The details of feeding history, sunlight exposure and dose of vitamin D supplementation were not recorded in the study. There were significant differences in the baseline variables among the study groups, but no statistical adjustments were made for the same.

In conclusion, a high incidence of hypovitaminosis D was observed among infants hospitalized with acute bronchiolitis and a significant association with worse clinical, respiratory, and cardiovascular status was recorded. Further studies are needed to better understand the exact mechanisms and significance of these relationships.

Ethics clearance: IRB, Puerta del Mar Univesity Hospital; No. 82.18, dated Oct 26, 2018.
Contributors: EM, CM, RG: conceptualized and designed the study, drafted the initial manuscript, and reviewed and revised the manuscript; EP,RC,RG,FG: collected data and reviewed and revised the manuscript. All authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work.
Funding: None; *Competing interest*: None stated.

REFERENCES

1. Ralston SL, Lieberthal AS, Meissner HC, et al. Clinical Practice Guideline: The Diagnosis, Management, and Pre-vention of Bronchiolitis. Pediatricss. 2014;134:e1474-502.
2. Vo P, Koppel C, Espinola JA, et al. Vitamin D status at the time of hospitalization for bronchiolitis and Its association with disease severity. J Pediatr. 2018;203:416-22.e1.
3. McNally JD, Amrein K. Vitamin D deficiency in pediatric critical care. J Pediatr Inten Care. 2016;5:142-53.
4. Michos ED, Selvin E, Misialek JR, et al. 25-Hydroxyvitamin D levels and markers of subclinical myocardial damage and wall stress: The atherosclerosis risk in communities study. J Am Heart Assoc. 2016;5:e003575.
5. Callejo M, Mondejar-Parreño G, Esquivel-Ruiz S, et al. Total, bioavailable, and free vitamin D levels and their prognostic value in pulmonary arterial hypertension. J Clin Med. 2020;9:448.
6. Rodriguez-Gonzalez M, Perez-Reviriego AA, Castellano-Martinez A, et al. Left ventricular dysfunction and plasmatic NT-ProBNP are associated with adverse evolution in respiratory syncytial virus bronchiolitis. Diagnostics (Basel). 2019;9:85.
7. Rodriguez-Gonzalez M, Benavente-Fernandez I, Castellano-Martinez A, et al. NT-proBNP plasma levels as biomarkers for pulmonary hypertension in healthy infants with respiratory syncytial virus infection. Biomark Med. 2019;13:605-18.
8. Balaguer M, Alejandre C, Vila D, et al. Bronchiolitis score of Sant Joan de Déu: BROSJOD Score, validation and usefulness. Pediatr Pulmonol. 2017;52:533-9.
9. Rodriguez-Gonzalez M, Perez-Reviriego AA, Castellano-Martinez A, et al. The assessment of myocardial strain by cardiac imaging in healthy infants with acute bronchiolitis: A systematic review and meta-analysis. Diagnostics (Basel). 2020;10:382.
10. Mona Abd El-Gelel M, Khalid Mahmoud Z. Impact of acute bronchiolitis on cardiac functions and serum microrna-11 and 499. Am J Infect Dis. 2016;12:11-9.
11. Passeri E, Rigolini R, Costa E, et al. Serum NT-proBNP levels are not related to vitamin D status in young patients with congenital heart defects. Dis Markers. 2016;2016:3970284.
12. Rodriguez-Gonzalez M, Estepa-Pedregosa L, Estalella-Mendoza A, et al. Early elevated NT-proBNP but not Troponin I is associated with severe bronchiolitis in infants. Clin Chim Acta. 2021.
13. Ozer PK, Emet S, Karaayvaz EB, Elitok A, et al. Silent myocardial dysfunction in vitamin D deficiency. Arch Med Sci Atheroscler Dis. 2020;5:e153-e162.
14. Skaaby T, Thuesen BH, Linneberg A. Vitamin D, cardiovascular disease and risk factors. Adv Exp Med Biol. 2017;996:221-30.
15. Atamañuk AN, Litewka DF, Baratta SJ, et al. Vitamin D deficiency among patients with pulmonary hypertension. BMC Pulm Med. 2019;19:258.

RESEARCH PAPER

Long-term Persistence of Immunogenicity After Primary Vaccination and Response to Booster Vaccination With Typhoid Conjugate Vaccine: Results of a Phase IV Extension Study

AMBROSE KUMAR KANDULNA,[1] KHEYA GHOSH UTTAM,[2] SHRIKANT SHARMA,[3] M RAVI KUMAR,[4] K SIVARAM PRASAD,[5] VIMAL KANT GOYAL,[6] SANJAY KUMAR JANGID,[7] PAVANKUMAR DAULTANI,[8] RAVINDRA MITTAL,[8] KAPIL MAITHAL[8]

From [1]Department of Medicine, GCS Medical College, Hospital and Research Centre, Ahmedabad, Gujarat; [2]Department of Pediatrics, Institute of Child Health, Kolkata, West Bengal; [3]Department of Medicine, SMS Medical College and Hospital, Jaipur, Rajasthan; [4]Department of Medicine, Indo-US Superspeciality Hospital, Ameerpet, Hyderabad, Telangana; [5]Department of Pediatrics, Gandhi Medical College and Hospital, Secunderabad, Telangana; [6]Department of Pediatrics, Panchsheel Hospital, Yamuna Vihar, Delhi; [7]Department of Medicine, Hi-Tech Medical College and Hospital, Bhubaneswar, Odisha; [8]Cadila Healthcare Ltd., Ahmedabad, Gujarat.

Correspondence to: Dr Pavankumar Daultani, Deputy General Manager, Department of New Product Development, Cadila Healthcare Ltd., Zydus Corporate Park, Near Vaishnodevi Circle, SG Highway, Ahmedabad 382 481, Gujarat. drpavandaultani@gmail.com
Received: December 06, 2021; Initial review: January 04, 2022; Accepted: March 17, 2022.

Objective: To evaluate the persistence of antibodies three years after primary vaccination with typhoid conjugate vaccine (TCV) of either Cadila Healthcare Ltd. (Cadila-TCV) or Bharat Biotech International Ltd. (Bharat-TCV) administered in a previous phase II/III study, and to study the booster dose response to Cadila-TCV. **Methods**: This was an open-label, phase IV extension study conducted in tertiary care and multispecialty hospitals in India. 112 subjects (Cadila-TCV-57, Bharat-TCV-55) who had participated in previous study were enrolled. Of these, eligible subjects received a single-dose of Cadila-TCV and were followed-up for 28 days post-booster. Primary outcome was persistence of antibodies 3 years after primary vaccination and seroconversion (≥4-fold rise in antibody titre from baseline) 28 days post-booster. Safety was based on reported adverse events (AEs) post-booster. **Results**: The baseline GMT reported in the current study was significantly higher than pre-vaccination GMT reported in the previous study. 89/112 (79.5%) subjects had antibody titer ≥10 IU/mL at baseline; eligible subjects (*n*=17) who had baseline antibody titre <10 IU/mL were administered booster dose. All the vaccinated subjects showed seroconversion post-booster. The GMTs reported at 10 days and 28 days post-booster were significantly higher as compared to GMTs reported after primary vaccination in previous study. 4 (23.5%) vaccinated subjects reported 9 AEs; all were solicited and of mild/moderate intensity. **Conclusion**: There was a significant persistence of immunogenicity after primary vaccination with both the TCVs, and robust immune response after booster vaccination with Cadila-TCV.

Keywords: *Efficacy, Protection, Safety*.

Trial Registration: CTRI/2019/07/019996 ***Published online:*** March 28, 2022; ***PII***: S097475591600415

Typhoid conjugate vaccines (TCVs) have Vi capsular polysaccharide of *S. typhi* conjugated to different carrier proteins. Four such TCVs have been approved and marketed in India. Zyvac TCV (Cadila Healthcare Ltd.) was licensed based on the prelicensure phase II/III non-inferiority clinical trial, the results of which were published previously [1]. The current phase IV trial was conducted as an extension of the previous phase II/III trial in which the same subjects were followed-up three years after their primary vaccination to evaluate the long-term persistence of anti-Vi IgG antibodies, and response to the booster dose of TCV administered to the eligible subjects.

METHODS

This prospective, open-label, multicenter, post-marketing clinical study was conducted from September, 2019 to November, 2020 at the seven centers (tertiary care or multispecialty hospitals) which also had participated in the previous study. The study was approved by the Central Licensing Authority and also by the registered local Institutional Ethics Committees for individual sites. The study was registered with Clinical Trial Registry – India before initiation of enrollment in the study.

In the previous study, a total of 240 healthy subjects aged 6 months to 45 years were enrolled, out of which 236 subjects including 117 and 119 subjects who had received TCV of Cadila Healthcare Ltd. (Cadila-TCV) and TCV of Bharat Biotech International Ltd. (Bharat-TCV), respectively completed the study with both pre-vaccination and 6 weeks post-vaccination immuno-

genicity assessments [1]. The subjects who had participated and completed the previous study were considered for enrollment in the current extension study 3 years (±3 months) after their primary vaccination.

Prior to screening, a written informed consent was obtained from the adult subjects and parents of the pediatric (<18 years) subjects; additionally, an assent was also obtained from the pediatric subjects aged ≥7 years. Adult subjects or parents of the pediatric subjects were also required to be literate enough to provide written informed consent and fill the diary cards. The subjects were excluded if they had a history of typhoid fever or vaccination against typhoid fever after previous study; any ongoing clinically significant systemic disorder, immunological disorder, coagulation disorder or thrombocytopenia; any ongoing anticoagulant, immunosuppressive or immunostimulant therapy; history of administration of blood, blood products or immunoglobulins within past 3 months, participation in another clinical trial within past 3 months, or alcohol or drug abuse within past one year.

The subjects fulfilling the eligibility criteria were enrolled and their blood samples were collected to evaluate the baseline anti-Vi IgG antibody titre. The subjects whose baseline antibody titre was <10 IU/mL, which corresponds to the proposed seroprotective cut-off titre of 2 mcg/mL [2,3], were considered eligible to receive a booster dose of TCV. A 0.5 mL single-dose of Cadila-TCV was given as a booster dose within 90 days of their enrollment, irrespective of the TCV administered for primary vaccination in the previous study. The vaccine in each dose of 0.5 mL contained 25 mcg of purified Vi capsular polysaccharide of *S. typhi* conjugated to tetanus toxoid (TT) as carrier protein. The vaccine was administered intramuscularly in the deltoid region under aseptic precautions following which the subjects were closely observed for at least 30 minutes for occurrence of any immediate adverse events (AEs). Further blood samples were collected during follow-up visits at 10 (+3) days and 28 (+7) days after vaccination, for assessment of post-vaccination antibody titres. The immunogenicity assessment was performed at the central accredited laboratory. The assessment of antibody titers was performed using the commercial Vacczyme ELISA kits (Binding Site Group Ltd.). The antibody titres were also derived in IU/mL using the WHO International Standard for anti-typhoid capsular Vi polysaccharide IgG (human) (NIBSC code 16/138) [2].

The subjects were not administered the booster dose of TCV if they had received any typhoid vaccine after enrollment, received any vaccine within the past one month, history of fever or any infectious disorder of >3 days within the past one month, and fever (≥37.5°C) at the time of planned vaccination. Urine pregnancy test was performed prior to vaccination for females of child bearing potential to rule out pregnancy. Pregnant or lactating females, and females of child bearing potential not using acceptable contraceptive measures were also not administered the booster dose.

Adult subjects or parents of the pediatric subjects were dispensed diary cards to record the solicited local (pain, redness, swelling and induration) and systemic (fever, headache, nausea, vomiting, malaise, arthralgia and myalgia) AEs till 7 days after vaccination and unsolicited AEs till the completion of post-vaccination follow-up. Any abnormality reported in vitals or physical examination was also planned to be dealt as an AE. The intensity of AEs was graded as mild, moderate or severe as described earlier [1], and causality was assessed based on the World Health Organization (WHO) criteria for AEs following immunization [4].

The primary immunogenicity variables were long-term persistence of anti-Vi IgG antibodies 3 years after primary vaccination and seroconversion, which was defined as a four-fold or greater rise in antibody titer at 28 days after vaccination, as per the WHO recommendations [2,5] . The secondary immunogenicity variables were seroconversion at 10 days after vaccination and geometric mean titer (GMT) of antibodies at 10 days and 28 days after vaccination as compared to the baseline. The safety variables were local or systemic AEs, serious AEs (SAEs) reported, if any, and overall tolerability evaluation by the investigators based on the reported AEs as follows: Excellent - no AE, Good – mild AE(s), Fair - moderate AE(s) and Poor - severe or serious AE(s). The maintenance of seroconversion at the baseline, defined as four-fold or greater rise in antibody titre at baseline in the current study as compared to antibody titre reported before primary vaccination in the previous study has also been evaluated as an exploratory variable.

Since this was an extension study, no minimum sample size was defined for this study. All the 236 subjects who had participated in and completed the previous study were considered for enrollment in the current study.

Statistical analysis: The immunogenicity analysis represents the data of full analysis set which included all the subjects with applicable immunological assessments including those with protocol deviations. The safety analysis represents the data of all the subjects who had received booster dose of TCV. The GMTs (95% CI) were computed for description of antibody titers. The GMTs between the groups were compared using unpaired *t* test

while the GMTs within the groups were compared using paired *t* test after log transformation of antibody titers. Continuous data was compared between the groups using unpaired *t* test. Categorical data was compared between the groups using Fisher exact test.

RESULTS

A total of 121 subjects were screened in this study, out of which 112 subjects (76 adults, 56 males) were enrolled while the remaining 9 subjects, who had received another dose of typhoid vaccine prior to this study, were excluded (**Fig. I**). The mean (SD) age, height, weight and body mass index of the enrolled subjects were 24.7 (12.6) years, 148.7 (22.9) cm, 51.5 (19.9) kg and 22.1 (5.2) kg/m^2, respectively. The baseline characteristics of the enrolled subjects are presented in **Table I**.

The GMT of antibodies reported at baseline in the current study (**Table I**) was significantly higher as compared to those reported before primary vaccination in the previous study (P<0.001). A total of 100 (89.3%) subjects, 50 subjects each who had received Cadila-TCV and Bharat-TCV, for primary vaccination in the previous study had maintained seroconversion at baseline in the current study (P=0.76). Based on the cut-off titer, 23 (20.5%) subjects were eligible to receive the booster dose vaccination, out of which 17 subjects were vaccinated. All the subjects followed-up on day 10 and day 28 after vaccination had shown seroconversion with a significant rise in antibody titers after vaccination as compared to baseline (P<0.001) (**Table II**). The GMT of antibodies reported at day 10 and day 28 after booster vaccination were significantly higher as compared to that reported after primary vaccination in the previous study (P<0.05) (**Table II**).

Table I Baseline Characteristics of Participants Who Had Received Typhoid Conjugate Vaccine (TCV)

Parameter	*Previous TCV Received*	
	Cadila-TCV (n=57)	*Bharat-TCV (n=55)*
Age (y)[a]	24.7 (13.1)	24.7 (12.2)
Age group		
Adult	37 (64.9)	39 (70.9)
Pediatric	20 (35.1)	16 (29.1)
Male gender	28 (49.1)	28 (50.9)
Baseline titer (EU/mL)[b]	140.8 (93.9, 211.2)	203.6 (144.3, 287.5)
Baseline titer ≥10 IU/mL	44 (77.2)	45 (81.8)

Data presented as no. (%) or [a]mean (SD) or [b]GMT (95% CI). All P>0.05.

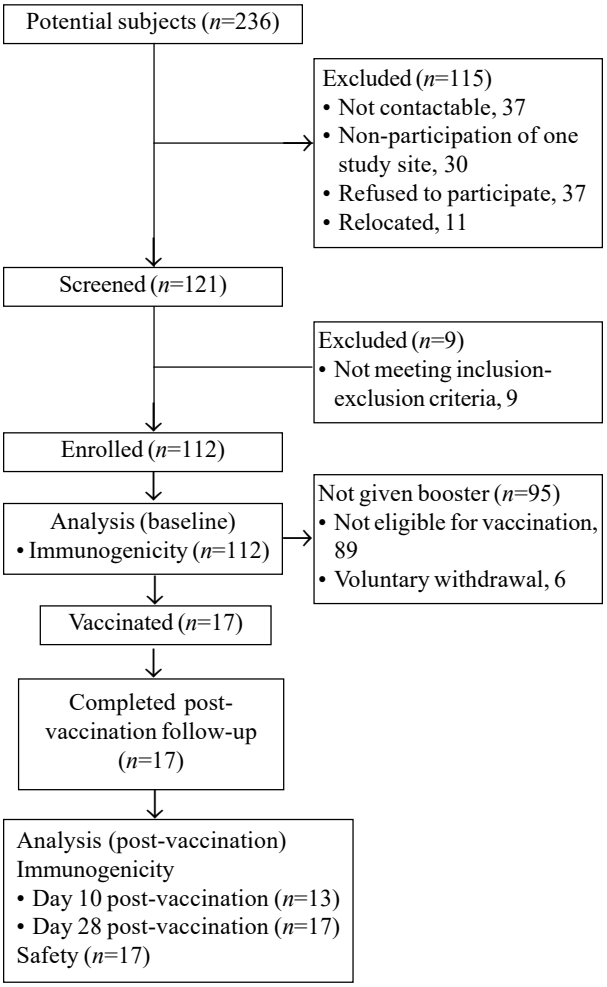


Fig. 1 Study flow chart.

A total of nine AEs were reported in four out of 17 (23.5%) vaccinated subjects; local pain in four participants, local swelling in two participants, and local redness, fever and headache in one participant each. All the AEs were of mild intensity except local pain in one participant, which was of moderate intensity. No severe or serious AE was reported for any subject. All AEs were solicited in nature, considered certainly related to the vaccination, and resolved completely within three days of their occurrence with/without supportive medications. Based on the reported AEs, an excellent, good and fair grade of tolerability was given for 13 (76.5%), 3 (17.6%) and 1 (5.9%) participants, respectively.

DISCUSSION

In the current study, we demonstrated that there was a significant persistence of antibodies at three years after primary vaccination with TCV, and the eligible subjects who received the booster dose mounted an antibody response which was significantly higher on day 10 and day 28 than that reported after primary vaccination. The

Table II Antibody Titers at Various Time Points for Participants Receiving a Booster Dose of Typhoid Conjugate Vaccine

Study, timepoint	*Previous TCV received*		*Overall*
	Cadila-TCV	*Bharat-TCV*	
Current phase IV study			
Baseline	*n*=8, 17.5 (7.1, 43.3)	*n*=9, 34.0 (22.9, 50.3)	*n*=17, 24.9 (15.9, 38.9)
Day 10 post-booster	*n*=7, 2734.3 (1066.1, 7013.2)	*n*=6, 1891.8 (795.3, 4500.4)	*n*=13, 2306.9 (1326.1, 4012.8)
Day 28 post-booster	*n*=8, 2107.1 (1004.5, 4420.0)	*n*=9, 1733.9 (1043.4, 2881.5)	*n*=17, 1900.5 (1288.3, 2803.7)
Previous phase II/III[a] study			
Post-primary	*n*=8, 829.5 (315.4, 2181.4)	*n*=9, 952.8 (429.5, 2114.0)	*n*=17, 892.6 (517.1, 1541.0)

Data presented as GMT (95% CI) in EU/mL. TCV: typhoid conjugate vaccine. [a]Full results available in reference 1.

booster dose administered to the selected subjects was also well tolerated.

One potential limitation of the study was that only around 50% of the subjects who had completed the previous phase II/III study were enrolled in this study. Other possible limitations could be selection of 10 IU/mL (2 µg/mL) as the cut-off titer for considering subjects eligible for booster vaccination, and non-evaluation of other immune response parameters such as IgG subclass response, functional antibodies, antibodies targeting other antigens apart from Vi capsular polysaccharide, cell mediated immune response etc.

To the best of our knowledge, there is no seroprotective titer defined for anti-Vi antibodies till date. From an earlier efficacy study of Vi-rEPA conjugate vaccine, 4.3 µg/mL was initially presumed as the seroprotective cut-off titer, which on subsequent re-examination based on the statistical modelling was estimated between 1.4-2.0 µg/mL [3], and based on the same evidence, 2 µg/mL has been used as the cut-off titer in this study. In a previous study, all the subjects who had received single-dose of Bharat-TCV and evaluated at 2 years after vaccination had maintained antibody titer ≥2 µg/mL [6]. In a further long-term follow-up study, all the subjects in a subset population aged 6-23 months at primary vaccination and who did not receive the booster dose subsequently (*n*=25) had maintained antibody titer ≥2 µg/mL at 7 years after vaccination [7]. The proportion of subjects maintaining the antibody titer ≥10 IU/mL (2 µg/mL) in the current study was relatively lower; however, it was comparable for both the TCVs.

Long-term persistence of serum anti-Vi antibodies has already been demonstrated upto 7 years after primary vaccination with TCVs in the earlier clinical studies [6-8]. In a study conducted with Bharat-TCV, the proportion of subjects maintaining seroconversion at 2, 3 and 5 years after primary vaccination was 59.5%, 73.5% and 73.2% in 6-23 months age group and 74.1%, 76.2% and 69.2% in 2-45 years age group [9]. In a subset of subjects from the former age group, 44% had maintained seroconversion at 7 years after vaccination [7]. Likewise, in a study of another TCV, the seroconversion was maintained in 83% subjects in the immunogenicity subset at 12 months after primary vaccination [8]. Although the data of current study cannot be directly compared with that of previously published studies, the baseline GMTs and the proportion of subjects maintaining seroconversion at 3 years after primary vaccination reported in our study was found comparable for both the TCVs.

Although the number of subjects receiving the booster vaccination was small, the robust booster response seen with TCVs can be explained by generation of immunological memory owing to conjugation of Vi polysaccharide with TT carrier protein which renders the antigen T-cell dependent leading to production of plasma cells and memory B cells [10]. Administration of the same vaccine for booster dose may be a possible explanation of a higher booster response observed in the subjects who had received Cadila-TCV for primary vaccination. The safety data of the booster vaccination reported in this study is also consistent with that reported with the marketed TCVs [11-13], even though our study had limited numbers.

Overall, this study provides useful insights on the long-term immunological persistence and response to booster dose of TCV. Further long term studies are warranted to confirm waning of antibody titres and to evaluate efficacy/effectiveness of TCVs over the years of follow-up. Pursuant to limited availability of long-term follow-up data, there is currently no recommendation provided for booster dose of TCVs [2,14,15]. The data derived from the current study and that generated from long-term follow-up studies of other TCVs will help policymakers to take appropriate decision on the requirement and timing of booster dose of TCV.

Ethics clearances: Institute's Ethics Committee, Indo-US Superspeciality Hospital, Hyderabad; No. Nil dated June 22, 2019. Institute's Ethics Committee, Panchsheel Hospital Pvt. Ltd., New Delhi; No. Nil dated July 02, 2019. Institute's Ethics Committee, Sparsh Hospitals and Critical Care Pvt. Ltd, Bhubaneswar; No. Nil dated July 15, 2019. Institute's Ethics Committee, Gandhi Medical College, Secunderabad; No. IEC/GMC/2019/05/05 dated September 11, 2019. Institute's Ethics Committee, GCS Medical College, Hospital and Research Center, Ahmedabad, No. GCSMC/EC/TRIAL/APPROVE/2019/2291 dated September 28, 2019. Institute's Ethics Committee, Institute of Child Health, Kolkata, No. ICH/IEC/81/2019 dated October 10, 2019. Institute's Ethics Committee, SMS Medical College and Attached Hospitals, Jaipur; No. 625/MC/EC/2019 dated November 1, 2019.

Contributors: AKK,KGU,SS,MRK,KSP,VKG,SKJ: study conduct, medical care of the study participants and data acquisition; PD,RM: study concept and design, overall study coordination, data analysis and interpretation; KM: study concept and design, and manufacture of the vaccine. All authors had full access to clinical trial data. PD, RM: prepared the manuscript and other authors provided their feedback for revising it for the intellectual content. All authors have approved the final version of this manuscript. All authors agree with the interpretation of data and its representation in the manuscript.

Funding: The monetary and material support including the vaccine supplies was provided by M/s. Cadila Healthcare Ltd., Ahmedabad.

Competing interests: AKK,KGU,SS,MRK,KSP,VKG,SKJ: were the clinical trial investigators and they received honorarium from the sponsor for the conduct of the study. PD, RM, KM are employees of M/s. Cadila Healthcare Ltd.

REFERENCES

1. Kundu R, Kandulna AK, Nayak U, et al. Immunogenicity and safety of typhoid conjugate vaccine in healthy Indian subjects: A randomized, active-controlled, comparative clinical trial. Indian Pediatr. 2020;57:625-30
2. World Health Organization. Recommendations to Assure the Quality, Safety and Efficacy of Typhoid Conjugate Vaccines. Replacement of Annex 3 of WHO Technical Report Series, No. 987. Accessed March 11, 2022. Available from: *https://cdn.who.int/media/docs/default-source/biologicals/vaccine-standardization/typhoid-fever/typhoid-conjugate-vaccine-trs-1030-a2-2021.pdf?sfvrsn=29505818_5&download=true*
3. Szu SC, Klugman KP, Hunt S. Re-examination of immune response and estimation of anti-Vi IgG protective threshold against typhoid fever-based on the efficacy trial of Vi conjugate in young children. Vaccine. 2014;32:2359-63.
4. World Health Organization. Adverse Events Following Immunization (AEFI): Causality Assessment. Accessed March 11, 2022. Available from: *http://apps.who.int/iris/bitstream/handle/10665/191391/;jsessionid=929398B2DAB017358AD11BF CDFBDB1B9?sequence=1*
5. World Health Organization. Guidelines on Clinical Evaluation of Vaccines: Regulatory Expectations. Replacement of Annex 1 of WHO Technical Report Series, No. 924. Accessed March 11, 2022. Available from: *https://cdn.who.int/media/docs/default-source/prequal/vaccines/who-trs-1004-web-annex*9.pdf?*sfvrsn=9c8f4704_2& download= true*
6. Mohan VK, Varanasi V, Singh A, et al. Safety and immunogenicity of a Vi polysaccharide-tetanus toxoid conjugate vaccine (Typbar-TCV) in healthy infants, child-ren, and adults in typhoid endemic areas: A multicenter, 2-cohort, open-label, double-blind, randomized controlled phase 3 study. Clin Infect Dis. 2015;61:393-402.
7. Vadrevu KM, Raju D, Rani S, et al. Persisting antibody responses to Vi polysaccharide-tetanus toxoid conjugate (Typbar TCV®) vaccine up to 7 years following primary vaccination of children <2 years of age with, or without, a booster vaccination. Vaccine. 2021;39:6682-90.
8. Mitra M, Shah N, Ghosh A, et al. Efficacy and safety of Vi-tetanus toxoid conjugated typhoid vaccine (PedaTyph™) in Indian children: School based cluster randomized study. Hum Vaccin Immunother. 2016;12:939-45
9. Bharat Biotech International Ltd. Typbar TCV®. The original typhoid conjugate vaccine. Accessed March 11, 2022. Available from: *https://www.bharatbiotech.com/images/typbartcv/Typbar-Tcv-Brochure.pdf*
10. Rappuoli R, De Gregorio E, Costantino P. On the mechanisms of conjugate vaccines. Proc Natl Acad Sci USA. 2019;116: 14-6.
11. Bharat Biotech International Ltd. Typbar TCV™. Accessed March 11, 2022. Available from:*https://www.bharatbiotech.com/images/typbartcv/Typbar-TCV-Package-Insert.pdf*
12. Biological E Limited. TYPHIBEV. Accessed March 11, 2022. Available from: *https://extranet.who.int/pqweb/content/typhibev%C2%AE*
13. Bio-Med Private Limited. PedaTyph™. Accessed March 11, 2022. Available from: *http://www.biomed.co.in/peda-typh/#*
14. World Health Organization. Typhoid vaccines: WHO Position Paper, March 2018 - Recommendations. Vaccine. 2019;37:214-6.
15. Kasi SG, Shivananda S, Marathe S, et al. Indian Academy of Pediatrics (IAP) Advisory Committee on Vaccines and Immunization Practices (ACVIP): Recommended Immunization Schedule (2020-21) and Update on Immunization for Children Aged 0 Through 18 Years. Indian Pediatr. 2021;58:44-53.

REVIEW ARTICLE

Dermatological Manifestations of COVID-19 in Children

MAITREYEE PANDA,[1] AKASH AGARWAL,[1] TRASHITA HASSANANDANI[2]
From [1]Department of Dermatology, IMS and SUM Hospital,Bhubaneswar, Orissa; [2]Department of Dermatology, Amaltas Institute of Medical Sciences, Dewas, Madhya Pradesh.
Correspondence to: Dr Maitreyee Panda, Department of Dermatology, IMS and SUM Hospital, K-8 Kalinga Nagar, Bhubaneshwar, Odisha. pandamaitreyee@gmail.com

Context: The clinical picture of pediatric severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection differs from adults as do the cutaneous manifestations. In this review, we summarize the varied morphological manifestations of SARS-CoV-2 infection in the pediatric population. **Evidence acquisition:** A comprehensive literature search was conducted (23 September, 2021) across multiple databases (PubMed, EMBASE, MEDLINE and Cochrane) with the relevant keywords. An additional filter of age group between 0-18 years was kept in each of the searches. **Results:** Chilblains constitute the most common cutaneous manifestation of pediatric coronavirus disease (COVID-19). Other commonly reported manifestations include maculopapular rash, urticaria, erythema multiforme, and papulovesicular eruptions. Majority of children with these manifestations are asymptomatic, highlighting the need to clinically suspect and appropriately manage such patients. A subset of pediatric patients develop severe multisystem involvement termed as multi-system inflammatory syndrome in children (MIS-C) that has varied mucocutaneous manifestations. **Conclusion:** A wide variety of dermatological manifestation of SARS-CoV-2 infection is reported, and both the pediatrician and dermatologist need to be aware of the same to suspect and diagnose COVID-19 infection in children.

Key words: *Chilblains, Erythema multiforme, MIS-C, SARS-CoV-2.*

Published online: March 10, 2022; ***PII:*** S097475591600412

The mucocutaneous manifestations of corona-virus disease (COVID-19) in children are still evolving, with varied presentations, which are relatively milder [1]. Moreover, they can often go undiagnosed when presenting as a sole cutaneous manifestation of pediatric COVID-19.

The incidence of dermatological manifestations in pediatric COVID-19 is diverse, varying across ethnic groups and geographical regions. Chilblain like lesions, urticaria and confluent maculopapular rash are commonly observed in children in European countries [2]. Chinese literature suggests ischemic lesions and urticarial rash as common manifestations [3]; whereas, reports from Thai-land have observed dengue like rash in certain patients [4]. Multisystem inflammatory syndrome in children (MIS-C) is already an established entity, which has its own mucocutaneous manifestations. We, herein, summarize all relevant literature on the mucocutaneous manifestations of pediatric COVID-19.

METHODS

A comprehensive literature search across multiple databases (PubMed, EMBASE, MEDLINE, and Cochrane) was carried out with keywords "COVID-19" OR "corona-virus" AND "rash", "skin rash", "cuta-neous", "chilblain", "chilblain-like", "pseudo-chilblain", "pernio like", "urticaria", "urticarial rash", "vesicular", "papulovesicular, "maculopapular", "morbilliform", "erythema multiforme", "varicella like", "vesicular rash", "dengue-like", "purpura", "hair", "nail", "mucosa", "Multisystem inflammatory syndrome in children", "MIS-C"," Multisystem inflammatory syndrome in neonate", and "MIS-N". An additional filter of age group between 0-18 years was kept in each of the searches.

CUTANEOUS MANIFESTATIONS

Chilblain

Chilblains represent the most common cutaneous manifestation reported among children affected with COVID-19. When the coronavirus pandemic began in March, 2020, a sudden surge in cases of chilblain-like lesions was noticed. A French study even revealed a rising trend regarding web searches of chilblains, fingers, toes and COVID-19 infection during the initial months of the pandemic [8]. The cases observed were not true chilblains, differing in terms of sex distribution (equal sex distribution), triggering factors (warm climate), and distribution of lesions (fingers and toes). Despite there being a temporal relation between the onset of chilblain-like lesions with the pandemic, there is still a debate regarding its association with SARS-CoV-2, as majority of patients test negative for infection both by reverse transcriptase - polymerase chain reaction (RT-PCR) or serology [9,10].

Clinical features: Multiple erythematous, violaceous and/or purpuric macules are observed predominantly over the

finger and toes (Covid toes). Majority of cases affect the toes with lesions not crossing the meta-tarsophalangeal joint. The reported mean age of patients is above 10 years [2]. Swelling of the surrounding skin may also be noted, with severe cases showing secondary vesiculation. They appear towards the end of COVID-19, and last for about 12 days on an average, but can last for up to 4-5 weeks. Unlike adults, where systemic association with chilblain-like lesions is common (up to 45%), children are commonly asymptomatic [11]. The chilblain lesions may; however, show local symptoms such as pain or itching. Co-existing erythema multiforme (EM) like lesions in pediatric patients with chilblains has also been noted [12]. A case of recurrent chilblain-like lesions in a patient with mild COVID-19 was also reported [13].

Dermoscopy of chilblain-like lesions in children has revealed the following features viz., a red or purplish background area, with red to purple globules in majority of cases, and a grey brown reticular network on the periphery in 30% of cases [14]. In an Italian report studying nail videocapillaroscopy in children with chilblain, peri-capillary edema and microhemorrhage were striking features, especially in toe lesions, along with capillary dilation common to both finger and toe nails [15].

Histopathology: Chilblain-like lesions are characterized by lymphocytic vasculitis ranging from endothelial swelling to fibrinoid necrosis. Superficial and deep peri-vascular lymphocytic infiltrate along with peri-eccrine lymphocytic infiltrate is also seen, which is similar to idiopathic perniosis. SARS-CoV-2 virus has also been demonstrated on immunohistochemistry in the endothelial cells and epithelial cells of eccrine gland [2].

Pathogenesis: Various possible pathomechanisms have been put forth. They include:

i) Direct virus - induced endothelial damage: Association between direct endothelial damage and chilblain in COVID-19 was suggested by Colmereno, et al. [16] based on immunohistochemistry and electron micro-scopy findings of virus particles affecting endothelium and eccrine epidermal cells. However, this was later challenged by Brealey, et al. [17], who explained that the virus like structure was infact a clathrin-coated vesicle, a normal subcellular organelle [17]. Still, viral particles in endothelium along with evidence of vascular damage on histopathology support a causal association.

ii) Role of interferon type 1: Interferon type 1, a part of the innate immunity, is responsible for the first line of defense against viral infections. Compared to adults, the levels of INF-1 are higher in infants and children. Hubiche, et al. [18] found that there was a significantly higher IFN-á response in chilblains patients (associated with mild disease) compared to patients with moderate or severe COVID-19. In severe SARS-CoV-2 infections, there is impaired interferon response, explaining why chilblain-like lesions are seen infrequently [18]. Given the histopathological similarity of COVID-19 induced chilblain to type 1 interferonpathy induced chilblain, a causal association has also been suggested.

iii) Thrombotic or embolic hypothesis: COVID-19 infection is associated with raised D-dimer levels in moderate to severe cases and thus carry a high risk of thromboembolism. These fibrin micro-emboli may have tendency to block the smaller vessels leading to acral ischemia and subsequently chilblain-like lesions. In a study by Hachem, et al. [16], microthrombi were observed on histopathological analysis in three patients with chilblains. This supports the possibility of a thrombotic or embolic event precipitating chilblain-like lesions.

Investigations: Strikingly, majority of patients who develop chilblain-like lesions test negative for SARS-CoV-2 on a RT-PCR based test [19]. Given that chilblain develops during resolution of COVID-19 and in children having asymptomatic infection, a lower positivity can be explained. Antibody serology with IgM and IgG in children have also demonstrated lower positivity rates [20,21]. Interestingly, an Italian series has depicted 53.3% IgA-positivity against S1 spike protein, which establishes a causal-link between chilblain-like lesions and asymptomatic SARS-CoV-2 infection [9].

Treatment: In children, spontaneous resolution over days to weeks is the dictum. Symptomatic treatment with analgesics and antishistaminics usually suffices. More-over, chilblains, in children are associated with mild COVID-19, unlike adults. Counselling the patient regarding the benign nature of the condition is important.

Urticaria

Acute urticaria is characterized by transient pruritic wheals not lasting for more than 24 hours, and for less than six weeks in duration. Among children presenting with acute urticaria, infections (mostly upper respiratory infections), drugs and food allergy are the most common precipitating factors [22]. During the ongoing pandemic, urticarial lesions have accounted for 13.5-26% of cutaneous manifestations arising due to SARS-CoV-2 infection in adults [23]. However, data in the pediatric age group is scarce and restricted to case reports (9 cases) (**Web Table I**). Majority of patients presented with urticaria as the sole symptom to the dermatologist or pediatrician; although, cases presenting with or after systemic symptoms have been reported. History regarding systemic symptoms and household contacts

gives a clue towards possible underlying etiology of SARS-CoV-2 infection. In the current pandemic, we feel that all children with acute urticaria must be subjected to SARS-CoV-2 testing (**Fig. 1**).

Multifactorial pathophysiological process has been put forth explaining the observation of urticaria in children. They include: affinity of SARS-CoV-2 with ACE2 receptor on endothelium may cause immune complex deposition leading to immune mediated urticaria; Cross-reactivity between viral IgM and IgG with mast cell IgE causing mast cell degranulation; Immune complexes stimulating basophils into producing vasoactive amines, which activates complement, leading to increased vascular permeability [32]; cytokine IL-6 (elevated in COVID-19 patients) directly stimulates mast cell degranulation, leading to urticaria [33]; and mediation via bradykinin due to activation of kinin-kallikerin system in conjunction with ACE2 receptor stimulation [34].

The diagnosis is primarily clinical. A possibility of drug-induced urticaria due to use of non-steroidal anti-inflammatory drugs (NSAIDs) and antibiotics in COVID-19 should be kept in mind. The treatment is symptomatic, with use of corticosteroids limited to resistant cases. The association of urticaria with COVID-19 infection in children has not been seen to be associated with any adverse effects.

Erythema Multiforme-Like Eruption

Erythema multiforme is an acute onset hypersensitivity disorder characterized by a distinctive rash called target lesions. Among children, infections viz., herpes simplex virus and mycoplasma pneumonia, and drugs are frequently associated [35]. During the COVID-19 pandemic, three different types of EM or EM-like rash have been reported: juvenile-virus related EM like rash (age <30 years), older classical EM rash (age >55 years) and drug-induced EM [36].

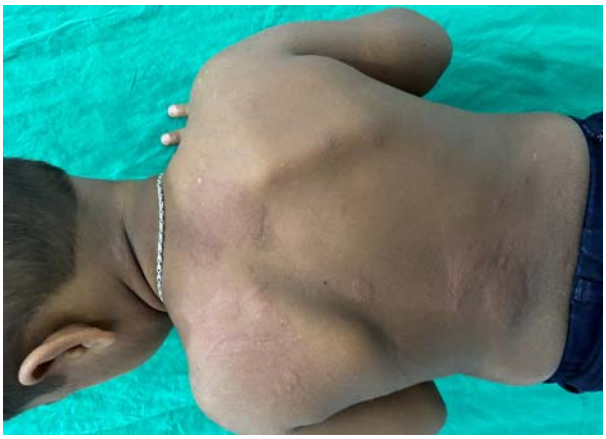


Fig. 1 Acute urticaria in a 7-year-old boy as presenting feature of COVID-19 infection.

In children, the rash is an atypical target lesion restricted to the palms and soles in majority of the cases (**Web Table II**). The lesions are smaller, less widespread and may be itchy or painful. A latency of few days to three weeks between COVID-19 infection and EM-like rash is seen. In a retrospective analysis of 132 patients presen-ting with acral lesions during the pandemic in Italy [37], 28% patients had EM-like lesions (*n*=37) with only two patients having sites other than palms and soles involved. Torello, et al. [13] reported four patients with EM-like rash among 22 patients with chilblains.

Histopathologically, mild superficial perivascular and perieccrine lymphocytic infiltrate was observed without the characteristic findings of EM such as keratinocyte necrosis and eosinophils. The correlation with COVID-19 was confirmed in one case by a positive RT-PCR report while in two patients, immunohistochemistry revealed antibodies to SARS-CoV-2 spike glycoprotein in the vascular endothelium and eccrine epithelial cells [13]. Thus, it is fair to say that the lesions are not true EM lesions but EM-like or rather resembling chilblains clinically and histopathologically. Overall, EM or EM-like rash is not associated with a severe course of SARS-CoV-2 infection among children. Symptomatic therapy with topical or oral corticosteroids is enough in most of the cases.

Papulovesicular Eruption

Papulovesicular eruption as a cutaneous manifestation of COVID-19 is well documented in adult literature. Average age of onset is 45-60 years with the eruption seen during early infection. The underlying pathogenesis is sus-pected to be the direct effect of SARS-CoV-2 virus on basal keratinocytes leading to acantholysis [41]. Two distinct morphological variants are described: localized monomorphic variant and diffuse polymorphic variant. In comparison to adults, wherein incidence rates of 7.2% have been reported [42], only a single case has been documented in pediatric literature. The case was that of an 8-year-old girl who presented with a three-day history of papulovesicular lesions along with six-day history of cough. A positive contact history led to suspicion of COVID-19 in this case which was confirmed by a RT-PCR test. The exanthema resolved in 7 days time without any need for intervention [43].

The authors also encountered of papulovesicular eruption in an 11-year-boy who was asymptomatic; however, he and his parents later tested positive for SARS-CoV-2 infection.

Multisystem Inflammatory Syndrome in Children

Multisystem inflammatory syndrome in children (MIS-C) is a relatively common complication of COVID-19 that presents clinically resembling incomplete Kawasaki disease and toxic

shock syndrome. Majority of children have positive serology for COVID-19 and negative polymerase chain reaction, supporting the hypothesis that this condition is related to immune dysregulation after acute infection has subsided.

In a large series of MIS-C patients across the United States, mucocutaneous findings were identified in 74% of children, out of which 59% had nonspecific eruption, 55% bilateral conjunctivitis, 42% oral mucosal changes, and 37% peripheral extremity changes. [52]. Commonly reported cutaneous features include diffuse non-specific eruptions, palmoplantar erythema, hyperemia of lips, strawberry tongue, periorbital and malar erythema [53]. EM has also been reported in a child with MIS-C [54]. The skin findings associated with MIS-C are more common in younger children and decrease with age (**Fig. 2**).

Pathogenesis: No literature with regards to pathogenesis of mucocutaneous manifestations in MIS-C is available.

Differential diagnosis: At the beginning of the COVID-19 pandemic, MIS-C was confused with Kawasaki disease, toxic shock syndrome and secondary hemophagocytic lympho-histiocyosis as the clinical features were overlapping. However, differences exist in the geographic distribution, pathogenesis, cardiac and gastrointestinal manifestations and hematological parameters. Moreover, majority of MIS-C patients are seropositive for SARS-CoV-2 antibodies.

Treatment: The mainstay of MIS-C treatment is intravenous immune globulin (IVIG), and adjunctive high-dose corticosteroids. The cutaneous manifestations are transient and resolve with treatment of MIS-C.

Non-Specific Cutaneous Lesions

Maculopapular or morbilliform rash has been reported in nearly 47% of adult patients with cutaneous mani-festations; however, in children, they are infrequently documented [61] (**Fig. 3**). Other nonspecific cutaneous manifestation reported in children include retiform purpura [62], cutaneous vasculitis (**Fig. 4**), dengue-like exanthem [4] (**Fig. 5**), livedoid lesions [63], acral ischemia [64] and petechial rash [65].

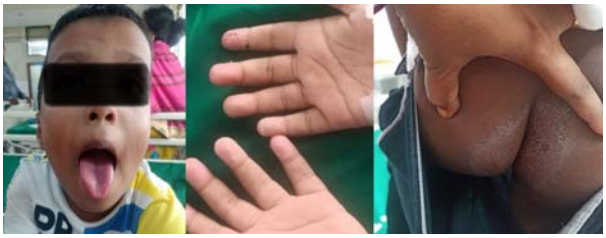


Fig. 2 Cutaneous manifestations of multisystem inflammatory syndrome in childhood (MIS-C) (strawberry tongue, periungual desquamation, and desquamation around buttocks) occurring in a 6-year-old boy four weeks after COVID-19, associated with IgG SARS-CoV-2 antibody positivity.

Hair and Nail Changes

Post-COVID hair changes have been commonly reported in adults; however, there is paucity of literature for hair changes after COVID-19 in children. Hayran, et al. [66] presented two children with different hair loss patterns post-MIS-C. The first patient was a 10-year-old boy who developed telogen effluvium. The second patient who was a 13-year-old boy who developed alopecia areata. The duration in both the cases was nearly four weeks after COVID-19-associated MIS-C [66]. The cause of hair loss in COVID-19 patients is unknown but stress and anxiety were the proposed precipitating factors. In a series of five patients, fluorescence of hair and nails was observed under Wood lamp in patients of COVID-19, who were treated with favipiravir [67].

Nail changes have been observed in patients with SARS-CoV-2 infection in the form of red half-moon nails, transverse orange nails, Mee lines and Beau lines. In a study by Thuangton, et al. [68], two patients had nail changes in the form of brittle nails and chromonychia. No documented reports are available for nail changes after SARS-CoV-2 infection in the pediatric population. However, clinicians should keep an eye out for these nail changes in children too, as these are also commonly seen in other viral infections.

INFERENCE IN SKIN OF COLOR

A lack of representation in skin of color have been well documented in COVID-19 cutaneous manifestations. The ethnic and racial disparities pose a problem in clinical imaging of the dermatological manifestation, and may impact the health outcomes as well as create a gap in the educational resources.

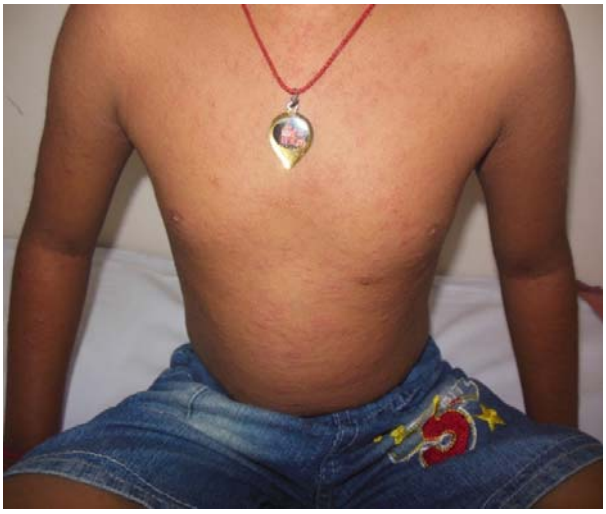


Fig. 3 Maculopapular rash appearing 3 days after diagnoses of COVID-19 infection in an 8-year-old boy.

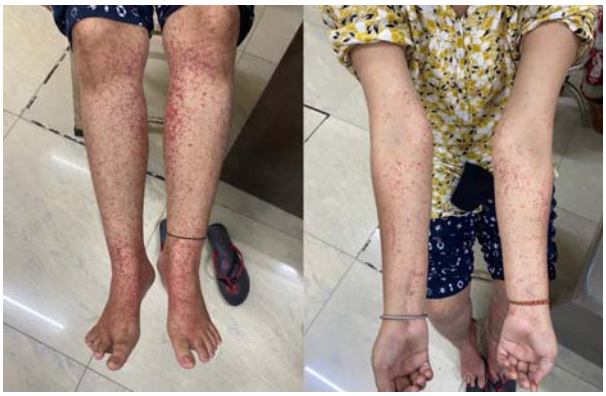


Fig. 4 Palpable purpura on all four limbs as presenting feature in a 13-year-old girl later diagnosed with COVID-19 infection. The biopsy was suggestive of leucocytoclastic vasculitis.

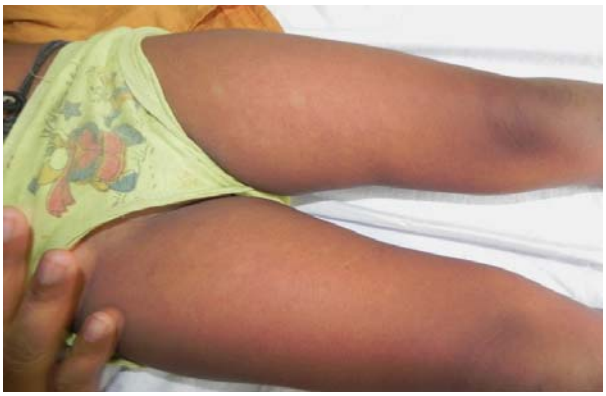


Fig. 5 Dengue-like rash with classical islands of sparing in a 6 year old girl diagnosed with COVID-19 infection.

Some authors have emphasizd the under represen-tation of skin manifestations of COVID-19 and paucity of images in Fitpatricks IV, V or VI types [69,70]. A study by Dalal, et al. [71] described the dermatological mani-festation in 12.7% of COVID-19 cases from Northern India, in which 7.8% had pruritus without cutaneous findings, 2.9% had maculopapular rash and 1.9% had urticarial rash. The largest series from Southern India consisting of 1065 SARS-CoV-2 positive patients had cutaneous manifestations in only 4.5% cases [72]. Urticaira and itching were the commonest, followed by maculopapular rash, vesicular rash, acral erythema, apthous ulcer, herpes zoster and others. Another cases series from India [73] had similar manifestations of urticaria, maculopapular rash and herpes zoster. However, none of these series reported any pediatric cases.

CONCLUSION

Cutaneous manifestation may present before, at or after the diagnosis of COVID-19 among children. Chilblains, the most common cutaneous manifestation, is associated with mild SARS-CoV-2 infection, unlike the adult population. Literature on urticaria, erythema multiforme like, papulovesicular eruptions and maculopapular rash is limited compared to adults, probably due to under-reporting and the fact that these manifestations are often associated with asymptomatic or mild infection. A subset of pediatric patients develop varied muco-cutaneous manifestations along with as MIS-C, which has not been observed in adults. We feel that acute urticaria and maculopapular rash in a child should prompt testing for SARS-CoV-2 infection in the current pandemic.

Given the relaxations on COVID-19 norms worldwide and vaccination status among children particularly, a surge in the cases of paediatric COVID-19 may be on the horizon. An in-depth knowledge regarding cutaneous manifestations of COVID-19 is necessary for both the pediatrician and dermatologist to suspect and diagnose COVID-19 infection in children early. Data regarding such manifestations is still evolving and further information is needed to diversify our understanding regarding the subject.

Contributors: MP: contributed to initial conceptualization and prepared the initial draft of the manuscript; AA,TH: contributed to literature review and drafting of final manuscript. All authors prepared and approved the final manuscript.
Note: Supplementary material related to this study is available with the online version at *www.indianpediatrics.net*
Funding: None; *Competing interests*: None stated.

REFERENCES

1. Panda M, Dash S, Behera B, Sil A. Dermatological Manifestations Associated with COVID-19 Infection. Indian J Dermatol. 2021;66:237-45.
2. Andina D, Belloni-Fortina A, Bodemer C, et al. Skin manifestations of COVID-19 in children: Part 1. Clin Exp Dermatol. 2021;46:444-50.
3. Mirza FN, Malik AA, Omer SB, Sethi A. Dermatologic manifestations of COVID-19: A comprehensive systematic review. Int J Dermatol. 2021;60:418-50.
4. Joob B, Wiwanitkit V. COVID-19 can present with a rash and be mistaken for dengue. J Am Acad Dermatol. 2020;82:e177.
5. Toubiana J, Poirault C, Corsia A, et al. Kawasaki-like multisystem inflammatory syndrome in children during the covid-19 pandemic in Paris, France: Prospective observational study. BMJ. 2020;369:m2094.
6. Verdoni L, Mazza A, Gervasoni A, et al. An outbreak of severe Kawasaki-like disease at the Italian epicentre of the SARS-CoV-2 epidemic: An observational cohort study. Lancet. 2020;395(10239):1771-78.
7. Pouletty M, Borocco C, Ouldali N, et al. Paediatric multisystem inflammatory syndrome temporally associated with SARS-CoV-2 mimicking Kawasaki disease (Kawa-COVID-19): A multicentre cohort. Ann Rheum Dis. 2020;79(8):999-1006.
8. Kluger N, Scrivener JN. The use of google trends for acral symptoms during COVID-19 outbreak in France. J Eur Acad Dermatol Venereol. 2020;34:e358-60.
9. Diociaiuti A, Giancristoforo S, Terreri S, et al. Are SARS-CoV-2 IgA antibodies in paediatric patients with chilblain-like lesions

indicative of COVID-19 asymptomatic or pauci-symptomatic infection?. J Eur Acad Dermatol Venereol. 2021;35:e10-e13.
10. Caselli D, Chironna M, Loconsole D, et al. No evidence of SARS-CoV-2 infection by polymerase chain reaction or serology in children with pseudo-chilblain. Br J Dermatol. 2020;183:784-85.
11. Freeman EE, McMahon DE, Lipoff JB, et al. Pernio-like skin lesions associated with COVID-19: A case series of 318 patients from 8 countries. J Am Acad Dermatol. 2020;83:486-92.
12. Torrelo A, Andina D, Santonja C. Erythema multiforme-like lesions in children and COVID-19. Pediatr Dermatol. 2020;37: 442-46.
13. Neri I, Conti F, Virdi A, et al. Chilblains in a child with confirmed SARS-CoV-2 infection: A red flag for late-onset skin manifestation in previously infected individuals. J Eur Acad Dermatol Venereol. 2021;35:e357-e359.
14. Navarro L, Andina D, Noguera-Morel L et al. Dermoscopy features of COVID-19-related chilblains in children and adolescents. J Eur Acad Dermatol Venereol. 2020.
15. l Hachem M, Diociaiuti A, Concato C, et al. A clinical, histopathological and laboratory study of 19 consecutive Italian paediatric patients with chilblain like lesions: lights and shadows on the relationship with COVID 19 infection. J Eur Acad Dermatol Venereol. 2020;34:2620-29.
16. Colmenero I, Santonja C, Alonso-Riaño M, et al. SARS-CoV-2 endothelial infection causes COVID-19 chilblains: histopathological, immunohistochemical and ultrastructural study of seven paediatric cases. Br J Dermatol. 2020; 183(4):729-737.
17. Brealey JK, Miller SE. SARS-CoV-2 has not been detected directly by electron microscopy in the endothelium of chilblain lesions. Br J Dermatol. 2021;184:186.
18. Hubiche T, Cardot-Leccia N, Le Duff F, et al. Clinical, Laboratory, and Interferon-Alpha Response characteristics of patients with chilblain-like lesions during the COVID-19 pandemic. JAMA Dermatology. 2021;157:202.
19. Roca-Gines J, Torres-Navarro I, Sanchez-Arr aez J, et al. Assessment of acute acral lesions in a case series of children and adolescents during the COVID-19 pandemic. JAMA Dermatol. 2020;156:992.
20. Garcia-Lara G, Linares-Gonzalez L, R odenas-Herranz T, Ruiz-Villaverde R. Chilblain-like lesions in pediatrics dermatological outpatients during the COVID-19 outbreak. Dermatol Ther. 2020; e13516.
21. Docampo-Simón A, Sánchez-Pujol MJ, Gimeno-Gascon A, et al. No SARS-CoV-2 antibody response in 25 patients with pseudo-chilblains. Dermatol Ther. 2020;33:e14332.
22. Antia C, Baquerizo K, Korman A et al. Urticaria: A comprehensive review: Epidemiology, diagnosis, and work-up. J Am Acad Dermatol. 2018;79:599-614.
23. Do MH, Stewart CR, Harp J. Cutaneous Manifestations of COVID-19 in the Inpatient Setting. Dermatol Clin. 2021; 39:521-32.
24. Morey-Olivé M, Espiau M, Mercadal-Hally M, et al. Cutaneous manifestations in the current pandemic of coronavirus infection disease (COVID 2019). An Pediatr (Engl Ed). 2020; 92:374-75.
25. Rotulo GA, Signa S, Rosina S, et al. Giant Urticaria and Acral Peeling in a Child with Coronavirus Disease 2019. J Pediatr. 2021;230:261-63.
26. Chen V, Escandon Brehm J, Bellodi Schmidt F. Acute urticaria preceding other COVID-19– associated manifestations—A case report. Pediatr Dermatol. 2021;38:455-57.
27. Larenas-Linnemann D, Luna-Pech J, Navarrete-Rodríguez E, et al. Cutaneous manifestations related to COVID-19 immune dysregulation in the pediatric age group. Current Allergy and Asthma Reports. 2021;21(2).
28. Proietti I, Mambrin A, Bernardini N, et al. Urticaria in an infant with SARS-CoV-2 positivity. Dermatol Ther. 2020;33: e14043.
29. Le NK, Brooks JP. Acute urticaria as the initial presentation of COVID-19 in a pediatric patient. JAAD Case Rep. 2021;11: 137-38.
30. Özdemir Ö, Yýlmaz EA, Engin MMN. An infant with COVID-19 presenting with acute urticaria and angioedema. Int J Dermatol. 2021;60:e471-e472.
31. Khalili M, Iranmanesh B, Mohammadi S, Aflatoonian M. Cutaneous and histopathological features of coronavirus disease 2019 in pediatrics: A review article. Dermatol Ther. 2021;34:e14554.
32. Imbalzano E, Casciaro M, Quartuccio S. Association between urticaria and virus infections: A systematic review. Allergy Asthma Proc. 2016;37:18-22.
33. Criado PR, Abdalla BMZ, de Assis IC. Are the cutaneous manifestations during or due to SARS-CoV-2 infection/COVID-19 frequent or not? Revision of possible patho-physiologic mechanisms. Inflamm Res. 2020;69:745-56.
34. Kaushik A, Parsad D, Kumaran MS. Urticaria in the times of COVID-19. Dermatol Ther. 2020:e13817.
35. Siedner-Weintraub Y, Gross I, David A, et al. Paediatric erythema multiforme: epidemiological, clinical and laboratory characteristics. Acta Derm Venereol. 2017;97:489-92.
36. Bennardo L, Nisticò SP, Dastoli S, et al. Erythema multiforme and COVID-19: What do we know? Medicina (Kaunas). 2021;57:828.
37. Fernandez-Nieto D, Jimenez-Cauhe J, Suarez-Valle A, et al. Characterization of acute acral skin lesions in nonhospitalized patients: A case series of 132 patients during the COVID-19 outbreak. J Am Acad Dermatol. 2020;83:e61-e63.
38. Janah H, Zinebi A, Elbenaye J. Atypical erythema multiforme palmar plaques lesions due to SARS-Cov-2. J Eur Acad Dermatol Venereol. 2020;34:e373-e375.
39. Navaeifar MR, Ghazaghi MP, Shahbaznejad L. Fever with rash is one of the first presentations of COVID-19 in children: a case report. Int Med Case Rep J. 2020;13:335-40.
40. Labé P, Ly A, Sin C, et al. Erythema multiforme and Kawasaki disease associated with COVID-19 infection in children. J Eur Acad Dermatol Venereol. 2020;34:e539-e541.
41. Mahe A, Birckel E, Merklen C. Histology of skin lesions establishes that the vesicular rash associated with COVID-19 is not 'varicella-like'. J Eur Acad Dermatol Venereol. 2020;34: e559-e561.
42. Rekhtman S, Tannenbaum R, Strunk A. Eruptions and related clinical course among 296 hospitalized adults with confirmed COVID-19. J Am Acad Dermatol. 2021;84:946-52.
43. Genovese G, Colonna C, Marzano AV. Varicella-like exanthem associated with COVID-19 in an 8-year-old girl: A diagnostic clue? Pediatr Dermatol. 2020;37:435-36.
44. Riphagen S, Gomez X, Gonzalez-Martinez C, et al. Hyperinflammatory shock in children during COVID-19 pandemic. The Lancet. 2020;395:1607-08.
45. Dufort E, Koumans E, Chow E, et al. Multisystem Inflammatory Syndrome in Children in New York State. 2022. N Engl J Med. 2020;383:347-58.
46. Nakra NA, Blumberg DA, Herrera-Guerra A, Lakshmin-rusimha S. Multi-system inflammatory syndrome in children (MIS-C) following SARS-CoV-2 infection: review of clinical presentation, hypothetical pathogenesis, and proposed management. Children. 2020; 7:69.
47. Consiglio CR, Cotugno N, Sardh F, et al. The Immunology of multisystem inflammatory syndrome in children with COVID-19. Cell. 2020;183:968-981.e7.
48. Whittaker E, Bamford A, Kenny J, et al. Clinical characteristics of 58 children with a pediatric inflammatory multisystem syndrome temporally associated with SARS-CoV-2. JAMA.

2020;324:259-69.
49. Yasuhara J, Watanabe K, Takagi H, et al. COVID-19 and multisystem inflammatory syndrome in children: A systematic review and meta-analysis. Pediatr Pulmonol. 2020;56:837-48.
50. Ganguly M, Nandi A, Banerjee P, et al. A comparative study of IL-6, CRP and NT-proBNP levels in post-COVID multisystem inflammatory syndrome in children (MISC) and Kawasaki disease patients. Int J Rheum Dis. 2021.
51. Feldstein LR, Rose EB, Horwitz SM, et al. Multisystem inflammatory syndrome in US children and adolescents. N Engl J Med. 2020;383:334-46.
52. Young TK, Shaw KS, Shah JK, et al. Mucocutaneous manifestations of multisystem inflammatory syndrome in children during the COVID-19. JAMA Dermatol. 2021;157: 207-12.
53. Bapst T, Romano F, Müller M, Rohr M. Special dermatological presentation of paediatric multisystem inflammatory syndrome related to COVID-19: Erythema multiforme. BMJ Case Rep. 2020;13:e236986.
54. Sood M, Sharma S, Sood I, et al. Emerging evidence on multisystem inflammatory syndrome in children associated with SARS-CoV-2 infection: A systematic review with meta-analysis. SN Comprehensive Clinical Medicine. 2021;3:38-47.
55. Lee PY, Platt CD, Weeks S, et al. Immune dysregulation and multisystem inflammatory syndrome in children (MIS-C) in individuals with haploinsufficiency of SOCS1. J Allergy Clin Immunol. 2020;146:1194-1200.e1.
56. Berardicurti O, Conforti A, Ruscitti P, et al. The wide spectrum of Kawasaki-like disease associated with SARS-CoV-2 infection. Expert Rev Clin Immunol. 2020;16:1205-15.
57. Noval Rivas M, Porritt RA, Cheng MH et al. COVID-19-associated multisystem inflammatory syndrome in children (MIS-C): A novel disease that mimics toxic shock syndrome-the superantigen hypothesis. J Allergy Clin Immunol. 2021;147: 57-59.
58. Rodriguez-Smith JJ, Verweyen EL, Clay GM, et al. Inflammatory biomarkers in COVID-19-associated multisystem inflammatory syndrome in children, Kawasaki disease, and macrophage activation syndrome: a cohort study. Lancet Rheumatol. 2021;3:e574-e584.
59. Kwak JH, Lee SY, Choi JW. Korean Society of Kawasaki Disease. Clinical features, diagnosis, and outcomes of multisystem inflammatory syndrome in children associated with coronavirus disease 2019. Clin Exp Pediatr. 2021;64:68-75.
60. Català A, Galván-Casas C, Carretero-Hernández G, et al. Maculopapular eruptions associated to COVID-19: A subanalysis of the COVID-Piel study. Dermatol Ther. 2020; 33:e14170.
61. Magro CM, Mulvey JJ, Laurence J, et al. The differing pathophysiologies that underlie COVID-19-associated perniosis and thrombotic retiform purpura: A case series. Br J Dermatol. 2021;184:141-150.
62. Bouaziz JD, Duong T, Jachiet M, et al. Vascular skin symptoms in COVID-19: A French observational study. J Eur Acad Dermatol Venereol 2020;34.
63. Zhang Y, Cao W, Xiao M et al. Clinical and coagulation characteristics of 7 patients with critical COVID-2019 pneumonia and acro-ischemia. Zhonghua Xue Ye Xue Za Zhi. 2020; 41:E006
64. Olisova OY, Anpilogova EM, Shnakhova LM. Cutaneous manifestations in COVID-19: A skin rash in a child. Dermatol Ther. 2020;e13712.
65. Trüeb RM, Dutra Rezende H, Gavazzoni Dias MFR. What can the hair tell us about COVID-19?. Exp Dermatol. 2021;30:288-90.
66. Hayran Y, Yorulmaz A, Gür G, Aktaº A. Different hair loss patterns in two pediatric patients with COVID-19-associated multisystem inflammatory syndrome in children. Dermatol Ther. 2021;34:e14820.
67. Aslan Kayýran M, Cebeci F, Erdemir VA, et al. Fluorescence of nails and hair on Wood's lamp examination in Covid pandemic; Undefined effect of Favipiravir in humans. Dermatol Ther. 2021;34:e14740.
68. Thuangtong R, Angkasekwinai N, Leeyaphan C, et al. Patient recovery from COVID-19 infections: Follow-Up of Hair, Nail, and Cutaneous Manifestations. Biomed Res Int. 2021; 2021:5595016.
69. Lester JC, Jia JL, Zhang L, et al. Absence of images of skin of colour in publications of COVID-19 skin manifestations. Br J Dermatol. 2020;183:593-5.
70. Cline A, Berk-Krauss J, Keyes Jacobs A, et al. The underrepresentation of "COVID toes" in skin of color: An example of racial bias or evidence of a tenuous disease association?. J Am Acad Dermatol. 2021;84:e91-e92.
71. Dalal A, Jakhar D, Agarwal V, Beniwal R. Dermatological findings in SARS-CoV-2 positive patients: An observational study from North India. Dermatol Ther. 2020;33:e13849.
72. Sukhavasi B, Kilaru KR, Munnangi P, et al. Cutaneous manifestations among COVID 19 patients admitted to a COVID hospital in South India. Int J Heal Clin Res. 2021;4:9-13.
73. Goyal S, Prabhu S, US Pai SB, Mohammed A. Cutaneous manifestations of COVID-19 in skin of color: A firsthand perspective of three cases in a tertiary care center in India. Postgrad Med. 2021;133:307-9.

Web Table I Reported Cases of Pediatric Urticaria Associated with Covid-19 Infection

Article	*Age*	*Clinical features*	*Covid report*	*Contact history*	*Severity of covid-19*	*Timing of urticaria with respect to Covid-19 infection*	*Sequalae*
Miriam et al [24] 2020	2months / Female	Acute urticaria in whole body sparing palms and soles. No angioedema	Positive	2 covid positive individuals	Mild	Along with other covid-19 symptoms such as fever	Resolution within 24 hours with oral symptomatic therapy
Rotulo et al[25]	6 years/girl	Giant urticarial wheals followed by desquamation in distal extremities	Positive	Mother covid-19 positive	Mild	1 day before onset of systemic symptoms	Resolution within 24 hours with oral symptomatic therapy
Chen et al[26]	6 months/ male	Generalized urticarial rash	Positive	Aunt and grandmother covid-19 positive	Mild	Few days before onset of systemic symptoms	Resolution within 24 hours with oral symptomatic therapy
Larenas-Linneman n et al[27]	5 months/ male	Generalized urticarial rash	Positive	Contact with covid-19 positive patient	Mild	Along with systemic symptoms	Resolution within 24 hours with oral symptomatic therapy
Proietti et al[28]	6 months/ male	Giant urticarial rash over limbs and trunk	Positive	Parents covid-19 positive	Mild	2 weeks after covid-19 positive report	Treated with oral corticosteroids
Le et al[29]	5 years/ girl	Generalized urticaria sparing face	Positive	Father covid-19 positive	Mild	2 days before onset of systemic symptoms	Resolution within 24 hours with oral symptomatic therapy
Oner et al[30]	12 months/ girl	Urticaria with angioedema	Positive	No contact	Mild	Along with systemic symptoms	Intravenous corticosteroids required.
Khalili et al[31]	2 months/ girl	acute urticaria involving face, trunk, and upper and lower extremities with sparing of mucosa, palm, and sole	Positive	2 family members with covid-19 positivity	Mild	Asymptomatic	Resolution within 24 hours with oral symptomatic therapy

Web Table II Reported Erythema Multiforms or EM-like Rash in Pediatric Covid Patients

Authors	*No.*	*Age*	*Clinical features*	*Onset with respect to Covid-19 infection*	*Covid-19 confirmation*	*Severity of Covid-19*	*Sequalae*
Fernandez-Neito et al [37]	37	12 years (Mean)	Round erythematous macules and vesicles which were less widespread, smaller in size and atypical target lesions. The lesions were restricted to palms and soles	With or after covid-19 infection	Via contact with covid-19 positive patient, health worker or RT-PCR positivity	Mild , only 6 out of 37 patients had other systemic symptoms of covid-19	7.4 days was mean duration of EM like rash. Good prognosis associated.
Torrello et al[13]	4	11-17 years	Target and targetoid lesions in addition to chilblain like lesions were noticed. Mild itch and mild pain was associated	-	1 case was covid-19 RT-PCR positive. In 2 cases IHC revealed antibody against SARS-CoV-2 spike protein to be positive in endothelial cells and eccrine glands	Mild	Excellent prognosis with compleet resolution in 1-3 weeks
Janah et al[38]	1	17/Male	Painless erythematous maculopapular atypical targetoid eruption of palms only	15 days after Covid-19 infection	RT-PCR positive	Mild	Complete resolution
Navaeifar et al[39]	1	12 months	Fever with EM like lesions	Along with systemic symtoms	RT-PCR positive	Severe	ICU care required with improvement within 5 days
Larenas-Linneman et al[27]	1	6 years/ Female	2 targetoid lesions on thigh and abdomen	7 days after systemic symptoms	RT-PCR positive	Mild	Complete resolution with symptomatic therapy
Labe et al[40]	1	6 years/ Male	EM lesions with painful chelitis	Few days after onset of chelitis	RT-PCR positive	Mild	Discharged after 2 weeks

Bharati Vidyapeeth (Deemed to be University)

Medical College, Pune (Maharashtra)

Post Doctoral Fellowships in Pediatric Subspecialties

Greetings! Bharati Vidyapeeth Deemed to be University has been reaccredited "**A" grade by NAAC** and as on today is recognized by the UGC as one of the 20 universities in India for global promotion of education. Bharati Vidyapeeth Deemed to be University Medical College, Pune has an advanced Department of Pediatrics and state-of-the-art infrastructure with multiple well developed Pediatric sub-specialties that offer Fellowship training of a national standing. More than 200 fellows have passed out in last decade form our unit.

****Meritorious candidates after successful completion of their training program may be given the opportunity in select courses to pursue a 3-month "self-sponsored observership" at Birmingham Women's and Children's Hospital, NHS Foundation, UK.****

Courses Available

No.	Fellowship Courses	Duration
1	Fellowship in Pediatric Critical Care	12 months
2	Fellowship in Neonatology	12 months
3	Dual Fellowship in Pediatric Critical Care and Neonatology	18 months
4	Fellowship in Pediatric Epilepsy and Neurology	12 months
5	Fellowship in Pediatric Endocrinology	12 months
6	Fellowship in Pediatric Infectious Diseases	12 months
7	Fellowship in Pediatric Hemato-Oncology	12 months
8	Fellowship in Development and Behavioral Pediatrics	12 months
9	Fellowship in Pediatric Genetics & Metabolic Disorders	12 months
10	Fellowship in Pediatric Rheumatology	12 months
11	Fellowship in Pediatric Pulmonology	12 months

Eligibility: MD / DNB (Pediatrics) / Equivalent Degree, age preferably less than **35** years

Sponsored candidates from Medical colleges can also apply with relevant certificates and documents, last date of application submission being 15th August 2022.

The candidates will be selected on the basis of virtual interview which will be held in the 2nd week of September 2022 at BVDU Medical College, Pune. Selected candidates will receive shared accommodation and a monthly stipend as per the University policy.

Eligible and interested candidates may apply with complete biodata and relevant certificates to -

Dr. S. K. Lalwani, Vice Principal, Medical Director, Professor and Head – Pediatrics, 09th Floor, New Super Speciality Building, Bharati Hospital, Pune Satara Road, Katraj, Pune - 411 043, MAHARASHTRA. Phone: 020-40 55 55 55 Ext 3931.

OR

E-mail your application with Curriculum vitae to *bvpedfellowship@gmail.com* by **15th August 2022**

Diagnosis and Management of Global Development Delay: Consensus Guidelines of Growth, Development and Behavioral Pediatrics Chapter, Neurology Chapter and Neurodevelopment Pediatrics Chapter of the Indian Academy of Pediatrics

MONICA JUNEJA,[1] ARPITA GUPTA,[1] SMITHA SAIRAM,[1] RIDHIMAA JAIN,[1] MONIKA SHARMA,[2] ANJANA THADANI,[3] ROOPA SRINIVASAN,[4] LOKESH LINGAPPA,[5] SHABINA AHMED,[6] KS MULTANI,[6] PANKAJ BUCH,[7] NANDITA CHATTERJEE,[8] SAMIR DALWAI,[9] MADHULIKA KABRA,[10] SEEMA KAPOOR,[1] PRARTHANA KHAROD PATEL,[11] GIRISHA KM,[12] MADHURI KULKARNI,[13] PAM KUNJU,[14] PRABHBJOT MALHI,[15] ZAFAR MEENAI,[16] DEVENDRA MISHRA,[1] NANDINI MUNDKUR,[17] MKC NAIR,[18] SAMUEL PHILIP OOMMEN,[19] CHHAYA PRASAD,[20] ARUN SINGH,[21] LEENA SRIVASTAVA,[22] PRAVEEN SUMAN,[23] RAHUL THAKUR[24]

From [1]Department of Pediatrics, Maulana Azad Medical College, New Delhi; [2]Department of Pediatrics, Christian Medical College, Ludhiana; [3]Niramaya Hospital and Guidance Clinic, Chembur, Mumbai, Maharashtra; [4]Ummeed Child Development Centre, Mumbai, Maharashtra; [5]Rainbow Children's Hospital, Hyderabad; [6]Indian Academy of Pediatrics, Neurodevelopment Chapter; [7]Department of Pediatrics, MP Shah Government Medical College, Jamnagar, Gujarat; [8] Department of Pediatrics, MGM Medical College, Kolkata, West Bengal; [9]New Horizons Child Development Centre, Mumbai, Maharashtra; [10]Division of Genetics, Department of Pediatrics, All India Institute of Medical Sciences (AIIMS), New Delhi; [11]GCS Medical College, Hospital and Research Centre, Ahmedabad, Gujarat; [12]Department of Medical Genetics, Kasturba Medical College, Manipal, Karnataka; [13]Mumbai Port Trust Hospital, Mumbai, Maharashtra; [14]Department of Pediatric Neurology, Medical College Thiruvananthapuram, Kerala; [15]Department of Pediatrics, Post Graduate Institute of Medical Education and Research, Chandigarh; [16]Ummeid Group of Child Development Centers, Bhopal, Madhya Pradesh; [17]Center for Child Development & Disabilities (CCDD) Bengaluru, Karnataka; [18]NIMS-SPECTRUM-Child Development Research Centre (CDRC) NIMS Medicity, Thiruvananthapuram ,Kerala; [19]Christian Medical College, Vellore, Tamil Nadu; [20]ASHA, Centre for Autism and Intellectual Developmental Disorders, Chandigarh; [21]All India Institute of Medical Sciences, Jodhpur, Rajasthan; [22]Bharati Vidyapeeth Medical College & Hospital, Pune, Maharashtra; [23]Child Development Centre, Sir Gangaram Hospital, New Delhi; [24]The Children's Neurodevelopmental Centre, Patna, Bihar.

Correspondence to: Dr Monica Juneja, Director-Professor and Head, Department of Pediatrics, Maulana Azad Medical College and associated Lok Nayak Hospital, New Delhi. drmonicajuneja@gmail.com

Justification: Global developmental delay (GDD) is a relatively common neurodevelopmental disorder; however, paucity of published literature and absence of uniform guidelines increases the complexity of clinical management of this condition. Hence, there is a need of practical guidelines for the pediatrician on the diagnosis and management of GDD, summarizing the available evidence, and filling in the gaps in existing knowledge and practices. **Process**: Seven subcommittees of subject experts comprising of writing and expert group from among members of Indian Academy of Pediatrics (IAP) and its chapters of Neurology, Neurodevelopment Pediatrics and Growth Development and Behavioral Pediatrics were constituted, who reviewed literature, developed key questions and prepared the first draft on guidelines after multiple rounds of discussion. The guidelines were then discussed by the whole group in an online meeting. The points of contention were discussed and a general consensus was arrived at, after which final guidelines were drafted by the writing group and approved by all contributors. The guidelines were then approved by the Executive Board of IAP. **Guidelines**: GDD is defined as significant delay (at least 2 standard deviations below the mean with standardized developmental tests) in at least two developmental domains in children under 5 years of age; however, children whose delay can be explained primarily by motor issues or severe uncorrected visual/ hearing impairment are excluded. Severity of GDD can be classified as mild, moderate, severe and profound on adaptive functioning. For all children, in addition to routine surveillance, developmental screening using standardized tools should be done at 9-12 months,18-24 months, and at school entry; whereas, for high risk infants, it should be done 6-monthly till 24 months and yearly till 5 years of age; in addition to once at school entry. All children, especially those diagnosed with GDD, should be screened for ASD at 18-24 months, and if screen negative, again at 3 years of age. It is recommended that investigations should always follow a careful history and examination to plan targeted testing and, vision and hearing screening should be done in all cases prior to standardized tests of development. Neuroimaging, preferably magnetic resonance imaging of the brain, should be obtained when specific clinical indicators are present. Biochemical and metabolic investigations should be targeted towards identifying treatable conditions and genetic tests are recommended in presence of clinical suspicion of a genetic syndrome and/or in the absence of a clear etiology. Multidisciplinary intervention should be initiated soon after the delay is recognized even before a formal diagnosis is made, and early intervention for high risk infants should start in the nursery with developmentally supportive care. Detailed structured counselling of family regarding the diagnosis, etiology, comorbidities, investigations, management, prognosis and follow-up is recommended. Regular targeted follow-up should be done, preferably in consultation with a team of experts led by a developmental pediatrician/ pediatric neurologist.

Keywords: *Developmental assessment, Developmental screening, Early intervention, Intellectual disability.*

Published online: February 19, 2022; ***PII:*** S097475591600406

The global estimates of the prevalence of global developmental delay (GDD) range from 1-3% [1]. There are recent reports of much higher prevalence of 6.4% among children from Turkey and 8% from UAE [2,3]. In India, various studies report a prevalence ranging from 3-13%, depending upon the age group screened, tools used and geographical areas surveyed [4,5]; however, these estimates may not be a representative, as most of these studies were based only on developmental screening. GDD is reported to be 30% more common in boys as compared to girls, with the difference disappearing with increasing age [6].

The etiology of GDD is heterogeneous and can be divided into genetic and nongenetic causes, and categorized as prenatal, perinatal and postnatal according to the timing of exposure [7-9]. Genetic defects are the most common etiology occurring in nearly 30-50% of the cases, the proportion being similar in developed countries and India [10,11]. These can be classified into syndromic and non-syndromic GDD, wherein, syndromic delay clinically manifests as a typical phenotype (e.g., Down syndrome), dysmorphisms and congenital anomalies whereas, when the pathology is unknown and GDD is the only discernible feature, it is called as non-syndromic delay [12]. Potentially preventable conditions like hypoxic ischemic encephalopathy (HIE) and hypothyroidism are a more common cause in India as compared to developed countries [10,11,13-19] (**Table I**). As most Indian studies on GDD etiology are tertiary-center based, they may be associated with a referral bias, and hence, not a true indicator of etiology in the community.

Children with GDD commonly have comorbidities, like epilepsy, visual problems, hearing impairment, sleep disturbances, motor impairment, autism, drooling, constipation and, behavioral and psychiatric problems [20-24] (**Box I**).

OBJECTIVE

These guidelines aim to provide pragmatic clinical guidance for pediatricians on the diagnosis and manage-ment of GDD in the Indian settings.

PROCESS

The process of formulating the guidelines started in March, 2020. Subject experts and members of Indian Academy of Pediatrics (IAP) chapters of Neurology, Neurodevelopment Pediatrics, and Growth, Development and Behavioral Pediatrics, were divided into seven subcommittees based on the expertise. Each group comprised of a writing team and a reviewing team. The seven subcommittees evaluated

Table I Etiology of Global Developmental Delay

Timing of exposure	*Possible causes*	*Proportion of diagnostic yield*	
		India	*Other countries*
Prenatal			
Genetic	Chromosomal aberrations (e.g., Trisomy 21)	19-20%	5-10%
	Monogenic (including Fragile X syndrome)	1-25%	3-10%
	Multiple malformations or clinically recognizable syndromes	6-14%	3-10%
	Metabolic/ Inborn error of metabolism	3-4%	1-8%
	Cerebral dysgenesis / Central nervous system malformations	10-11%	2-18%
	Intrauterine infection	3-4%	0.4-2%
Environmental	Toxins/ teratogens (e.g., alcohol, valproate, cocaine)	1 %	2 - 9%
Perinatal			
Acquired	Hypoxic ischemic encephalopathy (HIE)	14-31%	9-10%
Environmental	Neonatal complications: Bilirubin encephalopathy/ Meningitis/encephalitis sequelae	1%	–
	Prematurity/ birth trauma	2%	–
Postnatal			
Acquired	Hypothyroidism[a]	3-11%	–
Environmental	Brain tumor	1%	1%
	Infantile tremor syndrome	1%	–
	Severe psychosocial deprivation/neglect	–	3-4%
	Nutritional deficiencies (e.g. iodine, vitamin B12, thiamine)	–	–
	Toxins (e.g. lead)	–	–
	Post Traumatic	–	–

[a] Hypothyroidism may be found overlapping with other causes such as Down syndrome, other genetic syndromes, some inborn errors of metabolism, and secondary to maternal antithyroid antibodies. Data from references 10,11,13-19.

Box I Comorbidities of Global Developmental Delay

Medical comorbidities

Neurological

- Visual deficits (15-75%)
- Hearing impairment (9 -17%)
- Epilepsy (5-30%)
- Cerebral palsy (8-30%)
- Pseudobulbar dysfunction (feeding issues) (20-47%)
- Sleep issues (40-80%)

Non-neurological

- Recurrent infections
- Protein energy malnutrition (40-70%)
- Drooling (45%)
- Constipation (30-60%)
- Nutritional anemia (5.5%)

Psychiatric/behavioral comorbidities

Attention deficit hyperactivity disorder (35-40%), Autism spectrum disorder (15-20%), stereotypic movement disorders (with/ without self-injurious behaviors), mood disorder, anxiety disorder, aggression and disruptive behaviors (26%).

evidence on definition, etiology, clinical evaluation, investigations, management, neurological comorbidities, and prognosis of children with GDD. Each subcommittee reviewed literature, developed key questions, analyzed published studies and prepared draft guidelines for their respective topic after multiple rounds of discussion. Subsequently, the guidelines and their evidence were discussed by the whole group in an online meeting held on 21 December, 2020. Points of contention were again discussed through multiple rounds of discussions via Google forms, online meetings and emails. Final guidelines were then formulated by consensus. These were approved by all experts, and then approved by the Executive Board of Indian Academy of Pediatrics.

GUIDELINES

Definition

GDD is defined as a significant delay in two or more of the following developmental domains: gross/fine motor, speech/ language, cognition, social/personal, and activities of daily living. Significant delay is defined as performance being two or more standard deviations lower than the mean, on age-appropriate, standardized norm-referenced testing [1,7]. However, strict adherence to this definition i.e., involvement of any two domains may allow children with developmental delays but intact cognition to be also labelled as GDD. Many guidelines, especially those on etiological workup, consider GDD to be a precursor of intellectual disability (ID). The term GDD has come into popular use as a surrogate label because of the difficulties in agreeing on the objective measurement of intelligence in a consistent, reliable, and valid fashion in the young child (<5 year). Typically, these children have delay across all domains.

The diagnostic category of GDD has been included for the first time as a subcategory under ID in the 5th edition of Diagnostic and Statistical Manual of Mental Disorders (DSM-5) and is to be diagnosed when an individual younger than five years of age fails to meet expected developmental milestones in several areas of intellectual functioning and is unable to undergo standardized testing for the same [25]. DSM-5 also recommends reassessment of these children after a period of time.

Although both GDD and ID share common features, and at their core, represent a disorder of cognition, it is important to understand that not all children who meet criteria for GDD go on to develop ID later. Reasons for this might include maturational effects, a change in developmental trajectory (due to an intervention), reclassification to a different disability category, or an imprecise use of the GDD diagnosis initially [26]. As lack of stimulation is a known key risk factor for poor child development and efficacy trials have shown structured psychosocial interventions to successfully mitigate developmental deficits, these children should be provided appropriate stimulation before being labelled as GDD [27,28]. **Web Table I** summarizes the differences between GDD and ID [1,8,9].

GDD has been earlier classified into three grades of severity based on developmental quotient (DQ) [19]; however, the Rights of Persons with Disability Act of India (RPwD Act) has classified it in line with ID as mild when social quotient (SQ) is 55-70, moderate: 36-54, severe: 21-35 and profound <20, respectively, based on adaptive functioning [29].

Guidelines

1A GDD is defined as significant delay (atleast 2 SD below the mean with standardized tests) in at least two developmental domains from the following: gross or fine motor, speech/language, cognition, social/personal and activities of daily living in children under 5 years of age. Even though cerebral palsy or other neuromotor impairments as well as visual impairment/hearing impairment are common comorbidities with GDD, children whose delay in two or more domains can be explained primarily by motor delay or severe uncorrected visual/ hearing impairment may need to be excluded from the diagnostic label of GDD.

1B Children with developmental delay and coexistent psychosocial deprivation should be provided stimulation and reassessed after 6-9 months, before being diagnosed as GDD.

1C Severity of GDD can be classified as mild, moderate, severe and profound based on adaptive functioning when social quotient (SQ) is 55-70, moderate: 36-54, severe: 21-35 and profound <20, respectively.

Developmental Surveillance and Screening

Developmental surveillance includes documenting the developmental history, eliciting parental concerns and performing developmental examination, whereas developmental screening refers to use of a brief standardized tool for identifying risk of developmental disorders [30]. For developmental surveillance, primary pediatrician can assess the age of attainment of milestones and presence of any developmental red flags [31]. Universal developmental screening can be done in community by non-specialists after undergoing training. For developmental screening, preference should be given to norm-referenced tests, which assess multiple domains. Psychometric properties and feasibility of use are other important parameters to consider while evaluating the suitability of the develop-mental screening tool for a given setting. For screening/surveillance of preterm babies, corrected gestational age is used till two years of chronological age. Developmental screening tools that are commonly used in India are listed in **Web Table II** [32-36].

Autism screening: Autism spectrum disorder (ASD) and GDD are not only common comorbid conditions but children with isolated ASD without involvement of cognition may even get a diagnosis of GDD, in view of delay in ≥2 domains (personal-social and language). Autism screening and early diagnosis is important for instituting specific interventions that have been docu-mented to improve the long-term prognosis of ASD.

Guidelines

2A For all children, routine developmental surveillance should be done till two years of age during every immunization visit using questions specifically related to current age-appropriate milestones.

2B In addition, developmental screening using standardized screening tools should be done at 9-12 months, 18-24 months of age, and at school entry.

2C For high risk infants, in addition to surveillance, developmental screening should be done at 4-6 months, 9-12 months, 18-24 months and yearly till 5 years of age; and once at school entry.

2D Children diagnosed as GDD should be screened for ASD at 18- 24 months (as per the routine ASD screening guidelines for all children), and if screen negative, again at 3 years of age.

Clinical Evaluation

Comprehensive clinical evaluation remains the cornerstone for identification of etiology, associated co-morbidities, and assessment of developmental status as well as for planning intervention in children with GDD (**Fig.1**). The assessment of developmental status in various domains by a pediatrician can be used to provisionally diagnose GDD as a delay in ≥2 domains (DQ < 70), for initiating timely management. The child should; however, be referred to the experts at the slightest suspicion, even if clinical assessment is not possible.

The definitive diagnosis of GDD requires norm-referenced standardized tests of development, which are to be administered by trained personnel. The choice of tests in the Indian context is limited because most of the tests are norm-referenced for the Western population, and the norms for available Indian tests have not been revised for more than 20 years. The severity of the GDD is assessed by using standardized tools for adaptive beha-viors. The adaptive behaviors are social, communication and motor skills used for day-to-day functioning of an individual. The commonly used developmental and adaptive behavior tests are given in **Web Table III** [37-44]. The ones most commonly in use in India are Developmental Assessment Scale for Indian Infants (DASII), Bayley Scale of Infant Development (BSID), and Vineland Social Maturity Scale (VSMS).

Guidelines

3A A detailed history and clinical examination for assessment of developmental delay, etiological risk factors and comorbidities should be recorded as accurately as possible.

3B Definitive diagnosis of GDD should be based on the results of standardized tests of development.

Investigations

The aim of investigations is to establish the etiology, especially the treatable conditions, understand the recurrence risk, and identify the co-morbid conditions. Investigations are prioritized for the identification of treatable conditions, keeping in mind the clinical clues, essentiality of the investigation, and availability of resources.

Vision and hearing: Severe visual and hearing impairment can manifest as delay in multiple domains, as well as exacerbate the existing developmental problems and impact results of developmental testing. Visual assessment includes a comprehensive ophthalmologic examination including visual acuity, fundoscopy and extra-ocular movements. Visual evoked potential (VEP) is indicated in suspected cases of cerebral visual impairment (CVI). The choice of hearing screen is based on the type of hearing loss anticipated and available resources. Otoacoustic emissions (OAE)/ brainstem evoked response audiometry (BERA) screener

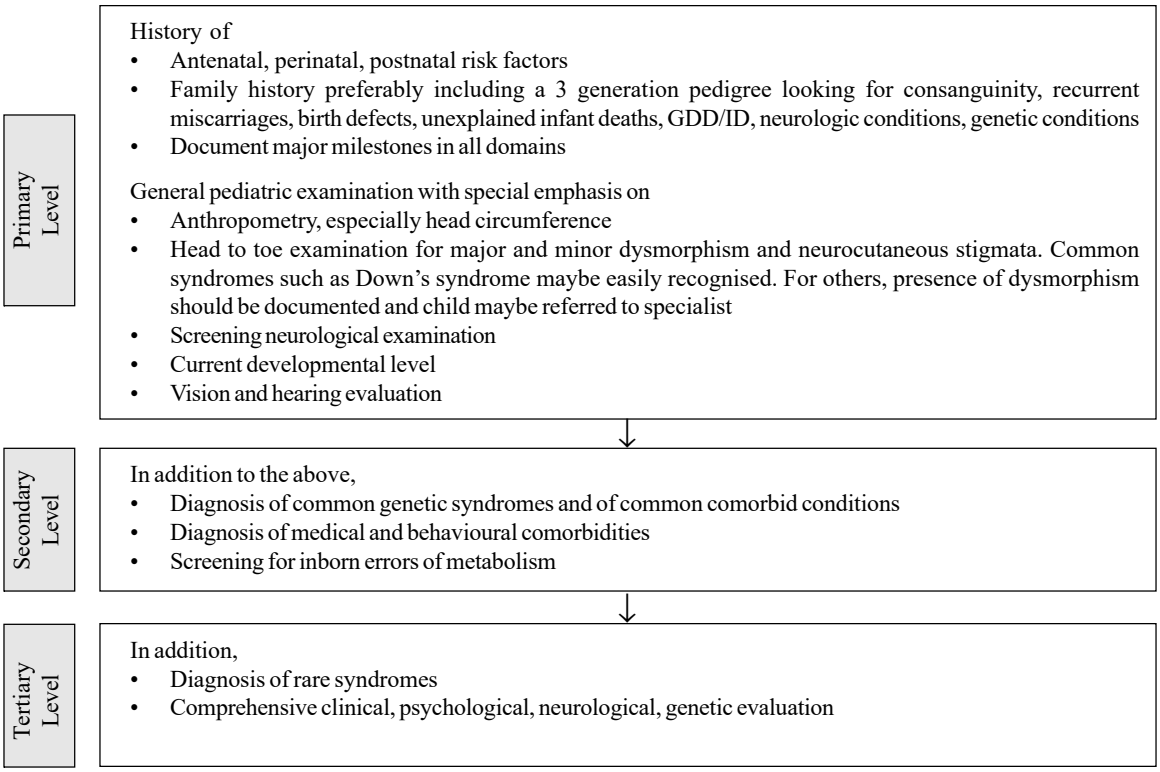


Fig.1 Clinical evaluation of a child with global developmental delay.

being objective and feasible methods are considered optimal screening tools [45]. Full diagnostic evaluation with auditory steady state response (ASSR)/BERA and/or behavioral audiometry is considered, if the child fails screening tests [46].

Blood investigations: These should be targeted towards identifying treatable conditions causing GDD, amongst which hypothyroidism is the most important. Congenital hypothyroidism accounts for around 1-3% cases of cognitive delay and due to limited reach of newborn screening programs in India, may be missed easily. In addition, many associated chromosomal abnormalities e.g., trisomy 21, 45X and 22q11 deletion, have an increased risk of hypothyroidism. Early identification of hypothyroi-dism and its timely treatment may markedly impact the prognosis and hence is an essential investigation in all cases of GDD [1,18]. Nutritional deficiency of vitamin B12, inborn errors of cobalamin metabolism, and iron deficiency may also be associated, especially in children having a restricted diet or pica [47,48]. Apart from these, around 20-30% of children with neuromuscular disorders such as Duchenne muscular dystrophy (DMD) have associated cognitive delay, which may present with GDD before other neurological deficits becomes obvious. Measurement of serum creatinine phosphokinase (CPK) may aid in screening for these disorders [49]. Serum lead levels should be done in cases where specific history of environmental exposure is present [1,49]. Studies have shown biotinidase deficiency as an important treatable cause of developmental delay and therefore, should be considered in these children even without characteristic clinical markers, more so in the absence of widespread routine newborn screening in India [50].

Neuroimaging: Abnormalities in neuroimaging may be seen in 30-70% cases with GDD [49]; however, its contribution towards an etiological diagnosis range from 10-40% [51]. Yield of neuroimaging increases two- to five-fold when neurological abnormalities like abnormal head size, seizures and abnormal neurological findings are present. For children with mild GDD without any motor abnormalities or specific clinical features, neuroimaging may be deferred. Plain magnetic resonance imaging (MRI) or computed tomography (CT) is usually sufficient for evaluation of GDD. MRI has been found to have a higher sensitivity than CT in detecting abnormalities and is the preferred modality. However, sequential use of CT followed by MRI should be discouraged. Mitochondrial disorders and cerebral creatine deficiency syndromes are additional treatable conditions, which may be picked on proton magnetic resonance spectroscopy (MRS) [49,51-53].

Electroencephalogram (EEG): It is indicated in patients where history or examination is suggestive of epilepsy or an epileptic syndrome. Although, the evidence is limited, children with CVI are at higher risk of underlying epileptic encephalopathy and may warrant an early EEG.

Metabolic testing: It is amongst the second line investigations and is considered in cases of GDD where neonatal screening has not been done, there is history of consanguinity, family history of a similar illness or unexplained miscarriages, developmental regression, episodic decompensation or examination findings suggestive of a specific etiology [54,55].

Genetic testing: Studies show that genetic etiology may be identified in 30-50% of cases of GDD based on the patient selection and techniques utilized [50]. Due to the presence of large number of tests in the armamentarium to identify a genetic etiology, often it is a challenge to choose an appropriate test and patients may undergo several tests before a conclusive diagnosis is reached [52]. The family needs to be counselled that despite undergoing all possi-ble tests, etiology may still not be established. This is important as many of these tests are expensive and may reveal inconclusive or uncertain findings [53,54]. These guidelines focus on choosing an appropriate test based on the history and clinical phenotype of the patients but with a caveat that there is a considerable overlap at times and clinical classification may be difficult in many scenarios.

It is important that the treating clinicians identify common genetic disorders early, based on the clues (**Web Box I**) and advise the parents regarding the need for genetic testing, which may be done locally, if available, and/or do timely referrals to experts who can interpret the results and provide information about variant interpretations and secondary findings. Some of these disorders may require early therapeutic intervention and majority may benefit by early intervention and management of comorbidities. Moreover, in a significant proportion of cases, recurrence can be prevented by counselling and prenatal testing if a specific genetic etiology is identified.

Web Table IV summarizes various techniques, indications of their use, yield and important advantages and pitfalls [51,52,55-58]. A simplified approach for genetic testing in GDD/ID is depicted in **Fig. 2** and approach towards genetic evaluation of GDD according to the level of care, is shown in **Fig. 3**.

Guidelines

4A Investigations should always follow a careful history and detailed examination to plan targeted testing, wherever required.

4B Vision and hearing screening are recommended in all cases especially before subjecting the child to standardized tests of development.

4C Neuroimaging is recommended when specific clinical indicators are present. If available, MRI/MRS should be obtained in preference to CT scan.

4D EEG is recommended only in children with clinical suspicion of epilepsy or epileptic syndromes.

4E Biochemical and metabolic investigations should be primarily targeted towards identifying treatable conditions causing/associated with GDD.

4E.1 Evaluation of thyroid function should be considered in all children with GDD, especially in the absence of documented newborn screening results. The tests may need to be repeated at regular intervals in children who are at high risk (e.g., Down syndrome) or have symptoms suggestive of hypothyroidism.

4E.2 Biotinidase deficiency should be ruled out, especially in absence of newborn screening.

4E.3 Iron and B12 deficiency should be considered, even in the absence of other pointers.

4E.4 Lead levels are recommended in cases with risk of environmental exposure.

4E.5 CPK is recommended in young boys with unexplained GDD.

4F Appropriate genetic tests are recommended in presence of clinical suspicion of a genetic disorder and/or in the absence of a clear etiology.

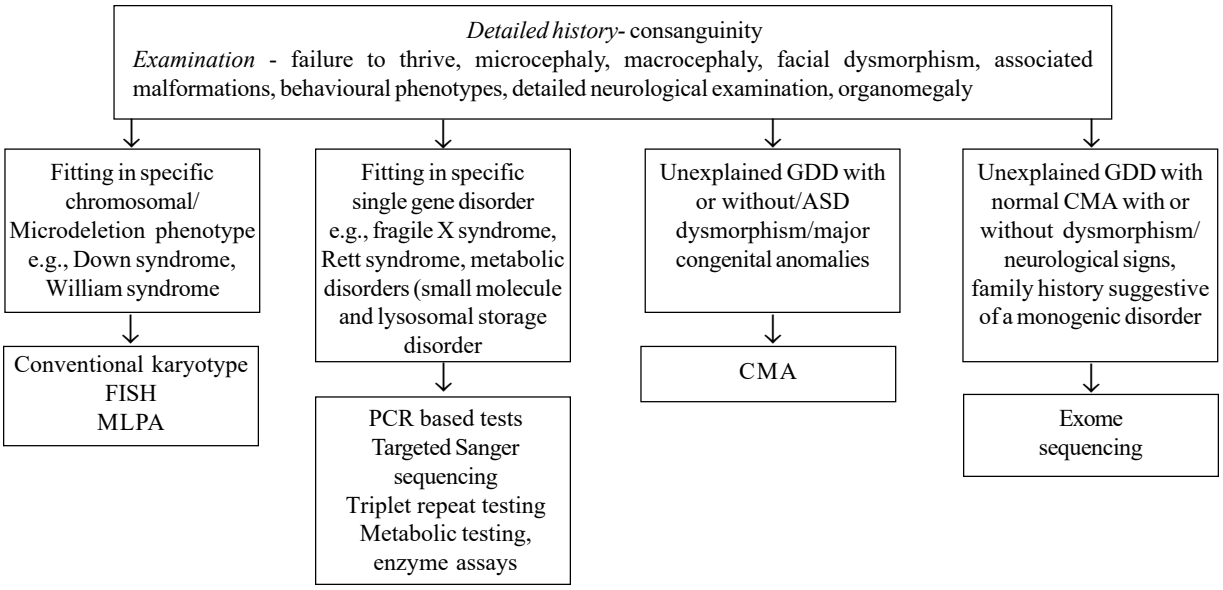
Management

Early intervention for high-risk newborns

In all cases, the mainstay of treatment is early detection and early intervention. Evidence suggests that developmentally supportive care in neonatal intensive care unit (NICU) setting could have significant effect on mental and motor development of preterm infants, at 12 and 24 months of age [59]. The core measures of developmentally supportive care include protected and maintained sleep rhythms, pain and stress assessment and management, positioning and handling, protecting the skin, provision of a healing environment, and family-centered care [60].

Management of treatable causes

In addition to common treatable causes like congenital hypothyroidism and nutritional deficiencies, around 116 treatable inherited metabolic disorders (IMDs) causing GDD/ID have been identified, and the number is increasing with the



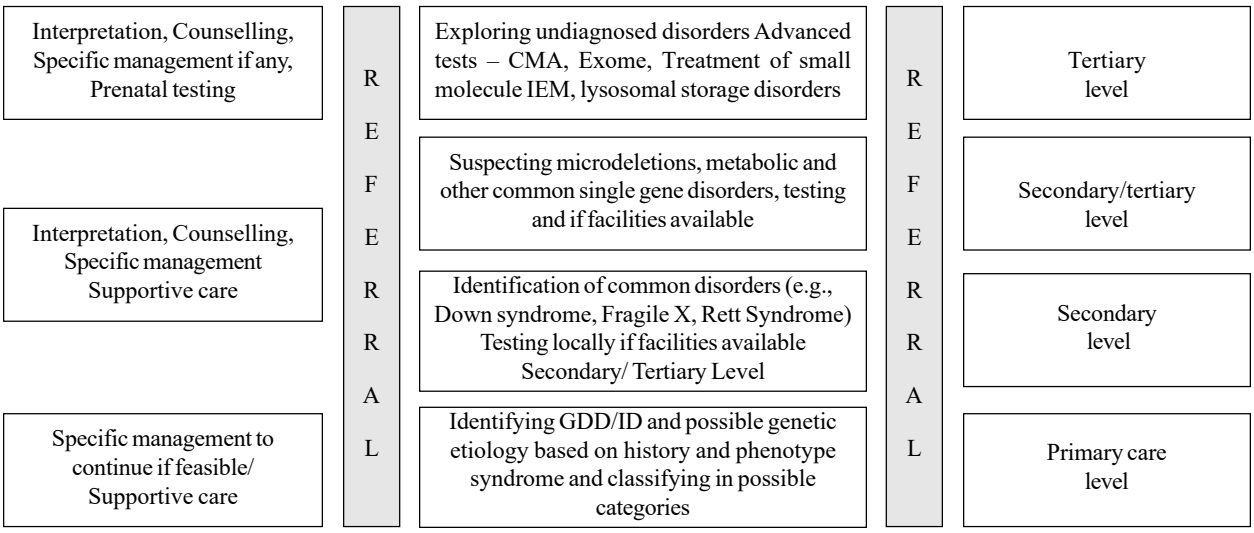
ASD: autism spectrum disorder, CMA: chromosomal microarray, GDD: global developmental delay, FISH: fluorescent in situ hybridization, MLPA: multiplex ligation probe assay.

Fig.2 Approach to genetic testing in global developmental delay.

advent of new technologies [61]. Even though these IMDs are rare, identification and early implemen-tation of specific therapy can improve developmental outcome, halt progression of an ongoing developmental delay and lead to improvement in associated comorbidities like seizures [62]. An app is available for the clinicians for more information on treatable IMDs (*https://treatable-id.org/about.html).*

Healthcare interventions

Infants and young children with developmental difficulties need access to primary healthcare just like other children of same age including components of early childhood development (ECD), which are good health (immunization, dental care and treatment during illness), optimal nutrition,



CMA: chromosomal microarray, GDD: global developmental delay, IEM: inborn error of metabolism, ID: intellectual; disability

Fig. 3 Genetic testing of global developmental delay at different health care levels.

opportunities for early learning, responsive parenting, and safety and security [63]. Growth monitoring is done using the usual growth charts; however, special growth charts are to be used in those with syndromes like Down syndrome and Prader-Willi syndrome [64-66]. **Table II** briefly outlines the health care interventions for children with GDD.

Early developmental interventions

Studies have shown that early intervention in children with developmental disabilities improves their develop-mental potential and functioning, and also benefits caregivers and families [67,68]. This requires a multi-disciplinary team consisting of developmental pedia-trician/pediatric neurologists, clinical psychologists, occupational therapists, physiotherapists, special edu-cators and speech therapists. The goals and specific treatment modalities should be individualized, depending on the cause and severity of GDD. In case of non-availa-bility of a multidisciplinary team, the primary pediatrician can advise parents about simple stimulation activities, till the child is seen by domain experts [69]. General principles of developmental intervention are outlined in **Box II**, and the activities for developmental intervention and stimu-lation in various domains have been described in the Mother and Child Protection (MCP) guidebook of Government of India (*https://nhm.gov.in/New_Updates_ 2018/NHM_Components/Immunization/Guildelines_ for_immunization/MCP_Guide_Book.pdf).*

Management of problem behaviors

Studies indicate that young children with developmental delays are 3-4 times more likely to exhibit behavioral problems as compared to their typically developing peers [70,71]. Common behavioral problems observed in these children include severe tantrums, aggression, non-compliance and hyperactivity [72]. These behavioral problems impede the child's learning, and add to the stress of the family [73]. A flowchart for managing behavioral problems is given in **Fig. 4**.

MANAGEMENT OF COMORBIDITIES

The common comorbidities of GDD are discussed here briefly and guidance on management is provided in **Web Table V**. For detailed information on each condition, specific guidelines should be followed or expert opinion solicited.

Epilepsy: Around 15-30% of children with GDD have a risk of developing epilepsy as compared to 4% in general

Table II Suggested Minimal Standard of Healthcare and Developmental Interventions for Different Levels of Facilities

Levels	*Level 1 (Pediatrician with/without access to one therapist)*	*Level 2 (District level hospitals/ DEIC/developmental pediatrician with access to multiple therapists)*	*Level 3 (Tertiary center with multidisciplinary team including developmental pediatrician/ pediatric neurologist and access to geneticist)*
Health Care Interventions (Routine pediatric medical and dental care)	Routine pediatric healthcare. Ensure compliance and provide follow-up care for children referred back from higher centres	Routine pediatric healthcare Ensure compliance and provide follow-up care for children	Routine pediatric healthcare Ensure compliance and provide follow-up care for children
Management of treatable causes of GDD/ IEM (inborn errors of metabolism)	Management of nutritional deficiency including iron and B12 deficiency. Screening and treatment for hypothyroidism	Screening and treatment for hypothyroidism, suspect and investigate for IEMs associated with GDD	Diagnosis/Medical management/ Specialized diets for IEMs
Identification and treatment of common dysmorphic and genetic syndromes	Identification, management and follow- up of Down syndrome	Identification, management and follow-up of common easily identifiable syndromes associated with GDD (e.g., Cornelia De Lange syndrome, neurocutaneous syndromes)	Identification, management and follow-up of all syndromic GDD
Developmental interventions	Mild GDD with no red flags. Advise appropriate stimulation activities[a] and follow-up Refer to higher level if no improvement	Management of mild to moderate GDD. Multi-domain intervention	Detailed evaluation and Intervention planning for all levels of severity by multidisciplinary team[b]

[a]Activities for developmental intervention and stimulation in various domains have been described in MCP card; [b]Child can be followed by referring pediatrician, and compliance with therapies and medications ensured. Children with severe problems may require continued follow-up at higher centres. DEIC: district early intervention centre, GDD: global developmental delay, IEM: inborn error of metabolism, MCP card-mother and child protection card.

population, and the prevalence increases with increasing severity of GDD. Refractory epilepsy and some epileptic encephalopathies can themselves contribute to develop-mental delay as well as impair the gain in milestones in those with manifest GDD. The diagnosis of seizure in a child with GDD may at times be difficult. Some seizure manifestations such as staring spells, myoclonic seizures and astatic seizures may be subtle and can be missed. On the other hand, dystonic posturing may be misdiagnosed as a seizure. Important associated epileptic encephalo-pathies include epileptic spasms (West syndrome) and Lennox-Gestaut syndrome. Early diagnosis of epileptic spasms is important as the time-lag from onset of symptoms to treatment significantly impacts develop-mental outcomes and response to treatment. Broad principles of treating epilepsy in children with develop-mental delay are the same as any child with epilepsy. The choice of antiepileptic medications depends on the ease of availability and safety profile of the drug. Usually, cognitive and behavioral abnormalities in these children are attributed to the effect of antiepileptic medications. Though, there is a lack of robust data on these effects, drugs with better neurocognitive profile may be preferred in these children.

Febrile seizures: Children with GDD are at a greater risk of having recurrent febrile seizures, febrile status epilepticus and progression to future epilepsy [74]. The risk of future epilepsy increases when there is presence of complex febrile seizures and/or family history of epilepsy, and in such cases, abnormal EEG may be helpful in establishing prognosis for development of later epilepsy [75,76]. Factors responsible for the increased risk of recurrence of febrile seizures are similar in these children when compared with their typically developing peers [77,78]. As for any other child, intermittent prophylaxis is considered when the risk of recurrence of febrile convulsion is high [79].

Visual deficits: The prevalence of visual problems in children with developmental delay has been reported to range from 15-75% [82]. Refractive errors are the commonest and seen in approximately 50% of cases [80]. Other common deficits include strabismus, optic atrophy, nystagmus and CVI [22,80,81]. As vision is central to early learning, social interaction and motor development; early identification and treatment of visual impairment is crucial. Studies have documented improvements in motor skills and social behaviors after correction of refractive errors in children [82]. At present, there is no standard treatment for CVI, and data regarding functional visual outcomes is limited; however, children with CVI and other low vision conditions may benefit from environmental modifications to promote visual functioning [46]. These include a simple visual environment to avoid overcrowding and utilizing objects with color, high contrast and motion to facilitate visual recognition [83].

Hearing impairment: Prevalence of hearing impairment in GDD ranges from 10-17% [20]. Few studies have shown that the children having mild to moderate GDD with comorbid hearing impairment may also have some improvement after timely cochlear implant, even though they may not achieve their full language potential [84].

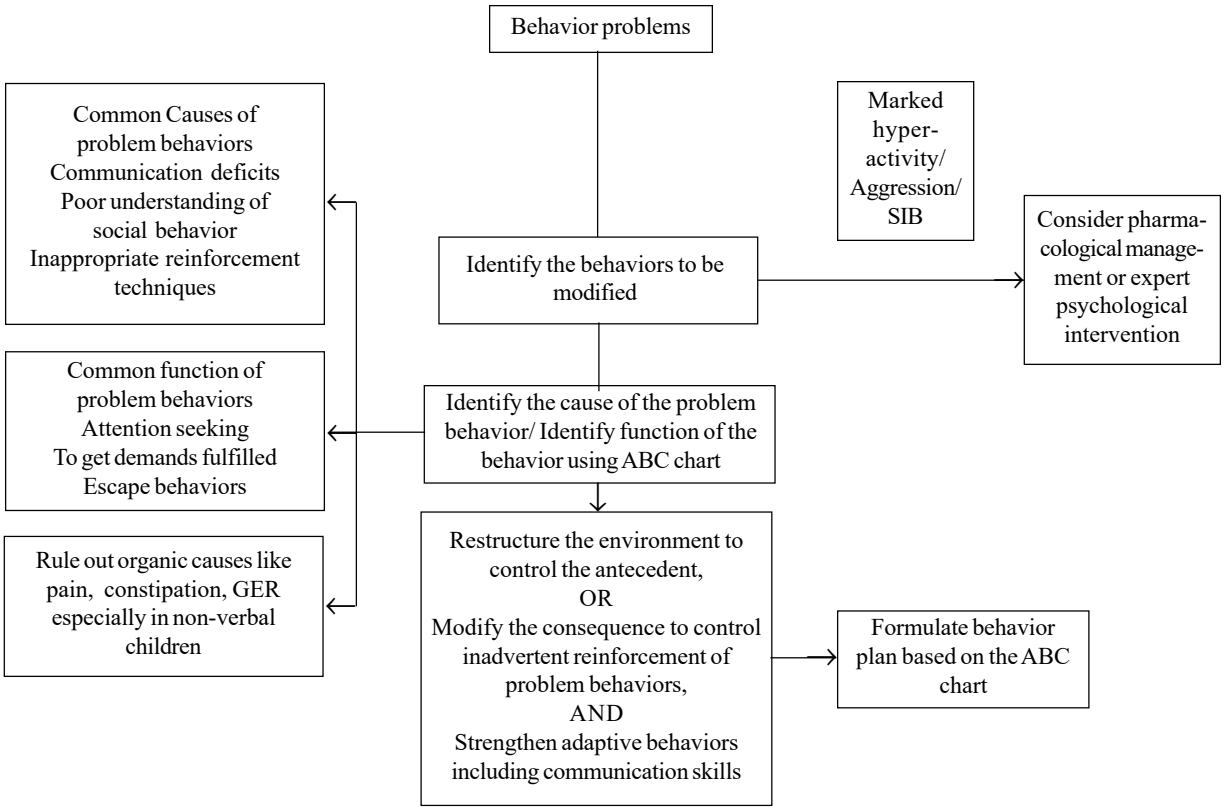
Sleep disturbances: These are common and predispose children to behavioral and cognitive impairments. Causes for disturbed sleep include regulation problems, alteration of sleep-wake cycle due to anti-seizure and sedative medications; and obstructive sleep apnea due to co-morbid conditions like Down syndrome, obesity, pseudobulbar dysfunction or hypotonia. The first line of management is promotion of improved sleep habits or sleep hygiene [85]. When these are not effective, melatonin administered around 3-4 hours before tentative bed time may be considered.

Cerebral palsy: It is a common comorbidity occurring in 8-

Box II General Principles of Developmental Intervention

- The cardinal principle should be early intervention using multiple modalities.
- Functional skills to be taught or addressed will depend on core deficits, needs of the child and family, and associated co-morbidities. Intervention plan should be individualized, keeping in mind the child's functional level in different domains. The next immediate milestone should be the target for intervention.
- As children gain skills in different areas simultaneously, congruent skills should be chosen from multiple domains at a time, therefore multidisciplinary intervention[a] is preferred. Pediatrician should ensure that all domains are being targeted during intervention.
- Toys and activities should be appropriate to child's developmental age. Play should be used to teach target skills, as this helps the child learn better. The activities should be chosen such that they are difficult enough to be interesting, but easy enough to be accomplished.
- Parents and other family members should be actively involved in the process, and implement the strategies at home during daily activities.

[a]*Multidisciplinary intervention requires involvement of multiple healthcare disciplines like developmental pediatrics/ pediatric neurology, special education, clinical psychology, occupational therapy/ physiotherapy and speech therapy.*



SIB: self injurious behaviors; ABC: Antecedent-Behavior-Consequence (where, antecedent is the event or situation that occurred before the behavior was shown, behavior refers to details of the behavior exhibited, and consequence to the sequence of events immediately after the behavior)

Fig.4 Management of behavioral problems in a child with GDD.

30% of children with GDD and should be managed as per standard treatment [20,86].

Learning issues: The underlying condition, subnormal DQ, and the comorbidities may hinder learning and delay schooling for children with GDD to a varying level. The Right to Education Act (RTE) stipulates that children with disabilities receive their educational services appropriate to address their educational needs in the least restrictive environment possible. The extent of inclusion may depend on the level of delay, the severity of associated comorbid conditions, and maladaptive behaviors like aggression and self-injurious behaviors. The services of special education teachers may be helpful for individualized intervention.

Guidelines

5A Early intervention for infants at risk of developmental delay should start in the neonatal intensive care unit (NICU) with neurodevelopmentally supportive care.

5B Potentially treatable causes of GDD should be identified and specific treatment started as early as possible.

5C Children with developmental delay should receive routine health care interventions at par with the typically growing peers, at all levels of care.

5D Early intervention should be initiated soon after the delay is recognized, instead of waiting for a formal diagnosis.

5E Screening for comorbidities like behavioral problems, epilepsy, cerebral palsy, visual and hearing impairment, and sleep disturbances with appropriate referrals should be done to ensure timely intervention.

5F For comorbid febrile seizures, EEG is indicated when associated with family history of epilepsy and/or complex febrile seizures. Intermittent prophylaxis is recommended in the presence of any one additional risk factor for recurrence of febrile seizure.

5G Children with GDD should receive preschool education services in the least restrictive environment that is possible and appropriate to address their needs.

Counselling

Counselling the family regarding the diagnosis, etiology,

anticipated comorbidities, investigations, management, prognosis and follow-up is an important aspect of GDD management. In addition, parents should also be made aware of social support and legal provisions available.

Disclosing the diagnosis: Various studies have suggested that the parent's adaptation to their child's condition may be modulated by the way in which the diagnosis is conveyed to them. It is important to communicate the diagnosis to the family clearly and directly, in a compassionate manner, emphasizing equally the child's strengths as well as deficits [71,87,88]. Also, possibility of improvement with consistent intervention, even in severely delayed children, must be reiterated while setting reasonable expectations.

Counselling regarding investigations: As etiology of GDD is complex, patients may need to undergo several tests before a conclusive diagnosis is reached. The family needs to be counselled that despite undergoing all possible tests, which may be expensive, etiology may still not be established.

Pretest counselling: This should always be done before ordering genetic tests, which should not only include information about the various tests available but also about the possibility of unrelated genetic condition being unveiled. Many a times, a genetic variant of uncertain significance may be found necessitating review of the findings few years later in light of new information available.

Guidelines

6A It is strongly recommended that the family should be counselled regarding the diagnosis, etiology, anticipated comorbidities, investigations, management, prognosis and follow-up; once at the time of initial diagnosis, and again whenever more etiological information is available/etiology is established.

Prognosis and Follow-up

There is a scarcity of published studies which have looked at long term prognosis of GDD and this limits the ability to predict developmental outcomes precisely in these children. Based on the available literature, several factors have been found to affect the prognosis including severity of GDD, its etiology, presence of comorbidities, family's socio-economic status, age at diagnosis and initiation of intervention, availability of specific treatment for underlying etiology, and compliance to therapy. The degree of delay is the most consistent predictor of long-term prognosis with mild cases doing well in comparison to moderate-severe cases of GDD. Early intervention has been documented to minimize developmental delays with gains in adaptive, academic and social functioning. However, nearly two-thirds children will get the diagnosis of ID later in life and another 20%; even though functioning well in society, may get an alternative neurodevelopmental diagnosis.

Follow-up: It includes tracking the child's development in all domains, screening for comorbidities on a continued basis, planning additional investigations and inter-ventions, whenever needed, parental training, and ensuring compliance. Documentation of the assessments, targets as well as therapy plan by all members of a multidisciplinary team is a must. Follow-up plan for those with manifest GDD should be customized as per the individual child's needs, as many children with GDD, especially those with moderate to severe GDD or multiple comorbidities, require frequent monitoring. Review of diagnosis should be carried out annually, at least in the initial years, to pick up possibly missed comorbid neurodevelopmental disorders. **Box III** enlists the key points to be assessed on follow-up.

Guidelines

7A Regular follow-up targeting all the developmental domains and associated comorbidities should be done. Consultation with a team of experts led by a developmental pediatrician/ pediatric neurologist may be considered, if possible. Apart from this, primary pediatrician may also play an important role in supporting the family and ensuring the compliance to therapies.

Rights of Persons With Disabilities Act 2016 and Disability Certification

The Rights of Persons with Disabilities Act, 2016 empowers persons with disabilities with certain rights and entitlements, legal provisions and provides a framework for assessment and certification [28]. GDD as a disability is included under the gambit of 'ID'. The Act defines ID as a condition characterized by significant limitation both in intellectual functioning and in adaptive behavior. Persons above the age of 5 years are given a diagnosis of ID, while children between the ages of 1-5 years are given a diagnosis of GDD. The minimum age for certification is one completed year. Children below the age of 5 years are issued a temporary certificate wherein a reassessment is required after a period of 3 years or at 5 years of age (whichever is earlier). Intellectual functioning is to be assessed by testing IQ on Binet Kamat test (BKT) and adaptive functioning through Vineland Social Maturity Scale (VSMS). Certification is done by the medical board headed by the medical superintendent/chief medical officer/ other equivalent authority as notified by the state government and comprises of a *i*) pediatrician or pediatric neurologist, *ii*) clinical or rehabilitation psychologist, and *iii*) psychiatrist.

Role of referring pediatrician in disability certification: The pediatrician should identify GDD as well as screen for associated comorbidities (hearing/vision/locomotor

Box III Key Points to Be Assessed During Follow-up

- Documentation of new milestones achieved.
- Improvement/ no change/ regression in the developmental domains involved in the child: involvement of a previously uninvolved developmental domain or any regression in milestones should prompt referral to secondary or tertiary level for detailed evaluation.
- Screening and monitoring for anticipated comorbidities (epilepsy, feeding problems, vision impairment, hearing problems, sleep problems, autism, behavioural issues, neurological problems etc.)
- Compliance with therapies and medications, and monitor for side effects, if any.
- New parental concerns
- Growth parameters and head circumference
- Routine pediatric management including nutrition, immunization, etc.
- Counselling
- Guide and advice regarding preschool education and disability certification and government benefits
- Guide families regarding parent/sibling training program, Non-government organizations, self-help groups
- Schedule next follow-up

impairments/epilepsy) and refer accordingly for detailed disability assessment.

CONCLUSION

Preventable causes of GDD should be addressed by providing adequate perinatal care including prenatal testing for genetic disorders, care during pregnancy and postnatal care in subsequent pregnancy, preventing infections and nutritional deficiencies in children, ensuring good health, providing opportunities for early learning and, focusing on child safety measures.

Pediatricians are often the first point of contact for all children, including those with developmental delay. Early identification of the developmental delay, its management, and multidisciplinary intervention are of paramount importance, as are establishing an etiological diagnosis, identifying and treating comorbidities, and guiding the prognosis. The role of pediatrician is central in collaborating with parents and multidisciplinary teams to provide seamless coordinated care to children and their families, so as to improve their medical outcomes and social functioning. **Fig.5** shows approach to a child with GDD.

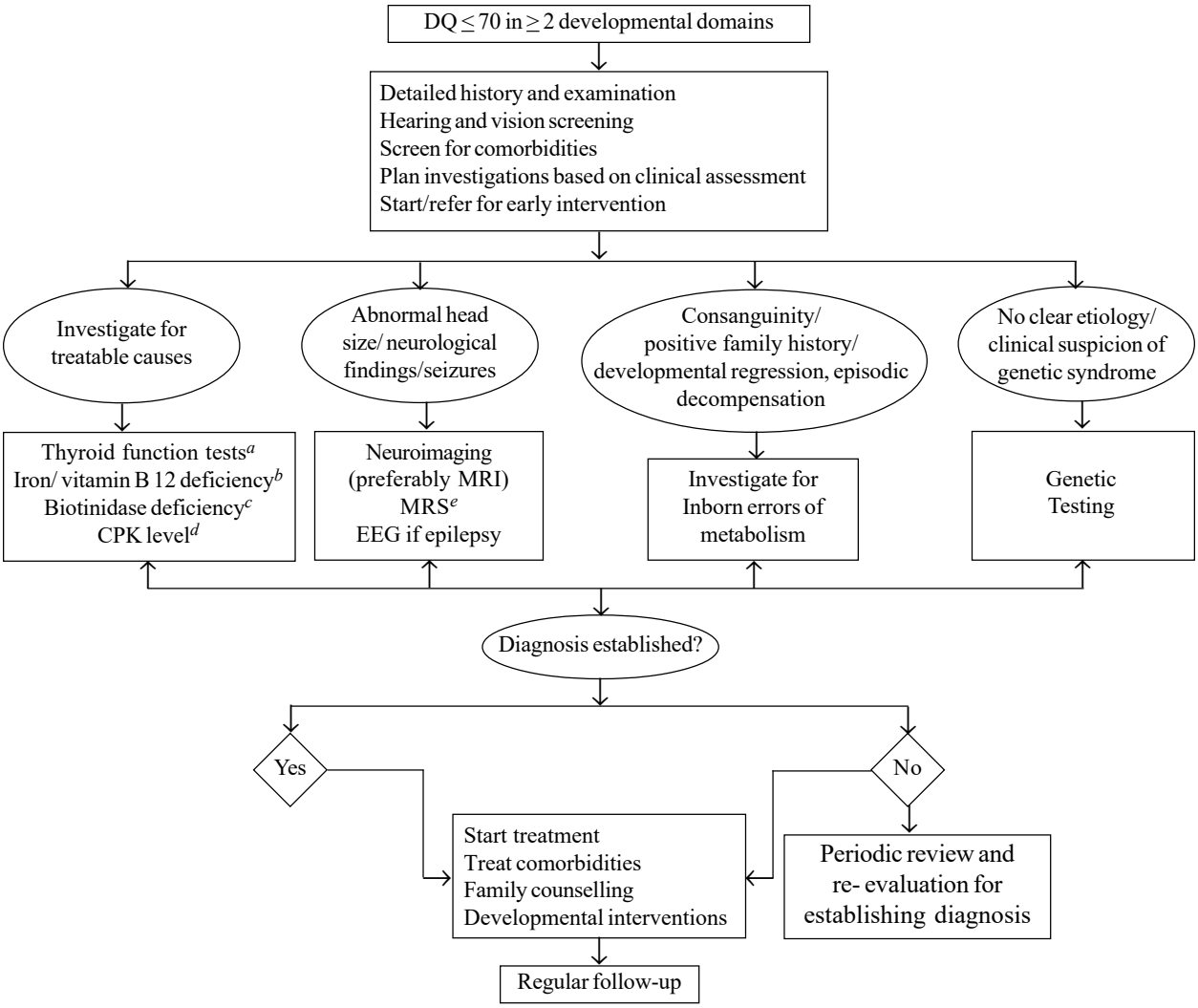
Contributors: All authors have contributed to the initial draft of the manuscript, made important intellectual contribution in the framing of the guidelines and finalization of the manuscript, and approved the final manuscript.

Funding: None; *Competing interests*: None stated.

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

REFERENCES

1. Shevell M, Ashwal S, Donley D, et al. Quality Standards Subcommittee of the American Academy of Neurology; Practice Committee of the Child Neurology Society. Practice Parameter: Evaluation of the child with Global Developmental Delay: Report of the Quality Standards Subcommittee of the American Academy of Neurology and the Practice Committee of the Child Neurology Society. Neurology. 2003;60:367-80.
2. Demirci A, Kartal M. The prevalence of developmental delay among children aged 3-60 months in Izmir, Turkey. Child Care Health Dev. 2016 42:213-9.
3. Eapen V, Zoubeidi T, Yunis F, et al. Prevalence and psychosocial correlates of global developmental delay in 3-year-old children in the United Arab Emirates. J Psychosom Res. 2006; 61:321-6.
4. Sharma N, Masood J, Singh SN, et al. Assessment of risk factors for developmental delays among children in a rural community of North India: A cross-sectional study. J Educ Health Promot. 2019;8:112.
5. Agarwal D, Chaudhary SS, Sachdeva S, et al. Prevalence of developmental delay and factors affecting the development status among under 5 children in an urban slum of Agra City. Natl J Community Med. 2018;9: 474–79.
6. Kishore MT, Udipi GA, Seshadri SP. Clinical Practice Guidelines for Assessment and Management of intellectual disability. Indian J Psychiatry. 2019;61:194-210.
7. Srour M, Shevell M. Genetics and the investigation of developmental delay/intellectual disability. Arch Dis Child. 2014;99:386-9.
8. Jimenez-Gomez A, Standridge SM. A refined approach to evaluating global developmental delay for the international medical community. Pediatr Neurol. 2014;51:198-206.
9. Wilska ML, Kaski MK. Why and how to assess the aetiological diagnosis of children with intellectual disability/mental retardation and other neurodevelopmental disorders: description of the Finnish approach. Eur J Paediatr Neurol. 2001;5:7-13.
10. Jain S, Chowdhury V, Juneja M, et al. Intellectual disability in Indian children: Experience with a stratified approach for etiological diagnosis. Indian Pediatr. 2013; 50: 1125-130.
11. Tikaria A, Kabra M, Gupta N, et al. Etiology of global developmental delay in young children: Experience from a tertiary care centre in India. Natl Med J India. 2010;23:324-9.
12. Ali YF, El-Morshedy S, Elsayed RM, et al. Metabolic screening and its impact in children with nonsyndromic intellectual disability. Neuropsychiatr Dis Treat. 2017;13:1065-70.
13. Jauhari P, Boggula R, Bhave A, et al. Etiology of intellectual disability in pediatric outpatients in Northern India. Dev Med Child Neurol. 2011;53:167-72.
14. Majnemer A, Shevell MI. Diagnostic yield of the neurologic assessment of the developmentally delayed child. J Pediatr. 1995;



[a]especially in absence of documented newborn screening results; [b]especially in children having a restricted diet or pica; [c]especially in the absence of newborn screening; [d]boys with history or findings suggestive of conditions like Duchenne muscular dystrophy; [e]where mitochondrial disorder is suspected or for diagnosis of cerebral creatine deficiency syndrome in children with unexplained GDD and normal MRI. MRI: magnetic resonance imaging, MRS-magnetic resonance spectroscopy, EEG-electroencephalogram, DQ-developmental quotient.

Fig. 5 Approach to a child with global developmental delay.

127:193-9.
15. Gupta N, Kabra M. Approach to the diagnosis of develop-mental delay - the changing scenario. Indian J Med Res. 2014; 139:4-6.
16. López-Pisón J, García-Jiménez MC, Monge-Galindo L, et al. Our experience with the aetiological diagnosis of global develop-mental delay and intellectual disability: 2006-2010. Neuro-logia. 2014;29:402-7.
17. Srour M, Mazer B, Shevell MI. Analysis of clinical features predicting etiologic yield in the assessment of global develop-mental delay. Pediatrics. 2006;118:139-45.
18. Ozmen M, Tatli B, Aydinli N, et al. Etiologic evaluation in 247 children with global developmental delay at Istanbul, Turkey. J Trop Pediatr. 2005;51:310-3.
19. Shevell MI, Majnemer A, Rosenbaum P, Abrahamowicz M. Etiologic yield of subspecialists' evaluation of young children with global developmental delay. J Pediatr. 2000;136:593-8.
20. Jauhari P, Bhargava R, Bhave A, et al. Comorbidities associated with intellectual disability among pediatric outpatients seen at a teaching hospital in Northern India. J Policy Pract Intellect Disabil. 2012; 9:10-16.
21. Einfeld SL, Ellis LA, Emerson E. Comorbidity of intellectual disability and mental disorder in children and adolescents: a systematic review. J Intellect Develop Disab. 2011;36:137-43.
22. Hegde V, Jain R, Bappal A, Shambhu R. Ocular manifestations in children with developmental delay at a tertiary center in South India. Saudi J Ophthalmol. 2021;35:1-4.
23. Shabnam S, Ravi SK, Swapna N. Feeding and swallowing issues in children with neuro-developmental disorders. In: Gupta S, Venkatesan S, Goswami S.P, editors. Emerging Trends in the Diagnosis and Intervention of Neurodevelopmental Disorders. 1st ed. IGI Global; 2019.p.56-75.
24. Wiggs L. Sleep problems in children with developmental dis-

orders. J R Soc Med. 2001;94:177-9.
25. American Psychiatric Association. Intellectual Development Disorders. In: Diagnostic and Statistical Manual of Mental Disorders. 5th ed. American Psychiatric Publishing; 2013.p.33-41.
26. Desmond P. Kelly MJN. Neurodevelopment and Executive function and dysfunction. *In*: M. Kliegman, Richard E. Behrman HBJ editors. Nelson Textbook of Pediatrics. 21st ed. Elsevier Inc;2020. p.1690-91.
27. Nelson CA 3rd, Zeanah CH, Fox NA, et al. Cognitive recovery in socially deprived young children: The Bucharest Early Intervention Project. Science. 2007;318:1937-40.
28. Andrew A, Attanasio O, Augsburg B, et al. Effects of a scalable home-visiting intervention on child development in slums of urban India: Evidence from a randomised controlled trial. J Child Psychol Psychiatry. 2020;61:644-52.
29. Gazette of India (Extra-Ordinary). The Rights of Persons with Disabilities Act, 2016. Accessed on Nov 27, 2021. Available from:*http://www.disabilityaffairs.gov.in/uploaad/uploadfiles/files/RPWD/ACT/2016.pdf*
30. Council on Children With Disabilities; Section on Developmental Behavioral Pediatrics; Bright Futures Steering Committee; Medical Home Initiatives for Children With Special Needs Project Advisory Committee. Identifying Infants and Young Children With Developmental Disorders in the Medical Home:An Algorithm for Developmental Surveillance and Screening. Pediatrics. 2006;118:405-20.
31. Scharf RJ, Scharf GJ, Stroustrup A. Developmental Milestones. Pediatr Rev. 2016;37:25-37.
32. Nair MK, Nair GS, George B, et al. Development and validation of Trivandrum Development Screening Chart for children aged 0-6 years [TDSC (0-6)]. Indian J Pediatr. 2013;80:S248-55.
33. Glascoe FP, Byrne KE, Ashford LG, et al. Accuracy of the Denver-II in developmental screening. Pediatrics. 1992;89: 1221-5.
34. Bal R, Karyakram S. Child Health Screening and Early Intervention Services. National Rural Health Mission, 2014. Accessed November 15, 2021. Available from: *https://nhm.gov.in/images/pdf/programmes/RBSK/Resource_Documents/RBSK_Job_Aids.pdf*
35. Phatak AT, Khurana B. Baroda development screening test for infants. Indian Pediatr. 1991;28:31-7.
36. Alpern Gerald, Boll Thomas, Shearer Marsha S. Developmental Profile II (DP-II) Manual. 2nd ed. Western Psychological Services, 1994.
37. Kamat V. A revision of the Binet scale for Indian children (Kanarese and Marathi speaking). British Journal of Edu-cational Psychology. 2011;4:296-309.
38. Sparrow S, Ciccheti V, Saulnier A. Vineland Adaptive Behaviour Scales (VABS). 3rd ed. Pearson, 2016.
39. Bharat Raj. Vineland Social Maturity Scale and Manual, Indian Adaptation-Enlarged Version, Swayamsiddha-Prakashanam 1992
40. Pathak P. Developmental assessment scales for Indian infants (DASII). Anand Agencies; 1998.
41. Albers, C. A., and Grieve, A. J. (2007). *Bayley, N.* (2006). Bayley Scales of Infant and Toddler Development, 3rd ed. Harcourt Assessment. P.180-190.
42. Shank L. Mullen Scales of Early Learning. *In*: Kreutzer JS, DeLuca J, Caplan B. (eds) Encyclopedia of Clinical Neuro-psychology. Springer. 2011. Available on *https://doi.org/10.1007/978-0-387-79948-3_1570*
43. Griffiths R. The abilities of young children: A comprehensive system of mental measurement for the first eight years of life. Association for Research in Infant and Child Development;1984. p.101-172.
44. Gesell Institute of Child Development. Gesell develop- mental observation-revised and Gesell early screener technical report ages 3-6. 2012.Available from *http://www.gesellinstitute.org*
45. Eiserman WD, Shisler L, Foust T. Hearing Screening in Early Childcare Settings. ASHA Lead 2008; 13: 34-37. Available at *https://www.infanthearing.org/earlychildhood/docs/ASHA%20Leader%20article.pdf*
46. Chang MY, Borchert MS. Advances in the evaluation and management of cortical/cerebral visual impairment in children. Surv Ophthalmol. 2020;65:708-24.
47. Ganesan S, Thanawala N, Hussain N. Vitamin B12 deficiency: a treatable cause of developmental delay in infancy. J Paediatr Child Health. 2013;49:E348-9.
48. McDonald L, Rennie A, Tolmie J, et al. Investigation of global developmental delay. Arch Dis Child. 2006;91:701-5.
49. Mithyantha R, Kneen R, McCann E, Gladstone M. Current evidence-based recommendations on investigating children with global developmental delay. Arch Dis Child. 2017;102: 1071-76.
50. Hsu, RH, Chien, YH., Hwu, WL. et al. Genotypic and phenotypic correlations of biotinidase deficiency in the Chinese population. Orphanet J Rare Dis. 2019;14:6.
51. Bélanger SA, Caron J. Evaluation of the child with global developmental delay and intellectual disability. Paediatr Child Health. 2018;23:403-19.
52. Moeschler JB, Shevell M. Committee on Genetics. Comprehensive Evaluation of the Child With Intellectual Disability or Global Developmental Delays. Pediatrics. 2014;134:e903-18.
53. Griffiths PD, Batty R, Warren D, et al. The use of MR imaging and spectroscopy of the brain in children investigated for developmental delay: what is the most appropriate imaging strategy? Eur Radiol. 2011;21:1820-30.
54. Cleary MA, Green A. Developmental delay: when to suspect and how to investigate for an inborn error of metabolism. Arch Dis Child. 2005;90:1128-32.
55. Shaffer, Lisa. American College of Medical Genetics Guideline on the Cytogenetic Evaluation of the Individual With Developmental Delay or Mental Retardation. Genet Med. 2005;7:650-54.
56. Kharbanda M, Tolmie J, Joss S. How to use… microarray comparative genomic hybridisation to investigate developmental disorders. Arch Dis Child Educ Pract Ed. 2015;100:24-9.
57. Savatt JM, Myers SM. Genetic Testing in Neurodevelopmental Disorders. Front Pediatr. 2021;9:526779.
58. Millichap J, Millichap John. AAP Genetics. Diagnostic approach to intellectual disability or global developmental delay. Pediatric Neurology Briefs. 2014;79.
59. Altimier L, Phillips R. The Neonatal Integrative Developmental Care Model: Advanced Clinical Applications of the Seven Core Measures for Neuroprotective Family-centered Developmental Care. Newborn Infant Nurs Rev. 2016;16:230-44.
60. Coughlin M, Gibbins S, Hoath S. Core measures for developmentally supportive care in neonatal intensive care units: theory, precedence and practice. J Adv Nurs. 2009; 65:2239-48.
61. Eva MM. Hoytema van Konijnenburg, Saskia B. Wortmann, Marina Johanna Koelewijn et al. Treatable Inherited Metabolic Disorders Causing Intellectual Disability: 2020 Review And Diagnostic App. Orphanet J Rare Dis.2021;16:170. Available as Treatable-ID App on *https://www.treatable-id.org/about. html*
62. Van Karnebeek CD, Houben RF, Lafek M, et al. The treatable intellectual disability APP www.treatable-id.org: A digital tool to enhance diagnosis and care for rare diseases. Orphanet J Rare Dis. 2012;7:47.
63. Bharadva K, Shastri D, Gaonkar N, et al. Consensus Statement of Indian Academy of Pediatrics on Early Childhood Develop-ment. Indian Pediatr. 2020;57:834-41.
64. Chandrasekhar P, Ramachandran S. Growth charts for Indian boys (0-36 months) with down's syndrome: a pilot study. Int J

Contemp Pediatr. 2018;5:2156.
65. Chandrasekhar P, Ramchandran S. Growth chart of Indian girls with Down syndrome from birth to three years-a pilot study. J Down Syndr Chr Abnorm. 2018;4:2.
66. Butler MG, Lee J, Manzardo AM, et al. Growth charts for non-growth hormone treated Prader-Willi syndrome. Pediatrics. 2015;135:e126-35.Erratum in: Pediatrics. 2015;135:946.
67. Hwang AW, Chao MY, Liu SW. A randomized controlled trial of routines-based early intervention for children with or at risk for developmental delay. Res Dev Disabil. 2013;34:3112-23.
68. Cameron RJ. Early intervention for young children with developmental delay: the Portage approach. Child Care Health Dev. 1997;23:11-27.
69. Ministry of health and family welfare ministry of women and child development mother-child protection card 2018. Accessed November 21, 2021. Available at *https://nhm.gov.in/New_Updates_2018/NHM_Components/Immunization/Guildelines_for_immunization/MCP_Guide_Book.pdf*
70. Baker BL, Blacher J, Crnic KA, Edelbrock C. Behavior prob-lems and parenting stress in families of three-year-old children with and without developmental delays. Am J Ment Retard. 2002;107:433-44.
71. Eisenhower AS, Baker BL, Blacher J. Preschool children with intellectual disability: syndrome specificity, behaviour prob-lems, and maternal well-being. J Intellect Disabil Res. 2005; 49:657-71.
72. Gadow KD, Sprafkin J, Nolan EE. DSM-IV symptoms in community and clinic preschool children. J Am Acad Child Adolesc Psychiatry. 2001;40:1383-92.
73. Hasnat MJ, Graves P. Disclosure of developmental disability: A study of parent satisfaction and the determinants of satisfaction. J Paediatr Child Health. 2000;36:32-5.
74. Tsai JD, Mou CH, Chang HY, et al. Trend of subsequent epilepsy in children with recurrent febrile seizures: A retrospective matched cohort study. Seizure. 2018;61:164-69.
75. Hofert SM, Burke MG. Nothing is simple about a complex febrile seizure: looking beyond fever as a cause for seizures in children. Hosp Pediatr. 2014;4:181-7.
76. Patel AD, Vidaurre J. Complex febrile seizures: A practical guide to evaluation and treatment. J Child Neurol. 2013;28: 762-7.
77. Pavlidou E, Tzitiridou M, Kontopoulos E, Panteliadis CP. Which factors determine febrile seizure recurrence? A prospective study. Brain Dev. 2008;30:7-13.
78. Canpolat M, Per H, Gumus H, et al. Investigating the pre-valence of febrile convulsion in Kayseri, Turkey: An assess-ment of the risk factors for recurrence of febrile convulsion and for development of epilepsy. Seizure. 2018;55:36-47.
79. Natsume J, Hamano SI, Iyoda K, et al. New guidelines for management of febrile seizures in Japan. Brain Dev. 2017;39:2-9.
80. Salt A, Sargent J. Common visual problems in children with disability. Arch Dis Child. 2014;99:1163-8.
81. Akinci A, Oner O, Bozkurt OH, et al. Refractive errors and ocular findings in children with intellectual disability: a controlled study. J AAPOS. 2008;12:477-81.
82. Smitha K, Patil V, Kamate M, et al. Impact of refractive error correction on mental and visual development in children with global developmental delay. Indian J Heal Sci Biomed Res. 2019;12:117.
83. Cohen-Maitre SA, Haerich P. Visual attention to movement and color in children with cortical visual impairment. J Vis Impair Blind. 2005;99:389–402.
84. Meinzen-Derr J, Wiley S, Grether S, Choo DI. Children with cochlear implants and developmental disabilities: A language skills study with developmentally matched hearing peers. Res Dev Disabil. 2011;32:757-67.
85. Jan JE, Owens JA, Weiss MD, et al. Sleep hygiene for children with neurodevelopmental disabilities. Pediatrics. 2008;122: 1343-50.
86. Patel DR, Neelakantan M, Pandher K, Merrick J. Cerebral palsy in children: a clinical overview. Transl Pediatr. 2020;9: S125-S135.
87. Sloper P, Turner S. Determinants of parental satisfaction with disclosure of disability. Dev Med Child Neurol. 1993;35:816-25.
88. Svarstad BL, Lipton HL. Informing parents about mental retardation: a study of professional communication and parent acceptance. Soc Sci Med. 1977;11:645-51.

EVENTS

☐ **1-13 August, 2022**

Nineteenth ICMR Course in Medical Genetics & Genetic Counseling, Lucknow

Contact:

Dr Shubha Phadke,
Phone: 0522 2494325
https://sgpgims.org.in/Home/recruit/Courses/ICMR%20Course_19th_ad.pdf

☐ **18-20 November, 2022**

MAHAPEDICON 2022, Nashik

Contact:

E-mail: *office@mahaiap.org;*
Phone: 0253-2313148

Web Table I Difference Between Global Developmental Delay (GDD) and Intellectual Disability (ID) [1,8,9]

	Global developmental delay	*Intellectual deficit*
Definition	DSM-5 reserves the term GDD for an individual who fails to meet expected developmental milestones in several areas of intellectual functioning and applies to those who are unableto undergo systematic assessments of intellectual functioning, including children whoare too young to participate in standardized testing. AAN defines GDD as subset of developmental disabilities defined as significant delay in two or more of the following developmental domains: gross/fine motor, speech/language, cognition, social/personal and activities of daily living.	DSM-5 defines ID as a neurodevelopmental disorder with onset during the developmental period that includes both intellectual and adaptive functioning deficits in conceptual, social and practical domains.
Age group	Children <5 years	Applies to older children when testing of intellectual functioning is valid and reliable (usually after 5 years of age). [a]The onset is usually before 18 years of age.
Diagnosis	Based on clinical assessment and standardized testing of a child in major domains of development requires diagnostic reassessment after a period of time.	Based on clinical assessment and standardizedtesting of intellectual and adaptive functioning. Stable diagnosis.
Natural course	Even though most children will have cognitive impairment subsequently, not all children go onto develop ID later.	It is a permanent disability requiring supportthroughout life.

[a]*Intellectual functioning can be tested from as early as 2 years using tests like Leiters, Wechsler Preschool and Primary Scale of Intelligence (WPPSI). However, data for validity and reliability is limited below 5 years of age.*

Web Table II Brief Description of Development Screening Tests for Pediatric Office Setting [32-36]

Development Screen test	*Domains tested*	*Age range*	*Time taken*	*Administration*	*Interpretation*	*Psychometric properties*	*Ease of use and feasibility*
Domain wise screening tools							
Ages and Stages Questionnaire (ASQ)	Gross motor, fine motor, communication, problem – solving, personal – social	1-66 months; 21 age-specific forms with 30 questions	10-15 mins	Parent reported questionnaire. Can be completed by parents/ caregivers independently or with the assistance of professionals.	Risk categorization: typical/ needs monitoring/needs further assessment	Moderate sensitivity and specificity	Includes intervention activities. Training required for interpretation. Indian studies available.
Denver Development Screening Test-II (DDST)	Gross motor, fine motor-adaptive, personal-social, language. Includes 5 "Test Behavior" items	0-72 months	10-15 mins	Directly administered by a professional in a standardized manner.	Item wise pass/fail/ caution Interpreted as normal/suspect or untestable	Low to moderate sensitivity and specificity	Low sensitivity limits applicability as a screening tool.
Developmental Profile (DP)	Physical, adaptive, behaviour, communication, cognitive, social-emotional	0-12 yrs 11 months (DP 3) 0-21 years (DP- 4)	25-30 mins	Can be completed by parents themselves-checklist or by parental interview including direct observation by professional.	Provides age equivalent and norm based standard scores in each domain and a general developmental score. Risk categorization: delayed/ below average/ average / above average / well above average.	Moderate to high; correlation with Vineland-II	Gives DQ. Includes intervention activities. Training required. Expensive.
RBSK screening tool	Vision, hearing, speech, motor, cognition, social	0-6 years		Directly administered by history and examination.	Domain wise pass/ fail	Not available	Not validated. Not norm referenced.
General Screening tools which do not screen domain wise							
Trivandrum Developmental Screening Chart (TDSC)	Gross motor, fine motor/adaptive, personal-social, language	0-6 years	10-15 mins	Directly administered by history and examination.	Pass/ fail for each item. Developmental delay considered if ≥ 1 fail obtained.	Moderate sensitivity and specificity	Minimal training required. Can be used as a community screening tool.
Baroda Development Screening Tool (BDST)	Motor and mental	0-30 months	15-20 mins	Directly administered by history and examination.	Pass/ fail for each item. Developmental age as per 50% and 97% pass placement each item.	Low sensitivity, high specificity	Can be used as a community screening tool.

Web Table III Developmental Assessment Tests [37-44]

Test	*Age*	*Description*	*Advantages*
Binet-Kamat Test of Intelligence (BKT)	3 years to adulthood	Includes both verbal and performance tests	Simple to score and administer available in Hindi, Marathi and Kannada
Vineland Social Maturity Scale (VSMS)	Birth to 15 years	Assessment of social and adaptive functions or social competency	Culturally appropriate and can be used in nonverbal children; Easy and quick to administer
Development Assessment Scale for Indian Infants (DASII)	Birth to 30 months	Gives mental and motor DQ	Uses indigenous material Norms have been developed for Indian population
Bayley Scales of Infant Development – IV (BSID-IV)	16 days to 42 months	Cognitive, language, motor, social emotional, adaptive behaviour	Internationally validated tool
Mullen Scales of Early Learning	Birth to 68 months	Five scales: Gross motor, visual reception, fine motor, expressive language, and receptive language	Helps in assessing visual and auditory learning thereby enabling the assessment of cognition
Griffiths Scales of Child Development – III (Griffith's III)	Birth to 6 years	Measures six areas of development including foundations of learning, language and communication, eye-hand coordination, personal-social-emotional, gross motor	Assessment is in line with latest research, new norms address Flynn-effect
Gesell Developmental Assessment (GDA)	2 year 6 months to 9 years	Direct observation to evaluate a child's cognitive, language, motor and social-emotional responses in five components (developmental, letter/numbers, language/comprehension, visual/spatial and social/emotional/adaptive)	The overall performance level (age appropriate, emerging or concern) can be used as a guide to customize curricula and/or identify need for additional diagnostic evaluation.
Vineland Adaptive Behaviour Scale (VABS)	Birth to 90 years	Correspond scales to the three broad domains of adaptive functioning-communication, daily living skills, and socialization	Helps in measuring the capabilities in dealing with everyday life; Identifies maladaptive behaviours which may be useful for planning the behaviour intervention

DQ - Developmental quotient

Web Table IV Genetic Testing in Children with Global Developmental Delay [51,52,55-58]

Test	*Yield*	*Indications*	*Remark*
Karyotype	3-5% excluding Down syndrome	GDD/ID with specific phenotype e.g. Down syndrome, Trisomy 13, 18. Dysmorphism, Multiple congenital anomalies	Detects abnormalities more than 5-10 kb in size, operator dependent Still used in resource poor settings as first line due to relatively easy availability and less cost. Can detect balanced chromosomal rearrangements. Chromosome analysis is also indicated when there is a family history of chromosome rearrangement or multiple miscarriages because it can detect balanced chromosomal abnormalities, which CMA does not detect.
Targeted Fluorescent in situ Hybridization (FISH)	Will depend on the phenotypic accuracy for identifying the disorder	Should be offered in situations with specific phenotypes indicating a known microdeletion syndrome e.g. William syndrome, velocardiofacial syndrome, 1 p36 del., etc.	FISH probes assess a specific copy number variant (CNV) associated with a specific syndrome. When there is a strong suspicion of a specific syndrome, FISH can be done; however, if the suspected diagnosis is not cot confirmed by FISH, it must be followed by CMA to establish the diagnosis. Metaphase FISH shows whether a duplicated region is at its normal location. Thus, FISH metaphase analysis is often used to assess the relatives of the affected patient for balanced rearrangements.
Testing for Fragile X syndrome	Yield will depend on case selection criteria, higher if scoring criteria are applied. Nonspecific testing yield is approximately 7%	Recommended as first line test in males with severe to moderate GDD, behavioural problems and autistic features with specific dysmorphic features described or with no obvious dysmorphology but with a normal or large head	Fragile X testing is responsible for significant proportion of cases with ID in males and mild to moderate delay in carrier females. This requires specific tests to pick up triplet repeats which are not picked up by routine sequencing or next generation sequencing (NGS) based tests
Testing for Rett Syndrome Gene sequencing & MLPA	With fulfilling RTT criteria nearly 100%. About 2% without a suggestive phenotype	Recommended in females fulfilling clinical criteria for Rett syndrome. MECP2 sequencing is also recommended if no etiology is found for GDD/ID with ASD in all females, and males with suggestive phenotypes.	Second-tier testing for GDD/ID includes MECP2 full gene analysis in females. Several guidelines suggest MECP2 testing along with sequencing and MLPA in girls with severe GDD.
Multiplex Ligation dependent Probe Assay (MLPA)	Depend on patient selection and disorders being tested e.g. microdeletion/duplications Rett syndrome	Assess a specific copy number variant (CNV) associated with a specific syndrome. Should be offered in situations with specific phenotypes indicating a known microdeletion syndrome e.g. William syndrome, Velocardiofacial syndrome, 1 p36 del, etc. Is at times used to evaluate for multiple microdeletion syndromes as kits are available and also to screen the CNVs in subtelomeric regions which are frequently associated with GDD/ID syndromes	Reliable and relatively low-cost method for specific phenotypes including common and rare microdeletion/microduplication syndromes. Specific MLPA kits are available for GDD patients suspected to have microdeletions/duplications which include many known syndromes Rapid turnaround time

Chromosomal Microarray (CMA)	~15-20% (~10% higher than the detection rate by karyotype analysis in the GDD/ID/ASD population).	Unexplained GDD, ASD, and multiple congenital anomalies (MCAs).	Able to identify submicroscopic deletions and duplications (less than ~ 5-10 Mb, the size of many of the deletions which cannot be detected by karyotype It also identifies regions of homozygosity, which can be scrutinized for autosomal recessive conditions and imprinting disorders Does not detect balanced rearrangements Counselling regarding getting inconclusive results as variants of unknown significance (VOUS) can be identified. TAT -2-3 weeks Cost high
Sanger Sequencing	Depending on patient selection	When a specific phenotype is identified	Expensive if the gene is big or caused by multiple disease-causing genes (genetic heterogeneity). In that situation NGS based tests are preferable.
Exome Sequencing	30-50% depending on patient selection	Developmental delay/intellectual disability, or multiple congenital anomalies not specific to a particular genetic syndrome. More useful if pedigree indicates a mendelian inheritance. There are uniformly followed guidelines for exome sequencing in general, which also apply to ID/GDD, but it needs to be kept in mind that at times there may be difficulty in clearly defining an indication which are as below: The phenotype or family history data strongly implicate a genetic etiology, but the phenotype does not correspond with a specific disorder for which a genetic test targeting a specific gene is available on a clinical basis A patient presents with a defined genetic disorder that demonstrates a high degree of genetic heterogeneity, making WES analysis of multiple genes simultaneously a more practical approach A patient presents with a likely genetic disorder, but specific genetic tests available for that phenotype have failed to arrive at a diagnosis	Exome sequencing detects variations in the coding regions of all known genes; WES or morbid genes which means genes with a known human phenotype; CES. Targeted panels can be used for a specific group of disorders (e.g. lysosomal storage disorders). Does not detect triplet repeat disorders, changes in intronic regions, large deletions & duplications Counselling regarding getting inconclusive results as variants of VOUS can be identified. Cost still prohibitive TAT 4-6 weeks WGS can detect variations in the intronic regions also but interpretation is more difficult and is not used routinely in clinical practice
Metabolic testing	Poor yield for small molecule diseases if isolated GDD/ID For large molecule diseases likely high if careful patient selection	GDD/ID in isolation (not common) but metabolic testing should be considered if it is in combination with autism, neurodegeneration, failure to thrive, lethargy, episodic symptoms such as epilepsy and encephalopathy, multiple organ dysfunction, dietary selectivity, unusual odours etc. Large molecule diseases present with GDD/ID coarse facial features, joint contracture, neuroregression etc.	Poor yield if cases with isolated GDD tested. Diagnosis may need confirmation by molecular studies. Selection of tests would depend on clinical suspicion. E.g. for small molecule diseases HPLC, TMS, GCMS, for large molecule diseases like storage disorders-enzyme assays

CES- clinical exome sequencing, TAT-Trans-activator of transcription, HPLC-high pressure liquid chromatography, TMS-tandem mass spectrometer, GCMS-gas chromatography mass spectrometry, VOUS-variants of unknown significance WES-whole exome sequencing, WGS-Whole Genome Sequencing

An Introduction to Qualitative and Mixed Methods Study Designs in Health Research

MANOJA KUMAR DAS

From The INCLEN Trust International, F1/5, Okhla Industrial Area, Phase 1, New Delhi.

Correspondence to: Manoja Kumar Das, Director Projects, The INCLEN Trust International, F1/5, Okhla Industrial Area, Phase 1, New Delhi 110 020. manoj@inclentrust.org

With the recognition of different population behavior and relevance of socio-cultural factors in health, health services and public health program contexts, qualitative research is increasingly being used in health research, including clinical trials. Qualitative research follows an inductive framework to explore and gain an in-depth understanding of the phenomena, especially why and how aspects, through techniques including interviews, focus groups and observations. It analyzes the textual data collected following one of the common analysis approaches: grounded theory, phenomenology, ethnography or participatory action research. Despite the divergence in principles, mixed methods research designs systematically combine the quantitative and qualitative methods for a comprehensive understanding on the issue. The commonly used mixed methods designs variably combine the purpose, priority, sequence, embedding and data integration. Mixed methods analysis requires strategic synthesis of the results to gain comprehensive knowledge for appropriate clinical or public health action.

Keywords: *Focus group, Implementation, Interviewing, Participatory research, Social research*.

Published online: 02 August, 2021; ***PII:*** S097475591600357

Traditional clinical research is dominated by quantitative study designs that document different variables (exposure, outcome and confounders) as measurable parameters and examines the relationship between them using statistical analysis principles. Much of the clinical and public health research happens within the clinical, social, population and interpersonal context, where the numeric data and statistical methods may be inadequate to document the patients, public and healthcare provider's experiences about the care and services. It is commonly observed that the outcomes and degree of associations vary across different populations or individuals, which are not explainable quantitatively. The quantitative study is unable to tell about 'why' people behave in the observed manner and the reasons thereof. It is important to move beyond 'what works' documented in quantitative research and understand 'what works for whom, why, how and when' to improve or customize the interventions or processes, and this is where qualitative research comes into play. The individual and population characteristics, experiences, behaviors and practices play a significant role in health and clinical practice. For documenting the possible reasons and identifying potential solutions for the observed patterns, qualitative research is needed. Moreover, qualitative research is critical for exploring new areas or issues of research, and identification of items of enquiry for documentation and quantification.

QUALITATIVE RESEARCH

Qualitative research is the systematic enquiry to obtain an in-depth understanding on the nature of phenomena in their natural setting, which may include but is not limited to, people's experience, individual and/or group behavior, and organizational function [1]. Several definitions for qualitative research have been proposed (**Suppl. material**). Qualitative research explores people's per-ceptions, experiences, attitudes, behavior, and inter-actions with others related to the phenomena or topic under study in the specific context. The researcher is the key instrument for data collection. It does not attempt to generalize the findings to the larger populations or other phenomena.

It was initially used by sociologists and anthro-pologists to study cultures and practices in their own and foreign contexts. Over the last few decades, it is increasingly used in clinical and public health research. As qualitative research does not attempt enumerating and generalizing the findings, some view this as the opposite of quantitative research. Although the methods are contrasting, the two research methods may overlap somewhat and complement each other. The key differences between quantitative and qualitative research are summarized in **Table I**. Qualitative research has been used in clinical trials and intervention studies to optimize interventions, trial procedures, improving the external validity, facilitating the interpretation of the findings, making trials human sensitive, and assisting in improving the

Table I Some Differences Between Quantitative and Qualitative Research for Various Domains

Quantitative research	*Qualitative research*
Theory orientation	
• Deductive; tests to confirm hypothesis about phenomena	• Inductive; generates theory or explores phenomena
Epistemology orientation	
• Positivism; only includes scientifically verifiable ones	• Interpretivist; interprets the meanings that humans attach to their actions
Ontology orientation	
• Objectivism; asserts the validity of objective phenomena over subjective experience	• Constructivism: allows people to actively construct their own knowledge • Reality is determined by researcher's experiences
Methods	
• Follows designs and tools fixed prior to data collection	• Allows flexibility in designs to emerge during study
Data collection	
• Precise measurement and objective data collection • Uses standardized tests, instruments and measurement tools • Close ended and objective questions • Often involves large sample sizes estimated by formula or software • Participants selected following randomness approach • Participant responses not affected by how questions are asked and sequence	• Accurate description of the processes and observations (words, texts, etc.) • Adopts interviews, discussions, observations and document reviews • Open ended questions • Often involves smaller sample sizes and observations, no sample size formula • Participants selected purposively • Participant responses are affected by how questions are asked and sequence
Interpretation	
• Conducts analysis after data collection • Numerical data (assigns values to responses as numbers, scales, categories) • Explores single truth and often measures single outcome • Performs data analysis in prescribed and standardized method(s) • Analysis attempts achieving significance level • Statistics complexities • Generalizes the findings from sample to population	• Conducts analysis during data collection • Accommodates complexities and multiple realities • Textual data (uses text, notes, audios/videos, observation narratives) • Flexible and iterative analysis approach • Can obtain multiple outcomes and accommodate multiple sources of data (triangulation) • Performs data analysis in creative, iterative, nonlinear and holistic manner • Analysis attempts insight and metaphor • Conceptual complexities • Specific to the sample and context, does not generalize to the population
Reporting	
• Quantifies the observations and variations • Follows standardized format	• Describes the observations and variations • Allows variability with expressive language and personal voices

Epistemology is a branch of philosophy that investigates the origin, nature, methods, and limits of human knowledge. Ontology is a branch of philosophy that explores the types, properties, and interrelationships of the entities that really or fundamentally exist for a particular domain of discourse.

effectiveness of future trials [2].

Qualitative Study Designs

The taxonomy of qualitative research has evolved over time [3-5]. In 2007, Cresswell proposed five qualitative study designs: Narrative research, Case studies, Grounded theory, Phenomenology and Participatory action research (PAR) [6]. Broadly, these are categorized into non-participatory types (the researcher only observes and does not participate in the activities) and participatory (the researcher participates in the process in the community and usually attempts to influence some activities). All study designs except PAR fall under the non-participatory category. The case studies have several subtypes: snapshot, comparative, longitudinal, pre-

post, and patchwork case studies. In health research, the grounded theory, phenomenology, ethnography and PAR are commonly used. The grounded theory explores a less-well-understood problem, situation, or context to generate a general hypothesis or explanation based on the views of a large number of participants. Phenomeno-logy focuses on the essence of the 'lived experiences' of the person (or group), regarding an issue that becomes embedded in the consciousness and what meaning that carries for the person or group. Ethnography focuses on the cultural and social systems (structure and function) of a particular group and may cover various aspects like religion, economy, politics, environment and history. This may require immersion of the researcher in the society to study everyday life and relies on participant observation along with interviews. PAR focuses on finding suitable solutions for a social problem through researcher-community collaboration at all levels. The PAR may involve mixed (both qualitative and quantitative) methods designs.

Choosing Qualitative Study Design

Qualitative research designs do not follow strict taxonomy with fixed boundaries or strict stages like quantitative research. The data collection, analysis, research question refinement, theory modification, and addressing validity proceed more or less simultaneously and each step influences the others. The researcher has the flexibility to revise or modify the design and methods during the study based on the new developments and experiences. It does not imply a lack of study design in qualitative research, but the concept of a broader design. While choosing a study design and methods, the researchers consider various aspects including research question, context, conceptual framework, appropriate data types, validity and ethical considerations. Apart from these, the researcher's skills, participant and community concerns, piloting experience, and larger goals also influence study design selection. **Table II** summarizes the characteristics of the qualitative study designs. Qualitative research question can focus on any one or combination of these: *i*) understanding the meaning of the events, situations, and participants actions based on their lived experiences; *ii*) understanding the specific context within which the participants live and operate for their action; *iii*) identifying unknown, unanticipated phenomena and influences to generate new theories; *iv*) understanding the processes of occurrence of events and actions and their interconnections; and *v*) developing causal explanations and relationships between the different segments [7].

Data Collection Methods

The most commonly used qualitative data collection methods in health research are interviews, focus group discussions (FGDs), observations, and analysis of documents.

Qualitative interviews: These are conversations between the researcher(s) and participant(s) to gain insights into their subjective experiences, perceptions, motivations and knowledge. The interviews may be of three types: structured, semi-structured and unstructured. In structured interviews, the interviewer refers to a predetermined list of questions, which allows consistency across participants and interviewers (multiple), but limits additional exploration. In semi-structured interviews, the inter-viewer(s) refer to some questions but have the flexibility to adapt and add questions based on the responses and context, which allows more intuitive and natural conver-sations with the participants. Unstructured interviews aim to gather in-depth information from key informants and usually do not have a pre-planned set of questions and are instantaneously generated during the interview. Thus, it resembles more with free-flowing conversation than an interview. The semi-structured and unstructured inter-views are also known as in-depth interviews (IDIs). For conducting IDIs, the interviewers must be researchers themselves or have a good understanding on the topic. The topics, questions, sub-questions and probes are developed based on the available literature, previous research and piloting, which may be revised during data collection. Interviews must primarily focus on being interactive and allowing unexpected issues to emerge and be explored. The interviewer can audio- or video-record the conversation with the consent of participants for transcription later [5,7.8].

Focus group discussion: This involves discussion among the participants (preferably of similar background) to explore their experiences, perceptions, knowledge and how and why people behave in certain ways. A FGD usually involves 6-10 people and is facilitated by an experienced moderator using a topic guide. Topic guide or interview guide is compilation of the list of topics or questions that the interviewer plans to cover during an interview. It is called a guide, because it is used to guide the interviewer, but not rigid like the questionnaire in quantitative studies. Topic guide include the topics or open-ended questions organized like a funnel; starting with warm-up discussion and easy questions, more detailed exploration, key areas of discussion, pulling out the essential insights, summarization. Additional obser-vers and note-takers may be involved to record the verbal and nonverbal expressions. The discussions preferably are audio- or video-recorded with participants' permission and transcribed later [9]. To review the quality of conversation, moderator's technique and compare between FGDs, a sociogram for each FGD is drawn. Sociogram reflects the flow of discussions in the group [10]. FGDs allow obtaining information from many people quickly representing the community view and supplement the interviews. FGD may be inappropriate for exploring sensitive topics.

Table II Qualitative Study Designs and Their Characteristics

Study designs	*Type of research question*	*Context*	*Unit of analysis*	*Data collection forms*	*Data analysis strategy*
A. Non-participatory research					
Narrative research	Questions about the life experiences of an/few individuals and how they unfold over time	Stories to understanding the problem	One or more individuals	Interviews, document review; chronological, story-oriented	Chronology, story elements Little set structure
Case study	Questions about developing an in-depth understanding about how different cases provide insight into an issue or a unique case	Explores a case bounded by time, place that inform a problem	An event, program, activityOne or more individuals	Interviews, observations, documents, artefacts	Case description, themes of the case and cross-case themes; Some structure
Grounded theory	Questions about experiences over time or changes that have stages and phases	No theory exists or existing ones are inadequate	Process, action or interaction involving many individuals	Interviews, may include others	Open coding, axial coding and selective coding; High level structure
Phenomenology	Questions about what is at the essence that all persons experience about a phenomenon	To understand the lived experiences of persons about a phenomenon	Several individuals with experience	Interviews, documents, observations, other items	Statements textual description on phenomenon; Structured approach
Ethnography	Questions about the structure and function of a group of people	Cultural and social system of a group; natural setting	Group of population	Observations, nterviews, docuiments, immersion	Themes, statements; textual description; High level structure
B. Participatory research					
Participatory action research	Questions about how changes occur in a community	To address community issues for bringing change	Entire community, multiple stakeholders	Interviews, documents, observations, other items	Combination of different options; Little set structure

Adapted from Cresswell, et al. 2007 [7]

Observation: This involves documentation of activities, behaviors, conversations, organization or community processes or other aspects of observable human experiences [11]. Observation may be reactive (with participant's knowledge) or non-reactive (without participant's knowledge). Observation is critical in both interviews and FGDs to identify nonalignment between verbal and nonverbal data. Observation can also be a stand-alone method with or without the participation of the researcher. Observation provides information about the setting, context and actual behavior, which may not be possible in reported behavior or opinions. Observations can be either participant (observer is part of the observed setting) or non-participant (observer neither participates nor influ-ences the setting) in nature. While observation allows deeper insights into the real-world setting and capturing issues not already considered, it also has the risk of the Hawthorne effect with the presence of an observer [12].

Written or audio-visual documents: These may be revie-wed to gain information about the issue under study [9].

Qualitative research assumes no objective hierarchy of evidence and methods and the selection of methods (single or combined) must be based on the research question and suitability and feasibility of method(s) for the question and setting. The selection of method must be justified and documented. The use of multiple methods may allow a more comprehensive understanding of the issue under study and comparison between findings from different methods, which is referred as triangulation.

Sample Size and Sampling

Unlike a quantitative study, qualitative research follows no fixed rule for sample size. The data collection usually continues until data saturation, i.e., no new information or opinion emerges. The sample size depends on the rich-ness

and type of participants. Usually, 15-20 participants per stakeholder category may be adequate, but the number may be decided depending on the research issue, context, stakeholders, anticipated differences in that phenomenon, and data saturation achieved during data collection [13]. The researcher may choose a small homogenous sample for in-depth study on the group and particular subgroups [11]. Sampling is usually non-probability and purposive. In qualitative research, the researcher attempts to gain in-depth understanding of the issues and dependent on the richness of the information shared by the participants/informants, not generalizability. Thus, the research selects the participants subjectively rather than random selection according to the likelihood of obtaining rich and in-depth information. The researchers often use purposive sampling and choose participants based on the specific expertise or insight regarding the phenomenon of interest.

Data Management and Analysis

The field notes and recordings of interviews, FGDs, observations are transcribed verbatim and checked for accuracy with the source documents. As needed, the narratives may be translated into the language of analysis, but care must be taken to ensure no loss of the essence and meanings.

Qualitative data analysis involves reading the texts and understanding its essence from the participant's perspective. The raw data (statements or segments of narratives) are coded either manually or using software. Coding is the process of organizing the sections of the data according to their meaning, sentiment and relationships. The codes bearing similar meanings are grouped into conceptual codes and categories. Linkages and connections between the conceptual codes and categories are explored (axial coding). Based on the emerging hypothesis, the conceptual codes and categories are organized under few themes (selective coding). The data is presented under the themes identified and compared across the data collection methods, stakeholders and contexts to identify the similarities and differences along with the potential reasons. The coding and data organization is an iterative process till an agreement between the researchers is achieved. The results are organized under the themes as the headings, the codes as the sub-headings with the researcher's interpretation and statements or narratives as 'quotable quotes'. Apart from textual format, data can be displayed in any form: boxed display, decision tree model, flow chart, ladder, matrix, metaphorical display, modified Venn diagram, network and taxonomy [14].

Use of Software and Challenges

Several free and license-based software programs (Ethnograph, NVivo, Atlas-Ti, NUD.IST, WInMAX, MAXQDA, HyperRESEARCH, HubSpot, FreeQDA, RQDA, etc.) are available [14]. International Clinical Epidemiology Network (INCLEN) qualitative data analysis software (IQDAS) is an in-house program and is being used by us. Although software programs improve the efficiency of data management, purists claim it to distance the researcher from the data.

Biases

Qualitative studies are at risk of biases from the data collection method, selection of participants or analysis based on the researcher's knowledge, preconception, theory and values. Although rigorous and standardized training may improve the quality of data collection, the influence of researcher and data collector cannot be eliminated [7].

Validity Tests

Validity of the data and credibility of the interpretation can be increased with: *i*) repeated observation over a longer period; *ii*) in-depth exploration using probes; *iii*) respondent validation (soliciting feedback on the conclu-sions from the study population to avoid misinterpre-tation); *iv*) data triangulation (comparison across parti-cipants, settings and methods); *v*) comparison (with the control group) [7].

Quality Assurance

Quality assurance is a systematic approach to review the practices and procedures followed in a research to document whether things are being done according to the standards/ best practices as well as they could/should be and identify possible improvements. It is a continuous and on-going process throughout the study and dissemination. Rigorous quality assurance measures must be adopted at all levels including *i*) selection of appropriate research methods and data collection techniques; *ii*) selection and appropriate training of the research team; *iii*) audio- or video-recording of the interviews and FGDs; *iv*) correct and complete transcription and translation; *v*) data analysis by multiple researchers and discussion to generate consensus on the code mapping; and *vi*) adopting validation methodology.

Ethical Issues

Although qualitative methods appear harmless compared to the biological sample collection, they may have unintended consequences at individual, group, organizational and societal levels. Thus, anonymity and confidentiality of participants must be ensured.

Some examples of qualitative research are given as Supplementary material.

MIXED METHODS RESEARCH

Mixed methods research (MMR) combines the quantitative

and qualitative methods (questions, data collec-tion, analysis, interpretation) in the same study. Despite the contrasting assumptions, principles and cultures, increasingly both quantitative and qualitative research methods are being used to complement and supplement the hypothesis and findings. Several definitions for this have been proposed (Supplementary material). For denoting the emphasis and contribution of research method types, the components are indicated as QUAL or qual and QUAN or quan (capital indicates primacy) for qualitative and quantitative research, respectively. The purposes of using mixed methods research include: complementarity, completeness, triangulation of results; development (one method informs the other), initiation (discovers new perspectives), explanation, expansion (expands the breadth and range of inquiry), instrument development, credibility and contextualization [15,16].

Designing Mixed Methods Research

Four issues must be addressed for planning mixed methods research: theoretical basis, priority, data collec-tion sequence and data integration. The theoretical basis and research question inform the dominance, sequence and integration of methods. Based on the dominance, MMR may be considered as qualitative dominant, quanti-tative dominant or equal status. MMR may use the methods in either a sequential or concurrent manner, based on the need. The qualitative and quantitative app-roaches may integrate at five possible points: planning, research question, tool development and data collection, analysis and result presentation [17,18]. The mixed methods research framework is summarized in **Table III**. MMR is broadly divided into six

Table III Mixed Method Research Design and Integration Framework for Each Level

Levels and types	*Characteristic*
Conduct	
Concurrent	Data collection and analysis for both methods done concurrently.
Sequential	Data collection and analysis of one method precedes the other.
Multistage	Multiple stages of data collection, variable combinations of methods.
Priority/ Dominance	
Dominant	One method is dominant based on the research question.
Equal	Both methods contribute equally according to the question.
Intervention	
Observational	No intervention.
Interventional	Intervention.
Hybrid	Observation and intervention combined variably.
Study designs	
Triangulation	Comparison and/or validation of the quantitative results with qualitative data or expand quantitative findings with qualitative data; Includes data transformation: one type of data is converted into the other type and integrated/compared with the data not transformed for analysis. Second method helps to explain the findings from first method.
Explanatory	Results of first method (usually qualitative) informs the second method (identify variables, develop instrument).
Exploratory	Second method (qualitative/quantitative) is needed to answer a research question within larger quantitative or qualitative study.
Embedded/ Nested Transformative	One type of data is converted into the other type and this data is integrated with the data not transformed for analysis.
Database linkages	
Connecting	One database is linked to the other through sampling.
Building	Results from one database informs the data collection approach of the other.
Merging	Both databases are brought together for analysis.
Embedding	Data collection and analysis are linked at multiple points.
Interpretation	
Narrative	Describe findings from both methods in same report. - Weaving: both findings presented together, either theme- or concept-wise. - Contiguous: findings presented in separate sections. - Staged: findings presented step/stage wise as conducted.
Transformative	One type of data is converted into the other type or consolidated into new variables for analysis.
Joint display	Data from both components integrated and presented together in figure, table, matrix or graph.

types: three concurrent or convergent (triangulation, embedded/nested and trans- formative) and three sequential (explanatory, exploratory and transformative). Additionally, sequential embedded and multiphase mixed methods research designs may also be adopted. The architecture of these mixed methods research study designs is shown in **Fig. 1**. These designs are selected according to the research purpose, conduct, priority, analysis, integration and presentation. Mixed methods research design involves several steps and considerations which guide the selection of the type of study design: research question, the purpose of mixed methods research, method priority, data collection seque-nce, embedding and data integration [19,20]. **Fig. 2** shows the steps in mixed methods research study design selec-tion. Mixed methods research is being used in health behavior, implementation researches and clinical research [2]. Some examples of MMR are given in the supple-mentary material.

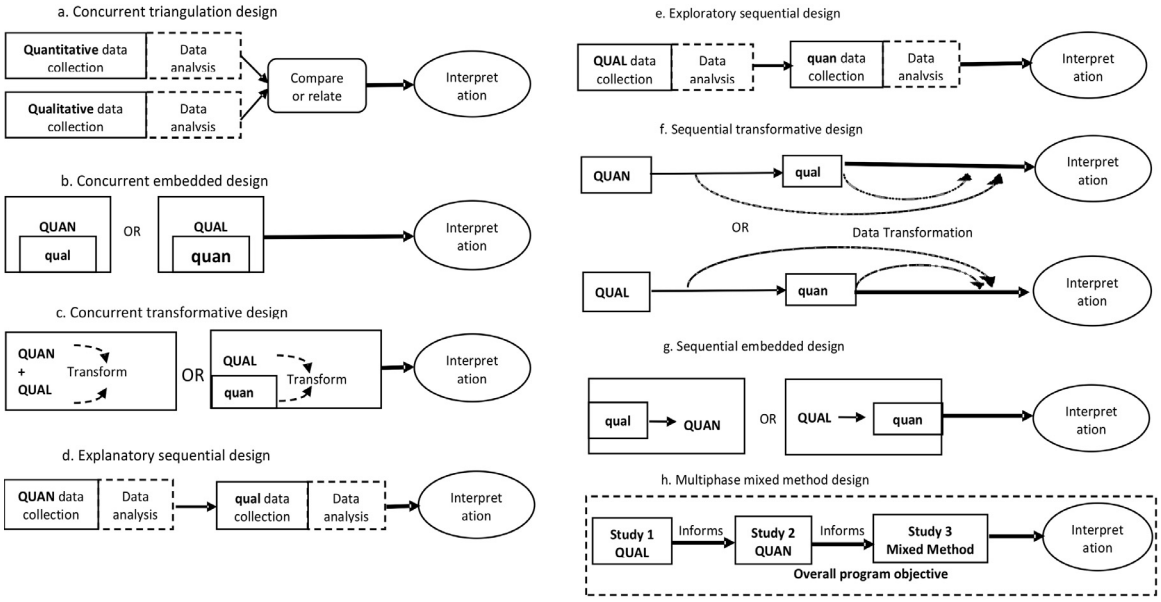
CONCLUSIONS

Qualitative research has a unique position in socio-behavioural research in health and significant value addition if used with the quantitative research. Qualitative research would enhance the etiological, risk factor and health behaviour understanding in clinical practice and public health programs. Qualitative research involves critical thinking and much dependent on the competence of the researcher(s). Despite the contrasting methodologies and dissimilarities, mixed methods research designs enable combining the qualitative and quantitative study designs in a meaningful and symbiotic manner to address the questions. While combining and selecting these study designs, careful planning must consider the research purpose, data dominance, depen-dence, sequence, sampling, data integration and analysis. While the research purpose and theoretical conceptual framework are the primary drives, the practical aspects like timing, context, sampling, feasibility and competency of the research team are to be considered.

Note: Supplementary material related to this manuscript is available with the online version at *www. indianpediatrics.net*
Contributor: MKD: Conceptualisation, data collation, data analysis, manuscript writing, final approval of the manuscript.
Funding: None; *Competing interest*: None stated.

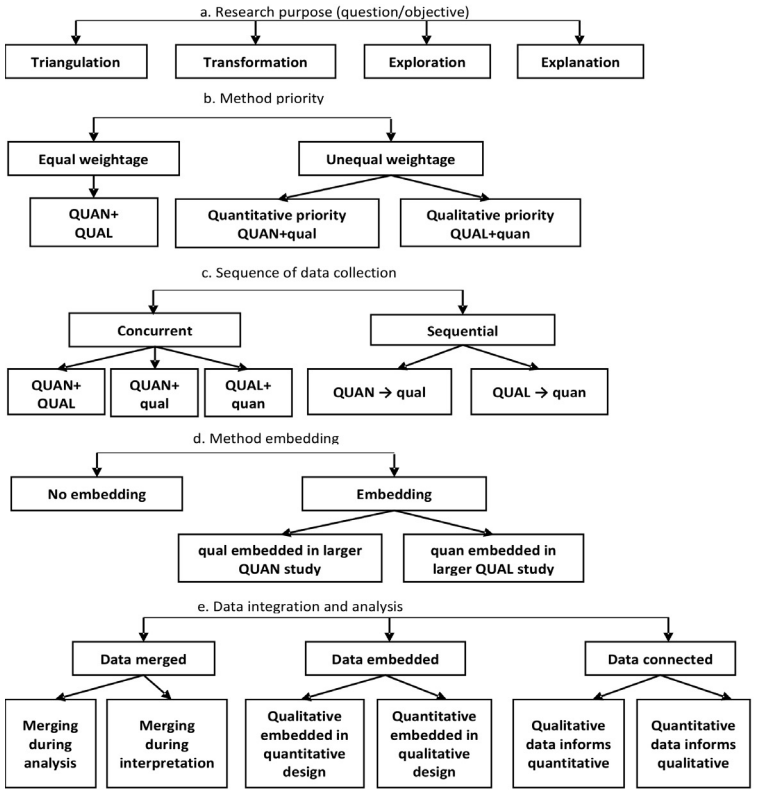
REFERENCES

1. Creswell J. Research Design: Qualitative, Quantitative, and Mixed Methods Approaches. 5th ed. Sage Publi Ltd; 2009.
2. O'Cathain A, Thomas KJ, Drabble SJ, et al. What can qualitative research do for randomised controlled trials? A systematic mapping review. BMJ. 2013;3:e002889.
3. Wolcott HF. Posturing in qualitative inquiry. *In*: LeCompte MD, Millroy WL, Preissle J, editors. The Handbook of Qualitative Research in Education. Academic Press; 1992. p. 3-52.
4. Norman KD, Yvonna SL. The Sage Handbook of Qualitative Research. 3rd Edition. Sage Publications Inc; 2005.
5. Tesch R. Qualitative Research: Analysis Types and Software. Routledge; 2013. Accessed October 8, 2020. Available from: *https://www.taylorfrancis.com/books/9781315067339*
6. Creswell JW, Hanson WE, Clark Plano VL, Morales A. Qualita-



QUAL: qualitative data is given higher priority; qual: qualitative data is given lower priority; QUAN: quantitative data is given higher priority; quan: Quantitative data is given lower priority.

Fig. 1 Types of mixed method research study designs (adapted from Cresswell, et al. [17] with permission).



QUAL: Qualitative data is given higher priority; qual: Qualitative data is given lower priority; QUAN: Quantitative data is given higher priority; quan: Quantitative data is given lower priority.

Fig. 2 The steps in mixed method research design selection.

tive research designs: Selection and implementation. Couns Psychol. 2007;35:236-64.
7. Maxwell J. Designing a qualitative study. *In*: Bickman L, Rog DJ, editors. The SAGE Handbook of Applied Social Research Methods. SAGE Publications; 2009.p.214-53.
8. Jamshed S. Qualitative research method-Interviewing and observation. J Basic Clin Pharm. 2014;5:87.
9. Busetto L, Wick W, Gumbinger C. How to use and assess qualitative research methods. Neurol Res Pract. 2020;2:14.
10. Drahota A, Dewey A. The Sociogram: A useful tool in the analysis of focus groups. Nurs Res. 2008;57:293-7.
11. Patton MQ. Qualitative Research & Evaluation Methods: Integrating Theory and Practice. 4th ed. SAGE Publi Inc.; 2014.
12. Austin Z, Sutton J. Qualitative research: Getting started. Can J Hosp Pharm. 2014; 67:436-40.
13. Malterud K, Siersma VD, Guassora AD. Sample size in qualitative interview studies: Guided by information power. Qual Health Res. 2016;26:1753-60.
14. Verdinelli S, Scagnoli NI. Data display in qualitative research. Int J Qual Methods. 2013;12:359-81.
15. Greene JC, Caracelli VJ, Graham WF. Toward a conceptual framework for mixed-method evaluation designs. Educ Eval Policy Anal. 1989;11:255-74.
16. Bryman A. Integrating quantitative and qualitative research: How is it done? Qual Res. 2006;6:97-113.
17. Creswell JW. Mixed methods designs. *In*: Creswell JW, editor. Educational Research: Planning, Conducting, and Evaluating Quantitative and Qualitative Research, 4th ed. Pearson; 2012. p. 534-75.
18. Ponce OA, Pagán-Maldonado N. Mixed methods research in education: Capturing the complexity of the profession. Int J Educ Excell. 2015;1:111-35.
19. Creswell JW, Clark VLP. Choosing a mixed methods design. *In*: Creswell JW, Clark VLP, editors. Designing and Conducting Mixed Methods Research. SAGE Publication; 2006. p. 58-88.
20. Hanson WE, Creswell JW, Clark VLP, et al. Mixed methods research designs in counseling psychology. J Couns Psychol. 2005;52:224-35.

Supplementary Document 1

The available definitions of qualitative research

"Qualitative research involves any research that uses data that do not indicate ordinal values" (Nkwi PN, Nyamongo IK, Ryan GW) (1). "Qualitative research is a situated activity that locates the observer in the world. It consists of a set of interpretive, material practices that makes the world visible. These practices transform the world. They turn the world into a series of representations, including field notes, interviews, conversations, photographs, recordings, and memos to the self. At this level, qualitative research involves an interpretive, naturalistic approach to the world. This means that qualitative researchers study things in their natural settings, attempting to make sense of, or to interpret, phenomena in terms of the meanings people bring to them" (Denzin NK, Lincoln YS) (2). "Qualitative researchers are interested in understanding the meaning people have constructed, that is, how people make sense of their world and the experiences they have in the world" (Merriam SB, Tisdell EJ) (3).

"Qualitative research is research using methods such as participant observation or case studies which result in a narrative, descriptive account of a setting or practice. Sociologists using these methods typically reject positivism and adopt a form of interpretive sociology" (Drislane R, Parkinson G) (4). "Qualitative research as an iterative process in which improved understanding to the scientific community is achieved by making new significant distinctions resulting from getting closer to the phenomenon studied" (Aspers P, Corte U) (5).

REFERENCES

1. Nkwi PN, Nyamongo IK, Ryan GW. Field research into socio-cultural issues/ : methodological guidelines. Yaoundé, Cameroon: International Center for Applied Social Sciences, Research, and Training; 2001.

2. Denzin NK, Lincoln YS. The SAGE Handbook of Qualitative Research. 3rd Edition. Thousand Oaks, California: SAGE Publications, Inc.; 2005.

3. Merriam SB, Tisdell EJ. Qualitative Research: A Guide to Design and Implementation. 4th Edition. San Francisco, CA: Jossey-Bass, A Willey Brand; 2016.

4. Drislane R, Parkinson G. Qualitative Research. In: Online Dictionary of The Social Sciences. Athabasca University and ICAAP; 2002.Accessed Jun 4, 2021. Available from: *https://bitbucket.icaap.org/dict.pl?term=QUALITATIVE%20RESEARCH*

5. Aspers P, Corte U. What is Qualitative in Qualitative Research.QualSociol. 2019;42:139-60.

Supplementary Document 2

Examples of published literature using Qualitative Research Method

Case study 1: Qualitative research using in-depth interviews and observations

Perceptions of the parents of deceased children and of healthcare providers about endof-life communication and breaking bad news

Parents of dying children face unique challenge and expect compassionate support from health care providers (HCPs). There is limited documentation from Indian context on the experiences of the parents and HCPs on end-of-life care for dying children and breaking the bad news around death. This study explored the experiences of the parents and HCPs about the end-oflife care and breaking bad news and related positive and negative factors in Indian context. This qualitative study was conducted at a tertiary care hospital. The data collection included in-depth interviews with the parents (n=49) and family members (n=21) of the children died at the hospital and HCPs (n=16; 6 doctors, 6 nurses and 4 support staffs) were conducted. The events and communication around death (n=8) for the children were observed. Data were inductively analysed using thematic content analysis method to identify emerging themes and codes.

The study observed that the doctors were the lead communicators for end-of-life communication. Majority of parents perceived the attitude, communication and language used as by resident doctors as brief, insensitive and sometimes inappropriate or negative. They perceived that the attitude and communication by senior doctor's as empathetic, positive and complete. Parents recalled the death declaration by resident doctors as non-empathetic, blunt and cold. Most parents received no emotional support from HCPs during and after death of their child. All doctors

expressed that death of their patients affected them and their emotions, which they coped through different activities. The overcrowded wards, high workload, infrastructural limitation and no formal communication training added to the emotional stress of the HCPs.

The study highlights the communication by HCPs and support for parents during the end-oflife communication and breaking bad news. Majority of the communication by the HCPs during the hospitalisation and end-of-life period were perceived as suboptimal by the parents. The HCPs were emotionally affected and faced end-of-life communication challenges. There is need for adoption of context specific communication protocol and materials and training of HCPs in communication to improve the quality of care and communication during the crisis periods.

REFERENCE

Das MK, Arora NK, Chellani HK, et al. Perceptions of the parents of deceased children and of healthcare providers about end-of-life communication and breaking bad news at a tertiary care public hospital in India: A qualitative exploratory study. PLoS ONE. 2021; 16(3): e0248661. Doi: 10.1371/journal.pone.0248661.

Case study 2: Multisite qualitative research using in-depth interviews, focus-group discussions and informal interactions to explore the determinants of undernutrition

The high levels of under-nutrition in India persists despite economic growth and multiple multisectoral interventions and continue to challenge political leadership and policy makers. This multisite qualitative research was conducted to map the perceptions of mothers and other key stakeholders, to identify emerging drivers of childhood undernutrition.

This multi-centric qualitative research was conducted across six states of India with high burden of undernutrition. The study sample included 509 in-depth interviews with mothers of undernourished and normal nourished children, policy makers, district level managers, implementer and facilitators. Sixty six focus group discussions and 72 non-formal interactions were conducted in two rounds with primary caretakers of undernourished children, Anganwadi Workers and Auxiliary Nurse Midwives.

Based on the perceptions of the participants, a model was inductively developed showing core themes as drivers of under-nutrition. The most forceful emerging themes were: multitasking, time constrained mother with dwindling family support; fragile food security or seasonal food paucity; child targeted market with wide availability and consumption of ready-to-eat market food items; rising non-food expenditure, in the context of rising food prices; inadequate and inappropriate feeding; delayed recognition of under-nutrition and delayed care seeking; and inadequate responsiveness of health care system and Integrated Child Development Services (ICDS). The study emphasized that the persistence of child malnutrition in India is also tied closely to the high workload and consequent time constraint of mothers who are increasingly pursuing income generating activities and enrolled in paid labour force, without robust institutional support for childcare.

The models identified from the data are shown in Figure 1 and 2.

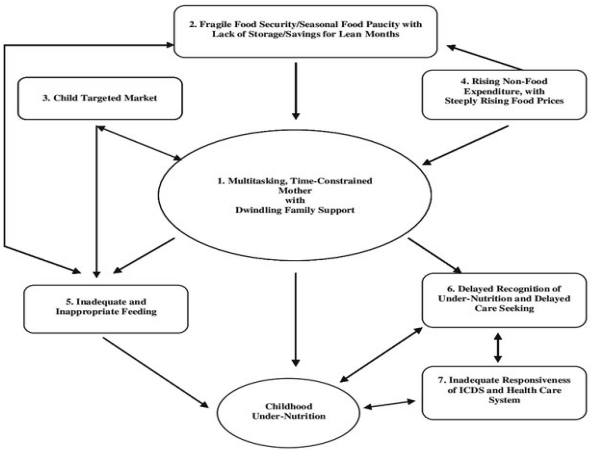


Fig. 1 Emerging model of childhood under-nutrition.

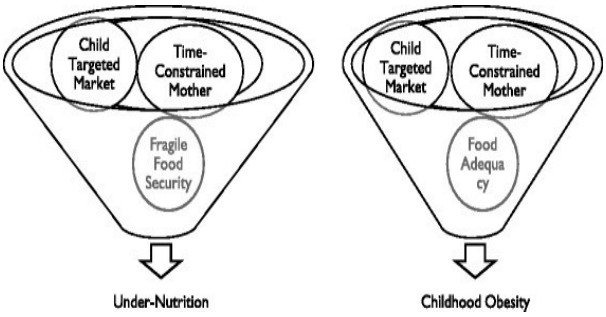


Fig. 2 The linkages between childhood under-nutrition and obesity (double Burden) with changing economic condition and food security at household level

The study findings identified the factors beyond the health sector with influence of the business and contextual issues on the food behaviour of the families and societies, which contribute to persistence of the child undernutrition burden.

REFERENCE

Chaturvedi S, Ramji S, Arora NK, Rewal S, Dasgupta R, Deshmukh V; for INCLEN Study Group. Time-constrained mother and expanding market: emerging model of under-nutrition in India. BMC Public Health.2016; 16: 632.doi: 10.1186/s12889-016-3189-4.

Case study 3: Using qualitative research for improving implementation of complex community intervention

The lay health workers (LHWs) are increasingly engaged to complement health services at community level. Their perceptions of the interventions they implement and their experiences in delivering community based interventions in India have been infrequently studied. A LHW led intervention was implemented to improve anemia cure rates in rural community dwelling children attending village day care centers in South India. To improve the implementation, a qualitative study was undertaken to understand the LHWs' acceptance of and perspectives regarding the intervention, particularly in relation to factors affecting daily implementation. The study used focus group discussions (FGDs) were conducted with the trained LHWs assigned to deliver the educational intervention. These were complemented by non-participant observations of LHWs delivering the intervention.

The study identified several factors related to the implementation of the intervention effort including pre-implementation training modules, intervention simplicity, and ability to incorporate the intervention into the routine work schedule. LHWs felt that the intervention impacted negatively on their preexisting workload. Fluctuating relationships with mothers weakened the LHWs position as providers of the intervention and hampered efficient implementation, despite the LHWs' highly valued position in the community. Modifiable barriers to the successful implementation of this intervention were seen at two levels. At a broader contextual level, hindering factors included the LHW being overburdened, inadequately reimbursed, and receiving insufficient employer support. At the health system level, lack of streamlining of LHW duties, inability of LHWs to diagnose anemia and temporary shortfalls in the availability of iron supplements constituted potentially modifiable barriers.

This qualitative study identified some of the practical challenges as experienced by LHWs while delivering a community health intervention in India. Methodologically, it highlighted the value of qualitative research in understanding implementation of complex community interventions.

REFERENCE

Shet A.S., Rao A., Jebaraj P., Mascarenhas M., Zwarenstein M., Galanti M.R., Atkins S. Lay health workers perceptions of an anemia control intervention in Karnataka, India: a qualitative study. BMC Public Health 2017; 17: 720. Doi: 10.1186/s12889-017-4758-x

Case study 4: Using photovoice for capturing the community perceptions on child health

The Sundarbans (West Bengal, India) has several inhospitable terrain and is at risk for frequent climatic shocks which challenge the access to healthcare for the inhabitants. Community members, and women in particular, have few means to communicate their concerns to local decision makers. Photovoice is one way in which communities can raise their local health challenges with decision makers. This study attempted to capture the mothers' voices on the determinants of their children's health to inform local level decision-making.

A photovoice action research was conducted in three blocks in the Sundarbans region. The project involved eight groups of eight to ten mothers who had at least one child below 6 years of age across four villages. The mothers were trained on photo documentation and ethical concerns before taking two rounds of photographs within 6 months, interspersed by fortnightly group meetings facilitated by researchers. Photographs and key messages were communicated to local decision makers during block and village level interface sessions with the mothers and researchers.

Mothers' photos focused on specific determinants of health, such as water and sanitation; health status, such as malnutrition and non-communicable diseases; service accessibility; climate conditions; and social issues such as early marriage and recurrent pregnancy. Some issues were not captured by photos but were discussed in group meetings, including domestic violence and the non-availability of medical practitioners. Differences in perceptions and photographs taken were observed according to the mother's educational status, livelihood and caste identity.

Photovoice has the potential to capture the voices of vulnerable and special group communities regarding their perceived health needs and challenges, which can help communicating these to the local decision makers for health policy and planning.

REFERENCE

Ghosh, U., Bose, S., Bramhachari, R., Mandal S. Expressing collective voices on children's health: photovoice exploration with mothers of young children from the Indian Sundarbans. BMC Health Serv Res. 2016; 16: 625. Doi: 10.1186/s12913-016-1866-8.

Supplementary Document 3

Examples of published literature using Mixed-Method Research

Case study 1: The relevance of qualitative research The poliomyelitis eradication effort in India

As a commitment under the Global Polio Eradication Initiative (GPEI), India accelerated its effort towards improving oral polio vaccine coverage through the routine immunization and Supplementary Immunization Activities (SIA, also known as the pulse polio immunization). With these intensified immunization efforts, the number of reported acute flaccid paralysis (AFP) cases decreased from 134 in 2004 to 66 in 2005. However, the cases resurged in 2006 and concentrated in western Uttar Pradesh and Bihar. The routine vaccination coverage with 3 doses of OPV was low in the polio-endemic states (Bihar, 27%; western UP, 38%; and eastern UP, 45%). (1)

The causes of the social resistance and low coverage of OPV vaccines could not be identified through the quantitative research approach. To document the determinants of social resistance and low OPV acceptance in the western UP districts, a qualitative research was conducted. This qualitative research used in-depth interviews (IDIs, with mothers, healthcare providers and community leaders), focus group discussions (FGDs, with mothers and healthcare providers), non-formal interactions (with community leaders, parents, businessmen, journalists, mobilizers, vaccinators and supervisors) and observations of the vaccination and mobilisation. The researchers documented a distinct machination of social resistance and rumors against OPV during the SIA in some minority dominated areas. While, most parents in minority areas supported the SIAs, a few clusters from extremely marginalized sections continued to evade SIAs, with an endemic pattern. The rumors circulated through varous channels reached majority community as well parents (2).

The findings of this research was used for appropriate programmatic modification and adoption of strategic communication approach targeting the resistant communities and pockets. With these refinements in the communication and community mobilisation approaches, the polio eradication was achieved in the country.

REFERENCES

1. Centers for Disease Control and Prevention. Progress Toward Poliomyelitis Eradication - — India, January 2005—June 2006. Morb Mortal Wkly Rep. 2006;55(28):772–6.

2. Chaturvedi S, Dasgupta R, Adhish V, Ganguly KK, Rai S, Sushant L, et al. Deconstructing social resistance to pulse polio campaign in two North Indian districts. Indian Pediatr. 2009;46(11):963–74.

Case study 2

Sequential mixed-method research- quantitative research followed by qualitative research and

Integrationat interpretation phase

Documenting the performance of electronic health records app and barriers in implementation

Electronic health record (HER) capturing is being promoted to improve the health services delivery, documentation and planning. A study documented the performance of "Comprehensive Public Health Management" application (CPHM App) in Karnataka forcing on the family based maternal and child health (MCH) services and the challenges.

This research compared the completeness and consistency of selected MCH indicators from paper-based records and the CPHM App and also the implementation enablers, barriers, and suggested solutions from the user perspective. A sequential mixed-method study design was followed. The first phase involved quantitative research focusing on the consistency of selected MCH indicators followed by in-depth interviews of healthcare providers (users). The quantitative research findings for consistency was expressed as percentages. In the qualitative phase, IDIs with various cadres of healthcare providers (ANMs, MHW, ASHA, and administrator) were conducted. The findings were integrated at the analysis phase to triangulate the findings from quantitative and qualitative phases and identify the potential reasons for the gaps and challenges faced by the users (3).

REFERENCE

Shilpa D, Naik P, Shewade H, Sudarshan H. Assessing the implementation of a mobile App-based electronic health record: A mixed-method study from South India. J Educ Health Promot. 2020;9(1):102.

Case study 3:

Sequential mixed-method research- qualitative research for tool development followed by quantitative research

Development of a composite Slum Adversity Index and factors affecting the mental health of individuals living in slums

The persons staying in slums are exposed to several adversities and have higher risk of common mental disorders (CMDs). There was no suitable tool and index to capture the risk for developing these CMDs in Mumbai slum. This mixed-method research used qualitative research (focus group discussions and in-depth interviews) to develop the tool and indices for slum adversity quantitative survey. The quantitative survey used the slum adversity questionnaire along with the other standard tools, which were used to create a composite Slum Adversity Index (SAI) score. The qualitative data were also used to identify the potential factors and their sources contributing and triggering the psychological distress in the inhabitants (4).

REFERENCE

Subbaraman R, Nolan L, Shitole T, Sawant K, Shitole S, Sood K, et al. The psychological toll of slum living in Mumbai, India: A mixed methods study. SocSci Med. 2014;119:155–69.

Breastfeeding Patterns and Stress Among Lactating Women in Pune During the COVID-19 Pandemic

Lactating mothers (*n*=126) residing in Pune, Maharashtra were interviewed to assess the prevalence of stress, rate of exclusive breastfeeding (EBF), and its association with different demographic factors. 75.4% mothers were found to be moderately stressed. Rate of EBF was 62.7%. Moderate stress and testing positive for COVID–19 were significantly negatively associated with EBF (*P*<0.001).

Keywords: *Exclusive breastfeeding, Employment, Maternal well-being.*

Published online: March 28, 2022; ***PII:*** S097475591600417

Prevalence of stress during and after pregnancy is directly related with breast milk synthesis and its expulsion, its composition and the desired duration of breastfeeding [1]. A pandemic like coronavirus disease (COVID-19), with the attendant lockdowns, may produce mental stress in mothers due to strictly advised social distancing, wearing masks and washing of hands frequently. Other possible reasons for stoppage of exclusive breastfeeding could also be delivery complications, hospitalization for delivery, and getting infected with the virus. Despite unequivocal encouragement from global as well as National healthcare stakeholders, there are many concerns regarding transmission from an infected mother to her infant, leading to increased perceived stress and subsequently to early cessation of breastfeeding [2]. During the early part of the current pandemic, there were questions on safety of breastfeeding, especially if the mother had severe acute respiratory syndrome coronavirus (SARS-CoV-2) [3]. Thus, the aim of this study was to assess the prevalence of stress caused by COVID-19 in lactating women, rate of exclusive breastfeeding, and its association with different demographic factors. For this cross-sectional study, mothers aged between 20-35 years having infants aged 0-12 month were enrolled through a maternity hospital in Pune by purposive sampling between May to July, 2021. Perceived stress scale (PSS) [4] was used to measure the level of perceived stress experienced in last one month by the mothers during a face-to-face interview with a single researcher (ZA). The mothers with stress identified during the study were counselled by the researcher and suggested relaxation therapies. Those who were not giving exclusive breastfeeding (EBF) to babies aged <6 months, were counselled regarding the importance and benefits of EBF. The demographic factors were studied using a questionnaire. Qualitative information was also collected using a semi-structured questionnaire.

Descriptive analysis was done and ordinal regression model was developed to analyze the data. The values were considered significant at the level of *P*<0.05.

A total of 126 lactating mothers were enrolled for the study [mean (SD) age 26 (3.7) year]. Of these, 54.8% were graduates; 30.2% had tested positive for SARS-CoV-2 by reverse transcriptase-polymerase chain reaction (RT-PCR) test; 46% of mothers were home makers and 33.3% full-time professionals; and 52.4% had delivered their baby via cesarean section. The rate of exclusive breastfeeding among mothers was 62.7%. Reasons for cessation of breastfeeding before six months were not enough milk supply (97.8%), and familial or social pressure (2.2%). Maximum mothers (75.4%) were moderately stressed and 20.6% were highly stressed; only 3.9% were 'little or not at all' stressed (**Table I**).

Demographic characteristics when measured against levels of perceived stress showed that being tested positive for SARS-CoV-2, being full-time employed, and cessation of EBF before 6 months were significantly associated with perceived stress levels. Housewives showed highest rates of EBF (34.1%), whereas rates of cessation of EBF was seen among full time professionals (17.4 %), which might be related to the post-partum stress arising due to less availability of time that the mothers dedicated for child care because of their work profiles. With respect to exclusive breastfeeding, against perceived stress levels it was observed that 3.9% had no stress, maximum women (57.1%) had moderate stress and only 1.5% reported high stress. Feeding techniques when analyzed against demographic factors and stress levels revealed that maximum of

Table I Characteristics of Lactating Mothers Enrolled in the Study (N=126)

Characteristics	No. (%)
Mother's age	
20-25	43 (34.1)
26-30	44 (34.9)
31-35	39 (31.0)
Education level	
Undergraduate	23 (18.3)
Graduate	69 (54.9)
Postgraduate	34 (27.0)
COVID-19 infection	40 (31.7)
Employment status	
House wife	58 (46.0)
Full time	42 (33.3)
Part time	12 (9.5)
Self employed	14 (11.1)
EBF for 6 mo	79 (62.7)
Cesarean section	66 (52.4)
Perceived stress	
No to little stressed	5 (4.0)
Moderately stressed	95 (75.4)
Highly stressed	26 (20.6)

the mothers (16.6%) who had tested positive gave formula feed by bottle, and those who had tested negative practiced latching of the baby directly at the breast. All the mothers who had little or no stress fed directly from the breast, whereas 21.3% mothers who were highly stressed fed formula feeds. On regression analysis, testing positive for SARS-CoV-2 infection (P<0.001) and moderate stress levels (P<0.01) were significant suppressants of practicing EBF among the mothers, whereas age, educational qualification and employment status did not have a significant effect.

The 2019-20 National Family Health Survey [5], reported the rate of EBF in Maharashtra as 71%, while in our study, which was carried out in urban Pune, it was 62.7%. The present study also reported high rates of perceived stress among mothers practicing EBF (58.6%), which is comparable to the findings of Sakalidis, et al. [6], who found that perceived stress was 62.6% amongst breastfeeding mothers living in Australian and New Zealand.

This study also found that those who tested positive for COVID-19 were more likely to cease EBF early. Testing positive for the virus was found to increase the chances of bottle feeds whereas those who tested negative fed their babies directly at the breast. Mothers perceived breastfeeding as a possible mode of transmission of the virus and hence may have resulted in early termination of EBF. This is despite the guidelines from Indian Academy of Pediatrics [7] and other bodies [8], recommending continued breastfeeding. Increased stress was also known to decrease the chances of having skin-to-skin contact and feeding the baby at the breast and as a consequence, babies were given formula feed by bottle. Since the baby is not being fed directly at the breast, this may result in early termination of EBF. Mothers also reported resuming with office work, having heavy work schedules and longer screen time acting as barriers to continue EBF. Hence these mothers used formula feeds and expressed breast milk while home-makers fed their babies directly at the breast.

Considering the adopted purposive sampling technique, the results of the present study cannot be generalized. Thus, the results direct towards a need to increase the awareness regarding the importance of breastfeeding during COVID-19 infection and precautions to be taken while breastfeeding. However, extended support to lactating mothers from antenatal care, social and family should be encouraged to practice EBF and decrease stress levels.

Ethical clearance: Institutional Ethics Committee, Symbiosis International (Deemed University), Pune; No. SIU/IEC/285, dated May 14, 2021.
Contributors: ZA: data collection, data analysis and manuscript writing; AM: development of protocol, supervision of the study, data analysis and manuscript writing; SM: manuscript review. All authors approved the final version submitted.
Funding: None; *Competing interest*: None stated.

ZAINAB AZAD, ARTI MULEY,* SRUJANA MEDITHI
Nutrition and Dietetics Program,
Symbiosis Institute of Health Sciences ,
Symbiosis International (Deemed University),
Pune, Maharashtra.
**asmuley@gmail.com*

REFERENCES

1. Lau C. Effects of stress on lactation. Pediatr Clin North America. 2001;48:221-34.
2. Vassilopoulou E, Feketea G, Koumbi L, et al. Breastfeeding and COVID-19: From nutrition to immunity. Front Immunol. 2021;12:661806.
3. Peroni DG, Fanos V. Lactoferrin is an important factor when breastfeeding and COVID 19 are considered. Acta Paediatr. 2020;109:2139-40.
4. Cohen S, Kamarck T, Mermelstein R. A global measure of perceived stress. J Health Soc Beh. 1983;24:385-96.
5. National Family Health Survey (NFHS-5), 2019-20. Accessed January 5, 2022. Available from: *http://rchiips.org/nfhs/NFHS-5_FCTS/COMPENDIUM/Maharashtra.pdf*
6. Sakalidis VS, Rea A, Perrella SL, et al. Wellbeing of breastfeeding women in Australia and New Zealand during the COVID-19 pandemic: A cross-sectional study. Nutrients. 2021;13:1831.
7. Bharadva K, Bellad RM, Tiwari S, et al. Breastfeeding in Coronavirus Disease 2019 (CoVID-19): Position statement of Indian Academy of Pediatrics and Infant and Young Child Feeding Chapter. Indian Pediatr. 2002;59:58-62.
8. World Health Organization. Breastfeeding and CoVID-19. Accessed March 19, 2022. Available from: *WHO.int/publications/i/itemWHO/2019-nCoV/Sci_Brief_Breastfeeding_2020.1*

Metagenomic Next-Generation Sequencing in Central Nervous System Angiostrongyliasis

Angiostrongylus cantonensis is a zoonotic pathogen that is the most common cause of eosinophilic meningitis worldwide. Due to the lack of a valid diagnostic method, most cases are diagnosed clinically. Although reverse transcription-polymerase chain reaction (RT-PCR) is recognized increasingly as the diagnostic method of choice, when it is not available, a serum enzyme linked immunosorbent assay (ELISA) is preferred. When these tests are negative, the diagnosis is difficult. Although *A. cantonensis* presents as eosinophilic meningitis and has a good outcome, there are still a few cases that may develop fatal meningoencephalitis. Metagenomic next-generation sequencing (mNGS) is an emerging method with a potential for pan-pathogen screening. We report two brothers with eosinophilic meningitis. Traditional microbiological and cytopathological detection methods failed to make a clear diagnosis. We detected *A. cantonensis* in the CSF of one of them using mNGS, enabling clear diagnoses for both and ensuring that they were cured in a timely manner.

A 8-year-4-month-old boy was admitted to our tertiary teaching hospital in western China with the chief complaint of paroxysmal headache for 22 days. It was a frontal lobe headache and accompanied by malaise. While he had no fever, he had blurred vision, paresthesia, vomiting, convulsions, and an altered state of consciousness. He was diagnosed with rhinosinusitis in two different hospitals and administered oral or intravenous anti-biotics empirically; however, there was no relief of symptoms.

Simultaneously, his 10-year-9-month-old elder brother developed dizziness and was admitted to our hospital. Both reported having consumed uncooked snails one week prior to the onset of symptoms, as part of traditional Chinese medicine.

On examination of the older child, temperature was 36.4°C, heart rate 116/min, respiration rate 24/min, and blood pressure 120/76 mmHg. He was conscious and cooperative. No peripheral lymphadenopathy was observed. Systemic examination of respiratory and cardiovascular system, and abdominal examinations were normal. There were no signs of cranial nerve palsy or focal neurologic deficits. Meningeal signs, including Kernig sign, Brudzinski sign, and nuchal rigidity were absent. Babinski sign was also absent. The hematological tests indicated a white blood cell count of 13.9×10^9/L, an eosinophil count of 54.0%, a hemoglobin level of 134 g/L, and a platelet count of 379×10^9/L. The C-reactive protein level was less than 0.5 mg/L. The liver and kidney function tests, and stool microscopy were normal. The magnetic resonance imaging (MRI) of the brain demonstrated scattered abnormal signals in the bilateral cerebral hemispheres and the left cerebellum. An abnormal signal was also noted in the right parieto-occipital sulcus. Cerebrospinal fluid (CSF) exami-nation revealed a white blood count of 560 cells per mm^3 (75% eosinophils), an elevated protein level (760 mg/L), and a decreased glucose level of (25 mg/dL). The chloride and adenosine deaminase concentrations were normal. The Gram stain, India ink stain, and acid-fast stain of the CSF were all negative. The CSF cultures for bacteria and fungi were negative. The results of the physical examination, blood tests, and CSF findings in the younger child were similar to the results of the older brother.

A diagnosis of eosinophilic meningitis was made based on the presence of CSF eosinophilia. *A. cantonensis* infection was highly suspected, as it is the most common parasitic etiology for eosinophilic meningitis and is due to known exposure to infective larvae. The serum antibodies for the common parasites were negative. The CSF sample was sent for mNGS. The specific DNA of *A. cantonensis* was detected in the CSF with mNGS, and 106 unique reads were identified. The coverage of the identified parasite genome, calculated by mapping the detected reads, was 0.0039%. Therefore, the younger brother was diagnosed definitively with CNS angiostrongyliasis.

The two patients were administered albendazole (15 mg/kg/day) in combination with prednisone (1 mg/kg/day, which was gradually discontinued over two weeks). The symptoms were relieved noticeably, and the patient did not complain of any headache or dizziness. One month later, a repeat routine blood test showed a white blood cell 6.6×10^9/L (8% eosinophils), and a repeated CSF analysis showed a decreased cell count of 165 cells per mm^3 (45% eosinophils), and normal levels of protein and glucose.

The existing literature shows that the severity of *A. cantonensis* disease and mortality in children is significantly higher than that in adults [2]. In humans, ingested third-phase larvae are not mature but are aggressive migrators. Neurological symptoms occur 2–35 days after infection. Eosinophilic meningitis caused by *A. cantonensis* is a self-limiting illness in which headaches, non-focal neurologic findings, and cranial nerve involvement are the most common symptoms and signs. Encephalitis is a relatively rare condition [3]. In a case report in which an infant with infection presented with long-term fever without other symptoms, the CSF also showed characteristic eosinophilia [4]. There have also been cases of transverse myelitis being reported [5]. In a prospective study that followed up on three previous studies, Chotmongkol, et al. [6] confirmed that a 2-week course of corticosteroids shortened the duration of headache and reduced the need for repeated lumbar punctures. The study concluded that corticosteroids plus albendazole were no better than corticosteroids alone. However, some scholars believe that corticosteroids are beneficial for severe patients, and that the dose can be increased when warranted [2]. The cases reported were not from an area that is knwon to be endemic to this parasite. The confirmation of the diagnosis underscores the utility of mNGS for CNS infections with unknown pathogens. Given the expanding endemic regions of *A. cantonensis* due to transportation logistics and global warming, clinicians should be aware of the possibility of the occurrence of *A. cantonensis* and

the utility of mNGS when etiological diagnosis is difficult.

Contributors: QL: drafted the manuscript, carried out the literature research, and prepared the illustrations; YZ,YW: conceived the idea of the study and carried out the final proofreading and editing of the manuscript. All the authors read and approved the final manuscript.

Funding: Grant from the Research Development Project of Sichuan Provincial Science and Technology Department (2020YFS0042).

Competing interests: None stated.

QIONG LIAO, YU ZHU, YANG WEN*

Department of Pediatric Infectious Diseases, West China Second University Hospital, Key Laboratory of Birth Defects and Related Diseases of Women and Children (Sichuan University), Ministry of Education, Chengdu, China

**289047544@qq.com*

REFERENCES

1. Cowie RH. Biology, systematics, life cycle, and distribution of *Angiostrongylus cantonensis*, the cause of rat lungworm disease. Hawaii J Med Public Health. 2013;72: 6-9.
2. McAuliffe L, Ensign SF, Larson D, et al. Severe CNS angiostrongyliasis in a young marine: a case report and literature review. Lancet Infect. Dis. 2019;9:e132-e142.
3. Slom TJ, Cortese MM, Gerber SI, et al. An outbreak of eosinophilic meningitis caused by *Angiostrongylus cantonensis* in travelers returning from the Caribbean. N Engl J Med. 2002;346: 668-75.
4. Flerlage T, Qvarnstrom Y, Noh J, et al. *Angiostrongylus cantonensis* eosinophilic meningitis in an infant, Tennessee, USA. Emerg Infect Dis. 2017;23:1756-57.
5. Defo A L, Lachaume N, Cuadro-Alvarez E, et al. *Angiostrongylus cantonensis* infection of central nervous system, Guiana Shield. Emerg Infect Dis. 2018;24:1153.
6. Chotmongkol V, Kittimongkolma S, Niwattayakul K, et al. Comparison of prednisolone plus albendazole with prednisolone alone for treatment of patients with eosinophilic meningitis. Am J Trop Med. 2009;81:443-45.

MIS-C Triggered by Omicron Variant of SARS-CoV-2

World Health Organization (WHO) designated the new variant of SARS-CoV-2 (B.1.1.529) as Omicron on November 26, 2021 [1]. Analyzing the initial cases of Omicron in South Africa to assess the clinical severity of cases, Walter and colleagues concluded that compared to Delta variant, the odds of hospitalization due to severe disease were less [2]. Even though the severity is likely to be mild, its impact on children and subsequent development of MIS-C is unknown.

Pediatric hospitalization due to Omicron in Gauteng Province of South Africa, was noted to be more when compared to the previous waves. During a six-week period, there were nearly 6,287 children with Omicron and four children in their series died, not because of COVID-19, but due to underlying comorbidity [3]. No case of MIS-C was reported in their series. India detected its first Omicron case on December 2, 2021, in Karnataka. We report what we believe to be the first case of MIS-C due to Omicron in India.

A 3-year-old male child presented to us on January 4, 2022 with fever for 6 days and maculopapular rash over the trunk and extremities, bilateral non purulent conjunctival congestion and abdominal pain with vomiting. Both the parents of this child had PCR confirmed mild COVID-19 a week before. Clinical examination did not reveal any features of tropical infections such as dengue or enteric fever. Since child had fever >3 days with mucocutaneous and gastrointestinal involvement, MIS-C was considered and further investigations were done. Complete blood count and inflammatory markers revealed leukocytosis and significantly elevated CRP and hypoalbuminemia (**Table I**). Given the epidemiology, reverse transcriptase polymerase chain reaction for COVID-19 was done, which was positive (Ct value – 12.9). Child had all criteria for WHO case definition for MIS-C [5]. ECHO and ECG were normal. He was started on intravenous immunoglobulin (2 g/kg) and intravenous steroids (methyl prednisolone 10 mg/kg/day for 3 days initially) which was then tapered and stopped over 2 weeks, and was also started on aspirin (5 mg/kg/day). He became afebrile within 24 hours and was well on follow-up after 2 weeks. Repeat ECHO at 2 weeks was normal.

This child presented to us after a lag period of around 4 weeks after the first case detection in our country. Whole genome sequencing of the SARS-CoV-2 from the nasopharyngeal aspirate confirmed it to be an Omicron variant (**Web Fig.1**).

There is a steep rise in the number of SARS-CoV-2 infections in South Africa, US and Europe and CDC has reported a proportionate surge in MIS-C with the increase in the number

Table I Laboratory Parameters in the Index Case

Laboratory parameters	*Value*
Leukocyte count	1.53×10^9 /L (N- 59%)
Hemoglobin	10.8 g/dL
Platelet count	271×10^9/L
C-reactive protein	64.2 mg/L
Serum sodium	130 mmol/L
Serum albumin	2.7 g/dL
Urine microscopy	Normal
D-dimer	2453 ng/mL
NT- Pro BNP	2774 pg/mL

of COVID cases in each of the previous waves [4]. Payne, et al. [5], in 2020 during the first wave of COVID 19 infection in US, reported the incidence of MIS-C per 1,000,000 person-months to be 5.1 (95% CI, 4.5-5.8) persons and MIS-C incidence per 1,000,000 SARS-CoV-2 infections was 316 (95% CI, 278-357) persons [5].

In children exposed for the first time to SARS-CoV-2 infection, when the Omicron variant was predominant, the disease severity has been observed to be significantly less than when compared to the period when Delta variant was predominant [6]. In a recent report from USA, the emergent Omicron cohort differed significantly from the Delta cohort in both pediatric and adult population in terms of emergency visits, hospitalization, ICU admissions and need for mechanical ventilation [7].

Though only minimal morbidity has been reported so far in children due to the Omicron variant, it is still not known whether the incidence of MIS-C triggered by Omicron is going to be more or less when compared to other variants of SARS-CoV-2.

Acknowledgment: Professor AV Ramanan, Professor of Pediatric Rheumatology, University of Bristol, for guiding in drafting of manuscript.

Funding: Med Genome Labs Ltd, Bangalore sequenced the genomic variant of SARS-CoV-2 free of charge.

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

S BALASUBRAMANIAN,[1] K DHANALAKSHMI,[1] S LAKSHAN RAJ,[1]* SARA CHANDY[2]

From [1]Department of Pediatrics, Kanchi Kamakoti CHILDS Trust Hospital; [2]Molecular Laboratory, The CHILDS Trust Medical Research Foundation; Chennai, Tamil Nadu.
**doc.lakshanraj2311@gmail.com*

REFERENCES

1. WHO. Classification of Omicron (B.1.1.529): SARS-CoV-2 variant of Concern. Accessed January 26, 2022. Available from: *https://www.who.int/news/item/26-11-2021-classification-of-omicron-(b.1.1.529)-sars-cov-2-variant-of-concern*
2. Wolter N, Jassat W, Walaza S, et al. Early assessment of the clinical severity of the SARS-CoV-2 omicron variant in South Africa: a data linkage study. Available from: Lancet. 2022; 399:437-46..
3. Cloete, Jeane & Kruger, Annelet & Masha, Maureen & Plessis, Nicolette & Mawela, Dini & Tshukudu et al. Rapid rise in paediatric COVID-19 hospitalisations during the early stages of the Omicron wave, Tshwane District, South Africa. 10.1101/2021.12.21.21268108.
4. CDC. COVID data tracker. *In*: Centers for Disease Control and Prevention, 2020. Accessed January 26, 2022. Available from *https://covid.cdc.gov/covid-data-tracker*
5. CDC. Responding to Covid – 19. Available from: *https://covid.cdc.gov/covid-data-tracker/*
6. Payne AB, Gilani Z, Godfred-Cato S, et al. Incidence of multisystem inflammatory syndrome in children among US persons infected with SARS-CoV-2. JAMA Netw Open. 2021; 4:e2116420.
7. Wang L, Nathan A. Berger E, et al. COVID infection severity in children under 5 years old before and after Omicron emergence in the US [pre-publication]. medRxiv 2022.01.12.22269179.

Artificial Pancreas: Pilot test

Almost a century after the discovery of insulin, NHS England has started a pilot test to check the effectiveness of a newer technology – "artificial pancreas" in achieving better control of blood sugar levels in patients with type 1 diabetes. In this technology, a sensor placed under the skin continuously monitor the blood glucose level and readings are sent to an automated insulin pump which calculates and delivers the amount of insulin required. Patient or caregiver can monitor these reading on their smartphones and can also enter the amount of carbohydrates taken during the meals. This whole system work in a closed loop, thus it is also known as 'hybrid closed loop technology'. Use of this technology eliminates the need of finger prick tests to check the blood glucose levels and the continuous monitoring can help in preventing the life-threatening episodes of hyper- or hypoglycemia. This will help in achieving a good control of blood glucose levels, thus improving the quality of life as well as reducing the long-term complications in people with type 1 diabetes, especially in the young children.

In this pilot study, NHS England distributed this 'technology' to 1000 persons suffering with type 1 diabetes including ~200 children to document its effectiveness and safety in the real-world. The data generated from this pilot study will be considered by National Institute for Health and Care Excellence in recommending the wider use of this technology in UK. Wider availability of this technology will change the long term outcome of the 537 million adults, and 1.2 million children and adolescents living with the type 1 diabetes globally at present. (*BMJ 1 April, 2022*)

Human Genome Sequence

Human Genome Sequence project was one of the most fascinating, ambitious and world's largest collaborative research work. At the completion of Human Genome Project in April, 200,3 more than the 99 percent of the euchromatic region of the human genome was sequenced with less than 400 gaps. Heterochromatic regions, which are found in the centromeres and telomeres were not sequenced in this project. Recently a group of researchers, known as the Telomere to Telomere (T2T) consortium, have published the complete, gapless 3.055 billion base pair sequence of human genome covering all chromosomes except Y. The T2T consortium has discovered more than 2 million additional variants in the human genome, this will add significantly to the existing knowledge about the segregation and division of chromosomes during the cell cycle. It will also provide a comprehensive knowledge about the variations in the human genome and its role in the development of certain diseases.
(*Science 31 March, 2022*)

Nafcillin for Empiric Therapy of Late Onset Sepsis

Increasing antimicrobial resistance is a topic of global concern. In order to prevent the further worsening of the situation, many institutions are running the Antimicrobial Stewardship Program across the world. In 2014, under the Neonatal Antimicrobial Stewardship Program, United States, nafcillin was recommended over vancomycin for empirical treatment of infants admitted in NICUs with possible late-onset sepsis (LOS) without a history of methicillin-resistant *Staphylococcus aureus* colonization or infection. The need for this recommendation arose from the concerns that widespread vancomycin use could lead to resistance in gram-positive bacteria causing LOS, including coagulase-negative staphylococci (CoNS). In a recently published paper, the authors have retrospectively analyzed the safety and efficacy of Nafcillin for empiric therapy of late onset sepsis in 3 NICUs located in Ohio, US. Authors have assessed the duration of blood culture positivity, recurrence of infection with the same previously identified pathogen in the 14 days after discontinuation of antibiotic therapy and mortality among 366 infants admitted with possible LOS. Results showed that empirical vancomycin was used in 84% (2013-2014) and 25% (2017-2019) infants before and after the implemen-tation of the Neonatal Antimicrobial Stewardship program respectively showing a 70% reduction. There was no difference in the recorded duration of blood culture positivity and infant mortality (9% vs 10%; OR 0.97, 95% CI 0.40-2.34) before and after the implementation of the vancomycin reduction guidelines. Authors concluded that nafcillin is a safe alternative to vancomycin for empirical treatment of possible late onset sepsis in NICU infants who do not have history of methicillin-resistant *S aureus* infection or colonization. (*Pediatrics 5 April, 2022*)

Exposure to Light During Sleep Affects the Cardiometabolic Health

Human circadian cycle is of 24-hours and primarily responds to the light and dark exposure patterns. With the global industrialization exposure to the artificial light, especially during the night, is increasing. Various studies have shown that the night time exposure to the artificial lights is deleterious for the human health. Researchers in USA studied the effect of exposure to the moderate ambient lighting (100 lx) during the night time sleep on the cardiovascular function compared to sleeping a dimly lit (< 3 lx) room. This was a parallel group study design involving 20 young adults [10 in each age, sex, body mass index (BMI) and race matched group]. Results showed increased insulin resistance in morning (higher fasting HOMA-IR and lower Matsuda index from the OGTT) in participants sleeping in the lighted room light compared to those sleeping in dim light condition though melatonin levels were similar in both groups. Participants sleeping in the lighted room were found to have higher heart rate and lower heart rate variability during sleep in comparison to dim light condition group. Authors thus concluded that night time light exposure during sleep can affect the cardiometabolic function, especially in those living in the modern cities where indoor and outdoor night time light exposure is common. This finding is worrisome in view of the increasing incidence of cardiovascular events in those living in the modern cities.
(*Proceedings of the National Academy of Sciences 14 March, 2022*)

Rajesh Kumar Meena
raj.mamc@gmail.com

CLIPPINGS

Combination therapy with anti-pd-1 or pd-1 antibody alone in Asian pediatric patients with relapsed or refractory cancer (*Front Immunol. 2021;647733*)

Prognosis for recurrent/progressive solid tumors in children remains unfavorable, with a 10-year OS and PFS of 24.5% and 18.4%, respectively. Many novel targeted therapies have come up in the recent years, inhibitors of PD-1, pembrolizumab and nivolumab have been extensively explored. The results indicated that they are tolerated well in pediatric patients and have shown benefit in lymphomas, especially in HL.

In this study, twenty-two pediatric patients with cancer who received PD-1 inhibitors between 2017 and 2020 were enrolled. Nine patients (6 with Hodgkin's lymphoma, 2 with Malignant melanoma and one with Burkitt's Lymphoma) received monotherapy with Anti PD-1. Amongst these, the Hodgkin's Lymphoma patients responded well. For PD-1 antibody combined with other standard chemotherapy regimens, no significant benefit was observed. Interestingly, among the patients treated with PD-1 antibody combined with decitabine, the effect was significant. The hypomethylating agent decitabine is suggested to increase tumor T-cell infiltration and the antitumor response, ultimately restoring immunosurveillance. Both the groups (monotherapy and combination therapy) tolerated Anti PD-1 well with minimal adverse events. Thus, combinatorial approaches are likely to be used in the future and have the potential to achieve therapeutic success, especially in relapse/refractory setting.

The phase 3 pediatric anticoagulant era (*Blood.2020;135:459-60*)

A phase 3 trial was done on patients from 3 months to 18 years with a history of provoked VTE (i.e. VTE associated with a clinical trigger such as central venous catheterization, etc) in whom 1 or more prothrombotic risk factors persisted after completion of a conventional course of anticoagulation and also patients with recurrent unprovoked VTE. The median duration of dabigatran administration (adjusted for age and body weight) in this study was 8 months. The investigators observed significant bleeding in only 2.5% of patients (5 of 203) and recurrent VTE in 1% (2 of 203) with no deaths reported.

These findings were consistent with studies on Riva-roxaban (Factor Xa inhibitor) in EINSTEIN Jr phase 3 trial of acute VTE in children. In this group clinically relevant bleeding was found in 3% of patients, and symptomatic recurrent VTE was found in 1%. Post throm-botic syndrome was observed by Brandão and colleagues in 1% of the children receiving extended anticoagulation with a DOAC. These studies suggest that dabigatran and Rivaroxaban are safe for extended VTE treatment in children and do not require routine laboratory monitoring. However, issues such as the optimal duration of antico-agulant therapy for pediatric VTE and the relationship between antiphospholipid antibodies and outcomes in young patients yet need to be evaluated.

2021 Update on clinical trials in β-thalassemia (*Am J Hematol.2021;96:1518-31*)

The treatment of β-thalassemia patients has witnessed a swift evolution from transfusions alone to novel targeted therapies, yet several unmet needs continue to persist.

Interim analysis of ongoing phase 3 trials using a refined transduction process showed transfusion independence in 30/34 (88.2%) evaluable patients (6/7 [85.7%] β0/β0 and 24/27 [88.9%] non-β0/β0) on receiving cells transduced ex-vivo with the LentiGlobinBB305 vector based gene therapy product betibeglogene autotemcel in 2019. Genome editing approaches are developed to inhibit BCL11A through enzymes like CRISPR-Cas9, transcrip-tion activator-like effector nucleases (TALENS), and zinc finger nucleases (ZFN). A phase 2 trial is underway in adults for PDE9 inhibitor IMR-687, which increases intracellular cGMP levels and stimulates the production of HbF. Erythroid maturation agents - Luspatercept (ACE-536) is a recombinant fusion protein of the human activin receptor type IIB fused to the Fc domain of human IgG1 which blocks SMAD2/3 signaling, and enhances erythroid maturation. The BELIEVE and BEYOND trial in adults have shown significant reduction in transfusion requirement. Pediatric trials on luspatercept are also underway. These therapies are promising but the key challenges to wide implementation are cost and the need for specialized centers and clinical expertise for application.

Palliative care in paediatric oncology: An update (*Curr Oncol Rep. 2022;24:175-86*)

Palliative care provides active, holistic care for children and young people with life-limiting illnesses, from the point of a child's diagnosis, throughout the child's life, death, and bereavement. Many deficiencies were realized while providing treatment to pediatric oncology patients in even tertiary care centers. Pain is a common symptom which is often not addressed adequately. Families felt that continuous communication between parents, family caregivers, and health care providers is lacking and they often felt unprepared to deal with their child's death.

Globally, children with cancer were infrequently referred to palliative care or referred late in the illness. Problems in low and middle income countries like limited access to opioids, lack of interdisciplinary care, and families less empowered to participate in decision-making; worsens the situation.

Palliative care input bettered end-of-life care support to children and their families. It facilitated less invasive diagnostic and therapeutic interventions at the end of life. The families appreciated the healthcare system's support beyond usual clinical management, also in terms of managing finances and assistance to manage the child's needs at home.

Ritika Khurana
ritangel247@gmail.com

Mammary-Digital-Nail Syndrome With Orofacial Changes

A 12-year-old premenarchal girl presented with the complaint of progressive increase in size of her breasts for one year. There was no family history of dysmor-phism, gigantomastia or onychodystrophy/anonychia. Her general examination showed dysmorphic facies with maxillary hyperplasia, maloccluded/ malaligned, hypo-plastic (conical) teeth, and prominent nose with flat nasal bridge, anteverted nares and short columella (**Fig. 1A, 1B**). Bilateral brachydactyly of all fingers except the middle fingers was noted in the form of hypoplasia of distal phalanges. Anonychia was seen in all fingers and toes. The big toe of both feet appeared hypoplastic (**Fig. 1C**).

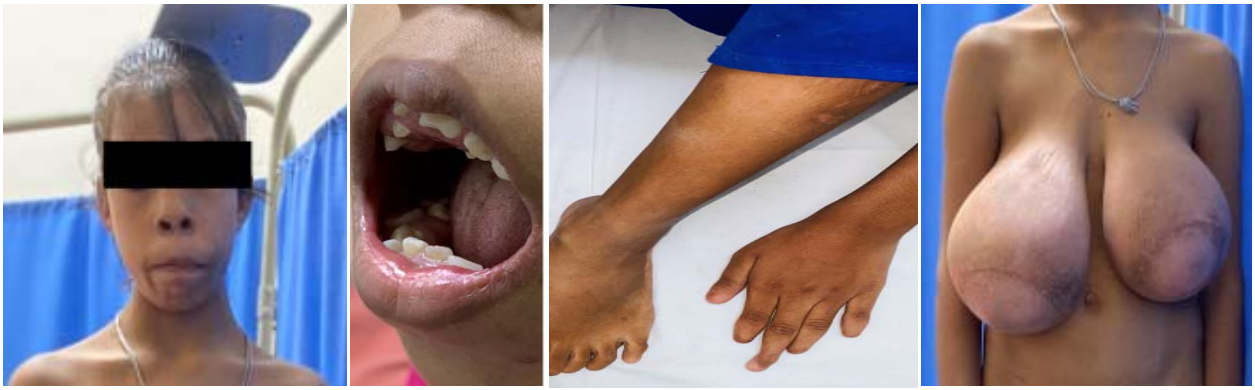
Her bilateral breasts were grossly enlarged, dispro-portionate to age (right more than left side) (**Fig. 1D**). A 8×5 cm mobile and non-tender lump was palpable in upper outer quadrant of right breast with no inflammatory changes. Ultrasound of breasts revealed excessive fatty parenchyma, without delineation of fibro-glandular portion. A well-defined lesion was confirmed in right breast, 9×5.5×8cm size, with benign characteristics.

Her hormonal profile, karyotype and ultrasound of abdomen and pelvis were normal. There was no history of intellectual disability in the girl. A phenotypic diagnosis of Mammary-Digital-Nail syndrome (MDN Syndrome; OMIM 613689) with additional orofacial changes was made. It is mapped to chromosome 22q12.3-q13.1. The patient was referred to surgeons for reduction mammo-plasty and management of benign breast lump.

Other etiologies for breast hypertrophy include fibrocystic disease, juvenile gigantomastia or juvenile hypertrophy of the breast, malignancies and hormonal disorders. Juvenile or virginal hypertrophy of breast (JHB) is a rare clinical entity of undefined etopatho-genesis, presenting with gigantomastia in peripubertal girls with no associated abnormality.

Preeti Priyadarshani,[1*] Manish Kumar[2]

*From Departments of [1]Obstetrics and Gynaecology, and [2]Pediatrics, All India Institute of Medical Sciences, Gorakhpur, Uttar Pradesh. *drpreeti.priyadarshani@gmail.com*



(*a*) (*b*) (*c*) (*d*)

Fig.1 *a*) Facial dysmorphism, *b*) dental abnormalities, *c*) toe abnormalities, and *d*) breast enlargment in a girl with Mammary-Digital-Nail syndrome.

Apollo HOSPITALS

India's first successful pediatric liver transplant recipient at Apollo Hospital, Delhi in 1998.

An Apollo doctor in 2022.

Baby Sanjay, in 1998

In 1998, 2-year-old Sanjay Kandasamy was brought to Indraprastha Apollo Hospitals with advanced liver failure. With his father as the donor, little Sanjay underwent a successful liver transplant. Sanjay grew up like any normal child, aspiring to become a doctor one day. In 2022, the young man has finally realised his dream as he now joins Apollo Hospitals, Bangalore as a doctor. The entire Apollo family welcomes Dr Sanjay Kandasamy with open arms and wishes him all the best as he begins his journey of saving lives.

Apollo Hospitals
pioneering work in Liver Transplantation

First successful pediatric and adult liver transplants in India	First international air rescue of acute liver failure patient followed by a successful liver transplant in India	ABO incompatible liver transplants
More than 3800 liver transplants; 435 in children	Liver transplants in very small babies weighing less than 4 kg	Liver transplants in patients from 50 countries, including children from 20 countries

Indraprastha Apollo Hospitals, Sarita Vihar, New Delhi - 110076 | Call: **011-71791090/ 91** | Email: infodelhi@apollohospitals.com

ApolloHospitalsDelhi apollohospitalsdelhi https://delhi.apollohospitals.com

Summer Time

Absence of Right Diet Shows Signals of GER

In Paediatric GER and GERD

℞ RANTAC® SYRUP

Ranitidine 75 mg/5 ml

Happy tummies…Happy kids

- Recommended as First Line Therapy by Nelson Text Book of Pediatrics[1]
- Established Safety from One Month of Age[2,3]
- Faster Onset and Sustainable Duration of Action[4]

References
1. Zantac prescribing information.
2. www.openanesthesia.org/h2-blockers_onset_time.
3. Dx.doi.org/10.5056/jnm.2013.19.1.25.
4. J Am Soceity nephrol 27, 2016- doi:10.1.1681/ASN.2015121377.
GERD: Gastro Esophageal Reflux Disease.

J.B. CHEMICALS & PHARMACEUTICALS LTD.

GER-Gastroesophageal reflux
Cnergy IT Park, Unit A2, 3rd Floor, Unit A, 8th Floor, Appa Saheb Marathe Marg
Prabhadevi, Mumbai - 400 025 • www.jbcpl.com

For the use of a Registered Medical Practitioner or a Hospital or a Laboratory only

Changing the Way the World Takes Temperature

Clinical accuracy of ± 0.1°F - Temperature range of 61-110°F - response time ~ 0.04 sec - LIFETIME warranty

More info on the **Temporal Artery Thermometer**

#1 thermometer used by pediatricians in the USA
Supported by 100 clinical studies
Exergen also has a professional light model: the TAT-2000

For more details you can contact:
Dr. Pushpa Goyal - Marketing Manager Exergen India | +91 98114 24550 | drpushpa@exergen.com

www.exergen.com

Mankind

Entromax

2 Billion *Bacillus clausii* Spores **Suspension**

THE RARE PROBIOTIC

For Billion Smiles

In

- Infectious Diarrhea
- Antibiotic Associated Diarrhea (AAD)

In RTIs, Typhoid Fever, Acute Otitis Media

Gudcef-100

Cefpodoxime 100mg/5ml **Dry Syrup**

Tasteful Choice for kids

Micronized Cefpodoxime for Faster Dissolution & Better Absorption