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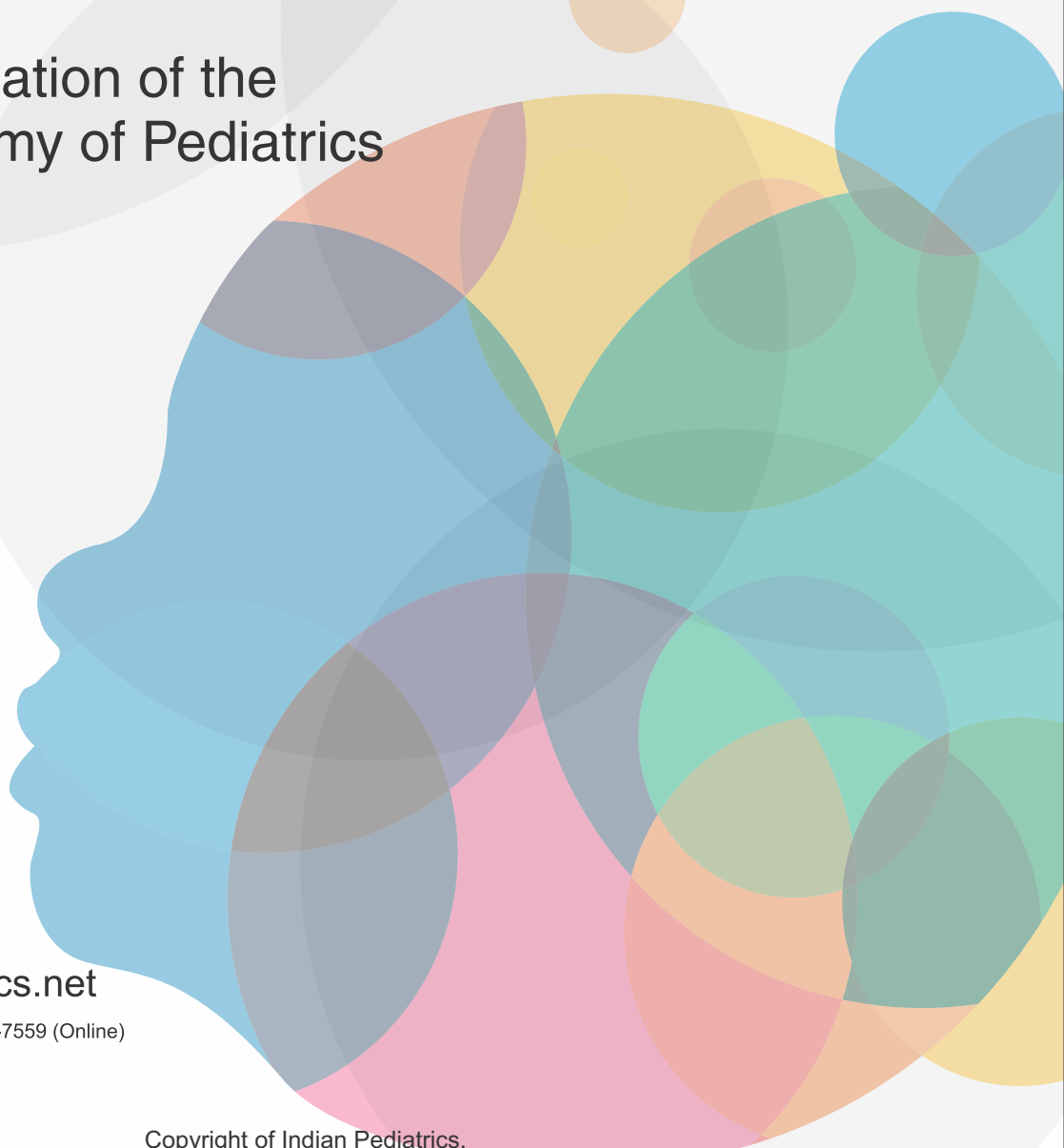

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Ref.: 1. Leung AK, Chiu AS, Siu TO. - Subcutaneous versus intramuscular administration of Haemophilus influenzae type b vaccine. -J R Soc Health. 1989;109:71-73.
SC* :- Subcutaneous | IM# :- Intramuscular | HAV :- Hepatitis A Virus
Ref.: 2.Chen Y et al. Immune memory at 17-years of follow-up of a single dose of live attenuated hepatitis A vaccine. Vaccine. 2018 Jan 2;36(1):114-121.

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COMPOSITION: Made from working seed virus, the H2 - attenuated strain of Hepatitis A Virus (HAV), cultured in Human diploid cells. Other Ingredients: Trehalose 24 mg; Dextran-40 4.5 mg; Sorbitol 9 mg; Mannitol 6 mg; Amino acids salts equilibrium solution 11.73 mg. **DESCRIPTION:** It is a freeze- dried, live attenuated Hepatitis A virus (HAV), propagated in human diploid cells through a series of technological process including culture, harvesting, purification, preparation, filling and freeze-drying. Pack of 0.5 ml vial of BIOVAC-A along with 0.5 ml ampoule of sterile water for injection. **PHARMACOLOGY:** It confers immunity against HAV infection by the induction of specific antibodies against the virus. The vaccine confers immunity against HAV virus by inducing antibody titres greater than those obtained after passive immunization with immunoglobulin. **INDICATIONS:** It is indicated for active immunization against Hepatitis A in children above 1 year age and adults. **CONTRAINDICATIONS:** Hypersensitivity to the vaccine or any component of the formulation, acute infectious disease, other acute illness, acute febrile illness with temperature above 37.5 degree centigrade, immunological deficiency states, a history of anaphylaxis or any other serious allergic reaction to vaccines. Usually no need to defer if minor afebrile illness or mild upper respiratory tract infection. **WARNINGS:** It does not provide protection against live pathogens or infections by hepatitis B, hepatitis C virus, delta virus, hepatitis E virus, or by other liver pathogens. Immunocompromised persons (from disease or treatment) may not obtain the expected immune response. If the person is in incubation period of Hepatitis A, the vaccine may be ineffective. **PRECAUTIONS:** The product is a live attenuated vaccine; the contact of the vaccine with any disinfectant should be avoided during manipulation. The product should not be used if vial has crack, unclear label, turbid dissolution or presence of foreign body. A separate syringe and needle must be used for each patient to prevent the transmission of infectious agents from person to person. After gamma globulin administration, the vaccine should not be given for 3 months. Epinephrine (1:1000) should be available for use in Case of anaphylaxis or anaphylactoid reaction. Prior to injection, with any vaccine, all known precautions should be taken to prevent adverse reactions, including review of the patient's history with hypersensitivity to the vaccine, etc. Use by pregnant women is not recommended. **ADVERSE REACTIONS:** are usually mild, confined to the first few days after vaccination with spontaneous recovery. Local - Pain at the site of injection, redness, swelling, hematoma, induration, oedema, pruritus which usually subsides spontaneously within 72 hours. Relevant treatment may be given if needed. Systemic -Fever (>37.5°C, axillary), asthenia/drowsiness, headache, myalgia/arthralgia, gastrointestinal disorders, behavioural changes, skin disorders. **DOSAGE:** Add 0.5 ml sterile water for injection; shake well till complete powder dissolution. Inject a single dose of 0.5 ml subcutaneously over the deltoid muscle insertion of upper arm. A single dose of the live attenuated Hepatitis vaccine imparts adequate immunoprotection in individuals above 1 year of age. booster dose is usually not required. **ADMINISTRATION:** The product should not be administered if extraneous particulate matter and/or discolouration before administration. Before injection, the skin over the site to be injected should be cleansed with a suitable germicide. Administer the vaccine subcutaneously. Over preferred site of deltoid muscle. Do not administer over the buttocks. After insertion of the needle, aspirate to ensure that the needle has not entered a blood vessel. Do not inject intravenously. **STORAGE & TRANSPORTATION:** Vaccine should be kept and transported at a temperature +2°C to +8°C in a dark place. Do not use vaccine beyond the expiration date. **SHELF LIFE:** 24 months. The vaccine should be used completely within 1 hr. after the vial is opened and/or reconstituted.

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Indian Pediatrics

Editor-in-Chief Devendra Mishra

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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 30,000 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*

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FROM THE EDITOR'S DESK

Following-on after an array of erudite personalities that helmed the academy's flagship journal, I find myself humbled by the onerous responsibility and too overwhelmed to express any thoughts. However, tradition demands and circumstances necessitate, so I herein delineate my vision for the journal's course over the coming few years.

Readers of *Indian Pediatrics* are our biggest strength, and also the most astute critics. With more than 30000 subscribed readers and more than 2 million web hits per month, we have one of the largest readerships among pediatric journals. We will continue with our tradition of publishing quality-research and topical reviews on issues related to child health in low- and middle-income countries.Providing free full-text access to all readers without charging authors for publication, the journal does have some financial constraints, but we would gradually try to improve the quality, and increase the volume and diversity of the content for readers.

We plan to start two new sections this year *viz*, *Pediatric Subspecialties*, and *Iconic Pediatric Institutions*. The former section is to encourage our colleagues working in pediatric subspecialties to conduct research relevant to the pediatrician and to provide them an avenue to showcase their research to the general pediatric readership. This section would provide our readers an exposure to research in these related fields. At present, we have short-listed four subspecialties *viz,* Pediatric Ophthalmology, Pediatric Orthopedics, Pediatric Radiology, and Pediatric Dermatology; more may be added later. The other section on iconic pediatric institutions is aimed to expose the readers to our shared history of child health in India. This section, which shall contain invited articles, would provide information about the beginning and development of some of the oldest pediatric departments/institutions in the country. For the time-being, five institutions have been shortlisted, and you will be reading their history in forthcoming issues.

For our authors, we plan to expedite the review process and aim for early editorial decisions. We also hope that the addition of new sections will stimulate more pediatricians to take up the pen and share their clinical experience with the readers. To give a wider exposure to the work of budding pediatricians, we will explore the possibility of publishing the abstracts of papers presented at PEDICON (National Conference of Indian Academy of Pediatrics) as a supplement to the journal from the year 2021.

It has been the editorial board's long-standing position that being the academy's journal, in addition to publication of scientific papers, our mandate also includes training novice researchers in the art of scientific writing. Thus, we would continue to hold the hugely popular 'Art and Science of Writing a Scientific Paper' workshop, which trains young (and the 'not so young') doctors of all specialties on writing for biomedical publication. We will also support any efforts to replicate this workshop at any institution in India for their in-house faculty. Postgraduate students and practicing pediatricians find the published collections of seminal journal articles extremely useful, and we will continue to publish further volumes of *Best of Indian Pediatrics* (in its third edition presently) and the *Art and Science of Writing a Scientific Paper*. Another article collection titled *Current Trends in Medical Education* will be out this month itself.

It has been my observation that most improvements in systems take time to achieve and require efforts to sustain. A long list of illustrious editors have brought the journal to its current position, and I hope to at least sustain the momentum, if not add on to it. The academy has always been providing full support to the journal and ensuring editorial independence, and I look forward to the same during my tenure.

I assure all that ethical publishing and editorial policies, objectivity in manuscript evaluation, and fiscal discipline would continue to be the guiding principles of all journal activities.

DEVENDRA MISHRA
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PRESIDENT'S PAGE

The Technology Empowered Academy – At Your Service

BAKUL JAYANT PAREKH
President, Indian Academy of Pediatrics, 2020
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"*Technology-driven Change is the New Reality***"**

A new year brings about new memories, new sciences and also let us not forget, new technologies! The world has seen a tremendous technology-driven change in the last couple of decades. Many things that were expensive and affordable only to the wealthiest people are now within reach of the common man.

The Internet, the World Wide Web, mobile phones and mobile smart phones have reduced the cost of communication to zero. Ready access to knowledge is no longer the privilege of the few. These technologies have also created an explosion of innovative services. These services include social media, search engines, online marketplaces, online stores, mobile payment, app taxis, online health services, and many more.

The next wave of change is even faster and more significant in impact. The wave is born of the convergence of the above technologies with the Internet of things, Artificial intelligence, Genetic engineering and Bio-technology.We have all experienced the change, and in media, we often hear and read of the impending acceleration of change. But what does it mean for clinical pediatrics? What does it mean for the pediatrician? Is this a great opportunity that we must grab or does it threaten to disrupt our purpose, our profession and our practice?

And what does it mean for our Academy? How can we better serve our members and society at large by riding this wave of change?

IMPACT ON CLINICAL PEDIATRICS AND THE PEDIATRICIAN

Technology enables us to diagnose faster and more accurately. Diagnostic algorithms, diagnostic support and advanced diagnostic technology are available at the point-of-care. We can treat better. Standard therapeutic guidelines, Prescription guidelines, Algorithms and Specialist advice are integrated into Point of Care systems.

The increased capabilities at the point of care lead to an increase in the number of services offered by the pediatric clinic. We are thus reducing the need for referring patients elsewhere for specialized diagnostics or treatment. Pediatricians will have robust patient education and telemedicine tools. So we can expect better-informed patients and improved health outcomes.

The democratization of mobile phone technology has improved the phone density in India and brought it to the same level as in developed countries. In the same way, the democratization of technology for clinical practitioners gives our pediatricians access to the same capabilities that their developed-world counterparts have.

THE TECHNOLOGY ENABLED ACADEMY

Our Academy is very well positioned to play a leadership role to accelerate the availability of technology and its benefits for our member pediatricians.

- We must integrate the output of our Academy's committees, chapters and experts into digital systems. The output must become instantly available to all our members. The creators must have total and direct control over creation, publishing, updating and distribution. All types of one-way or interactive content must be supported – guidelines, papers, courses, lectures, interviews, demonstrations or clinics.
- We must establish physical video conferencing and webinar infrastructure across the nation. This infrastructure will create a vibrant Academy with the in-house capability to have thousands of academic activities, round the year, accessible to all members
- We must leverage the Academy's reputation and large membership numbers to gain access to the latest and best technologies from anywhere in the world to serve our member-pediatricians.
- We must create technology infrastructure for inexpensive assimilation and rapid availability of cutting-edge services and technologies and make it available to all our members at the point-of-care.

dIAP IS PLANNED TO ACHIEVE THIS GOAL

We conceived and launched the dIAP program for IAP

Box I KEY COMPONENTS OF dIAP

- A financially self-sustaining professional education service for pediatricians. This service combines accredited courses, scientific reference material and a reservoir of searchable content; all created and published by IAP experts. Direct publishing and editorial interfaces are available to chapters and expert groups, ensuring ownership, credit and regular updates.
- A network of videoconferencing and webinar centers at IAP offices across the country. These centers allow for thousands of online lectures, clinics, webinars and more, accessible by all IAP members and available as an online searchable archive.
- A financially self-sustaining patient education service usable in a doctor/clinic personalized fashion.
- Academy created diagnostic support, specialist advice services, prescription guidelines, and diagnostic algorithms, embedded into a software system available for use at the point-of-care.
- A structured and formal Academy interface with technology innovation groups in the USA, Europe and India for early acquisition of the latest technologies for our members on attractive terms.

with precisely these objectives. dIAP is not only a window to IAP's services, but it is also the institutional, digital and financial architecture that will enable IAP to deliver on this promise – it is an integrated window to all existing IAP online services. The key components of dIAP are shown in ***Box* I.**

The Plan is Already Activated!

It gives me great pleasure to say that the Plan has already been activated. Some of these services are already available immediately to our members within the dIAP application. The dIAP application will be ready soon and I am sure that all of you will appreciate the hard work and the number of sleepless nights that have been spent by the entire team who have burnt the midnight oil just to turn our vision into a reality. I look forward to your support so that the rest of the dIAP Plan is implemented and made available to our members in the coming months.

Last, but not the least, I take this opportunity to welcome and congratulate all the elected Office Bearers, Executive Board members and most importantly, one of the past editor-in-chief of *Indian Pediatrics* and a great academician – Dr. Piyush Gupta, who has been elected as the President Elect. I feel truly honored and blessed to be working with such a dynamic team which is focused on taking IAP ahead. On seeing the wonderful team that has been elected by all of you, I can certainly say that IAP is going to achieve much greater heights in the coming year !

PERSPECTIVE

A Case for Expanding Thermochromic Vial Monitor Technology to Insulin and Other Biologics

ANJU VIRMANI[1] AND TCA AVNI[2]

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Insulin quality and efficacy determine glycemic control, which determines quality of life for people with diabetes. Insulin efficacy is reduced by heat exposure, especially in tropical climates, remote areas, and with improper handling. Insulin doses can be adjusted based on blood glucose monitoring, which may compensate for lack of viability. However, a measured response may be difficult with other biopharmaceuticals. Thermochromic vial monitor technology developed for oral polio vaccines (vaccine vial monitors) is an inexpensive, easily available, visible modality which can be used for insulin and other biopharmaceuticals to detect excessive heat exposure and thus reduced potency at any point in the cold-chain, till the end-users, thus improving patient care. Regulatory authorities must urgently consider the need to impose mandatory use of this technology for all biopharmaceuticals, including insulin, to ensure efficacy till end usage.

Keywords: *Drug stability, Drug storage, Efficacy, Temperature*.

Glycemic control determines quality of life (QoL) and the risk of acute and chronic complications in diabetes. Insulin quality is a major determinant of glycemic control, especially in type 1 diabetes. The efficacy of a biological product like insulin depends on its temperature during manufacture, transport, and storage till end-use. Exposure to high temperatures at any point reduces potency, resulting in blood glucose fluctuations, impeding diabetes care and thus QoL.

The effect of high temperatures on insulin has long been realized to be a problem in tropical countries. Vimalavathini, *et al.* [1] studied the effect of temperature on regular and biphasic insulin made by three manufacturers both *in vitro* and *in vivo*. They reported that storage at 32 ºC and 37 ºC decreased potency by 14%-18% by day 28. Carter, *et al*. [2] reported that the average intact insulin concentrations were only ~40 U/mL in regular and NPH 100 U/mL insulin vials, made by a US and a European manufacturer, and bought from retail pharmacies. They speculated that since manufacturing processes are tightly controlled, vagaries in the cold chain impacted insulin concentrations [2]. This study was criticized by pharmaceutical employees [3,4] for serious methodology flaws and by the American Diabetes Association (ADA) [5] for small sample size and methodology issues. The criticism; howsoever justifiable, does not take away from the issue that we can never be sure of insulin viability.

Blood glucose levels are affected by many factors, making day-to-day control difficult. Patients are educated to make extremely complex adjustments by frequent glucose testing, anticipating the effect of food and exercise and compensating for fluctuations. Technology, including continuous glucose monitoring systems (CGMS) and insulin pumps, has helped tremendously, but is expensive. However, a factor as important as insulin viability has not received enough attention. Patients can never be completely sure about potency and viability of insulin in any vial, and whether insulin storage at their end is optimum during usage. The ADA, and American and European regulatory authorities may be able to ensure that in wealthy, mostly non-tropical settings, insulin would get effective cold chain storage till usage, and feedback from CGMS would compensate when viability is reduced. However, the situation may differ, with gaps in cold chains, in tropical countries where summer temperatures can soar up to 48 ºC.

What happens after the insulin is purchased? In an observational study in summer 2015 from India, Patil, *et al*. [6] found that over 25% patients were keeping insulin vials outside the recommended temperature conditions (at room temperature or in the deep freezer) and 98% were transporting insulin during travel without maintaining the cold chain. Our experience in a pediatric diabetes clinic, with regular diabetes education and reinforcement, was similar. Between May and August 2014 (maximum temperatures 36-41 ºC), 9% patients had used no cooling

method during transport and insulin temperature ranged from 4-33 ºC, exceeding 25 ºC in 26% cases. If this was the situation with adequate patient diabetes education, the vast majority of insulin users would be considerably worse off with the resultant poor glycemic control leading to chronic and acute complications, including ketoacidosis.

Thus, knowing that, insulin loses potency on heat exposure, it is used by non-professionals with varying degrees of education and intelligence, that the majority of patients do not have access to sophisticated technology to monitor for the vagaries of insulin viability, and that the impact is clinically significant, the importance of maintaining insulin at the correct temperature till the end of usage, and the associated difficulties are incontrovertible. This is also true of all other biopharmaceuticals like teriparatide or growth hormone, which are equally vulnerable to heat, but have no mechanism like CGMS to help compensate if potency is redued. As the range of biopharmaceuticals expands, there is a greater need for ensuring these molecules are maintained in strict temperature conditions throughout their supply chain, till end-usage.

POLIO VACCINE AND COLD CHAIN

In 1988, the global initiative to eradicate polio was launched. The challenge of ensuring the oral polio vaccine reached every baby in every household, village and city across the world, with retained efficacy, was a critical concern as the vaccine is extremely vulnerable to high temperature, routinely seen in tropical and subtropical countries [7]. The response to the appeal by the World health organization (WHO) [8] and PATH [9] for an accurate, easy-to-use and cost-effective method to monitor the heat exposure of the vaccine, resulted in the development of the vaccine vial monitor (VVM) technology. The VVM is a small thermochromic label which adheres to the side of the vial. It is composed of chemicals that irreversibly change color on exposure to heat, with the rate of change dependent on the temperature and the length of time exposed. VVM technology has played a major role in eradicating polio from the world and is talked about as one of the great successes of science. It identified the vaccine vials overexposed to heat, so they could be discarded, ensuring safe and efficacious vaccination coverage, and also identified which had been exposed to heat but were still usable, preventing vaccine wastage [10,11]. This technology costs little and is already widely available and used. The limitations of VVM technology have been well-studied and discussed. Srivastava, *et al*. [12] pointed out that VVMs record only increases in temperature and not decreases; they do not respond perfectly to rapid fluctuations in temperature; and cannot provide information of all the temperature fluctuations experieced. However, these limitations do not impact the chief purpose of these thermochromic vial monitors, *i.e.* allow the end-user to simply, and directly evaluate whether to use a particular vial or not.

NEED FOR UNIVERSAL IMPLEMENTATION

Thermochromic vial monitor technology has not been widely adopted by the pharmaceutical industry in spite of the low cost, easy availability, and eminent desirability. If this technology is used, end point users like patients and health workers can discern the potency of the drug in any vial. When supply chains are properly maintained, the thermochromic monitor would serve as an indicator for optimum insulin storage. If insulin is not being stored or transported with adequate care, the breakdowns during handling can be identified, and improvements made. The utilization of more sophisticated technology such as electronic monitors and digital data loggers to track the minute-to-minute fluctuations in temperature in sealed consignments [13,14], while necessary for ensuring proper transportation of biopharmaceuticals, are not sufficient as their access and reach are limited. The simple visible thermochromic indicator would afford the patient a greater degree of control over purchase and discard, offsetting the cost increase of each vial with the societal benefits it confers, such as better medical outcomes. Perhaps it is optimistic to think that any single pharmaceutical company would initiate this process, which may increase prices and make its products uncompetitive. Therefore, the push can only happen when it comes from regulatory authorities, and applies to all the manufacturers.

The medical community needs to urgently wake up to this cost-effective, reliable, and configurable technology, and put pressure on regulatory authorities to demand universal application for all biological drugs. So far, this has not happened in the developed world. Perhaps it is time for governments in developing, tropical countries like India to take the lead in the matter, rather than waiting for the push to come from elsewhere. This would improve medical care not only for diabetes, but for several disorders; not only in the tropics, but across the globe.

Contributors: AV & TCAA: drafting, final approval, and agreement to be accountable for all aspects of the work.
Funding: None; *Competing interest*: None stated.

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EDITORIAL

Infant Pulmonary Function Testing: An Upcoming Modality for Evaluation of Respiratory Disorders

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Pulmonary function tests (PFTs) are used in adults and in children over six years of age to assist with monitoring a variety of lung conditions, and in some cases to aid diagnosis. The majority of available tests require the subject to perform complex respiratory maneuvers such as deep inspirations, forced expirations, or breath-holds. Such respiratory gymnastics are not possible for subjects younger than six years age. Respiratory physiologists divide this younger cohort into two groups: infants - who for this indication are defined as subjects less than two years age; and preschool children - who for this indication are defined as those aged between 3 to 6 years.The distinction is based upon the methodological approach to obtaining measurement. Succinctly, PFTs in infancy are obtained when the subject is asleep and the operator controls any necessary maneuvers. PFTs in the preschool years are obtained by a mixture of incentive techniques and distraction techniques, depending on the test being performed.

Infant pulmonary function testing (IPFT) has been performed by specialist centres for more than 40 years. Progress initially was slow, as individual tests are very time consuming, require two operators, and testing equipment had to be hand designed and built. Great progress was made, mainly due to the perseverance and dedication of a small number of committed physiologists. By the 1990s, laboratories had access to commercially produced IPFT equipment, and testing was being performed for clinical indications as well as for research. The progress has been such that a recent American Thoracic Society workshop report concentrated upon the clinical application of these measurements rather than simply upon the methodology [1].

All biomedical measurements need to meet certain criteria before they can be used in clinical practice. There need to be standardized procedures or approaches for equipment, staffing, data collection, data interpretation, and quality control. There needs to be adequate information on the variability of the measure, and crucially, reference ranges for normal values that are relevant to the local population. With regard to the last of these, in this issue of *Indian Pediatrics*, Kumar and colleagues [2] present data from repeated IPFT measurements in healthy children tested at their center between 2012 and 2017. The data presented here represent an extraordinary achievement. The authors have collected six-monthly data for three different IPFT modalities from birth to 36 months age in 281 healthy children. The three modalities are tidal breathing flow-volume loop (TBFVL), rapid thoracoabdominal compression (RTC), and raised volume RTC (RVRTC). The authors have generated centile curves using the LMS method, and gender-specific data.

The strengths of the study [2] are a prospective birth cohort design, excellent follow-up, and adherence to American Thoracic Society/European Respiratory Society criteria for IPFT testing [3,4]. The study does not have any funding from the manufacturers of the equipment. The limitations include being a single-center study from Northern India and lack of analysis by ethnicity. More importantly, and presumably unavoidably, the three modalities presented are not necessarily the three most useful to clinical care. TBFVL measurements are relatively easy to collect, but the data do not discriminate well between health and disease, and the test is now rarely used internationally. The RTC test is best considered a methodological precursor to the RVRTC test and has therefore been almost completely supplanted by the latter. At the same time the authors were unable to provide any data on infant plethysmography, multi-breath washout, or forced oscillation. That said, the RVRTC test – sometimes termed infant spirometry – is probably the single most widely used IPFT internationally, and the data presented here will be of great value to pediatric pulmonologists in India, and also to the wider international community.

What next for this area? At present the international consensus is that IPFT has rather limited clinical application. Some European and North American cystic fibrosis centers use IPFT (usually RVRTC plus multi-breath washout) as part of their clinical monitoring [5,6]. Even here, it is recognized that abnormalities can be very minor. Some centers with specialism in childhood interstitial lung diseases will use limited IPFT measurements in their practice. However, it should be noted that at present there are no commercially available systems for measurement of transfer factor in infants. With the current evidence, IPFT does not appear to be of value in the monitoring of chronic lung disease of prematurity or recurrent wheeze [1].

In our opinion, there is greater potential in the field of pediatric pulmonology research. It should not be forgotten that a ground-breaking IPFT study from the Institute of Child Health in London first identified the impact of maternal antenatal smoking upon infant lung development [7]. The effect of airborne pollution upon lung health is becoming a public health emergency, nowhere more so than in India. As pediatricians, we have a duty to determine whether airborne pollution is damaging the lungs of our youngest citizens. Perhaps the work of Kumar, *et al.* [2] can assist us with the next stage of this research.

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EDITORIAL

A Silver Lining in the HIV/AIDS Cloud

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Medical science has overcome insurmountable challenges. The physician's indomitable will and the potency of modern medicine have conquered the most intractable of illnesses. There are; however, a few adversaries that remain unsubdued. Of these, the most formidable is HIV/AIDS. India is one of its foremost victims. And amongst India's population, the mother and child are the most affected. Transmission of HIV from mother-to-child is a grim manifestation of this. Medicine; however, is relentless in its pursuit. The 'Prevention of Parent-to-Child Transmission of HIV (PPTCT) Programme', under the aegis of the National AIDS Control Organization (NACO) is a fine example of medicine's tenaciousness, to first bring down, and then finally eliminate the spread of HIV in India.

There is a large global evidence-base that substantial reduction in new pediatric infections can be achieved as a result of high coverage with highly effective interventions for PPTCT [1]. In 2013, the World Health Organization (WHO) published new guidelines which recommended providing life-long triple anti-retroviral therapy (ART) for all pregnant women living with HIV, irrespective of the CD4 cell count [2]; this new option termed (B+), would result in significant reduction in transmission of mother to child infections and would be helpful in maximising coverage for those needing treatment for their own health and long-term survival. This strategy would also prevent 'stopping and starting ART' with repeat pregnancies, providing early protection against mother to child transmission in future pregnancies, reducing the risk of transmission to sero-discordant male partners of these women, and reducing the emergence of drug resistance. Option B+ also offered other advantages, which included simplification of choice of ART regimen, and service delivery and harmonization with ongoing ART programmes.

The cost of antiretroviral drugs was a major determinant in a countries' choice of a particular drug regimen for PPTCT. In 2009, the average cost of ART offered under Option B was three to five times higher than the cost of providing single dose Nevirapine to the mother and child at delivery. However, by the end of 2011, this differential had diminished to two times higher. The cost of formulations of tenofovir with lamivudine and efavirenz has also decreased by 30% over the past three years [3]. The cost of these drugs is expected to fall further in future.

About 14,000 babies infected with HIV are born to an estimated 38,000 HIV-infected pregnant women in India. Mother to child transmission of HIV, which occurs during pregnancy, childbirth, or through breastfeeding, accounts for 4.7 percent of overall HIV transmission in the country (NACO Annual Report, 2013).It is the most important route of HIV-transmission among children in India. It is essential that these infected pregnant women are provided the package of PPTCT services to reduce transmission of HIV to the baby. The real challenge lies in reaching all pregnant women accessing antenatal care (ANC) services, at all health service delivery points, and to reach early in pregnancy, especially in line with the WHO guidelines that require women to attend ANC as early as possible.

In India, NACO has adopted the PPTCT component as an important service under National Aids Control Program (NACP) to respond to the challenge of controlling and reversing the HIV epidemic. PPTCT, that started in 2002, has witnessed a significant scale-up of HIV counselling, testing and treatment.The PPTCT used a single dose Nevirapine as the drug of choice which had the potential to reduce the risk of transmission to 12%-15%. Later, based on the WHO guidelines, NACO in September 2012, rolled out the triple drug ARV regimen (option B) in the 4 southern high prevalence states of Andhra Pradesh, Telangana, Karnataka and Tamil Nadu. This was later expanded to the entire country. Based on the new guidelines, NACO advocates initiating lifelong ART (triple drug regimen) for all pregnant and breast-feeding women living with HIV, regardless of CD4 count or WHO clinical stage, for their own health and also to prevent vertical HIV transmission [4].

In this issue of *Indian Pediatrics*, the exact transmission rates of HIV infection among pregnant women attending an ART centre in Delhi and the follow up of the infants at a Pediatric Centre of Excellence at Kalawati Saran Children's Hospital has been published [5].The study included mothers who had received single dose Nevirapine as preventive strategy for PMTCT as well as those receiving triple therapy as option B+. The overall transmission rate of HIV described in the study is 2% and an overall ARV cover in HIV-positive mothers of 94%. On analyzing the data before and after the change in PPTCT guidelines, no significant difference was found in terms of HIV-free survival or HIV transmission rate. Of the 155 infants, 10 (6.5%) died before 18 months of age. Of these, one had positive and three had negative HIV DNA-PCR at the age of 6 weeks (all 3 on exclusive replacement feeds), while the rest died before their HIV status could be ascertained. Out of the four children who were tested at 6 weeks only, one was positive and three were negative. They were apparently on replacement feeding and thus were perhaps uninfected. The fate of the other 6 is not known. It is possible that inclusion of these infants also in the final analysis (if their 18-month status was known) would have yielded a higher rate of HIV transmission.

It appears that single dose nevirapine was as effective as option B+ inprevention of HIV transmission in this study. Whether the triple therapy would have conferred various health benefits to the mother and birthweight of those children would have been better than those who received only single dose of nevirapine, needs further exploration. At 18 months age, 14% HIV-uninfected infants were wasted, 28% stunted, and 3% had microcephaly in this study. It is not known whether the mothers of these infants were on triple therapy or not. Recent evidence from trials in Botswana [6] revealed that both weight-for-age and length-for-age were significantly lower in HIV-exposed infants exposed to ART *in utero* compared to those that were only exposed to maternal single drug prophylaxis. It remains unclear whether these differences have a short term impact or whether they predispose the child for subsequent poorer growth, chronic disease and neurocognitive dysfunction [7]. The long term effects of antiretroviral drugs on the growing brain need further exploration.

At present, the benefits of ART in reducing vertical transmission and improving maternal health greatly outweigh the potential adverse effects of ART exposure to children [8]. However, as PMTCT coverage increases, the number of uninfected infants exposed to antiretroviral therapy (ARV) *in utero* or through breastfeeding will likewise increase, making it critical to continue monitoring for adverse effects.

As Hippocrates said wisely, *"Healing is a matter of time, but it is sometimes also a matter of opportunity."* An opportunity now beckons. Let this serve as a clarion call to physicians to pool their capabilities, knowledge and resources and engage with their patients with renewed vigour, enthusiasm and hope.The time starts now.

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RESEARCH PAPER

Normative Data of Infant Pulmonary Function Testing: *A Prospective Birth Cohort Study from India*

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Objective: To develop a normal reference range of Infant pulmonary function test (IPFT) indices for Indian children.

Design: Prospective birth cohort study.

Setting: Division of Pediatric Pulmonology of a tertiary-care institute in India from August 2012 to March 2017.

Participants: All neonates born at the institute during the study period were screened for eligibility.

Measurement: IPFT at baseline and every 6-month until 36-months of age.

Main Outcome Measure(s): Tidal breathing flow-volume loop (TBFVL), Rapid thoracoabdominal compression (RTC), and Raised volume RTC (RVRTC) indices at baseline and follow-up.

Results: 310 newborns were enrolled in the cohort; 281 of them (169 male) had completed 36-months of follow-up at the end of the study period. There was no influence of gender on the baseline IPFT indices. Tidal volume per unit body weight (V_T/kg) significantly increased from baseline to 36 months of age (P<0.001) while the peak ratio (t_{PTEF}/t_E) initially decreased in first 18-months of age (P<0.001), after that returned to the baseline value by 36 months of age. RTC indices did not change significantly from baseline values. In RVRTC, the ratio of forced expiratory volume in 0.5s to forced vital capacity ($FEV_{0.5}$/FVC) was significantly decreased from baseline to 36 months of age (P=0.002).

Conclusions: Normal values for various IPFT indices for TBFVL, RTC, and RVRTC from neonates to the age of 36-month are provided. These data may be used as normative data for healthy neonates and children of Indian origin.

Keywords: *Indices, Rapid thoracoabdominal compression, Tidal breathing flow-volume loop.*

While pulmonary function testing (PFT) is well established for older children (>5 years) and adolescents, it is still evolving for use in infants and preschool children [1]. Sophisticated equipments are now commercially available, which can measure pulmonary function even in premature babies [2].

PFT in infants (IPFT) may contribute to a better understanding of the nature and severity of respiratory illness, progression of the disease, and monitoring response to therapy [3]. Serial measurements of lung function since birth, especially in high-risk infants, may be helpful in recognition of early deviation from the normal pattern of lung development. Longitudinal studies have shown that many of the chronic respiratory disorders have their origin in childhood; hence, intervention at this stage may have an impact on the management of the chronic respiratory disease [4,5].

The measurement of PFT in infants and preschool children is a major challenge [6,7]. The values of pulmonary function indices vary with age, sex, body size, and ethnic groups [8,9]. Currently, there is lack of multiethnic global reference range for IPFT indices, so the development of regional ethnicity-specific normative data is the need of the hour, which will definitively expand its use in clinical practice [6].

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In this birth cohort, we performed IPFT from birth to 36 months of age. The normative data for various indices were generated, which may be used as reference range in a similar population.

METHODS

This prospective birth cohort study was conducted in the Department of Pediatrics of a tertiary-care institute in Delhi, India from August 2012 to March 2017. The study was approved by the Institutional Ethics Committee. Written informed consent was taken from the parents/ guardians. All neonates born at the institute during the study period were screened for eligibility. The inclusion criteria were age ≤4 weeks, full-term (≥37 weeks of

gestation), and appropriate for gestational age (weight 10^{th}- 90^{th} centile) babies [10]. The exclusion criteria were any perinatal insults (*e.g.* birth asphyxia, meconium aspiration, any amount of respiratory distress requiring respiratory support, pathological hyperbilirubinemia or seizure), known major congenital birth defect, required parenteral antibiotic or fluid, neonatal cholestasis, chronic kidney disease or inborn error of metabolism, and mother having antepartum or postpartum hemorrhage, preeclampsia or eclampsia, HIV infection, or parents' refusal for regular follow-up for three years. The enrolled babies were followed-up every 6 months (±8 weeks) and also whenever they had an acute respiratory infection or any other acute condition. The babies were clinically examined, and anthropometric measurements (weight, length, head circumference) were recorded at enrolment and each visit.

The IPFT were performed with Exhalyzer D (Eco Medics AG, Duernten, Switzerland) as per the American Thoracic Society/European Respiratory Society Task Force recommendations [11-16]. IPFT included tidal breathing flow-volume loop (TBFVL), rapid thoracoabdominal compression (RTC), and raised volume rapid thoracoabdominal compression (RVRTC) maneuvers (***Web Fig.* 1**). All the IPFT maneuvers were performed in the Pediatric Pulmonary Function Laboratory, which is well equipped with a central supply of oxygen and resuscitation equipment. The equipment was calibrated daily for atmospheric temperature and pressure and volume with a 100 mL calibration syringe (M30.9011) provided by the manufacturer. Weight and length/height were recorded using standard methods [17]. IPFT was postponed for 2-4 weeks if the child had an acute respiratory infection.

At enrolment and 6 months of age, IPFT were performed either in awake or natural sleep state after breastfeeding. Beyond six months of age, syrup Triclofos (25-50 mg/kg/dose) was used for sedation, whenever required. The maneuvers were performed in the supine position. Baseline TBFVL was completed within four weeks of birth, while RTC and RVRTC were first performed once the child was ≥8 kg as per recommendations of the manufacturer of IPFT equipment. IPFT were repeated at every follow-up visit. The IPFT indices and their physiological interpretation are described in ***Web Table* I**.

Statistical analysis: Clinical information during each visit was recorded manually into case record form; data were then entered into Microsoft Access software. IPFT data were automatically stored in software (SPIROWARE, Eco Medics AG, Duernten, Switzerland) after each procedure; data of each individual were extracted and managed in Microsoft Access software. Data were analyzed using Stata software v.13 (Stata Corp, College Station, TX, USA). Quantitative variables were summarized using mean and standard deviation if normally distributed; for skewed distribution, median (interquartile range) was used. Chi-square test was used for the analysis of categorical data. The change in IPFT indices from birth to 36 months of age were calculated with the Wilcoxon sign-rank test. Comparison of IPFT indices between gender and other parameters was analyzed with Wilcoxon rank-sum test (Mann-Whitney Test). A *P*-value of <0.05 was considered significant. For multivariate analysis, mixed-effects linear regression analysis was performed; IPFT indices were taken as the dependent variable, individual subjects as the random effect and gender, weight, length, and age as fixed effect. The LMS chartmaker Pro (Medical Research Council, UK) was used to model the expected median (μ or M), the coefficient of variation (σ or S), and skewness (λ or L) and to smooth the centile curves for IPFT indices against age, utilizing the method described by Cole TJ, *et al.* [18]. The goodness of fit for the model used was tested by the Q curve [18].

RESULTS

A total of 3412 neonates born in our institute from August 2012 to May 2014 were screened for eligibility; 310 neonates fulfilled the inclusion criteria and were enrolled. The median (IQR) age at enrolment was 4^{th} (3^{rd}, 5^{th}) postnatal day. A total of 281 children (90.6%) had completed 36 months of age by 31 March 2017; all IPFT (TBFVL, RTC and RVRTC) at this age were successfully performed in 225 infants (54.5% males) (***Fig.* 1**). The mean (SD) birthweight and length were 2.6 (0.6) kg and 47.7 (6.6) cm, respectively. Other demographic characteristics of enrolled children are summarized in ***Table* I**. TBFVL, RTC, and RVRTC were successfully performed in 1705, 948, and 875 occasions (baseline and follow up visits), respectively. The 5^{th}, 25^{th} 50^{th}, $75^{th,}$ and 95^{th} centile values of TBFVL and RTC as per the age, sex, mean weight and length/height of the enrolled children are described in ***Table* II** and **III**. The same for RVRTC indices are described in ***Table* IV**.

Tidal volume (V_T) per unit body weight (V_T/kg) increased significantly from birth to 36 months of age (P<0.001), which was more prominent in the first 12 months of age (P<0.001). The ratio of time to attained peak expiratory flow to expiratory time (t_{PTEF}/t_E) decreased significantly (P<0.001) from baseline till 18 months of age, then it began to increase (P<0.001) to achieve the baseline value at 36 months of age. V_{PTEF}/V_E

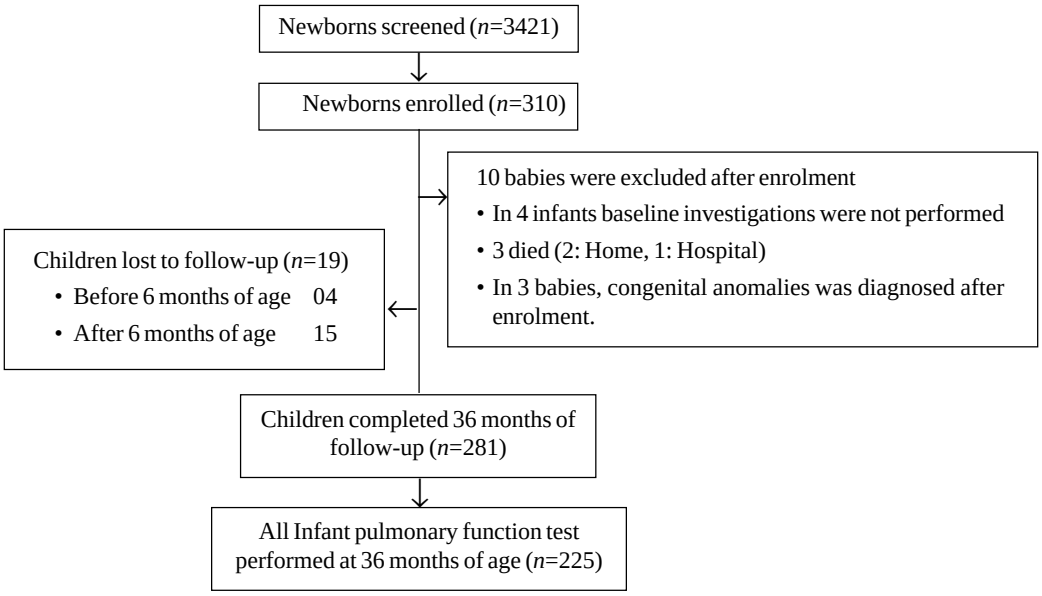

FIG. 1 *Enrolment and follow-up of the study cohort.*

also had decreasing trends from baseline to 18 months (P<0.001) then it gradually increased to achieve the baseline value by 36 months of age. The smoothed centile curves for V_T and t_{PTEF}/t_E are given in ***Fig.* 2** and **3**. At baseline, there was no significant association of gender with IPFT indices. However, on follow up, V_T was significantly more in boys at age of 6 months (P<0.001), 12 months (P<0.001), 18 months (P=0.02) and 36 months (P<0.001). The t_{PTEF}/t_E was significantly more in girls at 6 months (P=0.004) and 30 months (P=0.04) of age; for other ages it was similar in both sex. V_{PTEF}/V_E was significantly greater in girls at 6 months (P=0.004) and 30 months (P=0.02) of age. The t_E/t_{tot} was similar in both sexes at all ages.

TABLE I DEMOGRAPHIC CHARACTERISTICS OF ENROLLED INFANTS (N=310)

Characteristics	*Values**
Females	141 (45.5)
*Birthweight, g	2648 (689)
*Length, cm	47.7 (6.6)
*Gestation, d	267.9 (22.6)
Mode of delivery	
Vaginal	185 (59.6)
Caesarean	101 (32.5)
Instrumental	24 (7.8)
#Age at enrolment, d	4 (3, 5)
Age of parents, y	
Mother	26.5 (3.9)
Father	30.5 (4.3)
Family members in house	5.3 (2.8)
Urban accommodation	277 (89.3)
Smoking at home	93 (30)
Pets at home	31 (10)
Family history of allergy	144 (46.4)
Asthma	82 (26.5)
Allergic rhinitis	93 (33.1)
Atopic dermatitis	32 (10 .3)

*All values in no. (%) except *mean (SD) or #median (IQR).*

In RTC, $V'_{maxFRC,}$ $V'_{50,}$ and V'_{70} did not significantly differ from baseline to 36 months of age, while V_{PEF} gradually increased from baseline to 36 months of age (P<0.001). The smoothed centile curves for V'_{maxFRC} are presented in ***Fig.* 4**. $V'_{max,FRC}$, V'_{50}, $V'_{70,}$ and V_{PEF} were similar in both sexes at all ages except at 30 months, where V'_{70} (P=0.01) and V_{PEF} (P<0.001) were significantly higher in boys. In RVRTC, there were minor increases in FVC, $FEV_{0.5}$, FEF_{25-75}, and MEF_{25} from baseline till 24 months of age; after that, there were significant increases till 36 months of age (P<0.01).

PEF initially decreased from baseline to 12 months of age (P<0.001) then remained constant till 24-months of age, increasing again till 36 months of age (P<0.001). $FEV_{0.5}$/FVC was significantly decreased from baseline to 36-months of age (P=0.002), more during baseline to 18 months of age (P<0.001). The smoothed centile curves for $FEV_{0.5}$ and FVC are represented in ***Fig.* 5** and **6**.

TABLE II NORMAL VALUES (PERCENTILES) OF TIDAL BREATHING FLOW VOLUME LOOP INDICES

Indices	*Age, mo*	**Weight (kg)*	**Length/ Height(cm)*	*Boys, percentiles*					*Girls, percentiles*				
				5th	*25th*	*50th*	*75th*	*95th*	*5th*	*25th*	*50th*	*75th*	*95th*
V_T, mL	Baseline	2.8 (0.4)	48.2 (1.7)	5	6	7	9	10	5	6	7	8	10
	6	7.3 (1.1)	66.7(3.8)	10	17	24	49	79	6	13	18	23	60
	12	8.8 (1.0)	73.7(3.4)	35	51	69	92	119	10	28	53	83	109
	18	9.8 (1.2)	78.2(7.2)	35	53	86	121	145	32	52	71	98	130
	24	11 (1.3)	83 (7.5)	52	70	99	134	170	57	77	92	119	144
	30	11.7 (1.6)	86.4 (8.4)	58	94	126	150	184	59	85	120	143	178
	36	12.9 (1.7)	91.4 (7.0)	99	141	157	187	224	71	116	135	158	192
V_T per unit body	Baseline	2.8 (0.4)	48.2 (1.7)	1.6	2.1	2.7	3.1	3.9	1.8	2.2	2.6	3.1	3.6
weight, mL/kg	6	7.3(1.1)	66.7(3.8)	1.4	2.4	3.5	6.1	9.7	0.9	1.9	2.8	3.4	5.6
	12	8.8 (1.0)	73.7(3.4)	4.1	5.5	7.5	10.3	13.1	1.3	3.5	6.1	9.1	12.6
	18	9.8 (1.2)	78.2(7.2)	3.8	5.2	8.3	11.8	15.1	3.9	5.5	8.0	10.7	13.4
	24	11 (1.3)	83 (7.5)	4.5	6.4	8.6	11.2	14.9	5.5	7.3	9.4	11.3	13.2
	30	11.7(1.6)	86.4 (8.4)	4.9	7.5	10.4	12.8	15.6	4.9	7.6	10.9	12.7	15.3
	36	12.9 (1.7)	91.4 (7.0)	7.6	10.5	12.2	14.1	17.4	5.7	8.8	10.8	12.2	15.9
t_{PTEF}/t_E, %	Baseline	2.8 (0.4)	48.2(1.7)	22	32	37	45	56	22	29	36	43	58
	6	7.3(1.1)	66.7(3.8)	16	22	28	37	58	17	25	34	44	62
	12	8.8 (1.0)	73.7(3.4)	14	19	24	32	48	16	20	24	32	49
	18	9.8 (1.2)	78.2(7.2)	12	18	23	30	48	14	19	24	33	49
	24	11 (1.3)	83 (7.5)	15	21	30	40	51	15	21	28	39	58
	30	11.7(1.6)	86.4 (8.4)	19	27	34	44	61	19	29	38	51	65
	36	12.9 (1.7)	91.4 (7.0)	22	32	39	47	62	21	30	41	51	66
V_{PTEF}/V_E, %	Baseline	2.8 (0.4)	48.2(1.7)	20	34	40	46	57	22	30	36	43	55
	6	7.3(1.1)	66.7(3.8)	21	25	31	39	56	22	28	36	44	60
	12	8.8 (1.0)	73.7(3.4)	18	22	27	32	40	20	24	28	32	49
	18	9.8 (1.2)	78.2(7.2)	17	21	26	31	47	18	22	25	33	47
	24	11 (1.3)	83 (7.5)	11	21	32	45	73	16	21	26	39	80
	30	11.7(1.6)	86.4 (8.4)	22	29	36	43	57	22	31	40	48	61
	36	12.9 (1.7)	91.4 (7.0)	24	32	38	45	59	23	31	39	49	60
t_E/t_{tot}, %	Baseline	2.8 (0.4)	48.2(1.7)	47	50	53	55	61	45	49	52	56	60
	6	7.3(1.1)	66.7(3.8)	47	51	53	56	61	47	51	54	56	60
	12	8.8 (1.0)	73.7(3.4)	47	52	55	58	62	48	51	54	58	62
	18	9.8 (1.2)	78.2(7.2)	49	53	56	60	65	49	53	56	58	63
	24	11 (1.3)	83 (7.5)	49	53	56	59	64	49	54	57	59	62
	30	11.7(1.6)	86.4 (8.4)	49	53	56	59	62	47	54	56	58	62
	36	12.9 (1.7)	91.4 (7.0)	52	54	57	60	63	52	55	57	59	62

V_T: Tidal volume, t_{PTEF}/t_E : Ratio of time to peak tidal expiratory flow to expiratory time, V_{PTEF}/V_E : ratio of volume at peak tidal expiratory flow to tidal expiratory volume, t_E/t_{tot}: ratio of expiratory time to total respiratory time.

RVRTC indices were similar in both sexes at all ages except at 36-months, where FEV, MEF_{25}, FEF_{25-75}, $FEV_{0.5}$ and PEF, were significantly higher in boys (P=0.04, 0.01, 0.01, <0.001 and 0.02, respectively). $FEV_{0.5}$/FVC remained similar in both sexes at all ages.

On multivariate analysis, length/height, body weight, gender, and age were significantly associated with V_T. t_{PTEF}/t_E was significantly associated with gender, with values at any point being higher for females in comparison to males, after adjusting length/height and bodyweight.

TABLE III NORMAL VALUES (PERCENTILES) OF RAPID THORACOABDOMINAL COMPRESSION INDICES

IPFTIndices	*Age, mo*	**Weight (kg)*	**Height/ Length (cm)*	*Boys*					*Girls*				
				5th	*25th*	*50th*	*75th*	*95th*	*5th*	*25th*	*50th*	*75th*	*95th*
$V'_{max,FRC}$, mL/s	6	7.3 (1.1)	66.7 (3.8)	–	–	–	–	–	–	–	–	–	–
	12	8.8 (1.0)	73.7 (3.4)	0	0	50	70	125	0	0	40	70	120
	18	9.8 (1.2)	78.2 (7.2)	0	30	55	80	140	0	0	42	65	135
	24	11 (1.3)	83 (7.5)	0	0	50	90	205	0	0	47	75	90
	30	11.7 (1.6)	86.4 (8.4)	0	0	58	95	200	0	0	55	80	110
	36	12.9 (1.7)	91.4 (7.0)	0	0	50	105	180	0	0	60	105	210
V'_{50}, mL/s	6	7.3 (1.1)	66.7 (3.8)	55	80	125	190	335	50	95	105	135	250
	12	8.8 (1.0)	73.7 (3.4)	40	70	95	125	240	40	55	85	112	195
	18	9.8 (1.2)	78.2 (7.2)	45	65	85	125	310	40	60	85	115	235
	24	11 (1.3)	83 (7.5)	45	75	115	170	305	40	70	105	145	220
	30	11.7 (1.6)	86.4 (8.4)	45	80	125	195	315	35	70	103	180	305
	36	12.9 (1.7)	91.4 (7.0)	40	85	125	200	385	30	68	100	175	320
V'_{70}, mL/s	6	7.3 (1.1)	66.7 (3.8)	30	60	80	135	275	20	65	100	135	150
	12	8.8 (1.0)	73.7 (3.4)	25	50	77	105	180	20	55	75	99	140
	18	9.8 (1.2)	78.2 (7.2)	35	55	80	120	255	30	50	75	110	210
	24	11 (1.3)	83 (7.5)	20	60	85	125	210	25	50	80	100	170
	30	11.7 (1.6)	86.4 (8.4)	5	75	105	145	195	0	45	90	115	205
	36	12.9 (1.7)	91.4 (7.0)	0	60	105	150	280	0	55	95	133	270
V_{PEF}, mL/s	6	7.3 (1.1)	66.7 (3.8)	9	13	17	23	35	11	16	19	31	76
	12	8.8 (1.0)	73.7 (3.4)	8	13	18	24	45	8	13	18	27	43
	18	9.8 (1.2)	78.2 (7.2)	9	15	20	27	53	9	15	21	26	56
	24	11 (1.3)	83 (7.5)	9	16	24	37	61	9	16	21	29	42
	30	11.7 (1.6)	86.4 (8.4)	11	21	30	40	72	10	16	23	32	50
	36	12.9 (1.7)	91.4 (7.0)	10	18	31	42	79	10	18	30	37	58

**weight and height in mean (SD), $V'_{max,FRC}$: Maximum expiratory flow at functional residual capacity, V'_{50}: Maximum expiratory flow at 50% of expiration, V'_{70}: Maximum expiratory flow at 70% of expiration. V_{PEF}: Volume to peak expiratory flow; –: Not measured.*

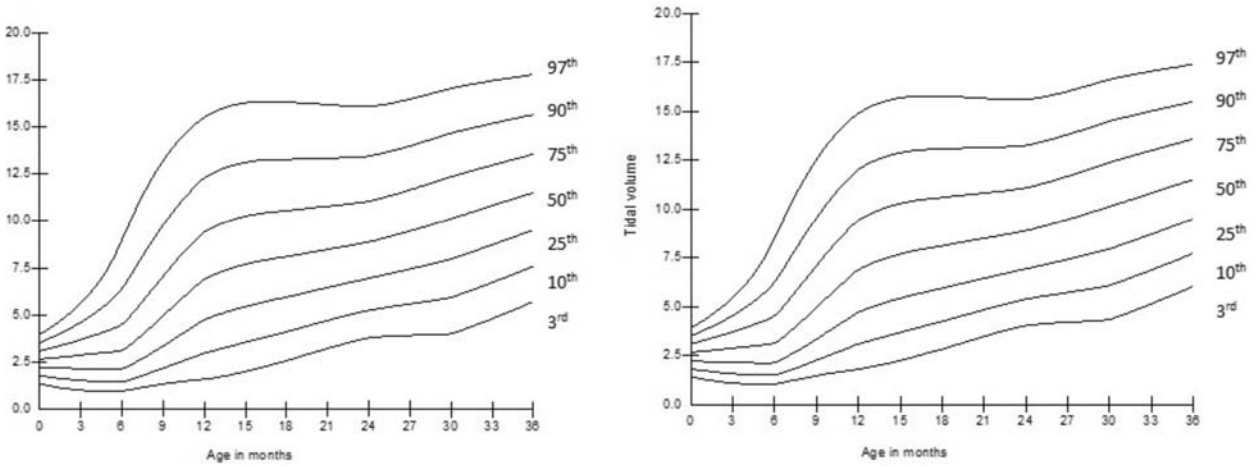

FIG.2 *(a) Change in tidal volume (V_T) with age in girls; (b) Change in tidal volume (V_T) with age in boys.*

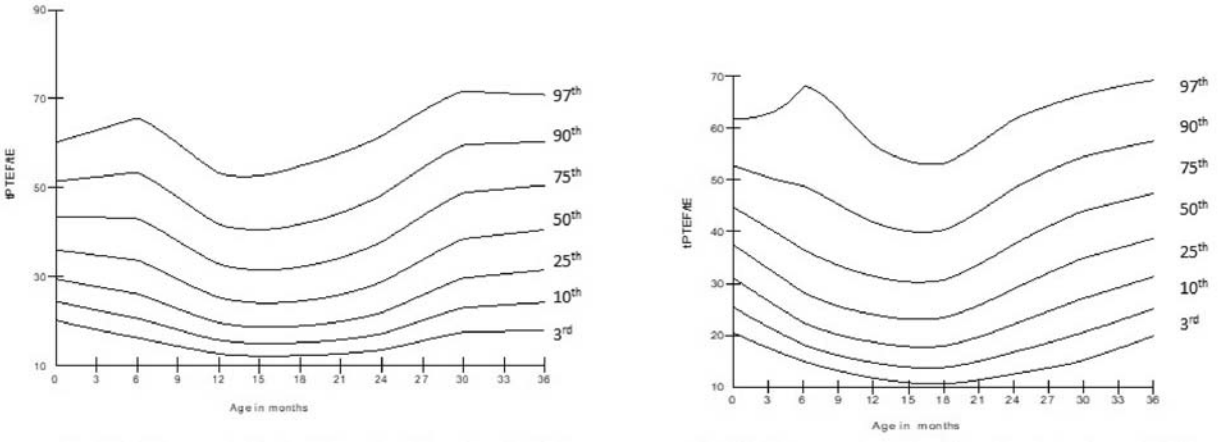

FIG. 3 *(a) Change in ratio of time to attained peak tidal expiratory flow to expiratory time (t_{PTEF}/t_E) with age in girls; (b) Change in ratio of time to attained peak tidal expiratory flow to expiratory time (t_{PTEF}/t_E) with age in boys.*

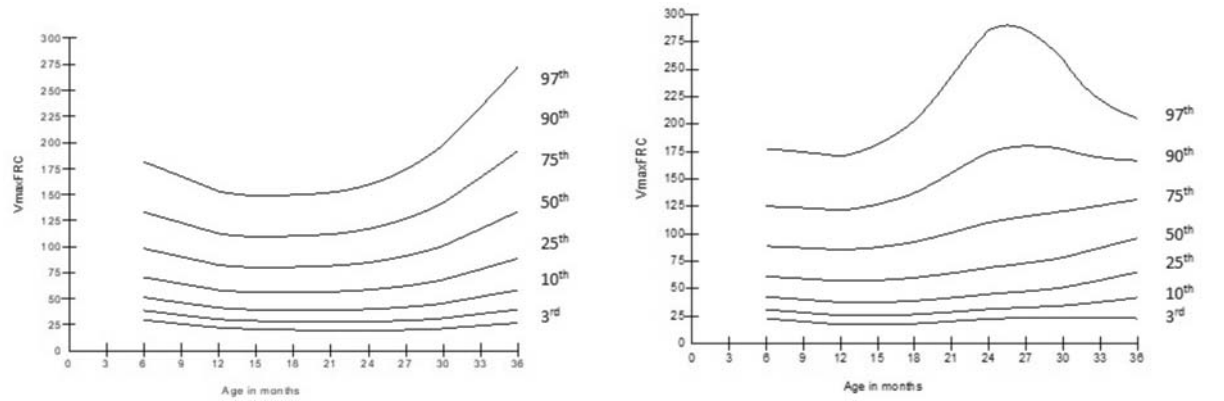

FIG. 4 *(a) Change in maximum expiratory flow at functional residual capacity ($V\dot{c}_{max,FRC}$) with age in girls; (b) Change in maximum expiratory flow at functional residual capacity ($V\dot{c}_{max,FRC}$) with age in boys.*

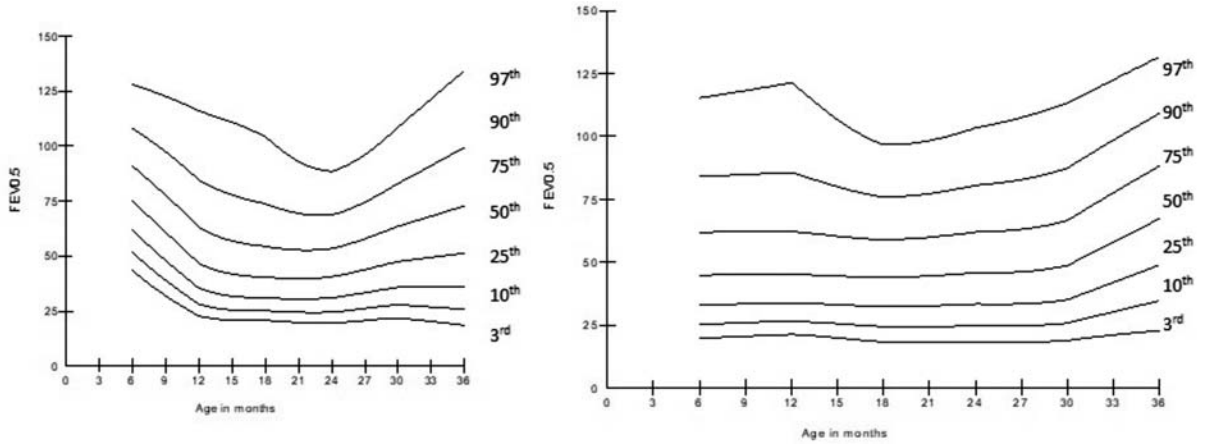

FIG. 5 *(a) Change in forced expiratory volume in 0.5 sec ($FEV_{0.5}$) with age in girls; (b) Change in forced expiratory volume in 0.5 sec ($FEV_{0.5}$) with age in boys.*

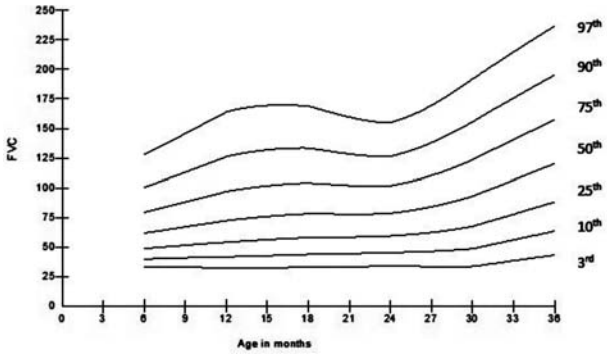

FIG.6 *Change in forced vital capacity (FVC) with age.*

DISCUSSION

IPFT is now widely used in the research context; however, its use in a clinical setting is still restricted by the non-availability of regional and ethnicity-specific reference values. In this prospective birth cohort study, we obtained normative data for TBFVL for neonates to 36 months of age, and RTC and RVRTC from 6 months to 36 months of age.

The major limitation of this study is that subjects were recruited from a single center, which may not be truly representative of the entire population. However, being a tertiary-care institute, subjects were referred from all around the country. Another limitation is that neonates with a family history of smoking or allergy were not excluded in this study, as we considered that they would be normally distributed, though to a different extent, in any given population.

The major strength of this study is the meticulously planned prospective birth cohort design of the study with frequent and regular follow-up. IPFT were performed as per ATS/ERS recommendation. Until one year of age, in the majority of the infants, TBFVL were performed without sedation. As there was a significant difference between genders at a particular age, so gender-specific data have also been presented. Centile curves have been constructed using the LMS method.

In comparison to studies on Caucasian population, V_T/kg was markedly lower at baseline in this cohort; however, it gradually increased with age, and by 12 months it became comparable to global values [19,20]. The rest of the TBFVL indices at baseline were comparable with other studies [19-21]. In this cohort, t_{PTEF}/t_E and V_{PTEF}/V_E were highest at baseline and then gradually decreased until 18 months of age. A study from Switzerland in 342 infants had also observed that t_{PTEF}/t_E gradually decreased in the first year of life [20]. However, a prospective birth cohort study from Taiwan did not observe any significant change in V_T, t_{PTEF}/t_E and V_{PTEF}/V_E from 5 to 26 months of follow-up [7]. Furthermore, in the present study, there was no significant influence of gender at baseline IPFT indices; however, on follow up, many of these indices varied significantly with gender. A study from Norway did not find any significant influence of gender on the baseline tidal expiratory volume; however, they observed that tidal flow and flow ratio were significantly higher in males in comparison to female babies [21]. Another study from Taiwan also did not observe any sex-related difference in IPFT indices [7]. $V'_{max,FRC}$ in this cohort remained similar throughout the follow-up period with no influence of gender. Studies from Taiwan [7], US [22], and a multicentric study from London, Indianapolis, and Boston [23] had observed that $V'_{max,FRC}$ correlates significantly with the height of the children. The measurement of $V'_{max,FRC}$ depends on accurate determination of FRC as a volume landmark, which is highly variable, especially in younger children, and this is a significant limitation in RTC measurement [24]. In this study, jacket pressure was kept fixed at 10 kPa while in other studies, it was used in the incremental range from 2-10 kPa [22,25]. Hence, it might be responsible for the deviation of our finding for $V'_{max,FRC}$ with age from other studies.

In this cohort, most of the RVRTC indices *viz*., FVC, $FEV_{0.5}$, FEF_{25-75}, MEF_{25} and PEF increased minimally until 24 months of age, after that they increased dramatically with age. $FEV_{0.5}$/FVC decreased from baseline until 36 months of age. There was no gender difference in any of the RVRTC indices except at 36 months where it was more significant in boys. A multicentric study from Indiana and Ohio [25] in children from 3 to 149 weeks had also observed that RVRTC indices were highly correlated with growth (height) of the child while $FEV_{0.5}$/FVC decreased with increasing length. They also did not find any influence of gender on RVRTC indices except for FEF_{75}, which was higher in girls [26]. Another study from London has documented that RVRTC indices increase with age [25].

In conclusion, this prospective birth cohort study provides reference values for various IPFT indices from neonates to 36 months of age. The median and centile values for boys and girl have been separately provided. Despite some limitation, the data will be useful as a reference range for Indian children for TBFVL, RTC, and RVRTC. These results will serve as normative data for neonates and preschool children of Indian origin.

Acknowledgments: Satish Thomas, Ritu Dubey and Rajat Prakash for their contribution in this study.

TABLE IV NORMAL VALUES OF RAISED VOLUME RAPID THORACOABDOMINAL COMPRESSION INDICES

IPFT indices,	*Age*	*Weight*	*Height/*	*Boys*					*Girls*				
unit	*(mo)*	*(kg)*	*length (cm)*	*5th*	*25th*	*50th*	*75th*	*95th*	*5th*	*25th*	*50th*	*75th*	*95th*
FEV, mL	6	7.3 (1.1)	66.7 (3.8)	36	47	61	81	102	36	47	61	81	102
	12	8.8 (1.0)	73.7 (3.4)	36	51	75	94	143	37	49	77	100	151
	18	9.8 (1.2)	78.2 (7.2)	43	60	85	104	146	32	54	81	94	135
	24	11 (1.3)	83 (7.5)	40	56	84	113	135	41	56	80	97	132
	30	11.7 (1.6)	86.4 (8.4)	36	67	106	133	171	41	67	91	109	149
	36	12.9 (1.7)	91.4 (7.0)	64	100	128	160	219	37	83	118	144	216
MEF_{25}, mL/s	6	7.3 (1.1)	66.7 (3.8)	30	45	65	110	275	30	45	65	110	275
	12	8.8 (1.0)	73.7 (3.4)	30	45	70	95	365	30	40	70	115	255
	18	9.8 (1.2)	78.2 (7.2)	30	50	70	95	125	20	45	65	90	110
	24	11 (1.3)	83 (7.5)	30	45	75	95	170	30	45	70	90	140
	30	11.7 (1.6)	86.4 (8.4)	30	65	90	140	195	30	50	85	120	150
	36	12.9 (1.7)	91.4 (7.0)	50	105	150	220	450	35	80	110	198	410
FEF_{25-50}, mL/s	6	7.3 (1.1)	66.7 (3.8)	39	51	75	134	273	39	51	75	134	273
	12	8.8 (1.0)	73.7 (3.4)	33	53	75	117	315	39	46	86	124	268
	18	9.8 (1.2)	78.2 (7.2)	33	59	80	116	159	24	53	81	108	161
	24	11 (1.3)	83 (7.5)	35	54	91	126	222	34	54	79	109	160
	30	11.7 (1.6)	86.4 (8.4)	35	71	120	177	253	38	66	106	145	194
	36	12.9 (1.7)	91.4 (7.0)	60	117	178	246	449	33	97	139	214	368
$FEV_{0.5}$, mL	6	7.3(1.1)	66.7 (3.8)	19	36	45	65	84	19	36	45	65	84
	12	8.8 (1.0)	73.7 (3.4)	22	35	45	55	134	23	25	51	64	116
	18	9.8 (1.2)	78.2 (7.2)	22	33	44	59	79	23	34	42	52	78
	24	11 (1.3)	83 (7.5)	19	34	44	60	93	18	32	42	56	75
	30	11.7 (1.6)	86.4 (8.4)	20	32	49	69	111	24	36	48	63	89
	36	12.9 (1.7)	91.4 (7.0)	28	49	68	89	111	20	37	50	74	114
$FEV_{0.5/FEV}$, %	6	7.3 (1.1)	66.7 (3.8)	37	54	71	94	100	37	54	71	94	100
	12	8.8 (1.0)	73.7 (3.4)	38	52	65	79	97	34	55	64	74	86
	18	9.8 (1.2)	78.2 (7.2)	32	46	54	67	80	34	48	58	67	87
	24	11 (1.3)	83 (7.5)	32	48	60	70	92	27	47	56	67	81
	30	11.7 (1.6)	86.4 (8.4)	27	42	53	69	90	32	44	59	68	88
	36	12.9 (1.7)	91.4 (7.0)	27	41	54	70	91	20	36	49	64	88
PEF, mL/s	6	7.3 (1.1)	66.7 (3.8)	65	145	200	275	405	65	145	200	275	405
	12	8.8 (1.0)	73.7 (3.4)	60	100	135	200	435	70	105	148	205	340
	18	9.8 (1.2)	78.2 (7.2)	60	105	150	205	360	70	105	140	190	285
	24	11 (1.3)	83 (7.5)	75	125	175	250	410	70	105	160	205	350
	30	11.7 (1.6)	86.4 (8.4)	95	145	195	285	450	95	140	180	235	355
	36	12.9 (1.7)	91.4 (7.0)	115	200	270	385	505	80	165	245	310	500

IPFT: Infant pulmonary function test; FEV: Forced expiatory volume; MEF_{25}: Maximal expiratory flow at 25% of FEV; FEF_{25-75}: Forced expiratory flow between 25-75 % of FEV; $FEV_{0.5}$: Forced expiratory volume in 0.5 sec, PEF: Peak expiratory flow rate.

Contributors: SKK, RL: conceptualized and designed the study, developed protocol and drafted the manuscript; PK,SR: enrolled patients, collected and analysed data, reviewed literature and prepared initial draft of the manuscript; AM: collected and analysed data, reviewed literature and manuscript preparation; KRJ: data analysis, reviewed literature and manuscript preparation. All authors critically revised and approved the final version of the manuscript.

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Competing interests: None stated.

WHAT IS ALREADY KNOWN?

- Infant pulmonary function test (IPFT) can help in the understanding of natural course and progression of respiratory disease and monitor the response to therapy in infants and preschool children.

WHAT THIS STUDY ADDS?

- This study provides the normative data of IPFT indices in healthy Indian children, and the data can be used as reference range for infant pulmonary function test in Indian infant and preschool children.

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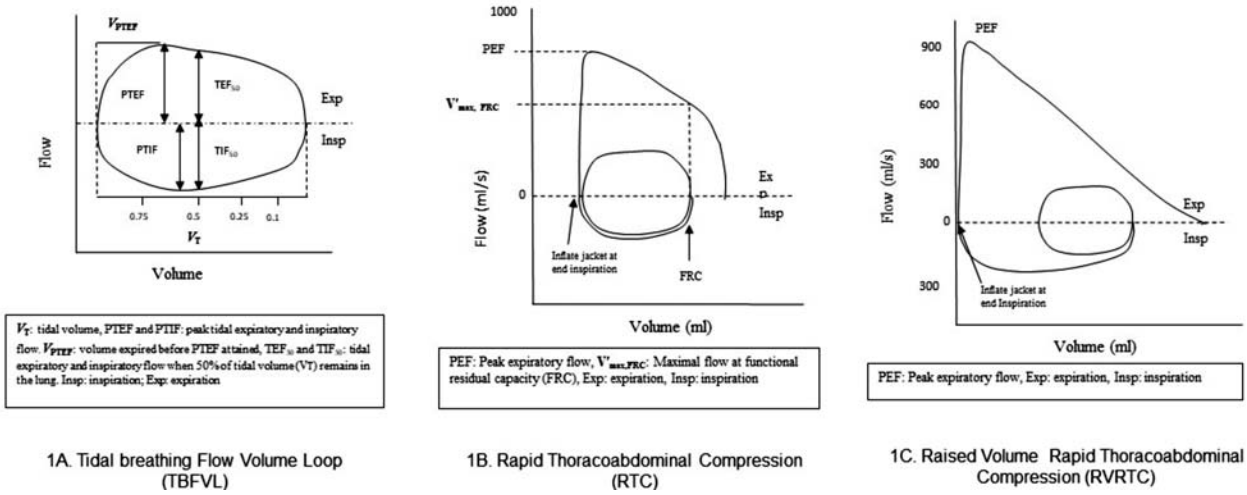

WEB FIG.1 *Normal infant pulmonary function test graphs; (a) Tidal breathing flow Volume loop (TBFVL); (b) Rapid thoracoabdominal compression (RTC); and (c) Raised volume rapid thoracoabdominal compression (RVRTC).*

WEB TABLE I INFANT PULMONARY FUNCTION TEST INDICES AND THEIR PHYSIOLOGICAL INTERPRETATION

Indices	*Unit*	*Physiological interpretation*
Tidal breathing flow volume loop		
• Inspiratory time (t_I), Expiratory time (t_E), Total respiratory time (t_{tot}), time to peak tidal inspiratory or expiratory flow (t_{PTIF} or t_{PTEF})	s	Time taken to complete individual maneuvers
• Tidal volume (V_T), Inspiratory or Expiratory tidal volume (V_I or V_E)	mL	Tidal lung volume
• Peak tidal inspiratory or expiratory flow (PTIF or PTEF)	mL/s	Flow rate
• Mid tidal inspiratory or expiratory flow (MTIF or MTEF)		
• Tidal expiratory flow (TEF) at 75, 50, 25 or 10% of tidal expiratory volume still have to be expired (TEF_{75},TEF_{50}, TEF_{25} or TEF_{10})		
• Ratio of inspiratory or expiratory time to total respiratory time (t_I/t_{tot} or t_E/t_{tot}), inspiratory to expiratory time(t_I/t_E),	%	Represent airway resistance
• Peak ratio: time to PTEF to t_E (t_{PTEF}/t_E)		
• Ratio of exhaled Volume to PTEF to tidal expiratory volume (V_{PTEF}/V_E)		
• Ratio of TEF_{75},TEF_{50}, TEF_{25} or TEF_{10} to Peaked tidal expiratory flow (PTEF)		
Rapid thorocoabdominal compression		
• Maximum expiratory flow at functional residual capacity ($V'_{max,FRC}$)	mL/s	Flow rate
• Maximum expiratory flow at 50% or 70% of expiration (V'_{50} or V'_{70})		
• Volume to peak expiratory flow (V_{PEF})	mL	Lung volume at partial expiratory flow- Airway resistance
Raised volume rapid throocoabdominal compression		
• Forced expiratory volume (FEV), FEV at 0.5, 0.75 or 1.0s of the forced expiration ($FEV_{0.5}$/$FEV_{0.75}$/$FEV_{1.0}$)	mL	Forced lung volume at given time period
• Maximum expiratory flow at 25% or 10% of FEV (MEF_{25} or MEF_{10})		
• Forced expiratory flow between 25% and 75% of FEV (FEF_{25-75})	mL/s	Forced flow rate –representing airway resistance.
• Peak expiratory flow (PEF)		
• Ratio of forced expiratory volume at 0.5 s to total forced expiratory volume ($FEV_{0.5}$/FEV)	%	Airway obstruction

IPFT: Infant pulmonary function testing, TBFVL: Tidal breathing flow volume loop, RTC: Rapid thoracoabdominal compression, RVRTC: Raised volume rapid thoracoabdominal compression.

RESEARCH PAPER

HIV-free Survival at the Age of 18 Months in Children Born to Women With HIV Infection: *A Retrospective Cohort Study*

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Objective: To assess HIV-free survival and nutritional status of HIV-exposed infants. **Methods**: This retrospective cohort study was conducted on infants born to woman with HIV infection born at our Institute between January 2011 to March 2016, and followed using current National guidelines. HIV transmission rate, HIV-free survival, and nutritional status were assessed 18 months age. **Results**: Of the 155 infants, 10 (6.5%) died before 18 months of age. Two of 145 surviving infants were confirmed HIV-positive, the remaining were HIV-negative at 18 months (HIV-free survival 92.3%). Of the 10 infants who died, one was confirmed HIV-positive and three negative; the rest died before their HIV status could be ascertained. HIV infection rate among the 149 infants for whom the test reports were available was 2%. At 18 months age, 14% HIV-uninfected infants were wasted, 28% stunted, and 3% had microcephaly. **Conclusions**: Infants born to mothers with HIV managed as per the current National guidelines have a good outcome at 18 months of age.

Key words: *HIV exposure, infants, Malnutrition, Outcome.*

Exposure to the same adverse environment places HIV-exposed infants at a higher risk of morbidity and mortality regardless of their own HIV status, as compared to infants born to women without HIV infection [1,2]. The survival and health of these infants are influenced by the feeding strategy adopted, higher exposure to infections, and HIV-status of the infant himself [3,4]. A high mortality in these children has previously been reported from this setting [5].

The current prevention of parent to child transmission (PPTCT) guidelines by National AIDS Control Organization (NACO), recommend lifelong anti-retroviral therapy (ART) to all pregnant and breast-feeding women with HIV regardless of clinical or immunological stage, anti-retroviral (ARV) prophylaxis to the baby, and safe infant feeding practices. A well-defined protocol has also been developed for care of the HIV-exposed infants [6]. The objective of this work is to report outcome of HIV-exposed infants born at a tertiary-case pediatric hospital, and provided standardized care as per the current NACO protocol.

METHODS

This retrospective cohort study was conducted in the Pediatric Centre of Excellence in HIV care located at a public teaching hospital in northern India. Infants born to women with HIV infection at the linked hospital and registered in the PPTCT program at our Center from January 2011 to March 2016 were included. We excluded infants born at other hospitals and subsequently referred to our Centre, those diagnosed with HIV after admission to our pediatric wards, or those who never attended the Centre after birth at the linked hospital.

Accompanying Editorial: Pages 23-24.

In accordance with the National guidelines, the HIV-exposed infants are registered at birth in our Centre and given a protocol-based care till 18 months of age [6]. This includes provision for early HIV diagnosis, safe feeding counselling, and access to routine infant care practices. Prior to January 2014, all women with HIV and their newborns were given a single dose of nevirapine (SDNVP) during labor and immediately after birth, respectively, in accordance with the national guidelines at that time [7]. After January 2014, all pregnant women with HIV are initiated on ART during pregnancy soon after detection of their HIV status. Infants born to these women are started on daily nevirapine prophylaxis at birth and continued for a minimum of 6 weeks [6]. This study included subjects registered both before and after these changes in the National recommendations. Determination of HIV status was done through HIV-1 DNA-PCR by dried blood spot (DBS) at ages 6 weeks, 6 months, and six weeks after stopping breastfeeding.

Infants testing positive on DBS testing were re-tested for DNA-PCR on whole blo od sample. In infants older than 18 months, serological tests (3 rapid antibody tests) were done for HIV diagnosis.

For the current study, information on maternal and infant characteristics was obtained from the records of all eligible infants maintained at our Centre. The nutritional status of children was determined by calculating Z-scores for weight for age (WFA), weight for length (WFL), length for age (LFA) and head circumference for age (HFA) using WHO growth reference standards [8].

The infants were considered to be HIV-infected if they tested positive on DNA-PCR any time before 18 months, or were found reactive on HIV serology at 18 months or beyond. They were considered HIV-uninfected if they had a negative DNA-PCR test and were not breastfeeding or had stopped it 6 weeks prior to the test, or had a non-reactive HIV serological test at or after 18 months performed at least 6 weeks after cessation of breastfeeding. The study was approved by the Institutional Ethics Committee for Human Research.

Statistical analysis: The data were analyzed using the SPSS statistical software package, Version 23. Chi square test, unpaired t test and Mann-Whitney U test were used to compare maternal and infant variables among HIV- uninfected infants at 18 months and those who died.

RESULTS

During the study period, 165 HIV-exposed infants were born at the linked hospital. Among these, 155 mother-infant pairs who were followed up at our centre till 18 months were eligible for the study. The clinical characteristics of these mother infant dyads are shown in ***Table* I**.

Of the 155 infants, 10 (6.5%) died before 18 months of age. Among the 145 surviving infants, two were confirmed HIV-positive. The rest 143 (92.3%) were surviving and HIV-free at 18 months. Of the 10 infants who died before 18 months, one had positive and three had negative HIV DNA-PCR at the age of 6 weeks (all 3 on exclusive replacement feeds), while the rest died before their HIV status could be ascertained (***Fig.* 1**).

HIV infection was reliably excluded in 146/155 infants (143 of those alive and 3 of those who died) while it was diagnosed in 3 infants (2 of those alive and 1 among those who died). Thus, HIV infection rate in the infants for whom the HIV test reports were available was 2% (3/149). On analyzing the data before (*n*=93) and after (*n*=62) the change in PPTCT guidelines, no significant difference was found in terms of HIV-free survival (92.5% *vs* 91.9%; P=0.9); or HIV transmission rate (2.2 *vs* 1.7 %; *P*=0.82).

The outcome in terms of survival and status of HIV infection stratified as per various maternal and infant factors is presented in ***Table* II**. Details of the three infants who were diagnosed with HIV infection are presented ***Web Table* I.**

At the age of 18 months, Z scores for WFL (*n*=108), LFA (*n*=108) and OFC (*n*=106) were -0.6 (1.2) , -1.2 (1.2), and -0.9 (1.2), respectively. At that time, 15 (14%) of uninfected infants were wasted, 30 (28%) infants were stunted, and 3 (3%) had microcephaly.

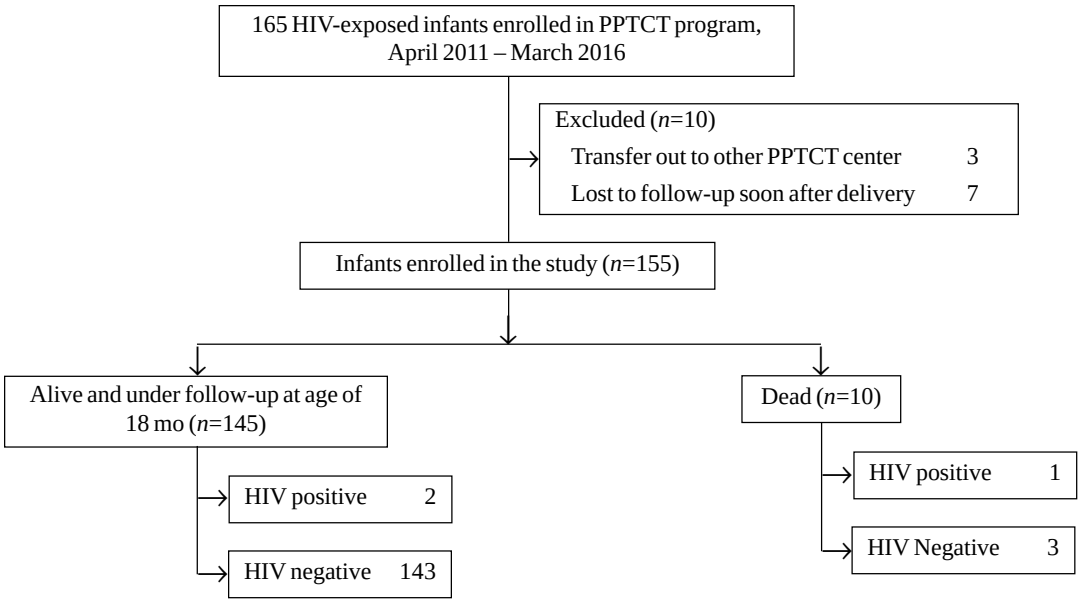
DISCUSSION

The present study has documented HIV infection rate and HIV-free survival among infants of women with HIV infection managed as per national PPTCT strategy. Parent to child transmission rate in the present study was 2%, with an overall ARV cover in HIV positive mothers of 94% (ART 79%, SDNVP 15%). We have previously reported a rate of 14.8% when the ARV cover in HIV-infected mothers was only 61.5% [5]. The current transmission rate is also much less when compared to studies from Africa [9,10], as well as few studies from India where the transmission rate has varied between 8-19% [11,12].

TABLE I CLINICAL CHARACTERISTICS OF INFANTS BORN TO MOTHERS WITH HIV INFECTION (*N=155*)

Characteristics	*No. (%)*
Male	82 (53)
Mother's HIV diagnosis	
Before pregnancy	72 (46)
During pregnancy	77 (50)
After delivery	6 (4)
Mother's therapy status at delivery	
*On triple ART	123 (79)
#Single dose nevirapine	23 (15)
No ART/ARV	9 (6)
Infant feeding status	
Exclusive breast feeding	82 (53)
Exclusive replacement feeding	71 (46)
Mixed feeding	2 (1)
Anthropometry at birth, mean (SD)	
WFL Z-score	-1.2 (1.4)
LFA Z-score	-0.7 (1.1)
OFC Z-score	-1.0 (1.1)

*Known HIV: infected before pregnancy: *68/72 and #4/72; HIV: positive detected during pregnancy: *55/77 and #19/77; OFC: Occipito-frontal circumference; LFA: Length for age; WFL: Weight for length.*



**Causes of death: In infant with HIV infection: severe malnutrition with sepsis; for the 3 infants who were HIV negative: prematurity with aspiration, prematurity with pneumonia and unknown in 1 case each; for the remaining 6 infants with unknown HIV status: sepsis, severe diarrhea, sudden infant death syndrome and road accident in 1 infant each and unknown in 2 infants.*

FIG. 1 *Outcome of HIV-exposed infants enrolled in the study.*

TABLE II OUTCOME OF INFANTS STRATIFIED ACCORDING TO MATERNAL AND INFANT CHARACTERISTICS (*N*=155)

Characteristics	*Total*	*Alive at 18 mo (n=145)*		*Death*
		Infected (n=2)	*Not infected (n=143)*	*(n=10)*
Maternal ART/ARV at the time of delivery, n (%)				
On ART	123 (79)	2 (100)	114 (80)	7 (70)
On ARV prophylaxis	23 (15)	0	23 (16)	0
Not on ART/ARV or started after delivery	9 (6)	0	6 (4)	3 (30)
*ART duration, mo	6 (2.3-24)	17.5 (1-17.5)	6 (3-23.3)	2 (0.8-35)
#Vaginal delivery	70 (45)	2 (100)	60 (42)	8 (80)
Maternal CD4 count, cells/mm^3, mean (SD)	359 (191.9)	259 (55.2)	365.7 (196.1)	300 (142.9)
‡*Infant prophylaxis, n (%)*				
Single dose Nevirapine	40 (26)		37 (26)	3 (30)
Nevirapine for 6 wk	86 (55)	1 (50)	81 (57)	4 (40)
Nevirapine ≥12 wk	28 (18)	1 (50)	24 (16.8)	3 (30)
Birthweight (kg), mean (SD)	2.6 (0.5)	2.6 (0.6)	2.6 (0.4)	2.1 (0.7)
$*Feeding during first 6 mo, n (%)*				
Exclusive breastfeeding	82 (53)	2 (100)	76 (53)	4 (40)
Replacement feeding	71 (46)		65 (45)	6 (60)
**Maternal CD4 < 350 cells/mm^3, *n* (%)^	70 (56)	2 (100)	61 (53)	7 (78)

**Value in median (IQR); HIV-uninfected surviving infants and dead infants*; *P* values of #0.02 and **0.002; ^*Available for 126 infants including the 2 infected infants, 115 alive uninfected infants and 9 dead infants*; ‡*One infant did not receive prophylaxis;* $*two infant were on mixed feeding.*

WHAT THIS STUDY ADDS?

- Implementation of the current Prevention of parent-to-child transmission (PPTCT) strategy and a structured follow up of HIV-exposed infants results in an HIV-free survival matching that observed in more developed countries.

TABLE III DETAILS OF INFANTS DETECTED HIV-INFECTED

Case number	*Mother's details*		*Infant prophylaxis*	*Feeding (total duration)*	*Infant HIV testing*			*Outcome*
	Prophylaxis (duration before delivery)	*Pre-delivery CD4 counts (cells/mm^3)*			*6 wk*	*6 mo*	*18 mo*	
1	ART (2 y 10 mo)	220	Nevirapine for 6 wk	Breastfeeding (13 mo)	DBS* negative	DBS negative	Serology positive	Alive/ on ART
2	ART (1 mo)	298	Nevirapine for 12 wk	Breastfeeding (16 mo)	Not done	Serology negative	Serology positive	Alive/ on ART
3	ART (4 mo)	334	Single dose nevirapine	Replacement feeding	DBS positive	-	-	Died at 3 mo of age (severe sepsis)

**DBS: Dried blood sample.*

These studies were conducted during the time when most mothers received SDNVP, ART being limited only to those eligible as per their clinical/immunological criteria. In a recent study, where 37% of HIV-infected pregnant women received ART, and 63% SDNVP, Seenivasan, *et al*. [13] have reported a HIV transmission rate of 4%. Another study from this region, where 92% of enrolled women were getting either ARV prophylaxis or ART, a HIV transmission rate of 3.4% was reported [14]. The results of the current and these other recent studies from India show that with current robust PPTCT strategy, the HIV transmission rate in India is approaching the rate observed in developed countries (1-2%) [15,16].

We observed a 92.3% HIV-free survival at 18 months of age, similar to a recent study from Rwanda [17] that reported a 24-month HIV-free survival of 93.2% in breastfeeding infants of HIV-positive mothers on lifelong ART. A systematic review including 18 studies published between 2005 to 2015 provided a pooled estimate of 18-month HIV-free survival of 89.0% with 6 months ART and 96.1% with lifelong ART [18]. The authors found that the HIV-free survival, though higher in the breastfeeding group, did not significantly differ by feeding patterns. Similar findings were also observed in the present study. At 18 months, the prevalence of wasting and stunting among infants was no different from that reported among Indian children of this age group as per NFHS-4 [19].

Several reasons contribute towards better outcome of HIV-exposed infants in terms of survival, HIV transmission and nutritional status in the current as compared to our previous study [5]. Unlike the previous study, the present work excluded infants diagnosed as HIV-exposed/infected after birth. A much higher proportion of mothers were on ART (79%) as compared to the previous study (17.4%). Provision of a protocol-based care with focus on repeated counselling to optimize health of mother-infant dyad also contributed to the improved outcome.

Due to a small number of infants who acquired HIV infection or died, our results give limited information regarding predictors of HIV infection transmission/ mortality in HIV-exposed infants. As routine viral load was not introduced in the national protocol during this study period, maternal viral load, that directly impacts upon the HIV transmission rate, could not be assessed.

We conclude that implementation of the current PPTCT strategy, which includes lifelong ART to all HIV-infected pregnant and breastfeeding women with ARV prophylaxis to their infants, and a structured follow up of HIV-exposed infants, has remarkably improved the outcome of these infants.

Contributors: NB: managed the cases, recorded the information and drafted the paper; AS: conceptualized the paper, drafted and edited the manuscript and was the consultant in patient management. She will be the corresponding author for this work; SS: contributed towards design of the work, data analysis and manuscript preparation; GS: provided clinical care to study subjects, and contributed towards record keeping and manuscript preparation; PK, JC: consultants in patient management and helped in drafting /editing the paper. All

authors gave their final approval for the submitted manuscript. *Funding*: None; *Competing interest*: None stated.

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RESEARCH PAPER

Diagnostic Yield of Pneumococcal Antigen Detection in Cerebrospinal Fluid for Diagnosis of Pneumococcal Meningitis Among Children in China

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Objective: To determine the diagnostic accuracy of pneumococcal antigen detection in diagnosis of pneumococcal meningitis in children. **Methods:** Purulent meningitis was diagnosed according to European Society for Clinical Microbiology and Infectious Diseases (ESCMID) guideline between July 2014 and June 2016. Along with a cerebrospinal fluid (CSF) culture, pneumococcal antigen detection in cerebrospinal fluid (CSF) was performed, and further identification of pathogens was done with 16S rDNA-PCR and high-throughput sequencing. **Results:** CSF samples collected from 184 children (median age of 1.92 mo). CSF culture was used as the gold standard. 46 (25%) had positive results for culture and 10 (5.4%) were pneumococci; 34 (18.5%) were pneumococcal antigen positive. The sensitivity and specificity of pneumococcal antigen detection were 100% (95% CI: 89.4%–100%) and 86.2% (95% CI: 96.4%–99.9%), respectively. 92.3% (12/13) were confirmed by nucleic acid detection to be pneumococci. **Conclusions:** Pneumococcal antigen detection in CSF has adequate sensitivity and specificity in diagnosing pneumococcal meningitis.

Keywords: *Etiology, Rapid diagnosis, Sensitivity, Specificity*.

Pneumococcal meningitis is a life-threatening disease with high incidence and case fatality rate (CFR) [1-2]. During 2000-15, the global incidence rate of PnM was 13/100,000 and CFR was 44%, and the burden was more in developing countries [1]. Early use of sensitive antibiotics is extremely important for improving its prognosis [3], which depends greatly on rapid etiological diagnosis. Usually, clinicians depend mainly on the cerebrospinal fluid (CSF) culture, which is time-consuming and can only detect the live bacteria in the specimen. The detection of the pneumococcal antigen or nucleic acid can improve the diagnosis of PnM [4-7]. Testing for pneumococcal urinary antigen helped identifying pneumococci as pathogen in patients with invasive pneumococcal diseases [4,6]; Immunochromatographic antigen test for the detection of pneumococci had high sensitivity and specificity in CSF samples from children with suspected bacterial meningitis [5]. However, till recently, testing for pneumococcal antigen in CSF was not available in China. The objective of this study was to determine the diagnostic accuracy of pneumococcal antigen detection in diagnosis of pneumococcal meningitis in children.

METHODS

We enrolled patients with purulent meningitis and hospitalized at our hospital between July 31, 2014 and June 30, 2016 after approval from institutional ethics committee. The inclusion criteria included: (*i*) The clinical characteristics including irritability, poor feeding, respiratory distress, marbling of skin and hyper- or hypotonia in neonates or very young babies [8]; fever, seizures, fontanell bulge, neck stiffness and vomiting in infants; and headache accompanied by fever in old children [8]; and (*ii*) mainly polymorphic leukocytes in CSF, elevated protein level, low glucose concentration, low CSF to blood glucose ratio [8]. Patients who had blood-tinged CSF that may affect the test results were excluded. From each patients, 4-5 mL CSF specimens were collected and divided in three portions: 1.0-1.5 mL for culture, 1-1.5 mL for cytology, and 1.5-2 mL for biochemistry, pneumococcal antigen detection and PCR. Microorganism identification and antimicrobial susceptibility test were performed by using the Vitek system (Mérieux, France). Pneumococcal antigen was detected by using the BinaxNOW Streptococcus pneumonia antigen detection kit (Alere, ME, USA).

Bacterial DNA was extracted from CSF, and bacterial 16S rDNA V3-V4 region was amplified by PCR using primer pairs: 341F: CCT AYG GGR BGC ASC AG and 806R: GGA CTA CNN GGG TAT CTA AT. The PCR products with sufficient quantity were

collected and purified. An OTU clustering analysis was carried out after high-throughput sequencing. The results on culture and pneumococcal antigen detection in CSF in patients with or without previous antibiotics were compared.

Statistical analysis: The collected data were compared using the chi-square test. $P<0.05$ was considered to be of statistical significance. Diagnostic accuracy testing was described by calculating sensitivity and specificity.

RESULTS

In this study, CSF samples were collected from 184 patients (36.4% neonates), aged from 1 day to 13 years and 8 months (median age of 1.92 months). Only 46 (25%) had positive culture results; with isolated bacteria being *Escherichia coli* (15 isolates), pneumococci (10 isolates), *Streptococcus agalactiae* (7 isolates), by *Staphylococcus aureus* (3 isolates), *Enterococcus faecium* (3 isolates, *Candida famata* was isolated in one of them), *Streptococcus mitis* (2 isolates), *Listeria monocytogenes* (2 isolates), *Streptococcus sanguis* (1 isolate), *Enterobacter cloacae* (1 isolate), *Haemophilus influenzae* (1 isolate), *Acinetobacter baumannii* (1 isolate), and *C. famata* (1 isolate). Fewer positive culture results were found in patients who had received previous antibiotics when compared with those who had not ($P<0.001$); but this difference was not seen for pneumococcal culture ($P=0.08$).

Pneumococcal antigen was tested positive in 34 specimens (18.5%), which included these 10 positive pneumococci cultures. No difference in the positivity rate of antigen detection was found between those with history of previous antibiotics and those without previous antibiotics ($P=0.09$) (***Table* I**). 46 (25%) had positive results for culture and 10 (5.4%) were pneumococci; 34 (18.5%) were pneumococcal antigen positive. The sensitivity and specificity of pneumococcal antigen detection were 100% (95% CI: 89.4%-100%) and 86.2% (95% CI: 96.4%-99.9%), respectively (***Table* II**).

Twenty-one CSF specimens were selected for 16s rDNA-PCR product sequence analysis. Among these were 13 positive and 8 negative for pneumococcal antigen testing. The distribution of the bacteria at the species level based on OTU is shown in ***Fig*. 1**. In one case with positive pneumococcal antigen, it was also positive for *E. faecium* and *C. famata* in the CSF culture and the abundance of pneumococcal OTU was low. As the sample with many species of OTU may be contaminated, pneumococcal infection could not be confirmed in this case.

Finally, thirty-three patients were diagnosed with PnM based on the combination result of pneumococcal antigen detection and PCR [median (range) age: (2 mo 16 d-9 y 11 mo) 11.1 mo]. Thirty (90.9%) were under the age of 5 years old, and one was a newborn (3%) 19 were boys. Twenty-eight were treated with β-lactams or β-lactams and other antibiotics in combination for 1-27 days (median: 3 days) before they received lumbar puncture. All of the 10 pneumococcal isolates were resistant to penicillin and erythromycin but were sensitive to ceftriaxone and vancomycin or linezolid. After admission to hospital, all 33 patients were treated with β-lactams antibiotics for 8-43 days (median: 18 days), including 81.8% (27/33) who received another antibiotic in combination (24 with vancomycin and 3 with linezolid). Thirty-one patients (90.9%) were cured and the incidence of complications was 27.3% (9/33). Two children did not survive (2/33, 6.1%).

DISCUSSION

The pneumococcal antigen test is a rapid diagnosis method in the diagnosis of pneumococcal meningitis [7,10-11]; Its advantage is the simplicity, rapidity, and usefulness in cases that have already received prior antibiotics. The sensitivity and specificity in our study were high, which was in accordance with the results from previous studies that have evaluated the diagnostic accuracy by detecting pneumococcal antigen in CSF specimens [7,9,10]. In the present study, 72.7% of all (24/

TABLE I PNEUMOCOCCAL ANTIGEN DETECTION IN CEREBROSPINAL FLUID OF PATIENTS WITH OR WITHOUT PREVIOUS ANTIBIOTICS (N=184)

	With previous antibiotics (n=136)	*Without previous antibiotics* (n=48)
All bacteria		
Culture positive	25 (18.4)	21 (43.8)
Culture negative	111 (81.6)	27 (56.2)
Pneumococcus		
Culture positive	5 (3.7)	5 (10.4)
Culture negative	131 (96.3)	43 (89.6)
Antigen positive	29 (21.3)	5 (10.4)
Antigen negative	107 (78.7)	43 (89.6)

TABLE II PNEUMOCOCCAL ANTIGEN DETECTION IN CEREBROSPINAL FLUID IN PATIENTS WITH SUSPECTED PNEUMOCOCCAL MENINGITIS

	Positive (n=10)	*Negative (n=174)*
Antigen positive, *n* (%)	10 (100)	24 (13.8)
Antigen negative, *n* (%)	0	150 (86.2)

WHAT THIS STUDY ADDS?

- Pneumococcal antigen detection in the cerebrospinal fluid has adequate sensitivity and specificity in diagnosing pneumococcal meningitis.

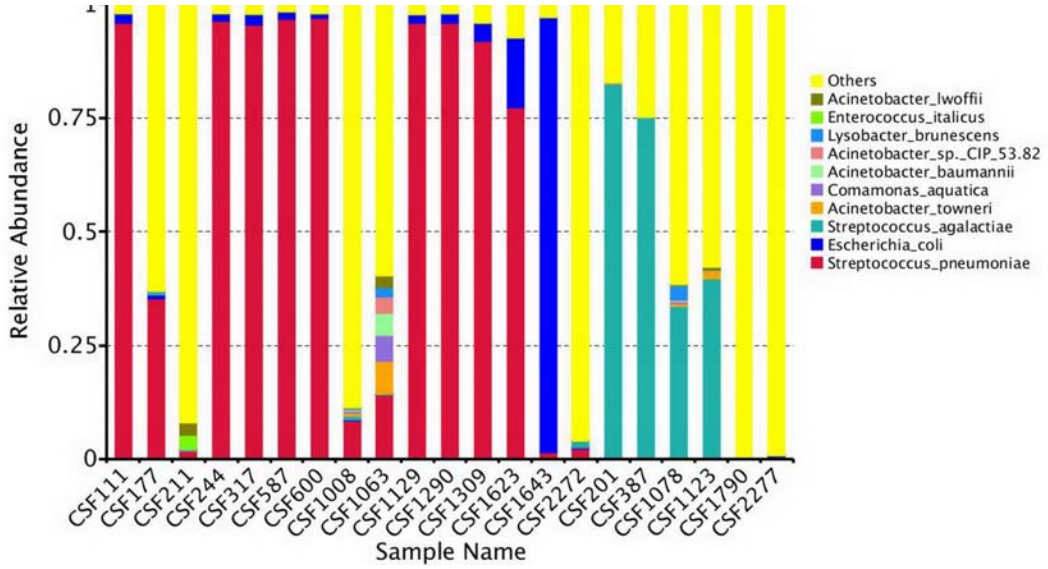

FIG. 1 *The distribution of bacterial species in 21 CSF samples by 16S rDNA-PCR high-throughput sequencing and OTU clustering.*

33) patients with pneumococcal meningitis were missed when the diagnosis was based on the CSF culture, which was mainly attributed to the fact that a majority of these patients had received antibiotics before the sampling. The introduction of the pneumococcal antigen test significantly improved the diagnosis of pneumococcal meningitis in our study. One advantage of pneumococcal antigen test is that the pneumococcal antigen might degrade slowly; it usually persists *in vivo* until 7 days (90%) to 4-6 weeks (40-48%) after recovery [11,12].

The pneumococcal antigen test was confirmed by PCR as a method with high accuracy. Bacterial DNAs are still detectable by PCR within several months after being killed by antibiotics; therefore, the diagnosis of pathogen based on the pneumococcal antigen and nucleic acid detection should be suggested in conjunction with clinical manifestations. However, both positive results of pneumococcal antigen detection and nucleic acid detection only provide the evidence of pneumococcal infection, rather than ongoing infection. There was one case positive for pneumococcal antigen testing but also positive for *E. faecium* and *C. famata* in the CSF culture. As contamination by *E. faecium* may lead to a false positive result of the pneumococcal antigen test [6] and the abundance of penumococcal DNA was not high, the exact pathogen in this case could not be determined and was not considered as PnM.

There are certain limitations in this study. The CSF specimen used for antigen detection and PCR were the same for biochemical tests and thus had a certain risk of contamination. The 16S rDNA sequencing could not be performed in all samples because of the inadequate CSF volume, which may cause biased results. Further studies are needed to confirm our conclusion with more patients.

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Contributors: YPX: collecting of clinical data; PCR of 16S rDNA; test the pneumococcal antigen; analysis and interpretation of data; drafting the article; CZH: design of the study; diagnosis of meningitis; analysis on 16S rDNA OTU; revising the article critically for important intellectual content; HJW: diagnosis of meningitis; collecting of cerebrospinal fluid (CSF) and clinical data; ANS: isolation and identification of the bacteria; drug-sensitivity test. JS: acquisition of consent and collecting specimen from the patients, collection and analysis of the clinical data, revising the article.
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Reporting of Basic Statistical Methods in Biomedical Journals: Improved SAMPL Guidelines

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Statistical methods have become an essential component of all empirical biomedical research. Science requires that these methods are fully reported with complete accuracy so that the evidence base could be fully appraised for validity, reliability, and generalizability. To meet this objective, Statistical Analyses and Methods in Published Literature (SAMPL) guidelines have been prepared for statistical reporting in biomedical publications. This communication proposes substantial improvement of these guidelines to make them more comprehensive, organized, compact, and easier to adopt.

Keywords: *Basic statistics, Guidelines, Statistical errors, Survival analysis.*

Reporting of research is done to apprise others of the new development. This objective is more effectively achieved when the communication contains enough details of the methodology and all other aspects so that the reader is convinced about the validity of the results, can assess their generalizability, and is able to replicate the results if needed.

Statistical methods have become an essential component of all empirical research publications, more so for biomedical research that confronts enormous uncertainties due to biological and environmental variability, sampling fluctuations, epistemic bottlenecks, and biases. Science requires that these methods are fully reported with complete accuracy so that the results could be fully appraised for validity, reliability and generalizability, and evidence-based medicine is strengthened. To meet this objective, Statistical Analyses and Methods in Published Literature (SAMPL) guidelines [1] have been prepared for statistical reporting in biomedical publications. However, these guidelines have some lacunae. For example, these guidelines mentioned about identifying the variables separately for Primary Analysis, for Reporting Hypothesis Tests, for Reporting Association Analysis, for Reporting Regression Analysis, and several others. Some of the essentials such as comparability of groups and robustness have been missed. These guidelines need to be reorganized on the lines of other reporting guidelines such as CONSORT. This communication proposes substantial improvement of these guidelines to make them more organized, compact, and easier to adopt.

ERRORS IN MEDICAL RESEARCH

Errors commonly creep into medical research endeavors, sometimes leading to false results [2-7]. Ioannidis [3] has expressed near inevitability of some false conclusions and has suggested designs to increase the chances of producing true results. *PLoS Medicine* editors [4] have opined that those involved in publication of research must make all efforts to reduce the chance of false conclusions. While some of this malaise can be attributed to the inappropriate methodology and questionable practices used in empirical research [5], some can be traced to poor reporting [7] that can happen even with otherwise good quality research. These deficiencies often render published results unusable [1,8]. Guidelines such as CONSORT, STROBE and STARD [9] have been developed for improved reporting of medical studies with different designs in the hope that adhering to these guidelines would reduce the chance of occurrence of these errors.

Statistical Errors

Many of the research errors are statistical in nature such as in design, elicitation of data, their processing and analysis, and the interpretation of the results [10-15]. Altman and Bland [16] in 1991 estimated that more than 50% papers at that time had some statistical errors and Wullschleger, *et al*. [17] found 64% (of a total 441) articles published in 2012 in three prime cardiovascular journals had inappropriate use of standard error of mean. Such errors often go unnoticed by the readers [18]. Sometimes, these errors can result in a statements that can jeopardize life and health of many people in course of time when

inadequately substantiated result is used to treat millions of patients [10]. This is accentuated when the future research is built on the existing inadequately proven results. Techniques to avoid such statistical errors have been described earlier [19,20].

Performing the appropriate analysis is different from accurately describing it, and there is no way for a third person to assess what was actually adopted except by reporting in the publications. It is expected that much of these errors can be avoided by improved statistical reporting.

STATISTICAL REPORTING

Statistical reporting in biomedical publication is an important part of the Material and Methods section but it also affects the way the results are understood and interpreted. Several studies have observed that the statistical reporting in some biomedical publications is inadequate [11-14]. These studies suggest that this inadequacy generally occurs at three levels: (*i*) incomplete reporting leaving out room for readers to impute guess: (*ii*) willful or inadvertent erroneous reporting that has potential to arouse suspicion about the results; (*iii*) and inadequate interpretation of the statistical results. Much of this deficiency can be effectively addressed if the publications adhere to a standard guideline of items for reporting of the statistical methodology so that it is fully reported in a proper manner without missing any essential component. This may also encourage researchers to use the right statistical methods at various stages of their research.

Much of the clarity in reporting comes from clear statements about how the data were collected; what analysis was done how; why that particular analysis was appropriate for the problem in hand; and how the conclusion was drawn. Statistical methods in an empirical research can be intricate multivariate and multilevel analyses or can be specialized such as time series analysis whose description is admittedly challenging, but many errors have been observed in basic methods used in biomedical publications [21]. As these are basic methods, there is a tendency to use and describe them without sufficient care [22]. The proposed guidelines are focused on these basic methods only.

Guidelines for Statistical Reporting

In view of the common occurrence of statistical errors in biomedical publications, attempts have been made in the past to present guidelines for statistical reporting [17,23,24]. Subsequently, Lang and Altman [1] compiled a set of guidelines for basic statistical reporting for articles published in biomedical journals. They called it "Statistical Analyses and Methods in the Published Literature" or the SAMPL Guidelines, and these are now part of the EQUATOR network [9]. The authors acknowledge that these guidelines are limited to the basic methods but consider them sufficient to prevent most of the reporting deficiencies as the basic methods are also the most commonly used methods. The first guiding principle for these guidelines is that the statistical methods should be described with sufficient detail for a knowledgeable reader to verify the reported results if the data are provided to him, and the second principle is to report enough details of the descriptive statistics from which other indicators such as relative risk and odds ratio are derived.

Besides that the current SAMPL guidelines have not included some of the basic methods such as comparability of groups and robustness of results,these are also repititive. They also need to be reorganized in a compact form just as are other statements such as CONSORT, STROBE and STARD. These statements have been revised from time to time as and when new knowledge is acquired and it is time to revise the SAMPL guidelines as well to make them more organized, compact and easy to adopt. We have undertaken this exercise and the guidelines have been substantially revised in content and format (***Table* I**). Most notable change is the complete reorganization of format to a numbered list for easy adoption. This also removes much of duplication. Other notable changes are inclusion of background information of the subjects, reporting of standardized rates (where needed) for comparability, robustness of results, not reporting mean and SD for extremely small sample size, and careful reporting of cause-effect inference. There are several other changes to make the guidelines more comprehensive and easy to understand. The reorganization is in terms of a list with 16 items, many with sub-items, which can also be used as a checklist. First 13 items will be required by almost any biomedical publication based on empirical data and the remaining 3 items are for specialized methods. To avoid duplication, there is no separate item on ANOVA and ANCOVA as reporting of these is included in other items. Bayesian analysis is also excluded as it is not a commonly used method in biomedical publications. Hazard ratio is excluded because of its specialized nature. Now, there is a clear demarcation of items to be reported for each analysis undertaken by the researcher although we continue to adhere to the principles enunciated earlier [1].

This revision is also restricted to the reporting of the basic methods. The advanced methods such as Cox regression, cluster analysis, and multivariate analysis of variance (MANOVA) are excluded in the hope that a qualified biostatistician will be involved when such

TABLE I IMPROVED SAMPL GUIDELINES FOR REPORTING BASIC STATISTICAL METHODS IN BIOMEDICAL PUBLICATIONS

Topic	*No.*	*Item*
Subjects under study	1	Identify the target population, state the method of selection of the sample, total sample size, stratification if any, and the groups under study.
Sample size	2a	State the sample size for each group and justify the size for the stated precision, alpha error, and/or power. For power, specify the smallest effect size considered medically important with reasons.
	2b	State the number of missing values, outliers and other exclusions with reasons, comment on the representativeness of the sample finally available for analysis, and describe possible biases with measures taken to control them.
Hypothesis	3a	State all the hypotheses keeping the study objectives in mind.
	3b	State the minimum effect size to be considered as medically important, if applicable, with its rationale (see Item 1b). For equivalence and non-inferiority studies, give the largest medically unimportant margin with reasons.
Variables under study	4a	State all the variables on which the data were collected and identify the ones on which the present analysis was done along with the rationale of the choice of variables. State the unit of measurement of each, and describe the validity of the methods of measurement for each variable.
	4b	Categorize continuous data for presentation of distribution if needed. If helpful, give histogram and comment on the distribution pattern, particularly of the outcome variables.
	4c	If dichotomous or polytomous categories have been used in analysis of continuous variables, explain the rationale of these categories in terms of clinical implication.
Antecedents and outcomes	5a	In the case of analytical studies, identify the antecedent factors under study, the outcomes of interest, and the covariates included.
	5b	Define the effect of interest in terms of the variables included in the study (the effect size can be difference between means or between proportions, odds ratio, correlation coefficient, phi coefficient, or any other measure).
Descriptive summaries	6	Summarize the data –Provide mean (SD) (and not mean ± SD) or median (IQR) of each continuous variable depending upon the Gaussian or (highly) skewed distribution, respectively (do not use SE here). For IQR, give the values of the first and third quartile. Do not give such summaries for groups with $n \leq 4$; give the original values instead. For categorical data, state actual frequency in different categories and the percentage if $n \geq 20$. All summaries should be with the appropriate degree of decimal accuracy as specified at the end of these guidelines*.
Modification of raw data	7	Describe transformation such as log and square-root, if any, with reasons and the method of calculation of scores, and rates and ratios, and fully specify the numerator, denominator and multiplier (per cent, per million, *etc*.) for each where applicable. For rates, specify the time period (per day, per year, *etc*.).
Baseline information	8	Summarize all important demographic and clinical features of the subjects in each group, particularly those that can affect the outcome (see Item 6).
Comparability of two or more groups	9	Before comparing two or more groups with respect to outcomes in terms of summaries such as means, proportions in different categories, and rates, confirm that the groups are comparable with regard to the baseline composition of the subjects for factors (such as the age distribution) that can affect the outcome. If not comparable, report the re-computed summaries after proper standardization. If standardization required but not done, state reasons and explain how the outcomes in various groups can still be compared.
Main method of analysis	10a	Describe the method for each analysis, confirm the validity of the underlying assumptions, and justify the parametric and non-parametric methods used for different variables. Provide reference or explain the methods not in common use. State the software used for analysis with version.
	10b	Identify post-hoc analysis if any, including sub-groups analysis, and interpret this as exploratory and not confirmatory.
Estimation	11	For descriptive part of the study, provide estimate of the mean, proportion, difference, *etc*. with 95% confidence interval (CI). Justify the Gaussian approximation in case this is used for computing the CI. In case any other confidence level is used, provide the rationale.

Contd...

TABLE I *(continued)*

Topic	*No.*	*Item*
Tests of statistical hypothesis	12a	State the statistical hypothesis for each test. Give the name of each test and its exact *P*-value with df where relevant. For $P<0.001$, state with less than sign and for $P>0.999$ with more than sign. Indicate whether the test is one-tailed or two-tailed with the reasons thereof. Avoid the use of the term statistical significance and do not mention significance level (such as $\alpha = 0.05$) for your results. Mention about any adjustment made for multiple comparisons and for using multiple tests for any conclusion. Distinguish between family-wise error rate and experiment-wise error rate. Also mention the CI for the effect size such as mean difference between the groups.
	12b	Report all the results and not just those that have low *P*-value. Interpret larger *P*-value as inconclusive and not as negative result unless the power is high to detect a specified medically important effect. Distinguish between results with low *P*-value (conventional statistical significance) and medical significance of the results.
Robustness of results	13	Comment about the statistical limitations of the study in addition to the other limita-tions. Statistical limitations could be due to imprecision of the measurements, restricted analysis because of the nature of the data or size of sample in different groups, not fulfilling the underlying assumptions, lack of representativeness of the sample, compromised design, lack of internal or external validations, and such other deficiencies.
The following are needed if these methods have been used in your paper		
Correlation and cause-effect	14a	Report the value of the relevant correlation coefficient. If described as low, moderate or high, give the categories with their biological implications. Interpret conventional Pearson correlation coefficient for assessing linear relationship and not for any general relationship between continuous variables. For association between categorical variables, include the full contingency table and explain if any categories were merged for analysis purpose.
	14b	Distinguish between association/correlation and cause-effect. If cause-effect is implied, rule out all possible alternative explanations such as the role of confounders and biases.
	14c	Distinguish correlation/association from agreement.
Regression analysis	15a	Describe the purpose of the regression analysis (explanatory or predictive), identify the response (outcome) and regressor (antecedent) variables with the selection process if any, assess colinearity between independent variables, and provide medical and statistical rationale of the chosen model (linear/nonlinear, simple/multivariable). State the size of sample available for running each regression and comment on its adequacy. In case the model is being used for prediction of individual values, give prediction interval and not the CI for mean. Do not predict for values much beyond the values actually studied.
	15b	Report the regression equation with comments on its adequacy based on indicators such as coefficient of determination (η^2, whose linear component is R^2) for quantitative and generalized R^2 for logistic regression, and report exact *P*-value for each regression coefficient with the associated CI. For quantitative dependent in simple linear or curvilinear regression, plot the regression line or curve with scatter where helpful and comment on the randomness of the residuals. For logistic regression, specify the reference category for categorical regressors, give odds ratio (OR) and the CI for each variable – adjusted as well as unadjusted. For cohort studies, state the number of subjects with positive and negative outcomes, and the relative risk with their CI – again adjusted as well as unadjusted. In the case of multivariable regression, interpret regression coefficient as adjusted only for the other variables in the model and give plausible biological explanation of the model obtained.
	15c	Specify whether and how the model was validated, or why it could not be validated.
Survival analysis	16a	Describe the purpose of the survival analysis, identify the beginning- and the end-point for the duration under study, specify censoring, name the survival analysis method with the confirmation of the assumptions, plot the survival curve and report the median survival time with the CI, and discuss the points of inflexion in the survival curve, if relevant.
	16b	Where helpful, give the table with the estimated survival probability at each follow-up with the CI.

Contd...

TABLE I *(continued)*

Topic	*No.*	*Item*
	16c	Specify the method used for comparing two or more survival curves if applicable and give exact *P*-value. Interpret it for overall survival pattern and not for specific time-points.

Decimal accuracy (rounded) as follows

Percentages - One decimal place if n <100 and two decimal places for n ≥100;
Mean and SD (Median and IQR) - One decimal place more than the original values;
Correlation coefficient - Generally two decimal places;
Odds ratio, relative risk and hazard ratio - Generally two decimal places;
P-values - Exact P-values to three decimal places and not as P <0.05 or P ≥0.05 (For extremely small values, write P <0.001, and for extremely high values, write P >0.999).

advanced methods are used and the reporting will be adequate. The basic methods covered by these guidelines are generally used by those also who use advanced methods. To retain the focus, other methodological aspects such as design, allocation and randomization as well as issues relating to proper graphs, diagrams and tables are excluded. These suggested guidelines continue to be described in a manner that a statistically literate medical researcher can adopt without much help of a statistician. As in the case of original version [1], this suggested revision too is not prepared by a 'formal consensus-building process' but is prepared after consulting various other guidelines [24-27].

CONCLUSION

We hope that the editors of the biomedical journals will incorporate these guidelines in their instructions so that the reporting of basic statistical methods can improve and evidence-based results are reported. The real solution to poor statistical reporting will come when authors and statisticians learn more about research methodology and appropriate analysis, and also learn to communicate it properly [11]. Deficient statistical reporting underscores the need to expose the medical researchers to detailed texts [28,29] and structured biostatistics courses so that the methodology and reporting can improve.

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REVIEW ARTICLE

Newborn Screening and Diagnosis of Infants with Congenital Adrenal Hyperplasia

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Congenital adrenal hyperplasia (CAH) is an autosomal recessive endocrine disorder which can manifest after birth with ambiguous genitalia and salt-wasting crisis. However, genital ambiguity is not seen in male babies and may be mild in female babies, leading to a missed diagnosis of classical CAH at birth. In this review, we provide a standard operating protocol for routine newborn screening for CAH in Indian settings. A standardization of first tier screening tests with a single consistent set of cut-off values stratified by gestational age is also suggested. The protocol also recommends a two-tier protocol of initial immunoassay/time resolved fluoroimmunoassay followed by liquid chromatography tandem mass spectrometry for confirmation of screen positive babies, wherever feasible. Routine molecular and genetic testing is not essential for establishing the diagnosis in all screen positive babies, but has significant utility in prenatal diagnosis and genetic counseling for future pregnancy.

Keywords: *17OHP, Cortisol, Fluoroimmunoassay, Tandem mass spectrometry*.

Congenital Adrenal Hyperplasia (CAH) is an autosomal recessive disorder with an incidence ranging from 1:10,000 to 1:20,000 births [1].The screen positive rate of CAH among a cohort of 104,066 babies screened at birth in India was 1 in 5762 as per a recent report [2].The most common defect in CAH is deficiency of enzyme 21-hydroxylase caused by mutation in *CYP21A2* gene, which comprises about 95% of all forms of CAH. Inadequate cortisol leads to an increase of ACTH which further stimulates adrenals, resulting in hyperplasia. A defective corticosteroid and mineralocorticoid enzymatic pathway shunts the steroid precursors to alternate derivatives like androgens and sex hormones.

The classical variety of CAH presents early as genital ambiguity in newborn females (due to excess sex hormones and their derivatives) or as adrenal crisis in both boys and girls. Adrenal crisis is characterized by insufficient corticosteroid and aldosterone production which causes hyponatremic dehydration and shock (salt wasting type). Patients with adequate aldosterone production without salt wasting who have signs of prenatal virilization are termed as simple virilizers classified under classical CAH. A mild non-classical form (NCCAH) of the disorder is also recognized in which presentation is in adolescence or later.

We herein provide guidance, in the Indian context, for diagnosis and referral of babies in early infancy with classical forms of CAH with 21-hydroxylase deficiency.

METHODS

A 2017 group of experts in the field of pediatric endocrinology and newborn screening (Delhi Pediatric Endocrinology Newborn Screening group- DePENS) used a semi-structured search strategy for preparing this review. The primary database used to search information was Medline through PubMed. The search was performed in September 2017 and updated till January 2019. Both MeSH and keyword based inputs were searched for articles pertaining to diagnosis and management of classical CAH with 21-hydroxylase deficiency in childhood. Systematic reviews, meta-analysis and randomized controlled trials were given priority. Articles pertaining to the management of CAH in adulthood were not included.

NEWBORN SCREENING

Incorporation of screening for 21-hydroxylase deficiency in to all newborn screening programs is recommended, wherever feasible.

Neonatal CAH is a disease which satisfies all the criteria

under newborn screening (NBS) checklist proposed by Wilson and Jungner [3]. NBS can help in early diagnosis, timely treatment and correct gender assignment of babies with classicalCAH [1,4]. Male babies with classical CAH may go undetected in the absence of genital ambiguity. Institution of specific steroid therapy can be life-saving in babies with salt-losing CAH where adrenal crisis may be misdiagnosed as sepsis. In addition, NBS can recognize simple virilizing forms in male newborn who would otherwise present later in childhood with features of precocious puberty. The final height of affected boys may be significantly compromised by that time due to advanced epiphyseal maturation [5]. However, NBS may not detect non-classical forms consistently when performed at birth [4].

The prevalence rate of CAH has shown an increase in the post-screening era. Sweden reported an increase in prevalence of salt-wasting CAH from 1 in 18,600 (1969-1986) to 1 in 12,800 (from 1989-1994) after introduction of NBS [6]. The incidence of CAH reported from Australia and Italy is variable from 1 in 15,488 or 18,105 births (in screened population) to 1 in 18,034 or 25,462 births (in unscreened population) [7,8].The incidence of screen positive CAH among cohort of 104,066 babies screened at birth in India was 1 in 5762 as per a recent report. There were marked regional differences with highest from Chennai (1:2036) to lowest from Mumbai (1:9983). The incidence of salt-wasting CAH was higher (1 in 6934) than simple virilizing type (1 in 20,801) [2]. Another study done on a cohort of 18,300 newborn in Andhra Pradesh showed an incidence of 1 in 2600.The screen positives were confirmed on recall in this study [9]. A prospective study on 11,200 newborns from Bangalore from 2007 to 2013showed a similar incidence of 1:2800 (confirmed cases) [10].

The mortality rate in CAH varies from 0-4% in unscreened cohort [6]. NBSwill reduce time to diagnosis, duration of hospitalization, severity of clinical manifestations, diagnostic uncertainty and reduce mortality in cases of salt wasting crisis. The importance of NBS to save lives in ethnic populations with high prevalence where timely clinical diagnosis is infrequent and CAH related deaths occur frequently, is undoubted. Thus, incorporation of screening for CAH should be considered as a component of NBS programme.

Standardization of first-tier screening tests with a single consistent set of cut-off values stratified by gestational age is recommended

It is recommended that first-tier screens for CAH employ fluoroimmunoassay to measure 17- hydroxy progesterone (17-OHP) in dried blood spots by heel prick methodon the same filter paper cards as are used for other tests in NBS [1].The use of cord blood is not recommended as the level of 17-OHP is significantly high immediately after birth [11]. Fluoroimmunoassay has supplanted radioimmunoassay and ELISA in most NBS programs [5,12]. It is recommended that the sample should be obtained between 24 to 72 hours of life as 17-OHP levels are normally high at birth and decrease rapidly in the first few postnatal days. Though in order to decrease the false positive rate it would be ideal to collect samples after 72 hours of birth, high birth rates necessitate screening after 24 hours or day 2 of life. Accessible births in the rural setting as collected by paramedical health workers maybe as delayed till 7 days. Hence it may be practical to collect samples between 24 hours and 7 days of life. In contrast, 17-OHP levels increase with time in affected neonates [4]. This makes diagnostic accuracy questionable in the first couple of days which can be an issue if newborns are discharged early. 17-OHP levels are higher in preterm, sick and stressed babies [4,12]. The cutoff values used should be adjusted for these factors to reduce recall rate. The combined use of gestational age and birth weight significantly improved predictive value in NBS for CAH [13]. However, 17-OHP, values correlate better with the gestational age rather than birthweight. Newborn screening programs in Switzerland and Netherlands have adopted the gestational age cut-offs which have improved the positive predictive value of screening [14,15]. The authors recommend the use of the gestation specific cut-offs (whole blood units in nmol/L) for Indian newborns as shown in ***Table* I,** which are based on data collected from a large multicentric study from Delhi

TABLE I GESTATIOAL AGE AND BIRTHWEIGHT-BASED CUT-OFFS FOR BLOOD LEVELS OF 17-HYDROXY PROGESTERONE FOR NEWBORN SCREENING FOR CONGENITAL ADRENAL HYPERPLASIA

Gestational age (completed wk)	*Birthweight < 2500 g*	*Birthweight ≥2500 g*
≤32 wk	81	51
33-36 wk	42	37.5
≥37 wk	37.5	37.5
Birthweight	*Preterm (<37 wk)*	*Term (≥37 wk)*
<1000 g	189	153
1000-1499 g	82	71
1500-2499 g	42	37.5
≥2500 g	37.5	37.5

Blood values when performed between 2-7th day of life; all values in nmoL/L (convert nmol/L to ng/mL by multiplying by 0.66).

[8].The use of birthweight-based cut-offs should be done only when accurate gestational age assessment by first trimester ultrasonography or record of last menstrual period is not available.

Blood 17-OHP values are considered borderline between 37.5-90 nmol/L and positive beyond 90 nmol/L, as per the fluoroimmunoassay kit-cut off values [2].The newborn screening programs in every country adopts its own cut-off value based on their population study. Cut-offs based on weight and gestational age are given in ***Table* I**. A multiplication factor of 0.66 with whole blood units (in nmol/L) may be used to obtain serum units (in ng/mL) of 17-OHP cut-offs [2].

It is recommended that infants whose mothers received antenatal corticosteroid treatment be retested after 2 weeks or at discharge, whichever is later

Antenatal corticosteroids used in preterm deliveries to facilitate fetal pulmonary maturation carry higher chances of interfering with CAH screening results as corticosteroids can cross the placenta and suppress the fetal hypothalamic pituitary axis. This may reduce the blood spot 17-OHP level thus leading to false negative result when performed at discharge or within 72 hours of birth. A reduction in serum 17OHP to upto 30% was seen after multiple courses of steroids [16], while inconsistent results have been obtained across otherstudies with a single course of steroids [17]. Thus, history of all institutional deliveries should be reviewed specially of the preterm babies for history of antenatal steroids. It is recommended that such infants (term or preterm) should be retested after two weeks of life, provided the baby is monitored carefully between the two screenings for salt losses and has easy access to health care services.For preterm babies, the cut-off should correspond to the cut-off for the corrected gestational age at two weeks, while for term babies the cut-off remains the same.

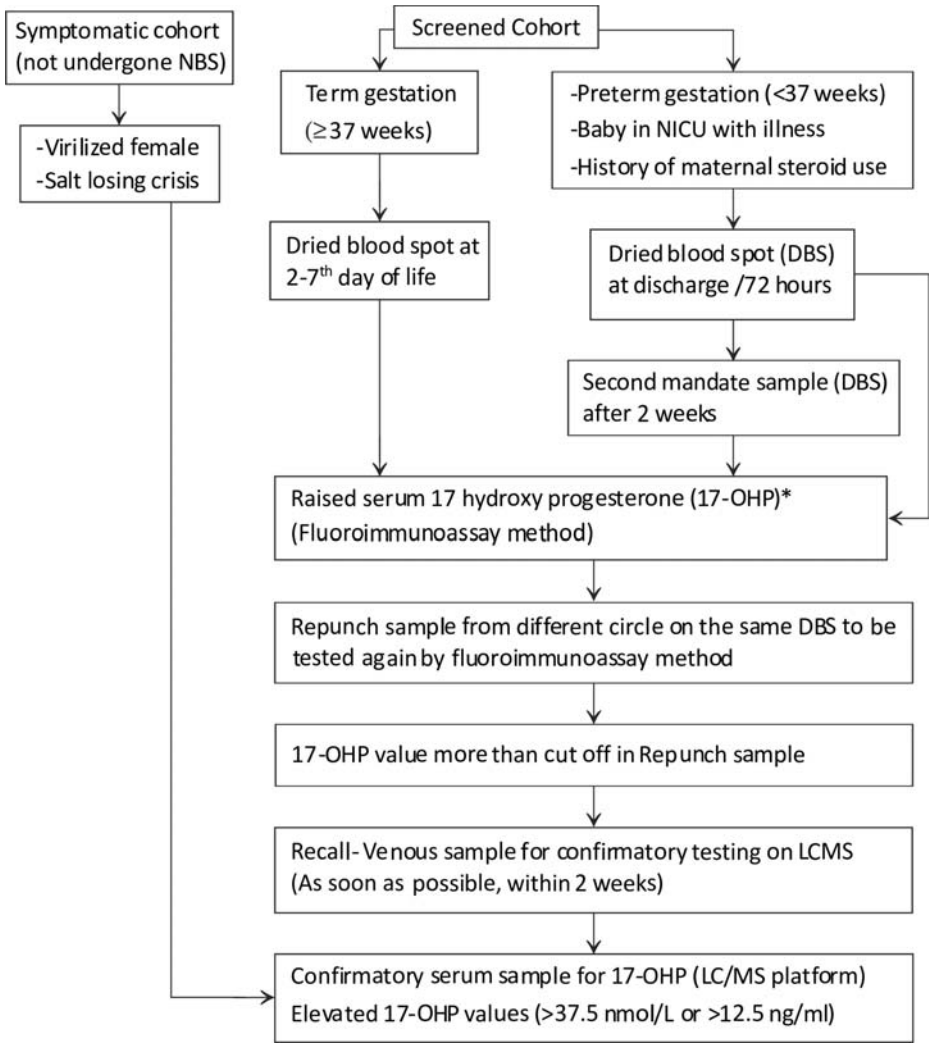
A two tier protocol (initial time resolved fluoroimmuno-assay with evaluation of positive tests by liquid chroma-tography-tandem mass spectrometry) is recommended for confirmation of all babies tested screen positive

The first sample for NBS should be collected on DBS after 24 hours till 72 hours of life to be processed by an initial fluoroimmunoassay. Babies admitted in intensive care, preterms and those whose mothers have received antenatal steroids should also have a mandatory repeat DBS tested after 2 weeks. In borderline and positive cases, a second repunch sample from a different circle on the same DBS is analyzed by fluoroimmunoassay (Repunch). In case the repunch spot also tests positive, the baby should be recalled immediately for a repeat venous blood sample for the second tier confirmatory testing, which is done by liquid chromatography-tandem mass spectrometry (LC-MS) (***Fig.* 1).** LC/MS is a diagnostic test for confirming the screen positives which is recommended by all the newborn screening programs. LCMS can profile the steroids separately into cortisol, 17-OH progesterone and 17-deoxycorticosterone and can thus differentiate the peak obtained in fluoro-immunoassay into its different components. Thus, it is both used as a diagnostic test and for confirming the screen positive cases.

In order to have high sensitivity, the cut-off levels for 17- OHP are typically set low enough to detect a positive proportion of approximately 0.3-0.5%. A study published from the New York screening program for CAH from 2007-14 reported a recall rate of 13,050 samples out of 1,96,2433 samples screened (0.66%). Out of this only 105 cases were confirmed positive (0.8% of the recalls) [18]. As the actual prevalence of CAH is approximately 0.01 to 0.02%, this effectively means that approximately 98% of screen positives would be false positive [19]. This in turn means that specificity of initial immunoassay is quite low and majority of screen positive cases are false positive. The cost of following up each false positive case could be avoided with use of a more specific second tier test. The specific test that should be ordered for confirmation of positive result on fluoroimmunoassay/immunoassay should be a biochemical assay on venous sample collected after immediate recall.

A similar two-tier protocol is being made feasible in resource-limited settings. Positive tested samples on immunoassay can be sent to the dedicated laboratory equipped to perform LC-MS/MS method with a priority label from centres that do not have similar facilities. It is recommended that LC-MS/MS be carried out on venous samples; however, in cases where venous sample is not available, DBS samples (whole blood) can also be used for the second tier testing.

Liquid chromatography followed by tandem mass spectrometry (LC-MS/MS) is an alternate option where steroid ratios are measured. This is a good confirmatory test which can be performed easily on serum samples. The principle of organic solvent extraction increases its specificity over an immunoassay [20,21]. Many CAH screening programs have reported an increase in positive predictive value, from 0.8 to 7.6% in Minnesota and 0.4 to 9.3% in Utah after implementing this approach [22,23]. A German program tested 1609 screen positive samples(out of 242,500 samples screened) using a modified LC-MS/MS protocol which used a ratio of the sum of 17-OHP and 21-deoxycortisol levels, divided by the cortisol level. They



Gestational age cut offs preferred instead of birth weight wherever available.

FIG. 1 *Suggested algorithm for screening of infant for congenital adrenal hyperplasia.*

concluded that a cut-off ratio of 0.53 had a positive predictive value of 100% [24].

The downstream cost of high recall rates with false-positive screen is difficult to estimate. The cost-effectiveness of NBS for CAH has not been well analyzed. A false positive screen may also be a cause of significant undue parental stress. However, justification for NBS in CAH scores over these minor issues. Use of better diagnostic tests will help to avert these logistic issues [5].

The use of molecular and genetic tests should be reserved for research settings where resources permit.They are recommended strongly for prenatal diagnosis and genetic counselling for future pregnancies

Molecular testing can be offered to babies who test positive on biochemical screening. The most common mutations detected are the *CYP21A2* gene mutations. One of the 10 common mutations is present in 90% of the affected patients, thus absence of these mutations would deem the diagnosis of CAH unlikely while presence of at least one mutation would warrant further workup [12]. Hotspots from both the northern and southern Indian cohort of patients with CAH have identified a panel of 9 to 10 common mutations which can be offered to screen positive patients in laboratories where molecular facilities are available [2]. Genotyping of screening samples has been proposed as a useful second tier test in lieu of LCMS in several studies [25]. Kosel, *et al.* [27] in their study

reported a decrease in number of retests by 90% when the screen positive 17-OHP values were screened by molecular means [26]. However, no large scale study has demonstrated cost wise efficacy of this strategy. It is currently more expensive than LC-MS/MS on a per sample basis and is not in use in any nationwide newborn screening program.

The genotype also helps in determining the degree of enzyme impairment, which in turn determines the severity of hormonal abnormalities. Studies have demonstrated genotypic-phenotypic correlation in 50% of the cases [12,27]. A deletion or intronic splice mutation in I2G is commonly associated with salt wasting CAH, I172N mutations and V281L were commonly associated with simple virilizing and non-classical CAH, respectively [28]. However, genotype may not always differentiate between salt wasting and non-salt wasting forms. For example, patients with V281L or P30L mutations, which have been traditionally associated with non-classical CAH may present with virilization [29].

Thus, genotyping carries implications for prenatal diagnosis and genetic counseling for next pregnancy.It is not required before initiation of treatment in index case of classical CAH. It is recommended for prenatal diagnosis or where diagnosis is questionable.

Urinary steroid profiling by Gas chromatography Mass Spectrometry (GCMS) is not recommended as a routine confirmatory test

Urinary steroid profiling (USP) is a biochemical analytical technique for the diagnosis of various types of steroidogenesis defects including those leading to CAH as it can identify and quantify a series of steroid metabolites both above and below the enzymatic block simultaneously in a single analysis [30]. Urine steroid profiling by GCMS requires time consuming preanalytical sample preparation. Apart from the technical challenges, an important limitation is the inability to perform these tests in a high through put format where large number of samples need to be processed in a short span of time. This has limited the use of GCMS to a few experienced research laboratories and is yet to be adapted to large scale commercial assays [31]. It can provide a rapid simplified differential diagnosis for CAH, where available [32].

DIAGNOSIS IN BABIES WHO HAVE NOT UNDERGONE NEWBORN SCREENING

A morning baseline serum 17 OHP is recommended in symptomatic individuals

A newborn who has not undergone genetic screening at birth should be offered screening for CAH anytime he/she presents during the neonatal period irrespective of whether symptomatic for CAH or not. Alternately, any neonate with ambiguous genitalia or suspicion of CAH on metabolic work-up should also be offered confirmatory testing on venous sample for CAH on priority basis. In these babies, a single measurement of serum 17-OHP prior to steroid administration must be sent and interpreted.

A complete adrenocortical profile is recommended to differentiate 21-OH deficiency from other enzyme defects and to diagnose borderline cases. Alternatively the steroid profiling done on tandem mass may help identify a subset of these disorders

The possibility of an alternative diagnosis other than 21-hydroxylase deficiency may be considered in neonates/infants with clinical or lab markers pointing to defects other than 21- OH deficiency. The other causes of CAH include 11β-hydroxylase deficiency, 17 a hydroxylase deficiency, 3b hydroxysteroid dehydrogenase deficiency and lipoid CAH. Only 21-OH deficiency and 11 β-hydroxylase deficiency are predominantly virilizing diseases. Patients with other causes of CAH have impaired production of cortisol by the adrenals as well as gonadal steroids. Male patients will have undervirilization, while female patients may or may not exhibit virilization. Clinical features may appear similar and basal serum 17-OHP may not be fully discriminatory in all such cases. Precursors to product ratios on LC-MS/MS are important in differentiating the various enzyme defects. In order to differentiate the various enzyme defects, serum 17-OHP, cortisol, 11-deoxycortisol, 17-OH pregnenalone, dihydroepiandrostenedione and andro-

TABLE II SUBTYPES OF CONGENITAL ADRENAL HYPERPLASIA

Subtypes	*Phenotype*	*Elevated metabolites*
11β-hydroxylase deficiency	Female virilization	Deoxycorticosterone, 11-deoxycotisol
17 α-hydroxylase deficiency	Male undervirilization Female virilization +/-	Deoxycorticosterone, corticosterone
3β-hydroxysteroid dehydrogenase deficiency	Male undervirilization Female virilization +/-	Dihydroepiandrostenedione, 17-OH pregnenalone

stenedione should be measured [12,27]. The metabolites elevated in the various subtypes are shown in ***Table* II**. Apart from 17-OH pregnenalone, the rest of tests are available on the LC-MS/MS platform. These tests can be performed on a venous sample collected as soon as possible but preferably within first two weeks of life. These disorders are less common and such children should be immediately referred to the endocrinologist for further workup. It should be noted that thepurpose of newborn screening is identification of babies with 21-hyroxylase deficiency, which is the commonest.

CONCLUSIONS

CAH is a disease associated with significant morbidity, mortality and long-term complications. The timely diagnosis and treatment is challenging in the absence of newborn screening. Screening for CAH with DBS using fluoroimmunoassays is recommended for all babies. The confirmation of diagnosis must be made using LC-MS method, which is getting widely available. Genetic diagnosis should be used for diagnostic confirmation where resources permit but definitely – for prenatal testing and counselling.

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Community-based Randomized Controlled Trial Evaluating Effect of Kangaroo Mother Care on Neonatal and Infant Outcomes

Source Citation: *Mazumder S, Taneja S, Dube B, Bhatia K, Ghosh R, Shekhar M, et al. Effect of community-initiated kangaroo mother care on survival of infants with low birthweight: A randomised controlled trial. Lancet. 2019; 394: 1724-36.*

SUMMARY

In this randomized controlled trial, 8402 babies weighing 1500–2250 g at home within 72 h of birth, if not already initiated in kangaroo mother care, irrespective of place of birth, who were stable and feeding were enrolled. Intervention group comprised of 4480 babies initiated on community-initiated kangaroo mother care (KMC) and 3922 were assigned to the control group. Mothers and infants in the intervention group were visited at home to support KMC and breast feeding. The control group received routine care. Primary outcomes were mortality between enrolment and 180 days. 81·4% occurred at a health facility and 36·2% had initiated breastfeeding within 1 h of birth, and infants were enrolled at an average of about 30 hours of age. From enrolment to 28 days, 73 infants died in 4423 periods of 28 days in the intervention group and 90 deaths in 3859 periods of 28 days in the control group (hazard ratio [HR] 0·70, 95% CI 0·51–0·96; p=0·027). From enrolment to 180 days, 158 infants died in 3965 periods of 180 days in the intervention group and 184 infants died in 3514 periods of 180 days in the control group (HR 0·75, 0·60–0·93; p=0·010). The risk ratios for death were almost the same as the HRs (28-day mortality 0·71, 95% CI 0·52– 0·97; p=0·032; 180-day mortality 0·76, 0·60–0·95; p=0·017). The authors concluded that community-initiated kangaroo mother care substantially improves newborn baby and infant survival.

COMMENTARIES

Evidence-based Medicine Viewpoint

Relevance: In low-income and middle-income countries, whether incorporation of kangaroo mother care for all infants with low birthweight, irrespective of place of birth, could substantially reduce neonatal and infant mortality is an important question to answer. A community-based randomized controlled trial (RCT) was undertaken in Haryana (India) to evaluate the impact of encouraging kangaroo mother care (KMC) initiated at home within 72 hours of birth, among babies weighing 1500-2250g [1]. The trial is summarized in ***Table* I**.

Critical appraisal: Overall, the trial qualified as having low risk of bias. The random sequence was generated by an off-site statistician using variably sized random permuted blocks. However, the method of generating the sequence was not specified. Although babies were individually randomized, there were certain exceptions. For instance, the second of twins was allocated the same group as the first twin who was randomized. Babies born in households where a previously enrolled infant resided (i.e sibling or member of a joint family) were allocated the intervention arm if the previous infant belonged to the intervention arm. If the previous infant belonged to the comparison arm, then the new infant was randomized to either arm.

Individual allocations were concealed in serially numbered, opaque, sealed envelopes and stored off-site. At enrollment, research staff contacted a central allocator who revealed the allocation. The trial participants and research staff were unblinded. The outcome assessors were intended to be blinded, but the nature of the outcomes to be assessed precluded effective blinding. All randomized participants were included in the primary intention-to-treat analysis. Detailed description of participants who were unavailable for follow-up was mentioned. Most of the clinically relevant outcomes were included. However, it can be argued that parameters reflecting temperature control in the neonatal period could have been included.

This trial has several strengths, notably meticulous pre-trial planning, baseline data acquisition, training of research staff for implementing the trial and data collection, large sample size, use of appropriate definitions for various outcomes measured, standardized tools for data collection, electronic data capture, and close follow-up. These measures reduced the risk of bias and ensure high internal validity. Other refinements included the Data Safety and Monitoring Board, meticulous data storage, appropriate data analysis, etc. Nevertheless, a few issues merit consideration.

TABLE I OUTLINE OF THE TRIAL

Clinical question	The research question in the PICOT format could be framed as: "What is the impact of encouraging kangaroo mother care (KMC) initiated at home and sustained through the neonatal period (*I=Intervention*), among babies with weight 1500-2250g recruited within 72 hours of birth (*P=Population*), compared to no KMC (*C=Comparison*), on mortality, anthropometric parameters, and serious childhood illnesses (O=Outcomes), measured at the end of 28 days as well as 6 months of life (*T=Time frame*)?"
Study design	Randomized controlled trial with allocation of individual participants to the trial arms.
Study setting	Community-based trial in two districts of Haryana state (India) with an estimated pre-trial population of 20 lakhs, birth rate of 26/1000, and neonatal mortality rate of 42 per 1000 live births.
Study duration	August 2015 to October 2018 (39 months).
Inclusion criteria	Newborn babies within 72h of birth, with weight 1500-2250 g, available at home. In this group, those weighing less than 1800 g were referred to hospital, and enrolled within 72 hours of birth, if hospitalization was refused, or hospitalized babies were discharged and sent home.
Exclusion criteria	Babies beyond 72 h, unknown weight, feeding difficulty, breathing difficulty, inadequate movements, or gross congenital malformations. Babies in whom KMC had been initiated already (for example in the delivery facility) were also excluded as also those whose mothers did not intend to stay in the area for 6 months.
Recruitment procedure	Pregnant women in the community were listed through active community-based surveillance carried out every 3 months. Potentially eligible women were approached more frequently closer to the delivery date, and eligible newborn babies identified within 72 hours of birth.
Intervention and Comparison groups	The intervention arm participants received counselling and encouragement for KMC and exclusive breastfeeding, provided by research staff including intervention workers and their supervisors. The mode of providing these was not described. Infants were visited by the research staff on days 1-3, 5, 7, 10, 14, 21, and 28 of life (i.e total 7 visits) for 30-45 minutes each. During these visits, research staff encouraged KMC 24 x 7, observed KMC practice, surveyed KMC and breastfeeding practices, and assisted mothers to resolve difficulties with these. The comparison arm did not receive the above-mentioned intervention. Both groups received standard newborn care delivered by ASHA workers, that comprised 5 visits of unknown duration in the neonatal period. These visits were on days of life 3,7,14,21,28 for babies born in hospital and an additional visit on the day of birth for home-delivered babies. Low birthweight babies were to receive additional ASHA visits at unspecified timepoints. During these visits, the ASHA workers encouraged exclusive breast-feeding, resolved difficulties with breastfeeding, and helped identify (and refer) babies who were ill.
Outcomes	Primary outcome: • Mortality within 28 days and 180 days of birth. Secondary outcomes: • Proportion of exclusively breastfed infants (at age 28 d, 90 d, 180 d). • Proportion of infants who were not breastfed (at age 28 d, 90 d, 180 d). • Weight for age z score (at age 28 d, 90 d, 180 d). • Length for age z score (at age 28 d, 90 d, 180 d). • Weight for length z score (at age 28 d, 90 d, 180 d). • Proportion with weight for age z score <-3 (at age 28 d, 90 d, 180 d). • Proportion with length for age z score <-3 (at age 28 d, 90 d, 180 d). • Proportion with weight for length z score <-3 (at age 28 d, 90 d, 180 d). • Head circumference (at age 28 d, 90d , 180 d). • Hospitalization for any reason (by age 28 d, 180 d). • Proportion with possible serious bacterial infection, local infection, or diarrhea/dysentery by age 28 d. • Proportion with diarrhea, or pneumonia, or severe pneumonia within a 2-wk window preceding the visit at 90 d of age. • Care seeking behavior for the morbidities described above.

Table 1 continue

Follow-up protocol	Research staff visited enrolled babies on days 28, 90 and 180 to obtain information on the pre-specified outcomes listed above. This was done through interviews (presumably with mothers) and objective measurements of anthropometric parameters.
Sample size	*A priori* sample size calculation was performed for a superiority trial, to detect a 30% reduction in neonatal morality from an estimated baseline of 42 per 1000 live births, with alpha error 0.05 and beta error 0.10. Assuming 10% attrition, the estimated sample size was 10500 infants. However, the intended sample size was not reached because a planned Data safety and Monitoring Board (DSMB) review approximately 3 years from the onset of the trial believed that additional recruitment was not required to answer the research question.
Data analysis	Intention-to-treat (ITT) analysis was performed for the primary outcomes, analysing participants in the groups to which they were randomized. Mortality rate was calculated in terms of person-time (i.e until the age of follow-up or death) as well as number enrolled. Thus hazard ratio (HR) as well as relative risk (RR) were presented. Secondary outcomes were measured *per protocol*. Multiple *a priori* as well as *post hoc* subgroup analyses were also performed.
Comparison of groups at baseline	The groups were comparable at baseline with respect to age at enrolment, weight and length at enrolment, gender ratio, birth order, frequency of twin birth, gestation, weight/gestation categorization, timing of initiating breastfeeding, place of delivery, mode of delivery, maternal age, maternal education level, family religion, caste, and income.
Summary of results	Please see **Box I**.

First, the trial title refers to 'low birthweight' infants, traditionally defined as <2500g at birth. Although all the enrolled infants were below this weight (hence low birthweight), not all low birthweight babies were included, as those weighing >2250g were excluded. Thus, strictly speaking, the trial results are valid for infants between 1500 and 2250g only (rather than all low birthweight). The distinction is more than semantic, because it is possible that inclusion of larger weight babies may have minimized the differences between the trial arms. The reason for excluding babies >2250g was that the investigators expected some loss of weight between birth and recruitment. However, it is highly unlikely to be of the magnitude of 250g. The other reason offered is that pre-trial analysis suggested that babies >2250g wriggled out of KMC before the age of 28 days. This reasoning seems implausible given that three quarters of enrolled babies weighed between 2000g and 2250g, hence that many of these would have attained a weight greater than 2250g within the neonatal period (hence wriggled out of KMC). Yet, the trial data showed that the median duration of KMC in the intervention arm was 27-28 days, suggesting that heavier infants did not wriggle out. Fortunately, the issue may not be critical as the authors reported that sub-group analysis showed that heavier weight babies in the intervention arm had outcomes similar to the others.

This trial [1] is not actually a comparison of KMC *versus* no KMC, but rather a trial of implementing a package of interventions (community outreach, motivation of mothers for exclusive breastfeeding and KMC, basic health education, trouble-shooting, practical support, belt binders to facilitate KMC, and close monitoring) to initiate as well as sustain KMC and breastfeeding in the community within 72 hours of birth for babies weighing 1500-2250g. Viewed in this light, two refinements could have been attempted in this trial [1]. First, the window period of participant recruitment for home-delivered babies could have been narrowed to within 24 (or even 12) hours after birth, rather than 72 hours. This is important because the authors suggested that about only 50% neonatal mortality occurs after the first day of life. Second, outcomes reflecting maternal and family perceptions of KMC, its feasibility at home, impact on other household activities, impact (positive or negative) on the care of other infants/children at home, socio-economic implications, etc could also have been considered.

Another extremely important issue is how much KMC contributed to the beneficial outcomes observed at various time points in this trial [1]. The investigators attributed mortality reduction to KMC, offering biologically plausible explanations in terms of better infant care, higher breastfeeding rates, closer maternal bonding, etc. However, it is important to note that the intervention was not merely the administration of KMC, but a package comprising motivation for KMC, encouraging exclusive breastfeeding, ensuring KMC and breastfeeding, resolving difficulties in these practices, close monitoring, etc- all of which were delivered through seven dedicated visits by an exclusive research team. These visits were over and above the routine five visits to be made by ASHA workers in both trial groups. Thus, the two arms of the trial differed in more ways than just KMC. This point is especially important as the data showed that over one-third infants (in both trials arms) were not visited even once by ASHA workers during

BOX I SUMMARY OF RESULTS (INTERVENTION *VS* COMPARISON GROUPS)

Primary outcome

- Mortality within 28d: 73 per 4423, 28d periods *vs* 90 per 3859, 28d periods; HR 0.70 (CI 0.51, 0.96)*
- Mortality within 28d: 73/4470 *vs* 90/3914; RR 0.71 (CI 0.52, 0.97)*
- Mortality within 180d: 158 per 3965, 180d periods *vs* 184 per 3514, 180d periods; HR 0.75 (CI 0.60, 0.93)*
- Mortality within 180 d: 138/3653 *vs* 166/3331; RR 0.76 (CI 0.60, 0.95)*

Secondary outcomes

Breastfeeding

- Exclusively breastfed at 28d: 3739/4470 *vs* 2125/3914; RR 1.54 (CI 1.49, 1.59)*
- Exclusively breastfed at 90d: 2239/3961 *vs* 1091/3521; RR 1.82 (CI 1.72, 1.93)*
- Exclusively breastfed at 180d: 127/3539 *vs* 13/3199; RR 8.83 (CI 5.0, 15.6)*
- Not breastfed at 28d: 116/4470 *vs* 160/3914; RR 0.63 (CI 0.50, 0.81)*
- Not breastfed at 90d: 123/3961 *vs* 175/3521; RR 0.62 (CI 0.50, 0.79)*
- Not breastfed at 180d: 254/3539 *vs* 343/3199; RR 0.67 (CI 0.57, 0.78)*

Anthropometric parameters, mean (SD) z scores

- Weight for age at 28d: -2.64 (0.92) *vs* -2.77 (0.91); MD 0.12 (CI 0.08, 0.16)*
- Weight for age at 90d: -2.43 (1.04) *vs* -2.50 (1.06); MD 0.07 (CI 0.02, 0.12)*
- Weight for age at 180d: -2.24 (1.10) *vs* -2.23 (1.13); MD -0.02 (CI -0.07, 0.04)
- Length for age at 28d: -2.43 (0.99) *vs* -2.49 (0.99); MD 0.06 (CI 0.01.0.10)*
- Length for age at 90d: -2.08 (1.04) *vs* -2.12 (1.06); MD 0.04 (CI -0.01, 0.09)
- Length for age z score at 180d: -1.91 (1.06) *vs* -1.86 (1.07); MD -0.05 (CI -0.10, 0.00)
- Weight for length at 28d: -1.02 (1.06) *vs* -1.16 (1.10); MD 0.14 (CI 0.09, 0.19)*
- Weight for length at 90d: -0.96 (1.13) *vs* -1.02 (1.17); MD 0.06 (CI 0.01, 0.12)*
- Weight for length at 180d: -1.30 (1.11) *vs* -1.32 (1.14); MD 0.03 (CI -0.03, 0.08)

Anthropometric parameters

- Weight for age z score <-3 at 28d: 1329/4380 *vs* 1363/3813; RR 0.85 (CI 0.80, 0.90)*
- Weight for age z score <-3 at 90d: 945/3772 *vs* 938/3340; RR 0.89 (CI 0.82, 0.97)*
- Weight for age z score <-3 at 180d: 763/3499 *vs* 718/3142; RR 0.95 (CI 0.87, 1.05)
- Length for age z score <-3 at 28d: 1092/4379 *vs* 1023/3812; RR 0.93 (CI 0.86, 1.00)
- Length for age z score <-3 at 90d: 630/3772 *vs* 598/3340; RR 0.93 (CI 0.84, 1.03)
- Length for age z score <-3 at 180d: 487/3499 *vs* 415/3143; RR 1.05 (CI 0.93, 1.19)
- Weight for length z score <-3 at 28d: 175/4275 *vs* 210/3725; RR 0.73 (CI 0.60, 0.89)*
- Weight for length z score <-3 at 90d: 149/3771 *vs* 158/3339; RR 0.84 (CI 0.67, 1.04)
- Weight for length z score <-3 at 180d: 216/3499 *vs* 232/3142; RR 0.84 (CI 0.70, 1.00)
- Mean (SD) head circumference at 28d: 34.0 (1.2) *vs* 33.9 (1.2); MD 0.07 (CI 0.01, 0.13)*
- Mean (SD) head circumference at 90d: 37.2 (1.3) *vs* 37.2 (1,2); MD 0.03 (CI -0.03, 0.10)
- Mean (SD) head circumference at 180d: 40.0 (1.3) *vs* 39.9 (1.4); MD 0.03 (CI -0.04, 0.10)

Hospitalization

- For any reason by 28d: 580/4470 *vs* 460/3914; RR 1.10 (CI 0.98, 1.24)
- For any reason by 180d: 852/3653 *vs* 793/3331; RR 0.98 (CI 0.90, 1.07)

Morbidity

- Possible serious bacterial infection by 28d: 919/4470 *vs* 904/3914; RR 0.89 (CI 0.82, 0.97)*
- Local infection by 28d: 390/4470 *vs* 300/3914; RR 1.14 (CI 0.98, 1.32)
- Diarrhea or dysentery by 28d: 235/4470 *vs* 334/3914; RR 0.62 (CI 0.52, 0.72)*
- Diarrhea in 2 wk preceding 90d visit: 640/4042 *vs* 609/3612; RR 0.94 (CI 0.85, 1.04)
- Diarrhea with dehydration or dysentery in 2 wk preceding 90d visit: 9/4042 *vs* 26/3612; RR 0.31 (CI 0.15, 0.66)*
- Pneumonia in 2 wk preceding 90d visit: 53/4042 *vs* 81/3612; RR 0.58 (CI 0.41, 0.82)*
- Severe pneumonia in 2 wk preceding 90d visit: 39/4042 *vs* 60/3612; RR 0.58 (CI 0.39, 0.87)*

Care seeking behavior

- Care sought from an appropriate provider for possible serious bacterial infection by 28d: 385/919 *vs* 297/904; RR 1.28 (CI 1.13, 1.44)*
- Care sought within 24h of identifying illness by 28d: 349/919 *vs* 261/904; RR 1.32 (CI 1.15, 1.50)*
- Care sought from an appropriate provider for local infection by 28d: 104/390 *vs* 37/300; RR 2.16 (CI 1.53, 3.05)*
- Care sought within 24h of identifying illness by 28d: 77/390 *vs* 29/300; RR 2.04 (CI 1.37, 3.05)*
- Care sought from an appropriate provider for diarrhea in 2 wk preceding 90d visit: 65/640 *vs* 53/609; RR 1.17 (CI 0.82, 1.65)
- Care sought within 24h of identifying illness in 2 wk preceding 90d visit: 46/640 *vs* 39/609; RR 1.12 (CI 0.74, 1.69)
- Care sought from an appropriate provider for pneumonia in 2 wk preceding 90d visit: 13/53 *vs* 21/81; RR 0.95 (CI 0.52, 1.73)
- Care sought within 24h of identifying illness in 2 wk preceding 90d visit: 11/53 *vs* 18/81; RR 0.93 (CI 0.48, 1.82)

**Statistically significant at P<0.05.*

the first seven days of life. While this would not matter for babies in the intervention arm (who would have received 3 visits by research staff within that period), it would severely impact breastfeeding initiation and maintenance in the comparison arm. Even beyond seven days, only about one-third to half the infants received scheduled ASHA visits. Could this be the reason for the stark differences observed in the trial groups for breastfeeding as well as outcomes positively affected by breastfeeding (such as immediate and longer-term mortality, sustained breastfeeding, reduced infections, etc)? This perception is further strengthened by the fact that most anthropometric parameters (except weight) did not show clinically significant differences between the trial groups. Thus, the higher proportions of exclusively breastfed babies in the intervention arm, could be the cause rather than the effect, of the findings in this trial.

The trial [1] showed some interesting findings that were not highlighted by the authors. First, the quantity of ASHA worker visits appears to be well below the scheduled plan as per government norms. ASHA worker visits are designed to promote exclusive breastfeeding and ensure compliance through practical assistance, counselling, and problem resolution. Second, the quality of these visits appears questionable considering that only half the babies in the comparison arm were exclusively breastfed during the neonatal period, with declining proportions over time. Other indicators are that less than 10% babies received breastfeeding within an hour of birth, and the mean time of initiation was over 4 hours. Even more worrisome is that all this happened in a setting wherein a highly controlled RCT was in progress. Since ASHA workers are the frontline health force for universal health care coverage in the community, this does not augur well for the community or the healthcare system. In addition, it has two implications for the trial [1] itself. The issue of compromised care of babies in the comparison arm (and imbalance *vis-à-vis* the intervention arm) has already been highlighted. The second issue is that replication and scale-up of the trial findings [1] in the real-world scenario would ultimately devolve to ASHA workers, in order to achieve the investigators' optimistic projection of thousands of lives saved. The experience from this trial [1] suggests that this is unlikely to happen given the current scenario. This view is strengthened by the fact that the number needed to treat (NNT) to prevent a single additional death was fairly high, suggesting that intensive efforts would be required on the part of ASHA workers.

CONCLUSION

This well-designed and well-executed RCT showed that home initiation and maintenance of KMC and breastfeeding, ensured by a dedicated team of trained workers, through 7 additional home visits (3.5 to 5.3 hours duration), in babies weighing 1500-2250g, reduced neonatal and early infant mortality by about 30%. There were additional benefits for outcomes influenced by better newborn care and exclusive breastfeeding viz reduced infection, less severe diarrhea & pneumonia, and better care-seeking behaviour in the community. The trial also indirectly highlighted the challenges to be overcome if a bundle of interventions (including KMC) were to be implemented in the real-world scenario.

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Neonatologist's Viewpoint

Kangaroo mother care (KMC) is one of the most cost-effective interventions in preventing deaths of low birthweight (LBW) neonates [1]. A recent Cochrane review (21 studies, 3042 infants) showed that facility-initiated KMC can result in decrease in risk of mortality, serious infection, hypothermia, improved exclusive breastfeeding and improved early weight gain as compared to conventional care [2]. Despite this fact, the global uptake of KMC among eligible babies is low, with estimated coverage being less than 5% [3]. The situation is even worse in the community.

Even though, both WHO as well as the Government of India (GOI) [4,5] have recommended uninterrupted and early KMC for all stable LBW infants in health facilities, no clear-cut guideline is available for community-initiated KMC. In lower-middle-income-countries including India, nearly half of all child births happen at home. In many cases LBW neonates are discharged early (within 24 hours of birth) from facility-care, before KMC can be initiated. In remaining, even if KMC is initiated in hospital, it is never sustained beyond discharge due to lack of awareness and involvement of family members at home. In a systematic review by Seidman, *et al.* [6], lack of a conducive environment and support from family members were two of the top five barriers to the practice of KMC. However, in the community, both these barriers can be alleviated. Mothers in a community are more likely to get support

from other family members, friends and relatives (listed as one of the top enablers of KMC).

(Hence, the authors need to be congratulated for conducting this rigorous randomized controlled trial on community initiated KMC (ciKMC) in rural India [7], which has important public health implications. In this first-of-its-kind RCT, the authors showed improved survival of enrolled LBW infants in both neonatal period (till 28 d, Number needed to treat 83) as well as early infancy (up to six months, 180 d, NNT 150) by 30% following initiation of KMC within 72 hours of life as compared to control group in rural Haryana. Some other noteworthy benefits of community initiated KMC (ciKMC) as proven from the study were decrease in incidence of severe infection, improved rates of exclusive breast feeding, improved growth parameters and increase in health seeking behavior for illness by parents of enrolled infants.

The results of this large RCT call the policy makers in India to incorporate ciKMC as part of national guidelines for home-based care of low birthweight babies. However, there are few issues in translation of this evidence in to practice. ciKMC requires active participation of community health workers (Accredited social health activists, Anganwadi workers and Auxiliary nurse midwives) as well as family members. Prior counselling of antenatal mothers and her family members by community health workers is also essential. Similar to rigorous training of intervention workers of the current trial, there is a need of strong capacity building of community health workers in basic essential newborn skills like skin-to-skin contact and exclusive breastfeeding through regular training (by neonatologists/pediatricians). Providing ciKMC can be challenging in settings where mother starts doing household chores soon after delivery. To avoid this, the entire family needs to be counselled about providing uninterrupted KMC in mother's absence. A recent quality improvement study [8] showed that duration of facility-based KMC can be improved by active participation of other family members (father, grandparents etc.), and ciKMC is not an exception. Simultaneously, the role of hygiene (daily bath and hand washing) needs to be underscored. The role of the neonatologist in community-initiated KMC; although minimal, nevertheless is vital, since many eligible LBW infants are discharged from hospital early (before KMC is initiated). It is the prime responsibility of treating neonatologist to utilize this vital window period for initiating KMC (in hospital) and also to teach the family about appropriate technique of skin-to-skin contact as well as explaining the monitoring parameters like neck position, breathing pattern and color of the baby during ciKMC. CiKMC should be advised to continue at home as long as possible. It is the primary responsibility of the neonatologist to ensure that none of these infants get discharged before initiation of KMC at facility.

With the combined efforts of administrators and healthcare providers, we expect ciKMC to get incorporated into national guidelines on care of LBW babies and imbibed in the community for improved survival of LBW infants in near future.

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Etiological Spectrum of Precocious Puberty: Data from Northwest India

We retrospectively analyzed clinic records of 55 children (36 girls) with precocious puberty. Majority (34, 62%) had central precocious puberty, out of which 19 were idiopathic. Peripheral precocious puberty was seen in 14 children. Congenital adrenal hyperplasia was the commonest cause of peripheral precocious puberty (6, 42.8%).

Keywords: *Congenital adrenal hyperplasia, Etiology, Idiopathic, Outcome*.

Precocious Puberty occurs due to premature activation of the hypothalamic-pituitary-gonadal axis, called central precocious puberty, or independent of it, called as peripheral precocious puberty. With the advancements in laboratory and radiological investigations, the etiological spectrum of precocious puberty has changed significantly in the past two decades with more cases of organic etiology being detected [1]. There is a limited data on etiology of precocious puberty from developing countries [2]. We retrospectively analyzed medical records of children with precocious puberty from a tertiary-care teaching hospital of Northern India between 2004 to 2018. Ethical clearance was obtained from institutional ethics committee.

Medical records of 55 children (19 boys) with complete information out of 80 children diagnosed as precocious puberty during the study period were analyzed. The mean (SD) age at onset of symptoms in boys and girls was 3.0 (3.2) y and 4.5 (2.5) y, respectively, There was a mean (SD) delay of 0.8 (1.4) y from onset to presentation to a health facility. The mean (SD) bone age was 8.2 (4.9) y in boys, and 7.8 (2.7) y in girls with mean (SD) advancement in bone age of 3.4 (2.9) y. The mean (SD) stimulated serum leutinizing hormone (LH) was 18.5 (1.9) IU/L in CPP group. The majority of patients (61.8%) had CPP, which was idiopathic in 56% (**Table *I***). Congenital adrenal hyperplasia (CAH) was seen in 42.8% of cases of PPP. Six patients with simple virilizing CAH had PPP at presentation and progressed to CPP in follow-up. Seven patients had incomplete PP variants.

The spectrum of diagnoses and patients' characteristics according to gender and type of precocious puberty are shown in ***Table* I**. Peripheral precocious puberty was less common in boys while incomplete variants were only diagnosed in girls. The delay in seeking medical attention and the consequent bone age advancement was more common in girls. Thirty-five out of 38 eligible patients were started on gonadotropin releasing hormone (GnRH) analogue therapy with leuprolide; remaining three had financial constraints. The mean change in height after 3 years of therapy was +0.6 SD score. Specific therapy for the underlying condition such as hydrocortisone and fludrocortisone for CAH, levothyroxine for hypothyroidism and surgery for adrenal tumor was provided. Four patients achieved final height during the study (mean height SDS -0.49) after mean (SD) duration of 6.7 (1.4) y of GnRH therapy. The worst height outcome (-2.72 SDS) was seen in a patient with central precocious puberty complicating CAH.

The spectrum of etiology in the present cohort of precocious puberty was similar to previous studies from developing countries [2,3]. Central precocious puberty was commoner than peripheral precocious puberty and was idiopathic in majority of the patients. Consistent with previous studies, central precocious puberty was more common than peripheral precocious puberty in boys indicating the critical role of neuroimaging in boys [4]. The female to male ratio of 1.9:1 in this study was lower than previous studies [4,5].

A significant finding was delay in seeking medical attention resulting in diagnostic delays, which was common amongst girls, especially those with peripheral precocious puberty and incomplete variants. Delayed medical attention resulted in significant advancement of bone age in the participants of present study. The late diagnoses and non-affordablility of treatment represent the challenges of managing precocious puberty in a developing country [6,7]. Bone age advancement at the initiation of therapy is associated with poor height prognosis [8]. The positive delta change in height SD score after of GnRH therapy indicated that GnRH therapy was unable to achieve the expected slowing of height velocity, if started late. In addition, the poor final height achieved in one of our patients also appears to be a consequence of delayed initiation of treatment.

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TABLE I PATIENT CHARACTERISTICS ACCORDING TO SEX AND TYPE OF PRECOCIOUS PUBERTY (*N*=55)

Characterisitics	*Males*		*Females*		
	Central (n=15)	*Peripheral (n=4)*	*Central (n=19)*	*Peripheral (n=10)*	*Incomplete (n=7)*
Age at onset, y	3.0 (3.2)	3.3 (3.7)	5.2 (2.5)	3.3 (1.9)	4.5 (3.2)
Age at diagnosis, y	3.6 (3.7)	3.4 (3.7)	5.8 (2.7)	4.3 (2.9)	6.4 (2.2)
Delay in diagnosis, y	0.6 (1.2)	0.2 (0.2)	0.7 (0.7	1.0 (1.6)	1.8 (2.8)
BA advancement, y	4.1 (4.0)	5.6 (2.2)	4 (2.6)	2.6 (2.6)	1.2 (1.7)
Height Z-scores	1.2 (1.9)	-0.03 (2.8)	1.5 (1.4)	0.3 (1.7)	0.6 (0.6)
Basal LH, IU/L	4.1 (4.3)	0.1 (0.0)	2.7 (2.5)	0.3 (0.4)	0.2 (0.2)
Basal FSH, IU/L	3.0 (5.7)	0.3 (0.2)	4.2 (2.0)	1.6 (2.1)	2.4 (0.8)
*Etiology	Idiopathic (7), Hypothalamic hamartoma (3), Hydrocephalus (3), Mega cisterna magna (1), Brain tumor (1)	Adrenal tumor (1), CAH (2), Hypo-thyroidism (1)	Idiopathic (12), Hypothalamic hamartoma (1), Hydrocephalus (2), Radiation-induced (2), Brain tumor (2)	CAH (4), Ovarian cyst (2), Hypothyroidism (2), McCune-Albright syndrome (1), Adrenal tumor (1)	Isolated thelarche (4), Isolated pubarche (3)

*All values in mean (SD); BA: bone age; CAH: Congenital adrenal hyperplasia; FSH: follicle stimulating hormones; LH: leutinizing hormone; *number of cases in parenthesis.*

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Nutritional Rickets with Severe Complications in Syrian and Iraqi Refugee Children

We investigated the presence of nutritional rickets in Syrian and Iraqi refugee infants who presented to hospital in Turkey in 2017. 25(OH)D levels were examined in 77 refugee children. Nutritional rickets was diagnosed in 22 (28.5%) children; 11 patients with rickets did not follow up.

Keywords: *Management, Prevalence, Vitamin D.*

The civil war in Syria in recent years has caused an enormous refugee crisis [1]. Nearly 4 million people have entered Turkey. Over 90% of whom are Syrian refugees. There are 142 thousand Iraqi refugees in Turkey [2]. In our country, routine use of a daily 400 IU vitamin D supplement is recommended for infants. Vitamin D has been provided free of charge to all infants during their first year since 2005 [3]. Syrians and Iraqi refugees benefit from health services free of charge if they register. This study aimed to investigate the presence of nutritional rickets in Syrian and Iraqi refugee infants who presented to our hospital.

In this study, results of 25(OH)D vitamin levels assessment were extracted from records of Syrian and Iraqi refugee children aged from 1 to 24 months who presented to our hospital in 2017. 25(OH)D levels were examined in 77 children (54 Syrian and 23 Iraqi) for various reasons. Vitamin levels of 25(OH)D were classified as <12 ng/mL, deficiency; 12-20 ng/mL, insufficiency; and >20 ng/mL, normal [4]. Serum calcium, phosphorus, alkaline phosphatase (ALP), parathyroid hormone (PTH) of all patients were evaluated. Nutritional rickets was diagnosed with inadequate vitamin D and/or calcium levels with elevated ALP and PTH, and radiological findings of rickets [5]. Postero-anterior radiographs of left wrist or knee were done. Widening, fraying and cupping of the distal radial, ulnar and femur metaphyses were considered compatible with the radiographic findings of rickets. The demographic and laboratory findings of these children are given in ***Table* I**. The present study was approved by the Ethics Committee of our institution.

Nutritional rickets was diagnosed in 22 of these children. Consanguineous marriages rate was 59% in families of children with rickets. However, vitamin D-dependent rickets type 1 or type 2 were not diagnosed in these patients. Rickets secondary to vitamin D deficiency was diagnosed in 21 patients. Calcipenic rickets was determined in one patient and his 25(OH)D, $1,25(OH)_2D$, calcium, phosphorus, ALP, PTH, albumin and magnesium levels were 29 ng/mL, 55.9 (25.1-153.8) pg/mL, 6.4 mg/dL, 3.9 mg/dL, 1204 IU/L, 721 pg/mL, 4.8 g/dL and 0.82 (0.70-0.86) mmol/L, respectively. The patient's clinical and laboratory findings of rickets recovered with calcium supplementation without $1,25(OH)_2$ vitamin D supplementation. Progressive familial intrahepatic cholestasis-2 (PFIC-2) was detected as a risk factor in only one patient with rickets. In other patients, there was no chronic disease that would constitute a risk for rickets.

TABLE I CLINICAL AND BIOCHEMICAL FINDINGS IN SYRIAN AND IRAQI REFUGEE CHILDREN (*N*=77)

Parameters	*Syrian (n=54)*	*Iraqi (n=23)*
Age	11.6 (6.9) [1-24]	12 (5.9)[2-23]
Males	32	10
25(OH)D (ng/mL)	28 (24) [2-119]	20 (22) [3-91]
*Vitamin D levels		
<12 ng/mL	18 (33)	13 (56.5)
12-20 ng/mL	9 (17)	3 (13.0)
>20 ng/mL	27 (50)	7 (30.4)
Calcium, mg/dL	9.5 (0.9) [6.4-10.9]	9.1 (1.3) [5.4-10.7]
Phosphorus, mg/dL	5.0 (1.0) [1.7-6.7]	4.4 (1.2) [2.2-6.6]
ALP	392 (346) [59-1955]	371 (298) [73-1014]
*Nutritional rickets	14 (25.9)	8 (34.8)

*All values in mean (SD) and [range] except *n (%); ALP: alkaline phosphatase.*

Infectious diseases were found in nine patients with rickets, and one child was diagnosed with type 1 diabetes mellitus. Dilated cardiomyopathy secondary to rickets was detected in one patient. The cardiac function had improved five months after treatment. Guillain-Barre syndrome was diagnosed in one patient on evaluation of recent-onset quadriparesis. Her calcium and potassium levels were normal.

After the diagnosis of nutritional rickets, a daily oral 2000-5000 IU vitamin D treatment was started in 20 patients [6]. Stoss therapy was implemented at a dose of 150,000 IU orally in two patients. One of them had dilated cardiomyopathy with severe findings and another one had problem with compliance with the daily regimen.

Rickets treatment was completed in 11 patients. Other 11 patients with rickets did not follow up. 10 patients who completed rickets therapy had normal calcium, phosphorus, alkaline phosphatase, parathormone and vitamin D levels after treatment. Alkaline phosphatase level of PFIC-2 patient regressed to 607 IU/L but did not normalize.

Vitamin D plays an important role in cellular and humoral immunity [7]. In the present study, 41% of patients with rickets had concomitant infectious diseases of which, and nearly 80% had lower respiratory tract infection. Several studies have revealed an association between vitamin D deficiency and the development of autoimmune disorders, including multiple sclerosis, Guillaine Barre syndrome, type-1 diabetes mellitus [8-10]. However, there is no conclusive evidence that low vitamin D levels are causally associated with autoimmune diseases.

Thirteen refugee children with rickets were hospitalized and 12 of them were younger than one year. The most common complaint in children aged 1-2 years was genu varum. These findings may suggest that rickets may be the cause of these clinical illnessess or may worsen the clinical findings in these children. Increased sun exposure and intake of vitamin D fortified foods and calcium-rich foods such as milk and dairy products should be encouraged to prevent rickets in refugee children.

In conclusion, most of the patients with rickets had presented to hospital with severe clinical findings or deformity. These results seem to be only the tip of iceberg

concerning vitamin D in refugee children. Moreover, stoss therapy might be considered in refugee children with rickets due to the problem about adherence to a daily regimen.

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Conveyor Belt Entrapment Trauma in Children: An Unreported Menace

A retrospective study was conducted including all the children who sustained motorized machine belt entrapment injuries. Six children included in study had mean (SD) Glosgow coma scale and pediatric trauma score of 5.7 (3.54) and 3.2 (1.21), respectively. Overall mortality and paraplegia rate were 33.3% each. Awareness and legislation both are important to curb this menace.

Keywords: *Belt entrapment, Head injury, Pediatric trauma, Thoracic trauma, Vertebral injury*.

Trauma is one of the most important causes of mortality and morbidity in children [1]. The injuries associated with the use of motorized machines is extremely common due to the lack of safety norms and their poor implementation [2]. The locally made machines with open conveyer belts are being frequently used in the villages. In recent years, we have been witnessing an emerging mode of trauma in children with extremely high case fatality rate and very high morbidity in children who are surviving. We present our experience of managing children with these injuries.

A retrospective review was conducted of the medical records at a level two trauma center of Northern India between May 2015 to April 2019. Clearance was taken from institutional ethics committee. The study included all the children presenting to our trauma center during the study period with a common mode of injury *i.e.* trauma due to entrapment in the open belt of motorized machine. All these children had sustained polytrauma. We classified these children into three groups having different spectrum of injury due to entrapment of different body parts: *viz*, Type I: Children pulled through their torso and had a blow to their head or face from the metallic wheel at the end of the belt; Type II: Children pulled through their torso with torso getting entrapped

between the belt wheel; and Type III: Children pulled through their upper or lower limbs.

Injury severity was assessed by a pediatric trauma score (PTS) and Glasgow coma scale (GCS) [3].

Total of six children (4 female) with belt entrapment injuries presented during the study period. The mean (SD) age of the studied patients was 5 (1.51) years. 66.6% of injuries occurred in rural areas. Two (33.33%) patients were shifted to a trauma center in an ambulance and only three got primary treatment at the health center. Others were directly referred to our trauma center with a median distance of 80 km (30-140). All the children were entrapped while playing near the machines. They were caught in the belt by their clothes while their parents were working nearby. All the patients had polytrauma (***Table* I**). The mean pediatric trauma score was 3.2 (1.21) (range 2-5) and mean GCS score was 5.7 (3.54) (range 3-13) at the time of presentation to the trauma center. Two children were dead at the time of arrival at the trauma center. Two of these had extradural hemorrhage with a parenchymal contusion. Four children had thoracic trauma and three children had associated abdominal and vertebral fractures. Two of these children had paraplegia at the time of presentation with mortality in one patient.

As a developing country with a large pediatric population, we have a huge burden of trauma [4]. We have witnessed an increasing incidence of trauma in children related to construction sites and use of machines. The assignment of children into three groups according to which body part got entrapped in the belt first, was extremely useful in predicting the pattern of injuries and overall morbidity and mortality.

Type I injury was most fatal as children had severe head and maxillofacial trauma. We propose the use of this classification for identifying the different injuries in these children with polytrauma. These injuries may compromise both airway and breathing which is rapidly fatal apart from the head injury itself. Type II injury were also severe in terms of both mortality and morbidity. All three children with these injuries had associated vertebral injuries with two children having paraplegia at the time of presentation. Type III injuries were least severe but also least common. The GCS and PTS scores were much lower when compared to scores reported in other studies [5,6]. This highlights the severe nature of this trauma and an overall mortality rate of 50% [6]. The use of Roller machines and conveyer belts have become extremely common, and they are a part of numerous manufacturing units. Though there are safety norms, but because of cost cutting their implementation is extremely poor [7,8]. Scarf used around the neck by females in our region has been reported to cause similar injuries [9].

The belt entrapment injuries in children have very high mortality and morbidity. The incidence may be higher because of very high prehospital mortality rate. Legislation for norms and their strict implementation is required for prevention of these injuries.

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TABLE I DESCRIPTION OF PATTERN AND DEMOGRAPHY OF CHILDREN WITH CONVEYOR BELT ENTRAPMENT TRAUMA

S.No	*Age/ gender*	*Type*	*PTS/ GCS*	*Head injury*	*Thoracic injury*	*Abdominal injury*	*Vertebral injuries*	*Bony fractures*	*Open wound*	*Mortality/ paraplegia*
1	6/F	II	4/6	–	+	+	+	+	–	–/+
2	7/M	IIw	4/6	–	+	+	+	+	–	–/+
3	4/F	III	5/13	–	+	–	–	+	+	–/–
4	5/M	II	2/3	+	+	+	+	–	–	–/–
5	4/F	I	2/3	+	–	–	–	–	–	+
6	4/F	I	2/3	+	+	–	–	–	–	+

PTS: Pediatric trauma score; GCS: Glasgow coma scale.

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Single Hepatitis B Booster Dose in High-risk Children with Suboptimal Surface Antigen Antibody Responses After 3-dose Primary Vaccine Series

This was a descriptive study of 30 children born to HBsAG positive mothers between June 2009 and December 2013. All children had anti-HBs response ≤100 IU/L after 3 doses of hepatitis B vaccine primary series. A single booster dose led to hepatitis B surface antibody titers ≥100 IU/L in (85%) of children.

Keywords: *Immunization, Prevention, Seroprotection.*

Approximately 10% of infants are non-responders or have suboptimal vaccine response with hepatitis B surface antibody (anti-HBs) titers ≤100 IU/L three months post-3 dose hepatitis B vaccine series [1-4]. Controversy remains over the need for booster dose in suboptimal responders with antibody levels 10-100 IU/L. None of the international guidelines address this, especially in high-risk infants born to hepatitis B chronic carrier mothers [1,5]. The study aimed to describe the change in anti-HBs titers in infants born to hepatitis B carrier mothers and anti-HBs titer of ≤100 IU/L after the 3 dose primary series; and to determine if for infants with anti-HBs titer of 10-100 IU/L, a single booster of 10 µg hepatitis B vaccine will increase the anti-HBs titers to >100 IU/L.

This was a descriptive study of children born between June 2009 to December 2013, to hepatitis B surface antigen (HBsAg) - positive mothers, at a tertiary university hospital in Singapore, with anti-HBs response ≤100 IU/L after completing 3 doses of hepatitis B 10 µg vaccine given at birth, and age of 1 month and 6 months. Vaccine response was defined based on anti-HBs level done 3 months after completion of the third vaccine dose *viz*. non-responder (anti-HBs <10 IU/L) or suboptimal responder (anti-HBs ≥10 IU/L but ≤100 IU/L). Occult HBV infection was defined as the presence of hepatitis B infection with undetectable hepatitis B surface antigen (HBsAg) [6].

Demographic data and details of maternal HBV infection were collected for all children. Baseline anti-HBs levels were checked for children who were suboptimal responders before administration of the fourth booster dose [intramuscular 10 µg monovalent hepatitis B (Engerix B, GSK, Wavre, Belgium)]. Eight weeks post-booster, HBsAg, HBV DNA, hepatitis B core antibody (anti-HBc) and anti-HBs titres were measured. Children who were non-responders received a repeat three dose vaccine series and were excluded from follow-up. Children whose mothers had hepatitis C virus or HIV infection, or children born before 37 weeks gestation, had a birthweight less than 2.5 kg, or known primary immuno-deficiency, were excluded. Informed consent was obtained from their parents and assent from those older than 6 years. Study was approved by the National Healthcare Group Domain Specific Review Board.

Data were analyzed with SPSS version 25.0. Comparisons were done using Mann Whitney test, and significance was taken as $P<0.05$.

Thirty-nine children (3 non-responders and 36 suboptimal responders) were eligible for the study; 30 (13 females) were recruited (3 non-responders and 27 suboptimal responders). Mean (SD) age at time of recruitment was 63 (31.5) months. Majority were Chinese (80%). Mean (SD) birth weight was 3.22 (0.26) kg. Twenty-four were breastfed until 9 months, 6 were born *via* Caesarean section.

Five (16.7%) mothers were HBeAg positive with HBV DNA viral load of >200,000 IU/mL in their third trimester prior to starting tenofovir. Two (6.7%) received tenofovir during the last trimester. There was incomplete data for 9 children; 4 (13.3%) declined booster vaccination and 5 (16.7%) declined blood tests post-booster for personal reasons. Hence, 21 children had both pre and post-booster serological results for analysis. No children had detectable HBV DNA or reactive anti-HBc.

Median (IQR) anti-HBs titers 3 months after completion of the primary vaccine series was suboptimal at 52 (22-77) IU/L. Median (IQR) anti-HBs titers just prior to booster vaccine further dropped to 7 (2-11) IU/L (P<0.05); 3 (10%) children had values <10 IU/L. Mean (SD) time from completion of three-dose vaccine series to booster vaccine was 62.6 (31.1) months. Median (IQR) anti-HBs titer rose significantly to 606 (134-1000) (P<0.05)) IU/L post-booster vaccine. Eighteen children (85.7%) demonstrated good anti-HBs response (>100 IU/L) after the booster dose. Three children (14.3%) continued to have suboptimal response post-booster vaccine (***Fig.* 1**).

Our study demonstrated that 85.7% of children with suboptimal immune response post-primary series achieved anti-HBs >100 IU/L after a single booster dose, supporting the 2018 ACIP guidelines that a single booster is sufficient instead of three repeat doses [5].

We also demonstrated that anti-HBs titers in infants born to hepatitis B carrier mothers, and who had suboptimal antibody titer three months after completing the three-dose primary series, declined further over the next four years. Occult HBV infection was not detected in this population as a cause of suboptimal response. Our small series contributes to supporting evidence for a single booster, which is cheaper and logistically easier, instead of repeating the three dose series. A single booster may also increase adherence to vaccinations and conserve public health resources involved in vaccine administration.

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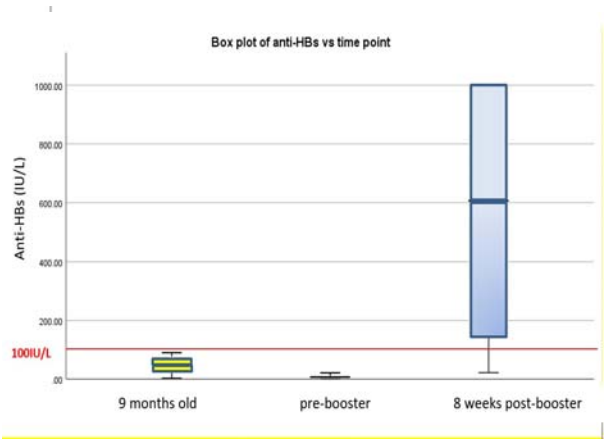

FIG. 1 *Box plot of anti-HBs versus time points.*

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Hepatitis A Virus Infection Associated with Cryoglobulinemic Vasculitis

Atypical symptoms, especially immune complex disorders, are uncommonly reported with hepatitis A virus (HAV) infection. We report an 8-year-old child who contracted HAV infection complicated by cryoglobulinemic vasculitis, and responded well to oral steroids. HAV infection may be considered in the etiology of cryoglobulinemia in children.

Keywords: *Acute hepatitis, Cryoglobulinemia, Vasculitis*.

Hepatitis A virus (HAV) infection in children is described as a mild condition, mostly asymptomatic. It is still a major public health problem in developing countries [1]. Atypical manifestations are very rare. We report a case of an 8-years-old girl who presented with acute hepatitis due to HAV infection complicated by cryoglobulinemic vasculitis.

An eight-years-old girl, with no significant medical history, presented to emergency department for an acute fever (up to 39 Celsius) (degee) and jaundice evolving for 5 days. She had white stools and very dark urine. The child was asthenic and anorexic. Serological screening was positive IgM anti"HVA antibodies. Management consisted of symptomatic measures, and the child was discharged home.

She persisted to have jaundice and pruritus. By eight weeks, she had an acute onset of epistaxis, arthritis of large joins (knees), and vasculitic rash (of legs, forearms, and the back), evolving for 2 days. The child was conscious with no neurological symptoms. She had normal body temperature and blood sugar. We found no other clinical abnormalities. Dipstick test was positive to proteins (2+) and blood (3+). Laboratory analysis showed cholestasis and hepatic cytolysis with no liver failure (prothrombin time = 84.4%, SGPT = 178 IU/l, SGOT=141 IU/L, total bilirubin = 66.1 mg/l, GGT = 101 IU/l, ALP=110 IU/l). Ultrasonography showed hepatosplenomegaly with acalculous gallbladder and thickened wall. The first hour, CRP=38.25 mg/L, she had normochromic normocytic anemia (hemoglobin, 10.1 g/dL), lymphocytosis (7220/mm^3), mild proteinuria (180 mg/24h), and features of inflammation (ESR=80 mm). HAV serological test identified negative IgM antibodies and positive IgG antibodies. Due to the atypical manifestations, we biopsied the vasculitic rash and found a leukocytoclastic vasculitis. Negative serodiagnosis of Epotein Base virus, hepatitis B, C and E virus, Cytomegalovirus HIV eliminated another viral cause of this condition. While, repeated blood cultures to look for a bacterial or fungal infection remained sterile. There were no additional clinical or biological abnormalities suggesting an autoimmune cause for the vasculitis (e.g. systemic lupus erythematous, systemic juvenile arthritis, or systemic vasculitis as Wegener granulomatosis and polyarthritis nodosa). In this context, anti-nuclear antibodies, anti-DNA antibodies, rheumatoid factor, and anti-nuclear anti"cytoplasmic antibodies were negative. Biological tests to look for Wilson disease and auto-immune hepatitis were normal. Cryoglobulin assay was positive to IgM, IgA, and IgG (mixed cryoglobulinemia type II). Management consisted of starting oral steroids (prednisone) at 1 mg/kg/day. Ten days later, we noted total regression of cutaneous, and osteoarticular symptoms. The regression of clinical and biological cholestasis was achieved by three months, and prednisone tapering started. Steroids were withdrawn by the sixth month. The child remains asymptomatic, with a normal liver function.

Cryoglobulinemia have been mainly described in Hepatitis B and C virus infections, there are rare case reports with HAV, mostly in adults [3]. The intrinsic mechanism by which viral hepatitis promotes cryo-globulin production is unclear. Virus persistence, may represent a continuous stimulus for host immune system unable to produce neutralizing antibodies; and cryoglobulins may represent the product of virus-host interactions in this context [4]. Treatment with oral steroids has shown benefit in adults [5]. Some authors suggest genetic predisposition to immune complex disorders after viral infections as the likely pathogenesis [6].

Treatment with steroids is effective in reducing the atypical manifestations of acute HAV infection in the form of cryoglobulinemia-related symptoms. Further studies are necessary to establish physio-pathogenesis and standardized protocols for the management of this rare condition.

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Cephalic Tetanus Presenting as Ptosis

A 7-year-old unimmunized boy developed cephalic tetanus following chronic suppurative otitis media. We wish to emphasize that possibility of cephalic tetanus should be considered in an unimmunized child presenting with ptosis.

Keywords: *Immunization, Management, Otitis media.*

Tetanus can be generalized or localized. Cephalic tetanus is a rare variant of localized tetanus, involving 1 to 3% of total reported cases [1]. The objective of this report is to describe a case of tetanus secondary to chronic suppurative otitis media in an unimmunized child, presenting with isolated ptosis, an unusual presentation of cephalic tetanus.

A 7-year-old boy belonging to a low socioeconomic status family, presented in the pediatric casualty. Five days prior to hospital admission, parents noticed child having oral ulcers secondary to tongue biting. Since 3 days, he was having multiple episodes of intermittent tightening of all 4 limbs lasting for few seconds and clinching of teeth during which he sustained multiple tongue bites. During these events, child was conscious, no facial deviation/asymmetry and no bladder bowel incontinence. There was no history of fever, trauma, animal bite or recent vaccination history. There was no history of difficulty in feeding, breathing or arching of body/opisthotonus. Child was having left ear discharge on-and-off since two years of age, for which he was taking treatment from local practitioner.

On examination at admission, child was conscious, following commands, and hemodynamically stable. On oral examination showed multiple cuts on tongue with oral ulcers. There was reflex spasm of masseters on touching the posterior pharyngeal wall (spatula test - positive). Left eye ptosis was present. Rest of the cranial nerves examination including pupillary reactions were normal. During examination child had tightening of all four limbs with teeth clinching lasting for less than a minute. He was conscious during the event which was self-aborted. CNS examination done after this event was normal. Baseline hematological work-up, liver and renal function tests and serum calcium and electrolytes were normal. Lumbar puncture and cranial computed tomography (CECT Head) were normal.

By the history, examination and his unimmunized status, tetanus was strongly suspected and was started on treatment for tetanus in the form of intravenous. Ceftrioxane and metronidazole, along with supportive management. Intramuscular and intrathecal tetanus immunoglobulin (TIG) along with one dose of tetanus toxoid was given. Intravenous diazepam was started to control spasms. He was monitored for neurological and respiratory deterioration. Child started improving clinically, tetanic spasms reduced and diazepam was tapered slowly and was discharged on oral diazepam on the 14th day of admission. The parents were counselled and planned for catch up immunization. He was followed-up one week after discharge, with full recovery of ptosis, no spasms. The otorrhoea had ceased.

Tetanus is strictly a clinical diagnosis, there is no laboratory test to confirm it. In our case the diagnosis was strongly suspected by the history, examination and unimmunized status of child.

Cephalic tetanus is defined as a combination of trismus and paralysis of one or more cranial nerves. The facial nerve is most frequently implicated but cranial nerves III, IV, VI, VII, and XII may also be affected [2,3]. Facial nerve palsy without trismus at presentation could be the first sign of

cephalic tetanus [4]. Cephalic tetanus usually follows middle ear infections like suppurative otitis media, as in our case or craniofacial injuries [1]. Such otogenic tetanus are common in the pediatric age group which may be explained by the immune status and frequency of middle ear infections. This case was rare in its type as it presented with isolated ptosis without any other cranial nerve involvement, unlike the cephalic tetanus reported earlier with trismus, ptosis and facial palsy mimicking Bell's palsy. Around 2/3rd patients with cephalic tetanus progress to generalized tetanus, which could be a possible reason fo the generalized spasms in this child.

The mechanism of cranial nerve palsies is not fully understood but few studies have given explanations like swelling of facial nerve under the influence of the toxin leading to strangulation in the stylo-mastoid canal, third-nerve lesions due to intense absorption of toxin from the orbicularis and ciliary regions, which are supplied by this nerve.

Survival rates in children receiving tetanus immunoglobulins *via* the dual route were significantly higher compared with children who received the intramuscular immunoglobulin only [5-7] and hence we preferred dual route for TIG administration.

High index of suspicion for tetanus should be considered in an unimmunized child presenting with ptosis without apparent trismus or facial palsy.

Contributors: MS: designed and drafted the manuscript; SG: concept and analyzed the manuscript; MY: helped in data analysis.

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An Infant with Milky Serum and a Rare Mutation

A 40-day-infant having milky serum, eruptive xanthomas, hepatosplenomegaly, lipemia retinalis, high cholesterol and triglyceride, was found to have lipoprotein lipase (LPL) deficiency on genetic workup. Triglyceride decreased with dietary fat restriction, medium chain triglyceride and fibrates. LPL deficiency in early infancy can be treated with pharmacological and dietary interventions.

Keywords: *Hypertriglyceridemia, Lipoprotein lipase, Outcome*

Familial chylomicronemia syndrome (FCS) is an autosomal recessive disorder of lipoprotein metabolism due to deficiency of lipoprotein lipase or Apo C II deficiency or presence of inhibitor to lipoprotein lipase. About 25% of FCS cases usually diagnosed during infancy [1]. We present a 40-day-old child with milky serum diagnosed to have lipoprotein lipase deficiency.

A 40-day-old female infant presented with 1 day history of poor feeding and lethargy. The baby was a product of non consanguineous marriage, born at term by vaginal delivery with birth weight of 2.8 kg. Antenatal and post natal period was uneventful. Child was exclusively breastfed since birth. Child has a healthy elder sister. On blood sampling for possible sepsis, child's blood was found to be pinkish white and viscous, which gradually turned into milky white after some time. She had hepatosplenomegaly and eruptive xanthomas over knee, face and buttocks. Liver and renal functions were normal. Sepsis markers were negative. Blood and urine cultures were sterile. Ultrasound abdomen revealed hepato-

splenomegaly with normal hepatic echotexture. Electrocardiography and echocardiography revealed no abnormality. On fundus examination, lipemia retinalis was found. Serum lipid profile revealed cholesterol 1467 mg/dL (Normal <200), triglyceride (TG) 5997 mg/dL (Normal <150), VLDL 1199 mg/dL (Normal <30), LDL 310 mg/dL (Normal <110), and HDL is 133 mg/dL (Normal 40-60**)**. Thyroid function, serum amylase and lipase were normal. Hemoglobin was low (7 gm/dL). Both parents had normal lipid profile, but her elder sister had moderately elevated triglycerides. No history of convulsion, jaundice, bleeding manifestation, skin rash. There was no known case of hyperlipidemia or premature sudden death in family.

Provisional diagnosis of familial chylomicronemia syndrome was considered because of extremely high triglyceride with moderately raised cholesterol with hepatosplenomegaly, eruptive xanthoma and lipemia retinalis. Blood was sent for genetic analysis and child was started on Fenofibrate, medium chain triglyceride containing oil and low fat diet (skimmed milk). Lipid profile repeated after 15 days revealed Cholesterol decreased to 297 mg/dL, triglyceride- 1793 mg/dL, VLDL -358 mg/dl and LDL-106 mg/dL. Fundus was normal on re-evaluation.

Genetic analysis revealed homozygous nonsense mutation in exon 5 of *LPL* gene in chromosome 8 resulting in premature truncation of the protein at codon 191 (p.Tyr191Ter) a rare mutation not reported previously in literature**.**

Hypertriglyceridemia is defined as having plasma triglyceride above the 95th percentile for age and sex [2]. Diseases having hypertriglyceridemia have a high risk metabolic dysfunction and cardiovascular diseases. Lipoprotein lipase is a key enzyme needed for hydrolysis of triacylglycerol in chylomicrons and LDL. Worldwide incidence is 1 in 1 million for LPL deficiency [3]. LPL deficiency in children usually has varied presentation. When TG >2000 mg/dL with eruptive Xanthoma mostly appear on shoulder, buttocks and extensor surface of limbs, lipemia retinalis is manifested when TG level surpasses 2500 mg/dl [4]. They are at increased risk of recurrent pancreatitis leading to pancreatic insufficiency which is the major morbidity of this disease and risk increases with level >1000 mg/dl [2]. Genetic analysis is the preferred and most readily applied method for diagnosis, as LPL mass assay is not easily available everywhere. Common *LPL* gene mutations reported in literature are Asp9Asn, Gly188Glu, Pro207Leu, Asp250Asn, Asn291Ser, Ser447X, Pro214Ser etc [5]

Differential diagnosis are Familial dysbetalipoproteinemia in which cholesterol and TG are elevated to a similar degree and presentation is in adulthood with tuberoeruptive xanthoma, Familial hypertriglyceridemia which presents without xanthoma with increased TG but normal cholesterol and Familial combined hyperlipidemia where cholesterol is more raised than TG without xanthomic eruptions.

Mainstay of treatment is severe dietary fat restriction for the lifetime [4]. MCT oil is recommended in chylomicronemia as it gets absorbed directly to portal circulation. The drugs studied and recommended for hypertriglyceridemia are fibric acid derivatives. These have the effect of both raising HDL and lowering triglycerides. Various case reports are there favouring use of fibrates without many side effects [6]. In our case we used fenofibrate which child was tolerating well till 3 months of follow-up.

Finding of lipemic serum during routine investigations should always be evaluated in detail. Early diagnosis, medical intervention by lipid-lowering agents and dietary modification can improve the prognosis and maintain a near normal lifestyle as the risk of pancreatitis and frequency of hospital admissions is significantly reduced.

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Pamidronate in Treatment of Calcinosis in Juvenile Dermatomyositis

Juvenile dermatomyositis is a rare systemic autoimmune disease wth calcinosis as its hallmark sequelae. We report three patients with juvenile dermatomyositis with calcinosis, who were treated with pamidronate. There was complete clearance of calcinosis in one child.

Keywords: *Management, Sequalae.*

Juvenile dermatomyositis (JDM) is an inflammatory disease of the muscle, skin and blood vessels with peak age of onset 5-14 years and female:male ratio 1.7:1 [1,2]. Calcinosis is a hallmark sequelae [3]. Pamidronate has been used earlier for this indication [4] but there is lack of reported experience in Indian set-up.

A 9-year-old boy presented to us with complaints of fever, pain in all limbs and difficulty in walking for one and half months. Child was initially treated with non-steroidal anti-inflammatory agents (NSAIDS) after which pain had initially subsided to recur again. On examination, there was peripheral myopathy, heliotrope rash and Gottron papules. Laboratory investigations showed hemoglobin 7.7 g/dL, elevated lactate dehydrogenase (399 U/L) and creatinine phosphokinase (321 ug/L), and normal electromyogram. A muscle biopsy from Vastus lateralis showed features of immune-mediated inflammatory myopathy. Child was diagnosed as having JDM, and was started on steroids (20 mg/day), hydroxychloroquine (100 mg/day) and methotrexate (7.5 mg once a week). The child improved over the next 3 years with good compliance and regular follow-up, and gradually steroids were tapered to 5 mg/day. He presented after fall from a bicycle with swelling of the little finger with chalky white discharge for 3 days (***Fig.* 1a)**. *X*-ray revealed calcium deposits in soft tissue of little finger (***Fig.* 1b)**. Pamidronate was infused at 1 mg/kg/day for 3 consecutive days every 3 months. On follow-up after one year, there was complete clearance of calcinosis of fingers without any new focus and good disease control without signs of myositis (***Fig.* 1c** and **d**).

An 11-year-old girl presented with complaints of swelling over right elbow and bilateral buttocks. She was diagnosed as having JDM at 8 years of age. She was initially treated with steroids, hydroxychloroquine and NSAIDS but had poor compliance to medicines. Following a fall from the stairs, she started developing calcinosis of buttocks followed by calcinosis of right elbow. There was presence of heliotrope rash and Gower sign. *X*-ray showed calcium deposits on affected areas. Child was started on 3-monthly pamidronate infusion after which there was significant decrease in calcinosis with no fresh foci. Complete resolution of disease process was not observed (***Web Fig.* 1a)**, but the compliance to drugs was also not optimal.

A 7-year-old girl presented with complaints of multiple swellings over the body and difficulty in walking for one year. The first swelling appeared in the waist region, followed by swellings in bilateral chest walls and scalp (***Web Fig.* 2 a)**. The swelling on the chest was excised by a local physician mistaking it to be an abscess. Following this, the child developed more swellings in the lateral chest near the site of excision (***Web Fig.* 2 b)**. On examination, there were nodules on left anterior chest wall with scar marks in bilateral infra-axillary area, Gottron papules and proximal myopathy. Laboratory investigations showed anemia with raised LDH and CPK. Hip *X*-Ray showed white nodular opacity around hip joint, suggestive of calcinosis (***Web Fig.* 2 c** and **d)**. Electromyogram showed membrane instability and fiber destruction. Child was diagnosed as a case of JDM with calcinosis and was started on steroids, hydroxychloroquine, methotrexate, folic acid and 3-monthly pamidronate infusion. Follow-up after 1 year showed significant decrease in scalp swelling with complete disappearance of swelling over chest wall and the waist region with no new calcinosis**.**

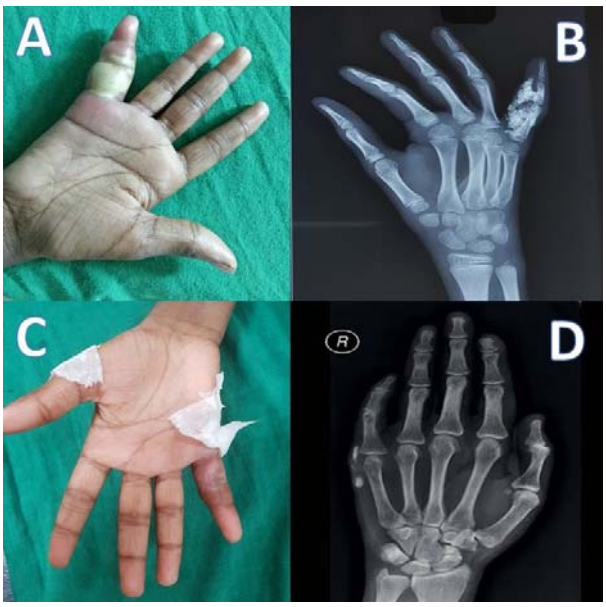

FIG. 1 *(a) Calcinosis in the little finger before treatment; (b) X-ray hand showing calcinosis before treatment; (c) clinical resolution of calcinosis after pamidronate; and (d) radiological resolution after pamidronate*

Calcinosis is a hallmark sequelae of JDM [1]. Alum, alendronate, diltiazem and rituximab are few drugs used for treatment of calcinosis [3]. Pamidronate is a nitrogen-containing bisphosphonate which inhibits bone resorption used to treat osteoporosis [4]. Although the mechanism of action of pamidronate is unclear, it was chosen based on available adult studies [1,4,6]. A significant decrease in calcinosis was found in two cases whereas there was complete clearance in one case. Aggressive treatment with disease modifying anti-inflammatory agents (DMARDs) early in the course of disease seem to be effective in good disease control as was evident from case 1 and 3. Prompt diagnosis and early intervention prevents further calcinosis. Our results suggest that treatment with pamidronate infusion may achieve good disease control in prevention of further calcinosis in JDM.

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Electrical Injury Causing Facial Nerve Palsy in a Toddler

Although electrical injuries are one of the common injuries encountered in clinical practice, low voltage electrical injuries presenting as focal neurological deficits are rare. We report the case of a 3-year-old boy who presented with right facial palsy and hemotympanum after electrical injury.

Keywords: *Childhood injuries, Electric shock, Paralysis.*

The curiosity of children to explore new things increases their risk of childhood accidents [1,2]. The most common sources for electrical injuries in children are electric sockets, faulty appliances, and live wires [3].

A 3-year-old boy was brought to us for consultation with deviation of angle of the mouth and inability to close his right eye for a day. Neurological examination showed deviation of angle of mouth to left side, absence of wrinkling of forehead on the right side, and incomplete closure of the right eye, with rest of the central nerve system and systemic examination was normal. Initially, idiopathic Bell palsy was considered as the diagnosis, but when a detailed history was elicited, a history of electric injury 1-day back was revealed which was thought to be irrelevant by the parents and hence was not initially revealed by them. The child did not have any history of trauma to the right ear or face, and there were no symptoms suggesting infection of right ear. Otoscopic examination revealed the presence of reddish-blue ear drum suggesting hemotympanum. Blood cell counts, creatinine kinase, urine analysis, renal and liver function tests, and electrocardiogram were within normal limits. Prothrombin time, activated partial thromboplastin time, bleeding time and clotting time were also found to be within normal limits.

The child was diagnosed as having Grade IV of House Brackmann lower motor neuron (LMN) type facial nerve palsy of right side with hemotympanum due to low-voltage electric current injury. Patient was started on low-dose oral steroids, eye lubricant and eye bandage to prevent exposure keratitis. The child was discharged after 3 days on low-dose oral steroids. On reviewing after one week, there was improvement of the facial palsy (grade 3). At follow-up, almost three months following the incident, the child had fully recovered with no residual facial nerve palsy or hemotympanum.

Low-voltage electricity, commonly used for household purpose, is the most common type of electric injury in children. Low-voltage current transmits through tissues like blood vessels and CNS tissue offering low resistance [4]. Low currents can induce fatal injuries, especially in children due to their increased body surface area to volume ratios, reduced overall fat content, thinness of skin and slowed withdrawal from the source of electric currents [5]. The injury sustained by the patient described in this report is unusual, the current being delivered at the right hand causing hemotympanum and facial palsy as it traversed the middle ear. Unilateral facial nerve palsy is commonly idiopathic, followed by traumatic, infectious, malignancy, familial and rarely congenital; it following a low voltage electric injury is extremely uncommon [6].

Electrical injuries are one of the commonest accidental household injuries in children and almost always preventable. Adequate adult supervision is always advised whenever the children are around potential electrical hazards [2]. Electrical injuries may present with an obvious external injury or it can remain hidden from an unsuspecting eye. Thus, it is important to elicit this history carefully from parents in suspected cases.

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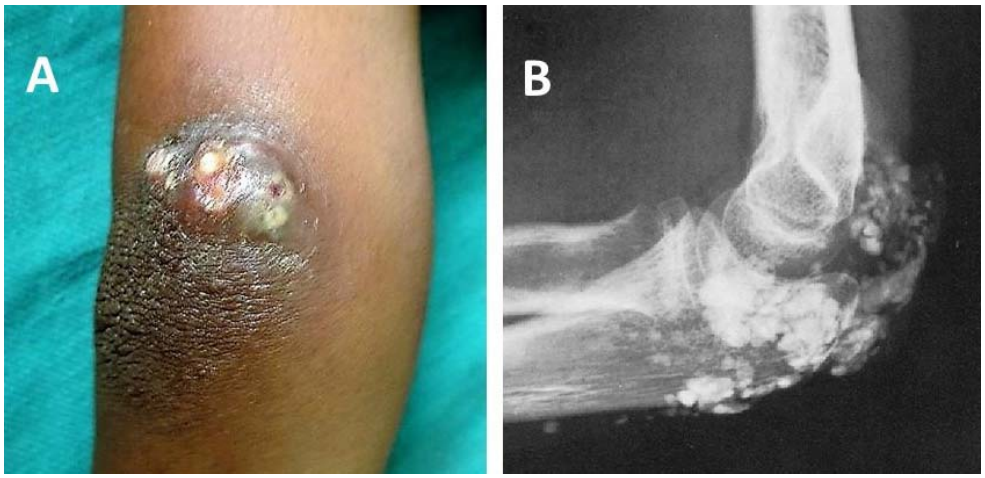
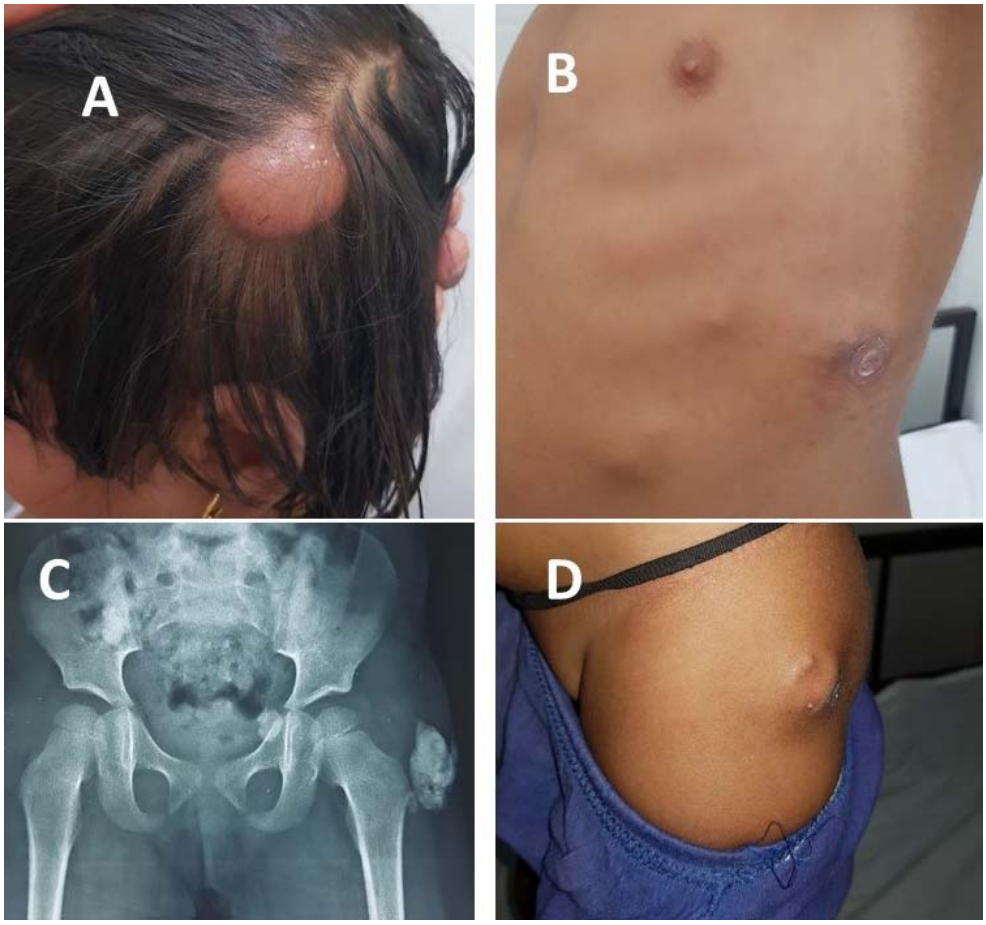


WEB FIG. 1 *(a) Calcinosis in the right elbow; and (b) X-ray elbow showing calcinosis before treatment.*



WEB FIG. 2 *(a) Scalp calcinosis; (b) multiple nodular swelling in lateral chest with scar mark; (c) X-ray pelvis with calcinosis; and (d) calcinosis in lateral aspect of the hip.*

When I Accepted the Denial

As a first year pediatric resident—sleepless, tired, and hungry–I distinctly remember a late night admission of an infant with severe dehydration, referred for securing an intravenous line. On examination, he appeared acidotic, and had evidence of the many veins that had already been punctured for cannulation. After trying to put-in an intravenous cannula with full dedication, I reported my failure to the registrar. "You cannot use an excuse of inability", was the curt response. "We are dealing with lives and 'not being able to' in any situation is simply not acceptable. Secure an intraosseous line and start fluids without wasting any time." I learnt a valuable lesson that our profession has no option of 'cannot', whatever the circumstances. Since we deal with living beings, there is a very high threshold for being unable to perform.

With each passing day of my years of training, we were continuously taught to evolve professionally; become more rational, more scientific, practice evidence-based medicine and follow guidelines. When I began my medical practice, I considered it my moral duty to treat all my patients with dedication and updated knowledge, win the trust of their parents, and educate them about the nature of the concerned illnesses and treatment. Then reality struck and I found all the knowledge and skills that I had learnt being put to test as I encountered people belonging to varied backgrounds, levels of education and diverse upbringing, and harboring their own traditions and beliefs.

Lots of children referred to my clinic present with speech delay, and turn out to have Autism. Parents are oblivious of the other symptoms of this disorder, besides the only too apparent speech issues. They are unable to recognize the social and non-verbal communication deficits in their child, and completely ignore the stereotypies and tantrums. I used to spend hours with parents explaining to them the nature of the illness, the importance of early intervention, and teaching them strategies to decrease the problem behaviors. However patiently I explained, disbelief was evident in their eyes, posture and expression, loudly proclaiming their reluctance to accept the diagnosis without saying a word. Their brains immediately started looking for some reason; however innocuous, that they could blame the problems on.

There is no dearth of reasons that materialize while parents attempt to rationalize; "My mother told me, I started to speak when I was 4 years old, he is only 2 years and 9 months. We have come only because our pediatrician insisted. We are not really concerned." During the third follow-up visit of the same boy, when I greeted him with his name, his father quickly interrupted, "Ma'am, please call him with a different name. Our *punditji* believes that his issues are due to the incorrect spelling of his name and has advised to add a "U" at the end. He has assured us that he will start improving now" (Incidentally, the child's father is a software engineer consultant and mother, lecturer of mathematics, in an premier university). One mother said, "He spoke perfectly well before he received a vaccine at fifteen months. I read on the internet that vaccination can cause speech regression. Now that I have decided to stop vaccinating my child, I think he will get better." Another explanation that was offered to me was; "I think the speech problems are because he has been exposed to a lot of television. Once I stop that, he will start talking normally." My efforts to demonstrate the absence of response to being called by a particular child's name, was met with a retort packaged in false pride, "Doctor, he is concentrating on his work so he won't respond.''

I was; however, programmed to apply evidence-based medicine to my practice. I found the ignorance displayed by the non-medical population exasperating and blamed their denial on their lack of scientific knowledge. My theory was subjected to a harsh reality-check when I encountered a colleague, whose son had red flags of autism. Being a pediatrician, she was aware of Illingworth's developmental cognizance. On sharing my concerns with her, she scoffed and said, "Like father, like son. My husband has no friends either and is also very shy. He is lost in his own world and hardly speaks with me. After his clinic, he will come home and just sit in front of the television. I have to call him four or five times before he listens. I don't see any abnormal behavior in him. I think the cause of my son's speech delay is because we speak in Bengali at home, and at school they use Hindi or English.''

I accepted the denial...

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Seizure in a Child with Guillain-Barré Syndrome: Association or coincidence!

A 6-year-old normally developing girl presented with progressive gait instability for the past three days followed by loss of ambulation along with paresthesia and pain in bilateral lower limbs. There was a history of probable viral upper respiratory infection two weeks prior to the presentation. On examination, she had flaccid weakness (power was MRC grade 2 in all limbs) and areflexia, without respiratory or bulbar involvement. Rest of the central nervous system (CNS) examination was unremarkable. Nerve conduction study showed motor-sensory axonal polyneuropathy. With a clinical diagnosis of Guillain Barré syndrome (AMSAN variant), she was started on IVIg (2 g/kg over 5 days). On third day of illness, she had an episode of generalized tonic-clonic seizure, lasting for two minutes. At that time, she did not have any fever. Her heart rate and respiratory rate, blood pressure, serum electrolytes and infection workup were normal. Magnetic resonance imaging of the brain and inter-ictal electroencephalogram were unremarkable. cerebrospinal examination revealed albumino-cytological dissociation (5 cells, protein: 74mg/dL), normal sugar, and sterile culture. Stool culture yielded no growth. She regained ambulation within two weeks and there was no seizure recurrence.

Guillain-Barré syndrome (GBS) is an immune-mediated polyradiculoneuropathy that primarily affects peripheral nervous system; however, rarely it can involve CNS [1]. The reported manifestations of GBS are encephalopathy, seizures, dystonia, myoclonus, visual disturbance, and nystagmus [2]. The pathogenetic mechanism of these CNS features are posterior reversible encephalopathy syndrome secondary to autonomic instability, watershed infarction, demyelination, adverse effects of immunoglobulin and hypoxic brain injury due to respiratory complications [3]. Isolated seizures without encephalopathy or aseptic meningitis are rarely reported in GBS.

Koul, *et al*. [4] reported a 10-year-old girl with Fischer variant of GBS who had recurrent myoclonic seizures during the course of the illness. The exact mechanism of seizures in GBS is unknown. Koul and colleagues [4] suggested a brain stem origin of the myoclonus. Seizures are a common manifestation in various autoimmune neurological and systemic disorders. GM1 is expressed both in central and peripheral nervous system; however, the pathological changes are evident only in peripheral nervous system as blood brain barrier is less permeable for autoantibodies [5]. The possible pathophysiological mechanism in the index case may be immune-mediated neuronal damage as other postulated mechanisms are unlikely to explain seizures in our case, or it can be merely a coincidence due relatively high prevalence of unprovoked seizures and GBS in the community.

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Levetiracetam is Still Not a First-line Treatment in Neonatal Seizures

I read with interest the research article by Gowda, *et al*. [1] and the accompanying editorial by Swami and Kaushik [2]. I would first like to commend the authors for conducting a randomized study to compare levetiracetam with phenobarbital in neonatal seizures. I completely agree with two particular observations documented in the editorial *viz*, the need for future robust trials before considering levetiracetam as the first-line therapy, and the need of continuous video EEGs for confirmation of cessation of seizures.

Moreover, it would also be important to assess and document the seizure severity (seconds of seizures/hour) before the study drug administration. As most neonatal seizures are symptomatic in nature and self-resolving, administration of the study drug during decreasing seizure trend can falsely mimic improvement from the study drug rather than the natural tendency of seizures to gradually decrease in severity and stop.

The authors in the research study used 20 mg/kg of levetiracetam as the loading dose, with a further loading dose of 20 mg/kg in the presence of continuing seizures. However, the results did not mention how many neonates in the study required this second dose. Additionally, this dose may be inadequate as a loading dose; a recent phase 2b randomized controlled study (NEOLEV2) showed that a higher levetiracetam dose (increase to 60 mg/kg from 40 mg/kg) had been associated with seizure remission in 7.5% of additional patients [3]. Additionally, in this study, phenobarbital (80%) was noted to be significantly more effective than levetiracetam (28%) [3].

In general, this cohort had a very high proportion of sepsis/meningitis neonates, almost close to the incidence of hypoxic-ischemic encephalopathy and much higher than other cohorts, including NEOLEV2 cohort. Moreover, the mean age of seizures in the study by Gowda, *et al*. [1] was 8-9 days. It is important to note that the high seizure burden in HIE is in the first 3 days of life and raises an uncertainty of generalizing the conclusion of the study to use levetiracetam as a first line treatment in neonates with HIE, especially during the first 72 hours. Besides, the authors did not mention how many of these patients received therapeutic hypothermia, as that may have some effect on the seizure control.

Although clinical seizure suppression is routinely considered as a good primary outcome measure, a long term follow up to assess neurodevelopmental outcome is necessary as effects of neuronal injury secondary to seizures *vs*. apoptotic injury due to antiseizure medicines are still unknown, and might be more clinically relevant rather than acute seizure suppression.

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AUTHOR'S REPLY

We thank the reader for critically evaluating our research study [1]. The queries raised are addressed below:

Video EEG was not done in our study and we have already mentioned it as a study limitation, and the same has also been highlighted in the accompanying editorial [1,2]. We agree that most neonatal seizures are symptomatic and do not require long-term medications. Our objective was to find out short-term outcome, and it was expected that randomization would have overcome any bias due to spontaneous seizure resolution or resolution due to medications, as it applies for both groups.

Following first dose of levetiracetam (LEV), seizures stopped in 30 (60%) neonates and following second dose, seizures stopped in 43 (86%) in our study [1]. The dose of LEV is not established in neonates and, we used a dose based on published studies, evidence available from off-label use, and our experience. The phase 2b randomized controlled study (NEOLEV2) was published after our study was completed [3]. As there are studies showing that both phenobarbitone (PB) and LEV are equally effective but LEV has lesser side-effects, we need more studies to find a definite answer in this regard.

Our study is on neonatal seizures in general and not specific to hypoxic ischemic encephalopathy (HIE), that may be the reason for mean age being 8-9 days. None of

our newborns received therapeutic hypothermia. We have proposed levetiracetam as an effective and safer alternative to phenobarbitone as a first line drug in neonatal seizures, and not in neonates with HIE [1]. We agree about the need for long term studies to look for neurodevelopmental outcome of these neonates, and the same has been acknowledged already as a limitation of our study.

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Early-onset Fulminant Subacute Sclerosing Panencephalitis in a Toddler

A 22-month-old boy born to non-consanguineous parents with pre-morbid normal development, presented with loss of previously acquired developmental milestones and recurrent head drops for the past 3 months. He was completely unimmunized and had a history of exanthematous febrile illness resembling measles at the age of 11 months. On examination, he was in a minimally conscious state, with generalized dystonia, intermittent choreoathetosis and repetitive myoclonic jerks.

Electroencephalography showed generalized periodic epileptiform discharges, with bursts comprising of high amplitude spike and slow-wave complexes. MRI brain showed patchy periventricular white matter signal changes. CSF measles specific IgG levels were elevated (1:625), confirming the diagnosis of subacute sclerosing panencephalitis (SSPE). He was started on isoprinosine and antiepileptic drugs. At 6 week follow up, myoclonic jerks had subsided; however, he was in vegetative state and had persistent extrapyramidal features.

Neurological syndromes caused by measles virus include primary measles encephalitis, acute post-measles encephalitis, inclusion-body encephalitis and SSPE [1]. SSPE is caused by latent smoldering infection of the brain by wild-type measles virus which has variable presentation and is frequently misdiagnosed [2]. The earliest documented case of SSPE following a postnatally acquired measles infection was at 10 months of age [3]. A total of 15 cases of SSPE have been diagnosed before three years of age [1] of which seven cases occurred following postnatally acquired measles infection.

The clinical course of SSPE was atypical, did not follow the classic four stages of the Jabbour Classification [1] and had history of pre-existing developmental delay or seizures. As compared to older children, course of the disease was fulminant with rapid progression to a vegetative state and fatal outcome [4]. Genetically determined immune dysfunction in the first 2 years of life preventing a successful cell-mediated immune clearance of measles virus has been implicated in this short latency and fulminant course [5]. Other putative genetic factors include genetic polymorphisms of Toll-like receptor 3, programmed cell death-1, MxA, interleukin-4, and interferon-1 genes [5]. Clinicians need to be aware of these important clinical observations to suspect atypical presentation of SSPE in young children. Although neuronal ceroid lipofuscinosis and other lysosomal storage diseases remain the most plausible clinical differentials for progressive myoclonic epilepsy with onset less than two years of age, SSPE should be considered in an unimmunized toddler who presents with cognitive decline, extrapyramidal signs and symptoms, myoclonus and a rapidly progressive fulminant course particularly in developing countries.

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Allgrove Syndrome and a Novel Mutation of *AAAS* Gene in a Boy

A 2.5-year-old boy presented with progressive darkening of lips for the past four months, and absence of tears noticed since early infancy. There was no history of dysphagia or neurological problems. Born to non-consanguineous parents, his two elder sisters had died at age 5 and 6 years after similar manifestations. On examination, he was underweight (9.2 kg, –3.07SDS), stunted (82.4 cm, –2.68SDS) and had small head (45.5 cm, –2.42SDS). Oral mucosa was deeply pigmented. The systemic examination was unremarkable. Laboratory investigations showed normal serum sodium and potassium but low morning serum cortisol (89.2 nmol/L) and elevated adrenocorticotropin levels (1470 pg/mL). Reduced tear production was confirmed by Schirmer test. Barium swallow and endoscopic evaluation showed no achalasia. Clinical exome sequencing showed a previously undescribed homozygous frameshift deletion c.762delC (p.Ser255Valfs* 36) at Exon 8 of the *AAAS* gene, further confirmed by Sanger sequencing. The mutation is considered pathogenic by prediction tools such as SIFT, Mutation Taster and Phenolyzer. The child was initiated on lubricant eye drops and hydrocortisone and showed improved growth over one year (weight 10.8 kg, –2.12 SDS and height 89.4 cm, –2.54 SDS).

Allgrove syndrome (or Triple A syndrome) is an autosomal recessively inherited disorder caused by mutations in the *AAAS* gene that encodes for a protein ALADIN involved in the movement of molecules into and out of the nucleus, probably affecting DNA repair mechanisms leading to cell death [1]. The syndrome may present with any one of the four cardinal features that include achalasia, Addison disease, alacrima and progressive neurological dysfunction, and the symptoms may evolve over a period of time [1]. Alacrima is often the earliest and most consistent finding. Presence of one more symptom warrants molecular analysis of the *AAAS* gene [2]. Although a lower prevalence of neurological dysfunction has been noted in Indian patients, this feature is known to manifest later [1]. The poor head growth in our patient probably indicates the beginning of neurological dysfunction. These patients are also prone to dermatological abnormalities such as hyperkeratosis. The *AAAS* gene mutations are known to affect siblings and may explain sibling deaths with adrenal failure [1,3]. A high index of clinical suspicion is required due to the rarity and presentation as incomplete triad of symptoms [4,5]. Molecular analysis of the *AAAS* gene helps in confirming diagnosis and prognostication.

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Abbreviated Instructions to Authors

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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 30,000 pediatrician members**.** The journal is being published regularly since 1964, and is indexed in PubMed, Current Contents/Clinical Medicine, Science Citation Index Expanded, Medline, Indian Science Abstracts, get CITED, POPLINE, CANCERLIT, TOXLINE, Psych Line and DERMLINE. The journal follows International Committee of Medical Journal Editors (ICMJE) Recommendations for the conduct, reporting, editing and publication of scholarly work in medical journals. Indian Pediatrics is also a member journal of Committee on Publication Ethics (COPE). The journal gives priority to reports of outstanding clinical work, as well as important contributions related to common and topical problems related to children and adolescents, especially those relevant to developing countries. Papers reporting research in Pediatric surgery, Pediatric orthopedics, Pediatric ophthalmology, Pediatric dermatology and Pediatric radiology are also published.

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Criteria for Acceptance

All manuscripts should meet the following criteria: the material is original, study methods are ethical and appropriate, data are sound, conclusions are reasonable and supported by the data, and the information is important; the topic has general pediatric interest; and the article is written in reasonably good English. The article should be submitted in the style of *Indian Pediatrics* (*vide infra*). Manuscripts conforming to ICMJE guidelines [1] will also be accepted and enter the review process; however, if accepted, the final version would need to conform to the journal's style. Manuscripts not prepared as per the journal guidelines or ICMJE guidelines would be sent back to authors without initiating the peer-review process. The current acceptance rate of submitted articles is around 20% overall, and 5% for Clinical case letters. All accepted manuscripts are subject to editorial modifications to suit the language and style of *Indian Pediatrics*. After modifications, they will be sent to the corresponding author for approval.

Review process: About half the submitted manuscripts are rejected after an initial Editorial board review. The usual reasons for rejection at this stage are insufficient originality, serious scientific or presentation flaws, major ethical issues, absence of a message, article not related to children or adolescents, not submitted in desired format, not of interest to majority of readers, or not in accordance with the current priorities of the journal. Decision on such papers is communicated to authors within two weeks. Remaining articles are sent to reviewers having sufficient experience on the subject, in a 'masked fashion'. Manuscripts are reviewed with due respect for authors' confidentiality. Authors should take care not to disclose their and their institution's identity in the text of the 'blinded manuscript.' The peer reviewer identity is also kept confidential.

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Categories of Articles

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Each manuscript should be accompanied with an 8-point structured abstract in not more than 250 words. The 8 subheadings of the structured abstract should be: background, objective, study design, participants, intervention, outcomes, results, and conclusion. The main text of the manuscript should be arranged in sections on Introduction, Methods, Results and Discussion. Key messages should be provided at the end of the manuscript in a box under headings: 'What is Already Known?' and 'What this Study Adds?.' Number of tables and figures should be limited to a maximum of 4 and 2, respectively. Extra tables and figures, subject to clearance by editorial review process, may be made available only at the journal website, as Web table or Webfigure. The typical text length for such contributions is 2500 words (excluding title page, abstract, tables, figures, acknowledgments, key messages and references). Number of references should be limited to 30.

Research Brief: Descriptive observational studies, epidemiological assessments, and surveys are published as Research Briefs. Knowledge, attitude, practice (KAP) studies are generally not preferred. Some of the manuscripts submitted as 'Research Papers' may also be considered for publication under this section at the discretion of editors. A reasonably large series of cases can also be considered for this section. Abstract should be limited to 150 words, and structured using the following headings: Objective, Methods, Results, and Conclusions. The text should contain no more than 1500 words, up to 3 illustrations/tables and up to 20 recent references. The text should be arranged in order of Introduction, Methods, Results and Discussion. Also include a box entitled 'What this Study Adds?' highlighting the main result of the study.

The distinction between Research Brief and Research Paper is purely the journal's prerogative and does not reflect on the originality of the research submitted. The manuscripts will be finally published under the heading of Research Papers.

Research Letter: These are reports of original research not exceeding 800 words of text and 10 references. They may have no more than five authors. Unstructured abstract of up to 50 words reporting the key findings should also be included. Letters must not duplicate other material published, submitted or planned to be submitted for publication. Although unstructured, the text should follow the general sequence of introduction, methods, results and discussion.

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Images: Only clinical photographs with/without accompanying skiagrams or pathological images are considered for publication. Images of radiographs/ histopathology slides alone (without accompanying clinical photograph) are not considered for this section. Image should clearly identify the condition and have the classical characteristics of the clinical condition. Clinical photograph of conditions that are very common, extremely rare, where diagnosis is obvious (e.g., penile agenesis), or where diagnosis is not possible on images alone would not be considered. A short text of about 150 words should be provided in two paragraphs; first paragraph having description of condition, and second paragraph discussing differential diagnosis and management. No references are needed. See guidelines for preparing and submitting figures/images (*vide infra*).

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The journal encourages submission of review articles addressing recent advances/controversies. These may be submitted as either Review Papers, Drug Review, Update or Perspective. Please note that as a routine all review papers submitted to *Indian Pediatrics* undergo a plagiarism check, and the articles are promptly sent back for revision or rejected depending on the extent of similarity with the published literature.

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Drug Review: *Indian Pediatrics* publishes state of the art reviews on drugs/agents meant for therapeutic or prophylactic use in children. It is expected that the authors have sufficient credible experience in the related field. Further guidelines for preparing a drug review are available at the website.

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The topic for this section should be specific and related to child health in general.The number of authors should usually be limited to three.The typical length is 2000 words and may include one figure and one table. Provide an unstructured abstract of up to 150 words. The views expressed should be supported by appropriate evidence and references. Number of references should be limited to a maximum of 25.

Rational Diagnostics: Articles under this section are usually solicited from experts. Authors of some of the articles submitted as reviews might be asked to modify it to submit it under this section. The manuscript should include an abstract (unstructured) of up to 150 words. The main manuscript should have about 2000-2500 words with about 25-30 references. The review should be a

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Other Categories

Clinical Practice Guidelines/Recommendations: In order to streamline the diagnosis, management and prevention of various childhood problems, *Indian Pediatrics* periodically publishes guidelines and recommendations formulated by various Chapters and Task Forces constituted by Indian Academy of Pediatrics (IAP), or other National Associations/Societies. The eight desirable attributes of practice guidelines are validity, reliability and reproducibility, clinical applicability, flexibility, clarity, documentation, development by a multidisciplinary process, and plans for review. In order to maintain uniformity of reporting and improve readability and applicability practice guidelines, a 10-point policy should be followed (*see website*).

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The methods section should describe, in logical sequence, how the study was designed (e.g. how randomization was done), carried out (e.g. how subjects were chosen or excluded, ethical considerations, accurate details of materials used, exact drug dosage and form of treatment) and data were analyzed (e.g. an estimate of the power of the study, exact test used for statistical analysis) [3]. For standard methods, appropriate references are sufficient, but if standard methods are modified these should be clearly brought out. Authors should provide complete details of any new methods or apparatus used. Commercial names of the drugs/equipment may be used once at first mention, with the initial letter capitalized and manufacturer's name and address in parentheses. Subsequently the scientific/non-propriety name is to be used throughout. © or TM in superscript after the propriety name is not required.

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All research studies should have obtained ethical clearance in writing from a formally constituted Institutional Ethics Committee, and the same should be stated in the manuscript (with name of ethics committee clearing the study, along with date and number in the title page; and a statement of ethical clearance without mentioning the identifying details in the Methods section). *Indian Pediatrics* reserves the right to demand a copy of the relevant document, whenever necessary. Even when a study has been approved by a Research ethics committee, reviewers/editors may be concerned about the ethics of the work. Editors may then ask authors for more detailed information and ask them about the ethical and moral justification of the work. Editors may also ask authors to provide the contact details of the research ethics committee that reviewed the work, so that the journal can request further information and justification from that committee. Editors may consult other editorial colleagues, the Committee on publication ethics (COPE), or more commonly the Ethical advisors of *Indian Pediatrics*, to evaluate the ethical aspects of any article, and reserve the right to reject a manuscript on ethical grounds, even if the research was cleared by the institutional ethics committee. Besides rejecting the manuscript, the journal reserves the right of explaining such concerns to the head of the authors' institution or the medical council in order to prevent unethical practices and to protect patients.

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in illustrative material. Written consent must be obtained from parents or legal guardians for publication (in print or electronic form) of clinical details or/and clinical photographs in all 'Case Reports' and 'Images'. (***Annexure* II**: Consent form, *see website*). This consent form need not be submitted with the manuscript but obtaining of consent should be confirmed on the title page. The identity of the patient in clinical photographs should be masked by suitable methods. Assent should be obtained for all children with developmental age above six years participating in clinical studies.

Statistics: Describe statistical methods with enough detail to enable a knowledgeable reader with access to the original data to verify the reported results [4]. When possible, quantify findings and present them with appropriate indicators of measurement error or uncertainty (such as confidence intervals). Provide actual P values, rather than stating as just <0.05 or >0.05. References for statistical methods should be to standard works when possible (with pages stated) rather than to papers in which the methods were originally reported. Specify any general use computer programs used. Define statistical terms, abbreviations, and most symbols. The relevant guidelines may be consulted for appropriate reporting.

Results

This section should include only relevant, representative data and not all information collected during the study. Major findings should be presented clearly and concisely [5]. It may also be useful to mention what the study did not find. Write units along with data at all places in the manuscript. Journal uses the format "mean (SD) ormedian (IQR)" rather than "mean ± SD or median ± IQR" for reporting summary measures. Text, tables, and illustrations should be used judiciously. Avoid repeating in the text the data depicted in the tables or illustrations; emphasize or summarize only important observations. Restrict tables and figures to those needed to explain the argument of the paper. Cite the tables sequentially in the text, and provide each table on a new page after the reference section. Do not insert figures or tables in the main text of the manuscript.

Units of measurement: Measurements of length, height, weight, and volume should be reported in metric units, i.e. meter (m), gram (g), or liter (L) or their decimal multiples. Milliliter or deciliter should be expressed as mL or dL and not ml or dl. Red and White blood cell counts are to be expressed as $\times 10^6$/L and $\times 10^3$/L, respectively. Temperatures should be given in degrees Celsius. Blood pressures should be given in millimeters of mercury (mmHg). All hematological and clinical chemistry measurements should be reported in terms of the International System of Units (SI) **(*Annexure* III**, *see website***)**.

Abbreviations and symbols: Use only standard abbreviations. Avoid abbreviations in the title and abstract, unless pertinent. The expanded form of the abbreviation should precede its first use in the text, unless it is a standard unit of measurement. Year, month, day, hour, minute and second should be abbreviated as y, mo, d, h, min, and s, respectively in tables and figures.

Discussion

Ordinarily it should not be more than one-fourth of the total length of the manuscript. Do not attempt a detailed review of literature [6]. This section should include (unheaded paragraphs in the order specified): (i) a summary of the major findings, (ii) limitations of the study, (iii) their relationship to other similar studies, and (iv) generalizability of the findings, and implications for practice/policy/research. Conclusions should be linked to the goals of the study. Avoid unqualified statements and conclusions not completely supported by the data. Authors should also refrain from making statements on economic benefits and costs unless their manuscript includes economic data and analyses.

References

Authors need to be accurate in citing and quoting references [7]. References should be numbered consecutively in the order in which they are first mentioned in the text. Identify references in text, tables, and legends by Arabic numerals in square brackets. References cited only in tables or in legends to figures should be numbered in accordance with the sequence established by the first identification in the text of the particular table or figure. Use the style of the examples provided with the full instructions at the website. The titles of journals should be abbreviated according to the style used in PubMed, write full names of journals not available on PubMed. Do not use unpublished observations and personal communications as references. References to papers accepted but not yet published should be designated as "in press"; authors should obtain written permission to cite such papers as well as verification that they have been accepted for publication. Do not cite foreign language references unless a certified English version is also available. The references must be verified by the author against the original documents. The Uniform Requirements style (the Vancouver style) is based largely on an American National Standards Institute (ANSI) standard style adapted by the NLM for its databases. Please take care that citations are not directly copied and pasted from websites; remove the hyperlinks

from the same. If the web version of a journal has been consulted instead of the print version, the same should be listed in the list of references. Avoid including any reference to the studies published in predatory journals [8]. Ensure that all hyperlinks have been removed from references directly copied and pasted from the Internet. The manuscript may be returned to authors for re-typing, in case this is detected during the final page-setting.

Tables

Type each table with double-spacing on a separate sheet of paper. Do not submit tables as photographs. Number tables consecutively (Roman numerals) in the order of their first citation in the text, and supply a brief but self-explanatory title for each. Tables with only two columns or those with more them 5 columns should be avoided. Also avoid tables with more than 20 Rows as these are likely to cross-over to the next page during printing. Detailed tables that cannot be adjusted in a single journal page will be incorporated as webtables, at editorial discretion. Give each column a short or abbreviated heading in italic font style. Place explanatory matter in footnotes, not in the heading. Explain in footnotes all abbreviations that are used in each table. For footnotes use the following symbols, in this sequence: *, #, $, ‡, ^, **, ##, $$, ‡‡, ^^, and so on. Identify statistical measures of variations such as standard deviation and standard error of the mean (Do not use ± sign). Ensure that each table is cited in the text.

If data are used from another published or unpublished source, obtain permission and acknowledge them fully. The source of the table should be in the footnote in full, and not by reference number alone. Obtaining the permission from the original copyright holder for reproducing already published material is the responsibility of the author, and any relevant queries will be directed to the corresponding author.

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Legends for illustrations: Type or print out legends for illustrations using double-spacing, starting on a separate page, with Arabic numerals corresponding to the illustrations. When symbols, arrows, numbers, or letters are used to identify parts of the illustrations, identify and explain each one clearly in the legend. Explain the internal scale and identify the method of staining in photomicrographs. Legends should be a part of main manuscript, and should not be sent as supplementary material.

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NEWS IN BRIEF

PROBIOTICS FOR INFANTILE COLIC

Despite years of research, the cause of infantile colic is still shrouded in mystery. There is a growing body of evidence to suggest that altered gut microbiota play a role in this distressing though self-limited disorder. An increase of *Proteobacteria* and a decline of *Bifidobacteria* have been noted in the stools of colicky babies. It is hypothesized that the *Proteobacteria* cause excessive fermentation of carbohydrates and proteins resulting in excessive gas production. Bifidobacteria also change the levels of immunomodulators like butyrate, beta defensive 2 and fecal calprotectin. Butyrate has been shown to modulate intestinal transit times, and visceral and central pain perception as well as produce an anti-inflammatory effect in the gut.

A new randomized controlled study in 80 infants from Italy has shown that *Bifidobacterium animalis subsp. lactis* when given orally daily for 28 days was effective in reducing duration of colic in 80% of the study group *versus* 31% of the placebo group. There was also an increase in fecal butyrate and calprotectin, though that could be tested only in a small number of patients. A previous study with this probiotic along with low lactose, partially hydrolyzed formula had shown good results in reducing colic. This study was done in exclusively breast fed babies, and the intervention may becomea valuable addition in the practicing pediatricians'armory against the frustrating problem of the colicky infant.
(*Aliment Pharmacol Ther. 2019 Dec 3*)

THE SURROGACY REGULATION BILL

India has long been considered the surrogacy capital of the world. Before 2008, commercial surrogacy was rampant in India. There were no legal guidelines till the case of a Japanese baby conceived by surrogacy in India hit the headlines in August 2008. The Japanese parents came to India to conceive by surrogacy but got divorced before the baby was born. In the ensuing confusion was born the seeds of the Surrogacy Bill in India.

The Surrogacy Regulation Bill was finally passed by the Lok Sabha in August 2019 and was then referred to the Rajya Sabha. In this bill commercial surrogacy has been completely prohibited. All foreign nationals, non-resident Indians or Persons of Indian Origin are forbidden to avail commercial surrogacy in India.

It allows altruistic surrogacy to Indian married couples who are childless. The surrogate mother and the couple who want to have children must be close relatives. Homosexuals, single parents or live-in couples are not allowed to have children by surrogacy. Couples who already have children will also not be allowed to use surrogacy services unless the child is severely physically or mentally challenged. A lady can be a surrogate mother only once.

Commercial surrogacy has been considered as the root cause of the prevalent exploitation, misdoings and irregularities, and the Bill is an attempt to reduce this. However, many feel the Bill seriously impinges on the rights of single parents, the LGBT community and couples in a live-in relationship. The Rajya Sabha has now referred it to a 23-member select committee for further refinements.
(*The Times of India 2019 Nov 22*)

VOXELOTORRECEIVES APPROVAL FOR SICKLE CELL ANEMIA

Voxelotor is a drug which aims to correct the root cause of the problems in sickle cell anemia. The drug inhibits polymerization of sickle hemoglobin. The FDA approved it after the phase 3 randomized controlled trial published this year. In the study, 274 patients were randomized to either Voxelotor or placebo. After 24 weeks, there was significant improvement in hemoglobin,and reduction in reticulocyte counts and indirect bilirubin - there were no serious adverse effects. It is now approved for use in patients 12 years and above.

The drug was discovered in 2017 and is an excellent example of basic science research reaching the bedside in a relatively short time. This was because it was granted fast track status and accelerated approval in view of the huge impact it will make on the lives of so many patients. However, caveats remain. Risk of delayed oxygen delivery in the brain leading to silent infarcts needs to be evaluated. Its use in variant SCDs such as sickle-thal and sickle cell disease with hemoglobin below 7 g/dLhave also not been studied.

As with all new drugs, the initial blush of enthusiasm may well fade with the reality of unanticipated side-effects, if we are not vigilant.
(*Medscape Medical News 2019 Nov 25*)

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CLIPPINGS

A big-data approach to producing descriptive anthropometric references: A feasibility and validation study of paediatric growth charts *(Lancet Digital Health. 2019;1:e413-e423)*

WHO international growth standards or national charts are not always found to be perfectly caliberated with the growth of contemporary children in many countries. This study used a novel big-data approachto generate new national growth charts for French children. 32 randomly sampled primary care pediatricians and ten volunteer general practitioners were recruited, who used the same electronic medical records software, from which all physical growth data for children born from Jan 1, 1990, and aged1 month to 18 years by Feb 8, 2018, with birth weight greater than 2500 g was extracted. Growth charts for weight and height were derived by using generalized additive models for location, scale, and shape with the Box-Cox power exponential distribution. It included 1458468 height and 1690340 weight measurements from 238102 children, in comparison to measurements for a median of 17000 children used for growth charts produced in the past worldwide. When compared, all height SD and weight percentile curves were distinctly above those for the existing French national growth charts, as early as age 1 month, with an average difference of 0·75 SD for height and 0·50 SD for weight for both sexes. Comparison with national cross-sectional surveys showed satisfactory calibration, with generally good fit for children aged 5-6 years and 10-11 years in height and weight and small differences at age 14-15 years.

The need to update height growth charts cannot be debated; however, caution should be exercised for updating weight growth charts, in the context of the increasing prevalence of childhood overweight and obesity worldwide.

Should paediatricians initiate orthopaedic hip dysplasia referrals for infants with isolated asymmetric skin folds?(*J Child Orthop. 2019;13:593*-9)

Asymmetric skin folds (ASFs) around the hips in children are often considered an early clinical sign for diagnosing developmental dysplasia of the hip (DDH). This study was done with the purpose to see the utility of isolated ASFs as a screening tool for DDH in a series of patient referred for evaluation. It was a retrospective review of 66 (mean age 6.4 months) consecutive patients between 0 and 12 months of age referred to orthopedic clinics for isolated ASFs. All received pelvic radiographs or ultrasound; 79% were found to have acetabular dysplasia. 36 (55%) were considered normal by their treating physician, and 25 (38%) were considered dysplastic and underwent brace treatment. One hip with an isolated ASF was found to have a dislocated hip on radiograph prior to their initial orthopedic visit. Thus, isolated ASFs can be an indicator of mild dysplasia and warrant further workup or referral.

Systematic review and meta-analysis of virtual reality in pediatrics: Effects on pain and anxiety (*Anesth Analg. 2019;129:1344-53*)

Medical procedures often evoke pain and anxiety in pediatric patients. Virtual reality (VR) is a relatively new intervention that can be used to provide distraction during, or to prepare patients for, medical procedures. The review assessed the effectiveness of VR on reducing pain and anxiety in pediatric patients undergoing medical procedures.The study showed that the overall weighted standardized mean difference (SMD) for VR was 1.30 (95% CI, 0.68-1.91) on patient-reported pain (14 studies) and 1.32 (95% CI, 0.21-2.44) on patient-reported anxiety (7 studies). It also showed a significant effects of VR on pediatric pain when observed by caregivers (SMD = 2.08; 95% CI, 0.55-3.61) or professionals (SMD = 3.02; 95% CI, 0.79-2.25). The results suggested that VR interventions for pain reduction were more efficacious for younger than for older children (P = .015). More specifically, the effect size of VR on pain decreased with 0.26 when age increased with 1 year. For anxiety reduction also, it were more efficacious for younger than for older children (P = .023). However, there was a difference in effect of VR for different medical procedures,so one should be careful when generalizing the suggested effect for VR to clinical practice.

Autonomous early detection of eye disease in childhood photographs (*Science Advances. 2019;5: eaax6363*)

The reflection of visible light by the choroidal and retinal blood vessels causes the human pupil to appear red when examined by a handheld direct ophthalmoscope or photographed with a camera flash. Absence of red reflex can be a symptom of common and rare childhood eye disorders.The authors of this study aimed to evaluate the clinical utility of screening photographic leukocoria by using a free Smartphone application (CRADLE: Computer-Assisted Detector of Leukocoria) available as White Eye Detector. They retrospectively analyzed 52,982 longitudinal photographs of children, collected by parents, casual in nature, before enrollment in this study. The cohort included 20 children with retinoblastoma, Coats' disease, cataract, amblyopia, or hyperopia and 20 control children. For 80% of children with eye disorders, the application detected leukocoria in photographs taken before diagnosis by 1.3 (95% CI, 0.4 to 2.3) years.

Eventhough, this may not replace routine newborn and pediatric eye evaluation, these appears no harm in asking the parents to keep clicking pictures of their kids.

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IMAGE

Scrub Typhus Eschar

An 8-year-old male presented with fever, pain abdomen and vomiting for 6 days. He had tachycardia, tachypnea, left cervical lymphadenopathy, hepato-splenomegaly and an eschar hidden behind left ear (***Fig.* 1**). Laboratory work-up was non-contributory except thrombocytopenia and elevated hepatic transaminases. Child was started on oral doxycycline. IgM-ELISA for scrub typhus was subsequently reported positive. He became afebrile within 36 hours, and was discharged after 7 days following normalization of clinico-laboratory parameters.

Eschar is a pathognomonic sign of scrub typhus and if sought carefully, seen in up to two-third (7% - 68%) of pediatric cases. It begins as a small-papule at the site of mite-bite, enlarges, undergoes central-necrosis and acquires a black crust with surrounding erythema, resembling a cigarette burn. Under appropriate epidemiological setting, painless eschar in a child with fever and multi-system involvement suggests scrub typhus. Other conditions associated with eschar formation are spider-bite (painful), tularaemia (usually on fingers), post-trauma, anthrax (pre-existing skin breach), some other rickettsiosis, and disseminated mycosis.

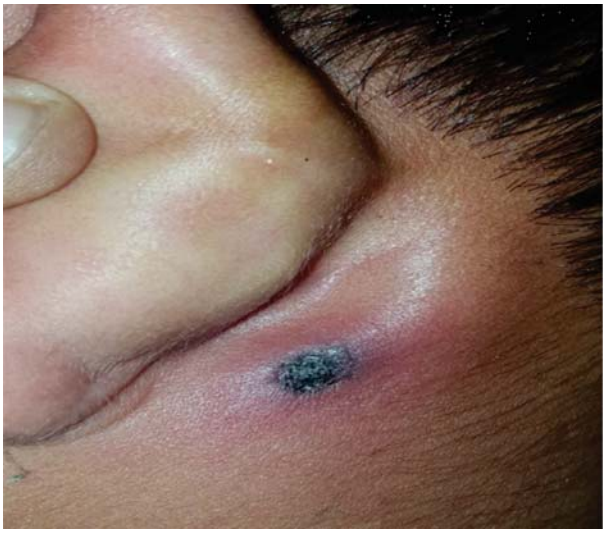

FIG.1 *Eschar behind left ear.*

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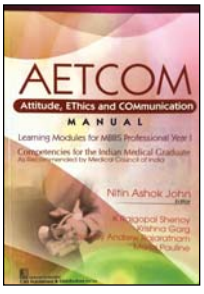
BOOK REVIEW



Attitude, Ethics and Communication Manual

NITIN ASHOK JOHN
CBS Publishers & Distributors Pvt Ltd.
Pages: 178; Price: Not mentioned.

This manual on Attitudes, Ethics and Communication (AETCOM) modules for MBBS first professional students is as per the guidelines provided by Medical Council of India. The best thing about the manual is the last section where Anatomy act, Cadaveric poem, history of anatomy dissection *etc* are provided as a way to understand the background of medical teaching. The manual has many blank pages to write students' notes, which is making this manual thick, otherwise whole exercise content is a replication of the original AETCOM manual provided by MCI. This book is small in size so may be easier to carry by students during AETCOM sessions. The idea of providing few questions or examples of role play at the end of every exercise is also laudable. What I liked most is the chapter on'Cadaver as our first teacher.' The foundation of communication-1 has 5A's Behavior model, and author has lucidly explained the reasons to make this chapter worth reading and practicing. Overall I liked the reading part and easy to use manual. If students also write their reflections in this manual, it will become their memoirs of first year MBBS.

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Infanrix *hexa*

Diphtheria, Tetanus, Pertussis(Acellular, Component), Hepatitis B (rDNA), Poliomyelitis (Inactivated)And Haemophilus Tybe b Conjugate Vaccine(Absorbed) Ph. Eur.

gsk

What really matters?

- Efficacy
- Preterms
- Real-World Experience
- Immune Persistence

The only 6-in-1* DTP vaccine with:[1-3]

- Up to 89% efficacy against pertussis in label[1-3]
- Multiple clinical trials specifically designed for preterm babies**,†,1-6
- The 6-in-1* vaccine with the longest follow-up of immune persistence[1-3]
- Evidence from more than 100 clinical trials and 17 years of real-world experience[1-3,7]

The very common adverse events seen with Infanrix hexa include Fever ≥38°C, local swelling at the injection site (≤50 mm), fatigue, pain, redness, appetite loss, abnormal crying, irritability & restlessness.[1]

*Against diphtheria, tetanus, pertussis, hepatitis B, poliomyelitis and diseases caused by Haemophilus influenzae type b. ** Preterm infants, born at 24–36 weeks' of gestation. Infanrix hexa can be given to premature infants, however a lower immune response may be observed for some antigens and the level of clinical protection remains unknown. † The potential risk of apnoea and the need for respiratory monitoring for 48-72h should be considered when administering the primary immunisation series to very preterm infants (born ≤ 28 weeks of gestation) and particularly for those with a previous history of respiratory immaturity.

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Infanrix hexa
For the use only of a Registered Medical Practitioner or a Hospital or a Laboratory
Abbreviated Prescribing information of INFANRIX HEXA [Diphtheria, tetanus, pertussis (acellular component), hepatitis B (rDNA), poliomyelitis (inactivated) and Haemophilus type b conjugate vaccine (adsorbed) Ph. Eur.]
ACTIVE INGREDIENT: Each 0.5 ml dose of reconstituted vaccine contains (i) Diphtheria toxoid ≥ 30 IU, (ii) Tetanus toxoid ≥ 40 IU, (iii) Bordetella pertussis antigens (Pertussis toxoid 25mcg, Filamentous Haemagglutinin 25 mcg, Pertactin 8 mcg), (iv) Hepatitis B surface antigen 10 mcg, (v) Inactivated Poliovirus [type 1 (Mahoney strain) 40 D-antigen unit, type 2 (MEF-1 strain) 8 D-antigen unit, type 3 (Saukett strain) 32 D-antigen unit], (vi) Haemophilus influenzae type b polysaccharide (polyribosylribitol phosphate, PRP) 10 mcg conjugated to tetanus toxoid as carrier protein (approximately 25 mcg). INDICATION: Primary and booster vaccination of infants against diphtheria, tetanus, pertussis, hepatitis B, poliomyelitis and disease caused by Haemophilus influenzae type b. DOSAGE AND ADMINISTRATION: Posology: Full-term infants: 3-dose primary vaccination: interval of ≥ 1 month between primary doses. Booster dose ≥ 6 months after last priming dose; preferably ≤ 18 months of age. 2-dose primary vaccination: interval of ≥ 2 month between primary doses. Booster dose ≥ 6 months after last priming dose; preferably between 11-13 months of age. Preterm infants (≥24 weeks gestational age): 3-dose primary vaccination; interval of ≥ 1 month between primary doses. Booster dose ≥ 6 months after last priming dose; preferably ≤ 18 months of age. Administered according to official recommendations. Can use in Expanded Program on Immunisation schedule (at 6, 10, 14 weeks of age) if hepatitis B vaccine given at birth. If hepatitis B vaccine given at birth, can use as replacement for supplementary doses of hepatitis B vaccine from age of six weeks. Safety and efficacy not been established in children > 36 months of age. Method of Administration: Deep intramuscular injection, preferably at alternating sites for subsequent injections. CONTRA-INDICATIONS: Hypersensitivity to any active substance or excipient or formaldehyde, neomycin and polymyxin. Hypersensitivity after previous administration of diphtheria, tetanus, pertussis, hepatitis B, polio or Hib vaccines. Encephalopathy of unknown aetiology, occurring within 7 days following previous vaccination with pertussis containing vaccine. Postpone administration in acute severe febrile illness. SPECIAL WARNINGS and SPECIAL PRECAUTIONS: Precede vaccination by review of medical history and clinical examination. Protective immune response may not be elicited in all vaccinees. Will not prevent disease caused by pathogens other than Corynebacterium diphtheriae, Clostridium tetani, Bordetella pertussis, hepatitis B virus, poliovirus or Haemophilus influenzae type b. Can be expected will prevent hepatitis D. Carefully considered decision to give further doses of pertussis-containing vaccines if any following events have occurred in temporal relation to receipt of pertussis-containing vaccine: temperature of ≥ 40.0°C (< 48 hours of vaccination), not due to another identifiable cause; collapse or shock-like state (<48 hours of vaccination); persistent, inconsolable crying lasting ≥ 3 hours (< 48 hours of vaccination); convulsions with or without fever, (< 3 days of vaccination). Appropriate medical treatment and supervision be available in case of rare anaphylactic event. Carefully weigh risk-benefit of immunising or deferring vaccination in infant or child suffering from new onset or progression of severe neurological disorder. Administered with caution in thrombocytopenia or a bleeding disorder. Do not administer intravascularly or intradermally. History of febrile convulsions, family history of convulsions or Sudden Infant Death Syndrome (SIDS) not a contraindication for use. Follow up closely vaccinees with history of febrile convulsions. Rate of febrile reactions higher when co-administered with pneumococcal conjugate vaccine, or with measles-mumps-rubella-varicella vaccine; reactions mostly moderate (less than or equal to 39°C) and transient. Initiate antipyretic treatment according to local treatment guidelines. Special populations: HIV infection not a contraindication. Expected immunological response may not be obtained in immunosuppressed patients. Can be given to preterm infants; however lower immune response been observed for some antigens. Consider potential risk of apnoea and need for respiratory monitoring for 48-72h when administering primary immunisation series to very preterm infants (born ≤ 28 weeks of gestation) and particularly if history of respiratory immaturity. Benefit of vaccination is high; vaccination should not be withheld or delayed. Interference with laboratory testing: Hib capsular polysaccharide antigen excreted in urine, positive urine test observed within 1-2 weeks. ADVERSE EFFECTS: Increase in local reactogenicity and fever after booster vaccination with respect to the primary course. Very common (≥1/10): Appetite lost, crying abnormal, irritability, restlessness, fever ≥ 38°C, local swelling at the injection site (≤ 50 mm), fatigue, pain, redness. Common (≥1/100 to <1/10): Nervousness, diarrhoea, vomiting, fever >39.5°C, injection site reactions, including induration, local swelling at the injection site (> 50 mm). Uncommon (≥1/1,000 to <1/100): Upper respiratory tract infection, somnolence, cough, diffuse swelling of the injected limb, sometimes involving the adjacent joint. Rare (≥1/10,000 to <1/1,000): Lymphadenopathy, thrombocytopenia, anaphylactic reactions, anaphylactoid reactions (including urticaria), allergic reactions (including pruritus), collapse or shock-like state (hypotonic-hyporesponsive episode), bronchitis, apnoea, rash, angioedema, swelling of the entire injected limb, extensive swelling reactions, injection site mass, injection site vesicles. Very rare (<1/10,000): Convulsions (with or without fever), dermatitis.
Version: IFX-HI/P/IN/2017/01 v01 dated 17 March 2018.
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GlaxoSmithKline Pharmaceuticals Limited, Dr. Annie Besant Road, Worli, Mumbai 400 030 (India).
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IN/INFH/0015/18(1)a, Date of preparation: Dec 2018

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