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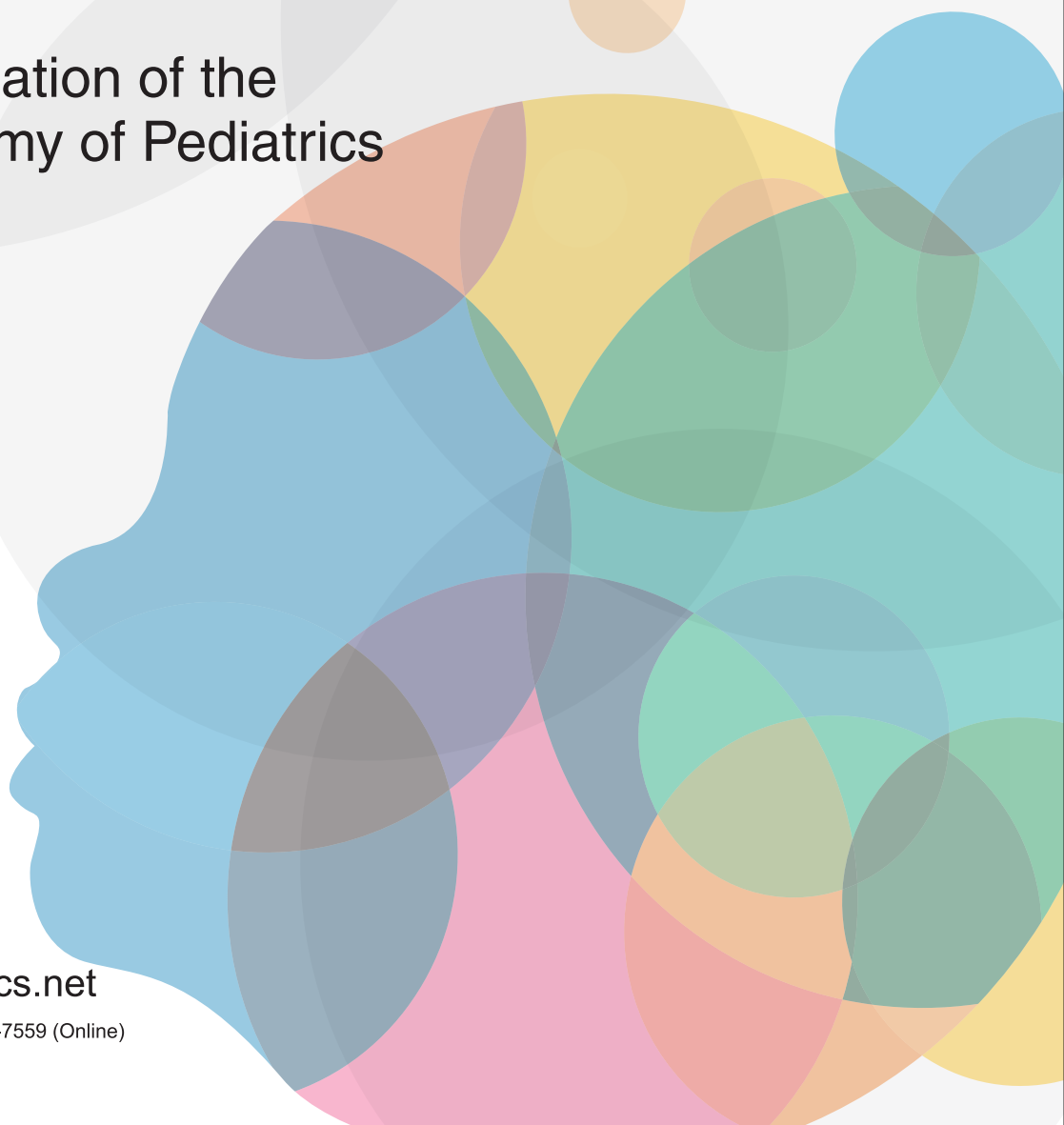

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2. Laquale, Kathleen M (2006). B-complex vitamins' role in energy release. In Movement Arts, Health Promotion and Leisure Studies Faculty Publications. Paper 25.

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RESEARCH PAPERS

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RESEARCH PAPER

Multicentric Hospital-Based Surveillance of Pertussis Amongst Infants Admitted in Tertiary Care Facilities in India

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Objective: To estimate the disease and economic burden of pertussis amongst hospitalised infants in India.

Design: Multicentric hospital-based surveillance study.

Participants: Hospitalised infants with clinical suspicion of pertussis based on predefined criteria.

Outcome measures: Proportion of infants with laboratory-confirmed pertussis, economic burden of pertussis amongst hospitalised infants.

Results: 693 clinically suspected infants were recruited of which 32 (4.62%) infants had laboratory-confirmed pertussis. Progressive cough with post-tussive emesis (50%) and pneumonia (34%) were the common clinical presentations; apnea in young infants was significantly associated with pertussis. Infants with pertussis were more likely to be younger (median age 102.5 days vs.157 days) and born preterm (42.9% vs 24.5%). Almost 30% infants with pertussis had not received vaccine for pertussis with 50% of these infants aged less than 2 months. Pertussis was associated with higher costs of hospitalisation, pharmacy and loss of working days by caregivers as compared to non-pertussis cases.

Conclusion: Younger infants, those born preterm and those inadequately immunised against pertussis are at higher risk of pertussis infection. Timely childhood immunisation and introduction of maternal immunisation for pertussis can help in reducing the disease burden.

Keywords: *Bordetella pertussis, Burden, Whooping cough*.

CTRI Registration No: CTRI/2018/07/014911

Despite the introduction of Diphtheria pertussis tetanus (DPT) vaccine in the expanded program on immunization, pertussis (whooping cough) caused by *Bordetella pertussis* continues to be an important public health problem with about 151000 cases reported globally in 2018 [1]. According to a recent modelling study on the global burden of pertussis, there are 5.1 million estimated pertussis cases and 85900 estimated pertussis deaths amongst infants [2] and India contributes to 26.7% (11,875 cases) of the global burden of pertussis [3,4].

In the recent years, resurgence in pertussis has been reported amongst infants and adolescents from many countries around the world including United States, England [5,6], Brazil, Argentina [7] and China [8]. The possible reasons for resurgence include waning immunity, inadequate vaccine coverage, failure to administer booster doses after the initial vaccination, differential herd immunity between whole cell (wP) and acellular (aP) vaccines, diagnostic and epidemiologic surveillance systems, and genetic changes in the pathogen [6,9,10]. Several hospital-based and community-based surveillance studies from developed countries have reported a high rate of hospital-admissions due to pertussis amongst infants, especially the youngest [11-13] and a significant economic burden in infants hospitalised with complications due to pertussis [14]. Maternal vaccination with the tetanus, diphtheria reduced dose and acellular pertussis (Tdap) vaccine in third trimester of pregnancy, neonatal vaccination, cocooning, adult and adolescent immunization, addition of new antigens to the existing vaccine are some of the strategies recommended towards reducing the resurgence of pertussis [5,15].

A recent systematic review from Asia has highlighted the burden of pertussis in neonates and the paucity of systematic data in this regard [16]. In India, although the reported incidence of pertussis has reduced significantly since 1987, due to lack of routine laboratory diagnosis and uniformity in the clinical definition of pertussis, large number of cases may go undetected and many non-pertussis cases may be getting misdiagnosed as whooping cough [4,17]. Thus, although India contributes significantly to global burden of pertussis, country-specific estimates on the burden of pertussis in infants at

community or hospital level are not available, which are important to inform the national immunization policy [17].

The national average for full immunization is only 62%, and nation-wide coverage for the 3rd primary dose of DPT/pentavalent vaccine (containing DPT with H. influenzae B and hepatitis B) is 78.4% as per National Family Health Survey-4 (NFHS-4) [18]. In line with WHO recommendations, the public health programs in India continue to use wP vaccines rather than aP vaccines [19]; although, the Indian Academy of Pediatrics recommends both wP and aP vaccines for primary immunization [4,20]. The present study was designed to estimate the disease and economic burden of pertussis amongst hospitalized infants in a network of four tertiary care hospitals in India.

METHODS

This cross-sectional, observational, multicentric hospital-based active surveillance of pertussis was conducted in four tertiary care hospitals in India – KEM Hospital, (KEMH) Pune, Maharashtra; Sri Ramachandra Medical College, (SRMC) Chennai, Tamil Nadu; Christian Medical College (CMC), Ludhiana, Punjab and Institute of Child Health (ICH), Kolkata, West Bengal. The sites were chosen from four different zones across the country to account for geographical, seasonal and socioeconomic variations. The study was conducted from October, 2018 to April, 2020 in the given four hospital sites.

The overall conduct of the multicentric study was coordinated by a team of investigators and project managers at KEM Hospital Research Centre, Pune (KEMHRC). This team was responsible development of study protocol and study tools, training of all site teams, site monitoring, data management and analysis. The sites teams were trained for the study protocol, case record forms and nasopharyngeal swab collection during investigators meetings arranged before initiation of the study.

The study protocol was approved by the institutional ethics committees of all the sites. The study was registered with Clinical Trial Registry of India. The study participants were recruited after obtaining written informed consent from their parents or guardians.

The study was conducted in hospitalized infants with clinical suspicion of pertussis. Three of the sites (KEMH, SRMC and ICH) pre-screened potential study participants from hospital registers before screening them using the study criteria whereas at CMC, all infants admitted were screened using study criteria. The clinical case definition for pertussis was adapted from the criteria published by Cherry, et al. [21], which were generally consistent with the case definitions from the United States Centers for Disease Control and Prevention (US CDC) [22], European Centre for Disease Prevention (ECDC) [23] and World Health Organisation (WHO) [24].

A laboratory confirmed case (LCP) of pertussis was defined as one with clinical criteria with at least one of the following laboratory criteria: *i)* Detection of *Bordetella pertussis, Bordetella parapertussis* or *Bordetella holmesii* nucleic acid in a clinical specimen using real-time polymerase chain reaction (RT-PCR); *ii)* Detection of *B. pertussis* or *B. parapertussis* in a clinical specimen using culture.

For the enrolled infants, information on demography, history of the present illness, vaccination records and socioeconomic status was collected from the caregivers by trained clinical coordinators on the case record forms at each site. The demographic variables collected included gender, date of birth, birthweight, gestational age and mode of delivery. Information on birthweight, gestational age and anthropometric parameters was collected from the hospitalisation records. The variables collected for vaccination status included number of doses of DPT or pentavalent vaccine received, type of vaccine (aP or wP) and the dates of vaccination. Details about the onset, duration and clinical course of the current disease were collected during the course of hospitalization till the child was discharged/transferred from the inpatient facility.

Data about economic burden of the present illness at household level were collected by interview method using a questionnaire which included costs on use of health care resources (cost of out-patient consultation, hospitalization, laboratory tests, medications, physician/ emergency room visits), use of non-health care resources (travel, food and miscellaneous expenditure) and productivity costs (loss of wages) for all clinically suspected cases for the given episode of illness. Cost data towards management of pertussis-related complications were collected till the end of present hospitalisation. In addition, the socioeconomic status of the household was determined using modified Kuppuswamy scoring [25]. The income of non-earning members of the family e.g., housewives was assumed to be equivalent to minimum daily wages of unskilled labour as per Government of India depending upon their geographical area [26].

Two posterior nasopharyngeal swabs were collected by trained clinical coordinators, nurse or laboratory technicians for all children with clinical suspicion of pertussis, not later than 72 hours following hospital admission and preferably before administration of systemic antibiotics. The swabs were transported dipped in Amies medium with charcoal/viral transport medium on dry ice in vaccine carrier to the local microbiology laboratory.

In the local laboratory, the swabs for cultures were immediately streaked on Bordet Gengou (BG) medium supplemented with 15% defibrinated horse blood and containing cephalexin to inhibit normal flora (40µg/mL). These culture plates were incubated for 7 days at 35-36°C and were inspected daily. Presence of any *Bordetella* colonies were identified based on colony morphology, colony smear showing Gram-negative coccobacilli and biochemical tests [27].

The swabs collected for RT-PCR were stored immediately after collection for refrigeration at –20ºC to -80ºC till further processing. The swabs were periodically (once in 2 months) shipped on dry ice to microbiology laboratory, KEM Hospital, Pune for analysis of RT-PCR (central laboratory). In the central laboratory, DNA was extracted from the submitted specimens using a QIAamp DNA mini kit (Qiagen) according to the manufacturer's recommendations. The assays for *B. pertussis* and *B. parapertussis* were done by RT-PCR using Taqman technology for the amplification of the insertion elements IS481 and IS1001 of *Bordetella spp*. Threshold cycle of ≥35 was considered positive for IS481. PCR assay for PtxA-S1 was carried out on all specimens that tested positive for IS481 to confirm the diagnosis of *B. pertussis*. Interpretation of results and identification of species was done using WHO algorithm for diagnosis of pertussis [28] (**Web Table I)**.

During the study period, the central laboratory completed a clinical proficiency testing program for *B. pertussis* with Wisconsin State Laboratory Hygiene (WSLH), USA as a part of external quality assurance.

Data management and analysis: At individual sites, the data including clinical and laboratory data from the case record forms were entered into a centrally managed electronic case record forms generated using Open Clinica (community version). Source data verification and quality control was managed by the central team at KEMHRC. The anonymized dataset for the entire study was extracted for analysis following source data verification. The dataset was archived at local servers at KEMHRC.

The demographic factors were compared between pertussis and non-pertussis cases. Young infants were defined as infants with age <60 days [29]. Age-appropriate pertussis vaccination was defined as vaccination within 4 weeks of the exact age of eligibility (i.e. for first dose of pertussis vaccine, vaccination within 10 weeks of age is considered age appropriate). The proportion of laboratory-confirmed pertussis was calculated and compared amongst different age sub-populations (i.e. <2 months, 2-6 months, and ≥6-12 months). Occurrence of total cases and pertussis positive cases per month was charted for total numbers as well as for site-specific cases.

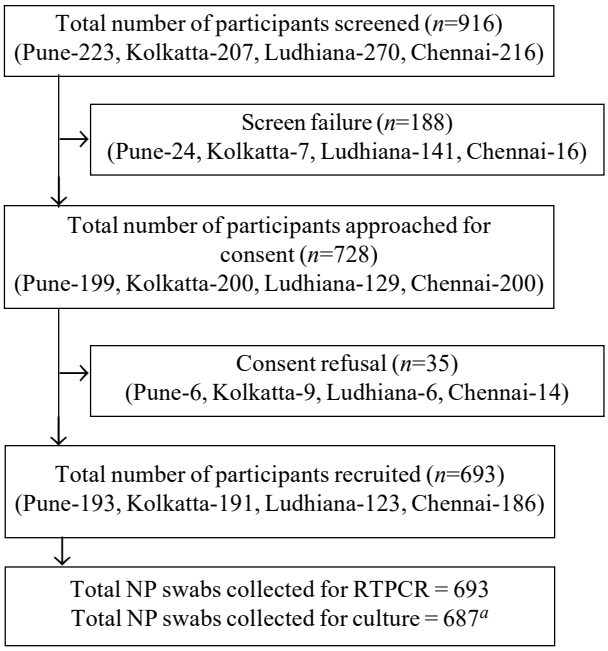
Pearson Chi-square test was used for comparing the proportions and Kolmogrov-Smirnoff test was used for comparison of numerical data (non parametric data). All the analysis was done using STATA 15.0.

RESULTS

A total of 916 infants were screened using clinical case definition criteria, and 693 study participants were recruited (**Fig. 1)**. Thirty two infants were detected with LCP; 8 from Pune, 17 from Kolkata, 2 from Ludhiana and 5 from Chennai (**Table I**). The median age of infants with LCP was about 3.6 months, and boys contributed 62.5% cases of LCP (**Table II**).

Web Table II shows characteristics of study participants recruited overall, and at each site. Approximately, two third of the study participants were boys with a median age of 5 months. About 85% of the participants were older than 2 months. Approximately, one-fourth of the recruited children had low birthweight and/or were born preterm.

About 75% of the study participants belonged to lower or lower middle socioeconomic class as per modified Kuppuswamy classification. About 50% (*n*=355) of



[a]Culture data not available for 6 swabs due to non-availability of culture plates. NP-nasopharyngeal.

Fig. 1 Study flow chart.

Table I Number of Infants With Laboratory Confirmed Bordetella Infection

Number of clinically suspected cases	*Total (n=693)*	*KEMH (n=193)*	*ICH (n=191)*	*CMC (n=123)*	*SRMC (n=186)*
Total Bordetella, n (%)	32 (4.6)	8 (4.1)	17 (8.9)	2 (1.6)	5 (2.7)
B. pertussis, n (%)	25 (3.6)	7 (3.6)	12 (6.3)	2 (1.6)	4 (2.1)
B. parapertussis, n (%)	7 (1.0)	1 (0.5)	5 (2.6)	0	1 (0.5)

the study participants had received age-appropriate vaccination for pertussis and 30% (*n*=214) of them had received less than adequate vaccination. A total of 124 study participants had not received any pertussis vaccination of whom 81 were aged less than 2 months. Amongst these, 39 were aged less than 6 weeks and thus were not eligible to receive first dose of pertussis vaccine.

Of the 687 cultures done, bacterial growth was detected in 164 cultures. None of the 164 cultures grew *Bordetella* species. Of 693 nasopharyngeal swabs collected for RT-PCR, *Bordetella* species were detected in 32 (4.62%) swabs, of which 25 were *B. pertussis* and 7 were *B. parapertussis* **(Table I).**

Presence of classical whoop was reported in only one child. Apnea was significantly more associated with pertussis especially in younger infants (aged <2 months). In addition to cough and fever, the presenting symptoms for LCP included worsening of symptoms at night in 59%, post-tussive emesis in 50% and pneumonia in 34% children. Although leukocytosis was reported in slightly higher proportion of children with LCP, this difference was not statistically significant (**Table II**).

Infants with LCP were significantly younger than those without LCP. Infants with LCP were more likely to have been born preterm and were smaller in size. About 68% of infants with LCP were not age-appropriately vaccinated for pertussis as compared to 48% of infants without LCP. Amongst children aged less than 2 months, all the 5 cases of LCP occurred in children who did not receive single dose of pertussis vaccine and only one of these was aged less than 6 weeks and was thus not eligible to receive first dose of pertussis vaccine (**Table II**). There were no significant differences in the gender, birth weight or socioeconomic status or receipt of antibiotic treatment in the two groups.

Fig. 2 shows age-wise proportion of laboratory-confirmed pertussis cases. In Pune and Chennai, the proportion of LCP was higher in infants aged less than 2 months whereas in Kolkata the highest number of cases was in the 2-6 months age group. As a result, overall, there were more cases of LCP in the 2-6 months age category as compared to less than 2 months and more than 6 months.

Table II Characteristics of Infants With and Without Laboratory-Confirmed Pertussis

Characteristics	*Pertussis (n=32)*	*Non-Pertussis (n=661)*
Age (d)[a,b]	102.5 (61-168)	157 (87-242)
Age <60 d	5 (15.63)	96 (14.52)
Male gender	20 (62.5)	439 (66.41)
Signs and symptoms		
Progressive cough	32 (100)	661 (100)
Presence of whoop	1 (3.13)	14 (2.12)
Apnea[d]	2 (6.25)	3 (0.45)
Post-tussive emesis	16 (50)	266 (40.2)
Cyanosis	0	7 (1.06)
Seizure	1 (3.1)	36 (5.4)
Pneumonia	11 (34.4)	320 (48.4)
Worsening of symptoms at night	19 (59.4)	322 (48.7)
Increased WBC counts	3 (9.4)	49 (7.4)
Weight (kg)[a,b]	5.25 (4.25-6.55)	6.2 (4.8-7.5)
Length (cm)[a,c]	59.5 (56-62.5)	62 (57-68)
Head circumference (cm)[a,b]	39 (36.5-4)	41 (39-43)
Low birth weight[e]	11 (34.4)	154 (23.44)[e]
Preterm[b,f]	12 (42.9)	150 (24.51)
Age-appropriate vaccination		
Full	10 (31.2)	345 (52.2)
Partial	12 (37.5)	202 (30.6)
None	10 (31.2)	114 (17.2)
Antibiotics in last 72 h	15 (46.9)	341 (51.6)
Socioeconomic status		
Upper	0	16 (2.4)
Upper middle	5 (15.6)	147 (22.3)
Lower middle	20 (62.5)	294 (44.5)
Lower	7 (21.9)	203 (32.8)

Data presented as no. (%) or [a]median (IQR). [b]P<0.05, [c]P=0.01, [d]P=0.001. [e]out of 657, [f]out of 28 for pertussis and out of 612 for non-pertussis. WBC: white blood cells.

Amongst the infants with LCP, 18% (*n*=6) required ICU admission as compared to 23.6% (*n*=156) amongst infants without LCP. Of these, only one infant with

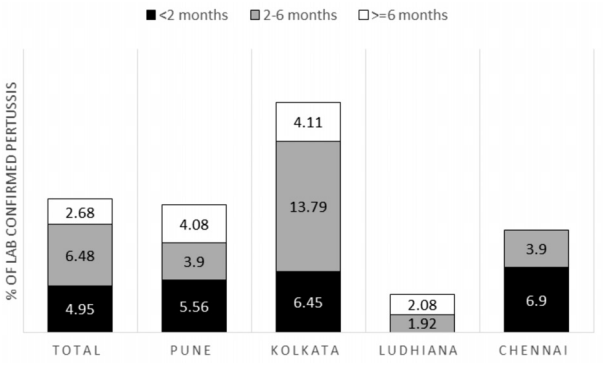


Fig. 2 Age-wise proportion of laboratory confirmed pertussis cases.

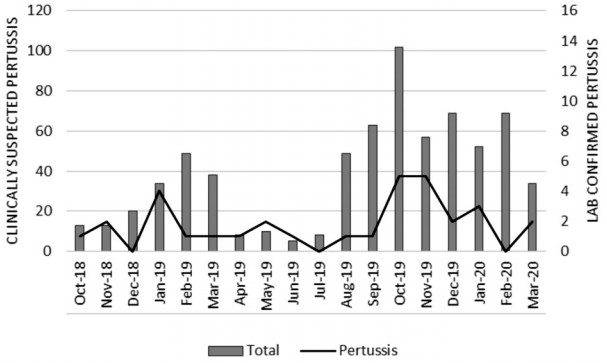


Fig. 3 Seasonality of occurrence of pertussis.

pertussis required mechanical ventilation. The remaining 5 infants were treated with oxygen therapy. Antibiotics were used in 23 infants, which mainly included macrolides and cephalosporins.

There was complete recovery at the time of discharge in 28 (87.5%) cases of pertussis. Two study participants with pertussis had partially recovered at the time of hospital discharge without any permanent debility and two children were discharged against medical advice. There were no death during hospitalization amongst infants with LCP as compared 8 deaths amongst infants without LCP.

Fig. 3 shows seasonal trends in occurrence of the total cases and pertussis. Clinically suspected pertussis as well as LCP cases were most frequent from October to January, which coincides with winter season in India.

Table III shows economic burden of pertussis at household level. Hospitalization of an infant with LCP resulted in a median hospitalization cost of approx. Rs.15000, median hospitalization duration of 5.5 days and a median loss of worktime of 2 weeks by the caregivers for taking care of the infant during the illness. This led to median loss of income of Rs 6921 to caregivers of infants suffering from LCP. The total cost for hospitalization including pharmacy cost was more in infants with LCP. The days spent away from work by the caregivers during illness were also significantly higher for LCP. For all the infants with LCP, the families used their savings to meet the expenses incurred. In addition, 15% families accepted donations from others and 28% families borrowed money to meet the expenses. It is noteworthy that 3% of the families had to sell their assets or use donations by non-government organizations or hospitals to meet the hospitalisation expenses.

Table III Economic Burden of Pertussis

Cost	*Laboratory confirmed pertussis(n=32)*	*Non-pertussis (n=661)*	*P value*
Direct costs (*in INR*), *median (IQR)*			
Pre-hospitalization expenses	590 (250-1050)	530 (250-1150)	0.92
Hospitalization cost[a]	10520 (8030-14685)	8950 (3499-19815)	0.06
Pharmacy cost	2270 (1150-3750)	1080 (0-3000)	0.01
Other costs (food, travel, accommodation)	1750 (700-2500)	1000 (500-2400)	0.18
Total cost	15035 (11959-21713)	12626 (5665-24976)	0.04
Indirect costs, median (IQR)			
Person time spent in outpatient consultation (h)	2 (1.5-4.5)	3 (1.9-5)	0.76
Days of hospitalization	5.5 (3.5-8)	4 (3-7)	0.53
Days of ICU hospitalization	1.5 (1-2)	2 (1-6)	0.6
No of person-days lost by caregivers	14 (2-21)	12 (8-22)	0.02
Income lost by caregivers (in INR)	6921 (5050-10446.67)	6065 (3441.67-10613.33)	0.08

[a]*Hospitalization cost includes cost of hospital stay, nursing and consultancy charges.*

DISCUSSION

This is the first hospital-based prospective surveillance study for LCP amongst infants in India. The earlier reported literature from India was an outbreak of suspected pertussis in Arunachal Pradesh in 2007, with 71% of the suspected cases of pertussis being under one year of age [30]. However, none of these children underwent laboratory confirmation for pertussis. Although two retrospective studies from tertiary care hospitals in India have been reported recently with 30 and 36 cases of LCP in infants and children, respectively; these studies present retrospective data from single centers [31,32].

In our study, 4.62% of Indian infants hospitalized with clinical suspicion of pertussis were found to have LCP. This is much less than the numbers reported from hospital-based studies conducted in Peru (39.5%) [12], Thailand (19%) [33], and a seven-country multinational study including Brazil, Germany, Spain, Costa Rica, Taiwan, Singapore and Uruguay (12%) [34]. A possible reason could be the difference in the clinical definitions used for diagnosis of pertussis. In the multinational study conducted by Kowalzik, et al. [34], infants admitted in pediatric wards with any one of the clinical symptoms i.e. respiratory failure, apnea, bradycardia, or cough accompanied by paroxysms, vomiting, whoop or cyanosis were included. Both the Thailand [33] and the Peruvian [12] study used a clinical definition similar to that of CDC [22]. However, the Thailand study recruited children presenting to the outpatient clinic, whereas Peruvian study recruited hospitalized children. In both these studies, children with chronic respiratory or cardiac diseases were excluded. Two community-based surveillance studies from other parts of South Asia have reported relatively low incidence of pertussis amongst infants (13.3 and 3.96 cases per 1000 infant-years from Nepal [35] and Pakistan [36]).

Amongst the clinical features of LCP, progressive cough with post-tussive emesis, pneumonia and worsening of symptoms at night were common presenting features whereas classical whoop was found in only one child with LCP. This highlights that inspiratory whoop, which is mainstay of clinical diagnosis for pertussis in older children and adults, may not present in infants [21]. Apnea and seizure were presenting features in young infants with LCP but leukocytosis with absolute lymphocytosis was present only in one child aged less than 2 months. This is not consistent with Cherry, et al. [21] and other hospitalized studies of pertussis [31,32,37,38] where leukocytosis with absolute lymphocytosis was largely reported in young infants with pertussis. Few studies have reported severe leukocytosis in critically ill patients with pertussis [39,40]. Pneumonia was found in over 30% of infants with LCP in our study; however, this is one of the many causes of pneumonia. Overall, per-tussis contributes to only a fraction of pneumonia hospitalizations amongst infants from low- and middle-income countries [38,41]. These observations point towards equivocality of clinical criteria and need for more frequent laboratory diagnosis of pertussis amongst children.

Almost 75% of the infants with LCP were aged less than 6 months and 15% were aged less than 2 months in our study. Retrospective studies from Indonesia [42], Philippines [38,43] and Singapore [37] have also reported pertussis cases with higher occurrence and mortality in infants aged less than 6 months. Bhattacharya, et al. [31] reported about 60% of cases in infants aged less than 16 weeks and 30% cases in infants aged less than 8 weeks. Our findings emphasize the earlier observation that pertussis can present with severe morbidity in younger infants requiring hospitalisation [40]. However, only 18-20% of our study participants with LCP required admissions in the intensive care and only one child required mechanical ventilation as against substantial morbidity and mortality reported from studies done elsewhere [37,38,40,42]. Children born as preterm presented as an additional risk factor for pertussis which has also been reported earlier [44]. This could partly be due to delay in the vaccination for preterm children (46.9% full vaccination in preterm as compared 54% amongst others).

In our study, inadequate vaccination or delayed vaccination for pertussis was found to be an important risk factor. Almost 30% infants with LCP had not received vaccine for pertussis, 50% of these infants aged less than 2 months. Another 30% had received less than adequate pertussis vaccination. Similar results were reported in earlier retrospective Indian study conducted by Kavita, et al. [32] and a recently conducted Chinese study by Wang, et al. [40]. Lack of timely vaccination has been reported to be an important preventable risk factor for pertussis amongst young infants globally [16,43,45] not only as a direct risk from lack of protection but also indirectly as infected young infants and children can contribute to increase circulation and cause infection of infants who are too young to get vaccinated and but at high risk of developing complications due to pertussis. This Indian scenario is different from the Western world where resurgence of pertussis has been documented despite high coverage of childhood pertussis immunisation and where the main postulated cause of pertussis is reported to be waning immunity from childhood vaccine in mothers [46].

WHAT IS ALREADY KNOWN?

- Pertussis can lead to severe manifestations in infants requiring hospitalization.

WHAT THIS STUDY ADDS?

- Laboratory confirmed pertussis was seen in 4.6% of children hospitalized with a clinically diagnosed pertussis.
- Younger age, prematurity and inadequate immunization against pertussis were the major risk factors for pertussis.

Introduction of maternal immunization with TdaP has been shown to protect young infants from pertussis and can be useful strategy in our setup as well [15].

Majority of the infants in our study had received wP vaccine and only 4-6% of them received aP vaccine for their primary immunization. National immunization program in India continues to use wP based on the WHO recommendations to continue wP vaccine in countries where it is part of the program in order to minimise the risk of pertussis resurgence associated with aP vaccines [45].

The costs associated with LCP were higher than that of non-LCP due to increased hospitalization and pharmacy costs. As almost 75-80% of the families belonged to lower or lower-middle socioeconomic status, the hospitalization posed significant economic burden on the households leading to stretching of the existing resources. Thus, 3% of the families had to resort to selling their assets or borrowing to meet the expenditure.

Our study has few limitations. Since it only focused on the hospitalized cases of pertussis, children admitted in the day care centres or visiting outpatient departments of the tertiary care centres with similar symptoms were not recruited. The clinical outcome after hospital discharge was not monitored. Although the nasopharyngeal swabs were collected within 72 hrs of hospitalization and preferably before administration of antibiotics, large proportion of the infants had received antibiotics before hospitalization. Although uniform clinical criteria were used for identification of clinically suspected pertussis cases, one of the study sites did not pre-screen potential study participants giving rise to higher screen failures as compared to the other three sites. The study did not collect information about household contacts for pertussis and does not provide population-based incidence of pertussis. None-the-less, the study emphasizes increased risk of pertussis amongst young Indian infants, especially those not fully vaccinated.

Our study provides the first systematic evidence for burden of pertussis amongst hospitalized infants in India. Younger infants, those born preterm and inadequately immunized against pertussis are at higher risk of infection. Efforts to reduce delay in primary immunization and introduction of maternal immunization for pertussis can clearly help in reducing the disease burden in young infants.

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Contributors: AA, SS: concept or design, supervision, data analysis and interpretation, drafting publication, critical revision, final approval, accountable for accuracy and data integrity; RS: data acquisition, project management, drafting publication, critical revision, final approval, accountable for accuracy and data integrity; MM,PVR,SJ: data acquisition, data interpretation, critical revision, final approval, accountable for accuracy and data integrity; JC: data acquisition, supervision, data interpretation, critical revision, final approval, accountable for accuracy and data integrity; JC,SP,AP: data acquisition, critical revision, final approval, accountable for accuracy and data integrity; RK, GK: data acquisition, supervision, critical revision, final approval, accountable for accuracy and data integrity; NJ: data acquisition, data analysis and interpretation, critical revision, final approval, accountable for accuracy and data integrity; PK: design or concept, critical revision, final approval, accountable for accuracy and data integrity; DM: design or concept, funding acquisition, critical revision, final approval, accountable for accuracy and data integrity; AB: design or concept, supervision, funding acquisition, critical revision, final approval, accountable for accuracy and data integrity. All authors approved the final version of manuscript, and are accountable for all aspects related to the study.

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Competing interest: Denis Macina is currently employed by Sanofi Pasteur SA and also reports holding of shares in the Sanofi group of companies as part of his employee remuneration. All other authors declare no competing interests.

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Web Table I WHO algorithm for pertussis

Species	IS481	IS1001	ptxS1
B.pertussis	+	-	+
*B.parapertussis**	-	+	-
B.holmesii	+	-	-
B.pertussis and B.parapertussis coinfection	+	+	+

**Specimen positive for IS1001 was considered to contain B. parapertussis but the possibility of B. bronchiseptica cannot be ruled out.*

Web Table II Demographic characteristics of study participants with clinically suspected Bordetella infection

Characteristics	Total *n*=693	Pune *n*=193	Kolkata *n*= 191	Ludhiana *n*= 123	Chennai *n*=186
Male Gender, n (%)					
Male	459 (66.23)	130 (67.36)	129(67.54)	84(68.29)	116 (62.37)
Age in (days), Median(IQR)	154 (85-239)	180 (100-258)	136 (79-228)	155 (83-228)	144 (81-245)
Age category, *n* (%)					
Age<60days	101(14.57)	18(9.33)	31(16.23)	23 (18.7)	29 (15.59)
Age≥60days	592 (85.43)	175 (90.67)	160 (83.77)	100 (81.3)	157 (84.41)
Outborn **young infants (<60 days)	50/101(49.5)	15/18 (83.33)	---	17/23 (73.91)	18/29 (62.07)
Low birth weight* , n(%)	165 (23.95)	51(26.42)	46 (24.08)	25 (21.01)	43 (23.12)
Premature (<37 weeks)[#], n(%)	162 (25.31)	67 (34.72)	51(30.72)	14 (11.97)	30 (18.29)
Received antibiotics , n(%)	356 (51.37)	170 (88.08)	24 (12.57)	118 (95.93)	44 (23.66)
Age-appropriate pertussis vaccination					
Full vaccination	355 (51.23)	92 (47.67)	95 (49.74)	51(41.46)	117 (62.9)
Partial vaccination	214 (30.88)	62 (32.12)	59 (30.89)	45 (36.59)	48 (25.81)
No vaccination[@]	124 (17.89)	39 (20.21)	37 (19.37)	27 (21.95)	21 (11.29)
Socioeconomic status[$]					
Upper	16 (2.31)	6 (3.11)	3 (1.57)	5 (4.07)	2 (1.08)
Upper-middle	152 (21.97)	32 (16.58)	24 (12.57)	27 (21.95)	69 (37.30)
Lower middle	314 (45.38)	92 (47.67)	121 (63.35)	41 (33.33)	60 (32.43)
Lower	210 (30.35)	63 (32.64)	43 (22.51)	50 (40.65)	54 (29.19)

***** *data available for 689 participants ; # data available for 640 participants, $ using modified Kuppuswamy SES scale 2018; @ Includes infants less than 6 weeks old which is the minimum age for vaccination; **outborn infants are the infants who were not born at the participating hospital sites.*

RESEARCH PAPER

Clinical Profile and Short-Term Outcome of Children With SARS-CoV-2 Related Multisystem Inflammatory Syndrome (MIS-C) Treated With Pulse Methylprednisolone

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Objective: To study the clinical profile and outcome of children with MIS-C treated with methylprednisolone pulse therapy and /or intravenous immunoglobulin (IVIG). **Method:** This prospective observational study included children satisfying CDC MIS-C criteria admitted from September to November, 2020. Primary outcome was persistence of fever beyond 36 hours after start of immunomodulation therapy. Secondary outcomes included duration of ICU stay, mortality, need for repeat immunomodulation, time to normalization of CRP and persistence of coronary abnormalities at 2 weeks. **Results:** Study population included 32 patients with MIS-C with median (IQR) age of 7.5 (5-9.5) years. The proportion of children with gastrointestinal symptoms was 27 (84%), cardiac was 29 (91%) and coronary artery dilatation was 11 (34%). Pulse methylprednisolone and intravenous immunoglobulin were used as first line therapy in 26 (81%), and 6 (19%) patients, respec-tively. Treatment failure was observed in 2/26 patients in methylprednisolone group and 2/6 patients in IVIG group. C-reactive protein levels less than 60mg/L by day 3 was seen in 17(74%) in methylprednisolone group and 2 (25%) in IVIG group (P=0.014). There was no mortality. At 2 weeks follow-up coronary artery dilatation persisted in 4 in methylprednisolone group and 1 in IVIG group. **Conclusion:** In patients with SARS-CoV-2 related MIS-C, methylprednisolone pulse therapy was associated with favorable short-term outcomes.

Keywords: *Coronary artery, COVID-19, IVIG, Kawasaki disease*.

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Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) related multisystem inflammatory syndrome in children (MIS-C) is a dreaded complication that is seen more often in children than in adults [1]. Intravenous immunoglobulin (IVIG) is considered as the treatment of choice for Kawasaki disease (KD) [2]. MIS-C has many dissimilarities with KD, like occurrence in older children (median age 10 years), presence of multi-organ involvement, commonly gastrointestinal tract, myocar-dial dysfunction and shock [3]. MIS-C has been treated empirically with IVIG and steroids [2]. Some studies have used biologicals like tumour necrosis factor inhibitor, interleukin 1 inhibitor, interleukin 6 receptor antibody etc. Most studies have used IVIG alone or in combination with methylprednisolone than methylprednisolone alone in the treatment of MIS-C [1,4]. Non-availability and high cost of IVIG precludes its use in many centers. Hence, this observational study was conducted to assess the clinical profile and treatment outcome of patients treated with pulse methylprednisolone.

METHODS

This observational study was conducted in a tertiary care teaching hospital in India. This was the preliminary analysis of an ongoing prospective observational study at the institute. Ethics committee clearance was obtained for the study and informed consent was taken from patient caretakers. Children admitted with MIS-C aged 1 month to 12 years of age from September to November, 2020 were included.

Patients who fulfilled the CDC criteria for diagnosis of MIS-C during the study period were included in the study [5]. Infective causes like dengue, leptospirosis, scrub typhus and bacterial sepsis were excluded by appropriate investigations. SARS-CoV-2 reverse transcriptase polymerase chain reaction (RT-PCR) was done in all patients, and SARS-CoV-2 antibody testing was done using Vitros CoV2T kit [6].

Choice of immunomodulation was decided by the treating unit based on patient demographics and Kerala State guidelines for treatment of children with MIS-C [7].

Bedside echocardiography was done in all patients with MIS-C with shock at admission. All patients were subsequently seen by a pediatric cardiologist to look for coronary artery status and cardiac dysfunction. Coronary artery diameter *z*-score >2 was considered as coronary artery dilatation/aneurysm [8]. Coronary artery changes like increased echogenicity and non-tapering in the absence of *z*-score >2 were taken as nonspecific coronary artery changes. Shock was defined when a patient required more than 20 mL/kg of intravenous (IV) fluid resuscitation or inotropic support to maintain blood pressure above the 5th centile.

Study variables collected using pre-designed pro-forma included patient demographic characteristics, initial symptoms and clinical signs, laboratory para-meters, type of immunomodulator used, time to deferve-scence, duration of ICU stay, need for inotropic support, duration of shock, duration and type of respiratory support, coronary artery changes at admission and 2 weeks follow-up and mortality. Patients who were treated with methylprednisolone received pulse dose of 30 mg/kg once daily for 3 days followed by oral prednisolone at 2 mg/kg for 1 week or till CRP normalized, whichever was later. Steroid was tapered and stopped over the next 2 - 3 weeks. Children who were treated with IVIG received 2 g/kg as a continuous infusion over 8-12 hours with longer duration in patients with cardiac dysfunction.

Time to fever defervescence was recorded at 12-hourly intervals. CRP and D-dimer were repeated on the third and seventh day after the start of IVIG or methylprednisolone. Treatment failure was defined as persistence of fever or worsening of clinical condition beyond 36 hours from the start of first-line therapy or recrudescence of fever within 7 days. Repeat immuno-modulation was considered if fever persisted beyond 36 hours of the first dose of immunomodulatory therapy or if there was a clinical deterioration, irrespective of time since finish of first therapy. Children with treatment failure with IVIG first dose were treated with a second dose of IVIG with pulse methylprednisolone according to the Kerala State guidelines [7]. Children with treatment failure with pulse methylprednisolone were treated with IVIG. All patients were followed up at two weeks after discharge.

All patients with shock were started on low molecular weight heparin (LMWH) at prophylactic dose, which was changed to treatment dose if thrombus was detected. Children on LMWH were transitioned to low dose aspirin once liver enzymes normalized and platelet count increased to more than 80×10^9/L. Children with thrombus were put on LMWH and anti-platelet dose of aspirin. Anti-inflammatory dose of aspirin (50 mg/kg) was given in refractory MIS-C with KD like presentation. Children receiving methylprednisolone also received prophylactic IV pantoprazole.

Statistical analysis: Data were entered in MS Excel and analyzed using SPSS 20. Independent sample *t* test was used for comparison of means. Categorical variables were compared using nonparametric tests. Logistic regression was done to assess the relationship between clinical variables and treatment outcome.

RESULTS

A total of 32 (males, 21) patients with a median (IQR) age of 7.5 (5-9.5) years were enrolled. Seventeen patients were antibody positive, 8 patients were both PCR and antibody positive, and two were only PCR positive. Five patients were negative for PCR and antibody but were epidemiologically related to COVID-19 positive cases.

All children presented with fever with a median (IQR) duration of 5 (3-6) days. The clinical characteristics are shown in **Table I**. The mean (SD) CRP was 141(72) mg/L and ESR 41(33.1) mm in the first hour. The mean (SD) age of children with shock was significantly higher than those without shock [7.93 (2.27) vs 5.67 (3.39) years; *P*=0.02]. Children with shock also had statistically significant higher D dimer [4.75 (3.3) vs 1.59 (0.982) mcg/mL; *P*=0.007], lower albumin [2.8 (0.40) vs 3.32 (0.5) gm/dL, *P*=0.008], higher CRP [152 (62.7) vs 120 (98.9) mg/L; *P*=0.049], higher lactate [2.35 (1.27) vs 1.01(0.212) mmol/L; *P*=0.012] and lower ejection fraction [53.5 (13.09) vs 65.1 (6.29)%; *P*=0.015]. Eighteen patients (56%) had transaminitis but hepatic failure was seen in only one child. Of the four patients with vascular thromboembolic events (VTE), three had thrombus in the left ventricle and one in the right popliteal vein. Even though 10 (31%) patients were PCR positive, antiviral therapy with remdesivir was offered only to one child in our series.

Table II shows comparative clinical features in children who received pulse methylprednisolone (*n*=26) or IVIG (*n*=6). Treatment failure was observed in 2/26 patients in the methylprednisolone group and 2/6 patients in the IVIG group. No child required additional immuno-modulation with immune-biologicals or died during the study period.

Logistic regression was done to assess the effect of clinical variables which were significantly different between the two treatment groups, on the likelihood of occurrence of treatment failure. Logistic regression did not show any effect of age (*P*=0.7), respiratory support (*P*=0.7) and five or more organ involvement (*P*=0.2) on the likelihood of occurrence of treatment failure.

Out of 11 patients with coronary artery dilatation at

Table I Demographic and Clinical Characteristics of Patients With MIS-C (*N*=32)

Characteristics	*No. (%)*
Underlying medical condition[a]	4 (12.5)
ICU admission	30 (94)
RT-PCR positive	10 (31)
Serology positive	22 (69)
Multiorgan involvement (>5 organs)	22 (69)
GI symptoms	27 (84)
Mucocutaneous manifestations	29 (90.6)
Conjunctival congestion	22 (69)
Conjunctival Haemorrhage	10 (31)
Oral mucosal changes	3 (9)
Rash	21 (66)
Coagulopathy	32 (100)
INR >1.5	4 (12.5)
D dimer >0.5 mcg/mL	32 (100)
Thrombocytopenia <150×10^9/L	19 (59.3)
CNS involvement	13 (40.6)
Seizure	3 (9.3)
Headache	4 (12.5)
Encephalopathy	6 (18.7)
Respiratory	14 (43.7)
Cough	2 (6)
Abnormal chest *X*-ray	7 (21.8)
Bilateral lung infiltrates	6 (18.7)
Liver	18 (56)
Aspartate aminotransferase >40 IU/L	18 (56)
Alanine aminotransferase >40 IU/L	16 (50)
Renal	8 (25)
Urea >40 mg/dL	8 (25)
AKI stage 2	2 (6.2)
AKI stage 3	2 (6.2)
Cardiac involvement	29 (90.6)
Myocardial dysfunction (EF <55%)	13 (40.6)
Elevated NT Pro BNP	28 (87.5)
Coronary artery dilatation / aneurysm	11 (34.4)
Arrhythmia	3 (9.3)
Clinical thrombosis	4 (12.5)
Serositis	8 (25)
CRP >60 mg/L	30 (94)
Elevated ESR	15 (47)

[a]*rhabdomyoma-1, obesity-2, hypothyroidism-2. MIS-C: Multisystem inflammatory syndrome in children temporally associated with SARS-CoV-2 infection. ICU: Intensive care unit; GI: Gastrointestinal; CNS: Central Nervous System; EF: Ejection fraction; AKI: Acute kidney injury; AKI stage 2: Doubling of serum creatinine from baseline; AKI stage 3: Tripling of serum creatinine from baseline or need for renal replacement therapy.*

Table II Comparison of Clinical and Outcome Measures in the Two Treatment Groups

Clinical characteristics	*Methyl prednisolone group, n=26*	*IVIG group n=6*
Age,[a,e]	8 (6-10.25)	3.5 (2.4-4.5)
ICU stay (d)[a]	4.5 (3-6.25)	4 .5 (2-10)
Hospital stay, (d)[a]	11 (10-14)	8.5 (2.7-21)
Shock[d]	20 (77)	2 (33)
Cardiac dysfunction	11 (42)	2 (33)
Coronary artery dilatation/ aneurysm	8 (30.8)	3 (50)
Coronary artery non-specific changes	6 (23)	0
≥5 organ involvement[d]	20 (77)	2 (33)
Inotropic support	18 (69)	2 (33)
≥2 inotropes	14 (52)	1 (17)
Respiratory support[d]	19 (72)	1(17)
Invasive ventilation	4 (16)	0
CPAP	14 (52)	1 (17)
NIV	1 (4)	0
CRP <60 mg/L on day 3	17 (68)	2 (33)
D-dimer decrease by day 3	19 (68)	4 (83)
Repeat immunomodulation	2 (7.7)	2 (33.3)
Persistent coronary artery dilatation /aneurysm at 2 wk	3 (12)[b]	1 (25)[c]
Persistent nonspecific coronary changes	4 (16)[b]	1 (25)[c]
Normal ejection fraction at 2 wk	23 (92)[b]	4 (100)[c]

Data expressed as no. (%) or [a]median (IQR). [b]n=25; [c]n=4. MIS-C: multisystem inflammatory syndrome in children temporally associated with SARS-CoV-2 infection; cardiac dysfunction: Ejection fraction <55%; NIV: non invasive ventilation; CRP: C Reactive protein;CPAP: continuous positive airway pressure. [d]P<0.05; [e]P=0.01.

admission, four had persistent dilatation at two weeks. Six patients (21%) had echogenic non-tapering coronaries but coronary artery diameter was less than 2 *z*-score. One patient in this group developed coronary dilatation with a *z*-score of more than 2.5 at 2 weeks. LV thrombus had resolved in two patients at 2 weeks follow up while one patient continued to have thrombus at 2 weeks follow up even though the ejection fraction had normalized at 2 weeks. Of the 13 patients with LV dysfunction, 11 (85%) had normal ejection fraction at 2 weeks follow up. LV systolic function normalized for the remaining 2 patients, at 6 weeks follow up. One child had developed mononeuritis of the right peroneal nerve after one week, which improved with the continuation of steroids and aspirin at antiplatelet dose.

WHAT THIS STUDY ADDS?

- Use of pulse methylprednisolone therapy as the first line treatment for MIS-C was associated with favorable immediate and short term follow up outcomes.

DISCUSSION

The present study reports favorable outcomes in MIS-C with pulse methylprednisolone therapy. MIS-C had dissimilarities to classical KD like higher age at presentation and higher incidence of GI symptoms and shock, as seen earlier [3,9,10].

Only 6% of children were referred with a suspected diagnosis of MIS-C, highlighting the fact that MIS-C continues to be a great masquerader. Clinical features in children with acute SARS-CoV-2 infection included fever in 49%, cough in 45% and GI symptoms in a few [11]. In contrast, all children with MIS-C, had fever with a higher proportion of GI symptoms, while cough was rare [12,14], as also seen in the present study.

As previously reported, more children in our study had conjunctival congestion than oral mucosal changes [12,13]; 31% of children also had conjunctival hemorrhage, which has not been reported in other studies. Breathlessness was also observed in a higher proportion of patients compared to cough [14]. Unilateral lung infiltrates are more frequently reported in acute COVID-19 infection in children [15]. While bilateral lung infiltrates were seen in a higher proportion of patients with MIS-C.

A higher seropositivity rate with or without SARS-CoV-2 RT-PCR positivity is reported in patients with MIS-C with shock and multiorgan involvement [14]. Presence of positive COVID-19 antibody in patients with positive SARS-CoV-2 PCR at admission probably indicates a greater role of immune-mediated inflam-matory response than acute SARS-CoV-2 viremia in the pathogenesis of MIS-C. As many children with MIS-C have hepatic derangement, use of antiviral therapy in these patients may be counterproductive [13].

Cardiac involvement is the most frequently reported organ dysfunction in MIS-C as also seen in the present study [1,10,12,6]. Occurrence of coronary artery aneurysm at follow up in a patient with nonspecific coronary artery changes without dilatation in the initial echocardiogram, highlights the need for meticulous follow up with echocardiogram. Thrombosis has not been reported in similar studies from India [16,17] but reported in studies from the US and UK [12,13].

Earlier studies [12,14] have shown the need for repeat IVIG and immunomodulators in almost 20% of those who received IVIG. In our study, only 2 patients who had received steroids subsequently needed IVIG. Logistic regression did not show any relationship between clinical variables like age, shock or multiorgan involvement with initial treatment failure. None of the children required any other alternative immunomodu-lators. There were no deaths or need for ECMO in our study. Earlier studies have reported a mortality of 1.2-2% [13,14] and need for additional cardiac support with ECMO in 4% of patients [12,13].

Studies have reported favorable short-term response to IVIG and steroid [3,14]. Currently proposed treatment modalities are derived from its similarity with KD and are based on expert opinion. Treatment with IVIG in resource limited settings is a challenge. In our study, children who received methylprednisolone were significantly older and had a higher number of organ involvement. Outcome measures showed a favorable role for pulse methylprednisolone in the treatment of MIS-C. A recent study [18] also found a more favorable outcome in those treated with IVIG and methylprednisolone than those treated with IVIG alone. Small sample size, observational nature and absence of matched cohorts are the main limitations of the study.

In patients with MIS-C with shock and multi-organ dysfunction syndrome, IV methylprednisolone pulse therapy was associated with favorable immediate and short term follow-up outcomes. Patients with nonspecific coronary changes like absence of tapering and increased echogenicity need to be meticulously followed up for occurrence of coronary artery dilatation even with a low initial *z*-score.

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drafting of manuscript, Critical revision of manuscript for intellectual content; NHR: acquisition, analysis and interpretation of data, drafting of manuscript; SKA: supervised the study and contributed to the critical revision of manuscript for intellectual content. All authors approve the final version of manuscript, and are accountable for all aspects related to the study.

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RESEARCH PAPER

Bed Utilization and Overcrowding in a High-Volume Tertiary Level Pediatric Emergency Department

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Objectives:To measure bed utilization rate and overcrowding in a high-volume tertiary level pediatric emergency department (ED) and correlate with outcome. **Methods**:All children beyond neonatal age attending the 22-bedded emergency were prospectively enrolled from February to December, 2019. Number of daily admissions, boarders, discharges, ward transfers, length of stay (LOS) and unfavorable outcomes (care discontinuation and deaths) were recorded. Daily bed occupancy rate (BOR) was calculated and correlated with unfavorable outcome. **Results**: A total of 17,463 children visited the ED during the study period. The median (IQR) daily attendance and admission rate was 58 (51,65) and 22 (17,26) patients, respectively. The median (IQR) number of boarders and BOR was 48 (40-58) and 218% (181-263%), respectively. The median (IQR) LOS was 42.7 (23-71.4) hours. Unfavorable outcome correlated positively with number of boarders and BOR (P<0.001). **Conclusions**: Overcrowding of the ED was associated with increased frequency of care discontinuation and mortality. This data calls for systemic changes to tackle overcrowding.

Keywords: *Boarders, Length of stay*, *Bed occupancy rate*

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The emergency department (ED) serves as the first interface for children with acute illnesses, the key objectives being rapid assessment and timely intervention. Overcrowding due to non-urgent ED visits, inappropriate referrals and bed boarders poses a major hurdle in realizing these objectives [1]. Globally, the data suggest that a substantial proportion of ED attendees do not require emergency care [2].

The causes of ED overcrowding are divided into input, throughput and output factors [3]. Input factors include causes that increase patient inflow into ED (e.g. lack of access to primary care and increased number of referrals from other sectors of health care). Throughput factors pertain to lack of space, understaffing and inadequately trained healthcare providers. Non-availability of inpatient beds and delayed transfer out from ED constitute the output factors.

Overcrowding stretches the ED resources (manpower, infrastructure and logistics), causing treatment delays, staff stress, adverse clinical outcomes and reduced patient satisfaction [3,4]. In a resource constrained set up, the supply demand mismatch gets further amplified. Several surrogates of ED overcrowding have been described which include length of stay (LOS), bed occupancy rate (BOR), number of patients leaving without been seen, and number of ED boarders [3]. Data from low middle-income countries (LMIC) is under-reported.

Our ED records over 20,000 patient visits per year, of which about 10,000 get admitted; most referred from adjoining states. This is compounded by inordinate delays in patient transfer out from ED to wards related to our hospital admission policy.

We designed this study to determine the bed utilization rate, quantify overcrowding, ascertain its reasons, and study its relation with patient outcome in our pediatric ED.

METHODS

A prospective observational study was conducted in the 22-bedded standalone pediatric ED of our hospital between February and December, 2019. Our ED uses a five-level triage in which patients are assigned levels, based on their disease acuity and physiological status [5]. Our emergency ward is categorized into 'red' (7 beds) and 'yellow' (15 beds) areas where critical and non-critically ill children are managed, respectively until they are transferred to inpatient wards. Doubling or trebling of patients on a single bed is practiced if the number of patients exceed the available bed capacity. The nurse-patient and doctor-patient ratios vary from 1:5 to 1:12 for red area, and 1:8 to 1:30 for yellow area. There is no provision for surge staffing in our ED.

We included data of children from 29 days to 12 years of age attending ED during the study period. The total number of ED visits, admissions, number of boarders (defined as patients in ED awaiting an inpatient bed), time of admission, sickness levels, final outcome [discharged, transferred to wards, died in ED, discontinued care and left against medical advice (LAMA)] and time of outcome were recorded. Length of stay (LOS) was calculated as time difference between admission and outcome. Daily bed occupancy rate (BOR) was calculated as total number of admitted children in the ED at midnight divided by the total number of beds in ED x 100. All ED deaths and care discontinuation were taken as 'unfavorable outcome'.

Statistical analysis: Analyses were performed using SPSS version 22. Correlation between LOS, admissions and other parameters was performed using Spearman correlation coefficient. *P* value <0.05 was considered significant.

RESULTS

A total of 17463 children visited ED during the 303 days study period with a median (IQR) daily attendance rate of 58 (51,65) patients. Over a third (*n*=6659; 38.1%) required admission [median (IQR) daily admission rate 22 (17,26)]. The median (IQR) age of admitted subjects was 24 (12,72) months. The triage levels of the children are summarized in **Table I**.

The median (IQR) number of boarders per day was 48 (40-58) (range, 21-81), while the median (IQR) BOR was 218% (181-263%) (range, 95-368%). Of the 6659 (38.1%) admitted children, 47.2% (*n*=3145) were discharged from ED, 7% (*n*=472) discontinued care and 4.9% (*n*=330) died.The remaining 2712 children (41%) were transferred to in-patient wards.

Although the overall median (IQR) LOS of admitted children was 42.7 (23-71.4) hours, it ranged from 1 day to >14 days (**Fig. 1**). Nearly half (43.8%) of the patients had an ED stay of >48 hours. The median (IQR) lengths of ED stay before transfer to PICU [23.7 (14.8-37.4) hours] and surgical wards [14.8 (7-28.7) hours] were significantly shorter as compared to ED stay before transfer to subspecialty wards [38.7 (23.1-66.9) hours] and ED discharge [52 (30.1-81.5) hours] (*P*<0.001). There was a significant but weak correlation between unfavorable ED outcome and new admissions (*P*<0.001; ρ=0.285), BOR (*P*<0.001; ρ=0.253), and number of boarders (*P*<0.0001; ρ=0.253).

Table I Triage Levels of Children Presenting to the Emergency Department

Triage levels	*All attendees*[a] *(n=17328)*	*Admission*[b] *(n=5669)*
Level 1 and 2	4908 (28.1)	2969 (52.4)
Level 3	3154 (18.1)	1872 (33)
Level 4 and 5	9266 (53)	828 (14.6)

Data in no.(%). [a]Of all ED attendees, 135 children were brought dead and hence not triaged; [b]triage forms complete in all aspects were available for 5669 out of 6649 admission.

DISCUSSION

Our data reveals significant overcrowding in the pediatric ED, as indicated by high number of non-urgent visits, boarders, higher bed occupancy rate and prolonged ED stay. The overcrowding correlated with increased frequency of care discontinuation and death on any given day.

A sizeable burden (53%) of our ED visits was contributed by children with low acuity illness (triage level 4 and 5), which could have been otherwise managed effectively in outpatient settings. Others have also reported 30-63% of pediatric ED visits being of low acuity or non-urgent [2]. In India, the reasons for inappropriate ED visits are diverse; lack of effective primary care, paucity of an organized referral network and inadequate triage system in most public hospitals [6]. Other reasons include non-availability of healthcare services beyond office hours and referrals for trivial conditions prompted by increa-sing violence against HCPs in peripheral hospitals [7].

Our ED admission policy is stringent and based on acuity levels. However, when patient numbers double and treble, there is no provision for surge staffing. Bed boarding and fixed staff ratios are known to compromise quality of care [1]. Output factors are reported to be the most important contributors to ED crowding [8], similar to our observations. The median length of ED stay seen in our study was much higher than that reported from developed countries (median LOS <4 hours) [9].

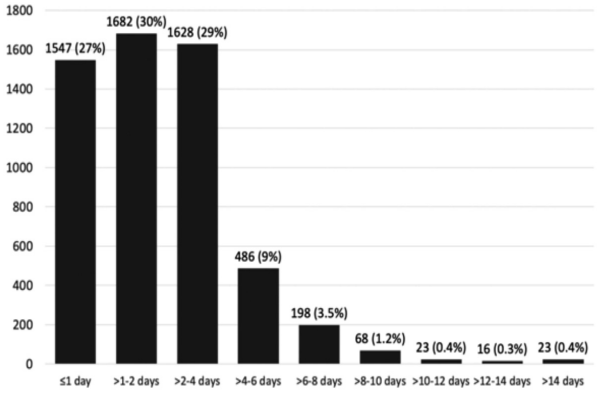

Fig. 1 Distribution of patients according to their length of stay in the emergency department.

WHAT THIS STUDY ADDS?

- All three factors viz., input, throughput and output contributed to the increased patient numbers in our ED.
- In a resource-limited set up, increased boarders, higher bed occupancy rate and prolonged ED stay affect patient outcome.

The proposed solutions to curtail input include strengthening of primary and secondary healthcare through capacity building and telemedicine services, establishing an organized referral network and protecting HCPs through proper laws. Trained medical or nursing staff conducting telephone triage system was shown to decrease the number of self-referred pediatric ED attendees [10]. Similarly, creation of parallel urgent care centers to handle non-sick cases during peak evening hours may take the burden off a crowded ED. Some strategies described to overcome output factors include computerized active bed management, creation of a discharge lounge, and reverse triage (patients with least need of a bed are discharged) [11]. Allocation of flexible beds (25-30% of total bed capacity of the hospital) in sub-specialty units can improve efficiency and reduce 'blocking probability'[12]. Creation of an acute medical unit (AMU) by pooling all flexible beds from every sub-specialty unit to enable ED transfers is another effective strategy followed in many Western units. AMUs function as an intermediary between ED and sub-specialty wards, with admission duration ranging from 24-72 hours [13].

Studies have reported that ED overcrowding diminishes the capacity of ED, affects quality of care, increases risk of adverse outcomes for cardiac and intubated patients, enhances risk of hospital-acquired infections and chances of medication errors [14,15]. Our findings also reflect a similar trend. Although the correlation between ED crowding parameters and unfavorable outcome was significant, the effect of socioeconomic status, duration of illness, and disease-specific factors were not evaluated in the present study.

The pediatric ED of a tertiary care teaching and referral hospital faces a significant burden of over-crowding related to input, throughput and output factors. Prolonged ED stay and bed boarders due to delayed ward transfers were associated with increased frequency of care discontinuation and mortality. This data provides a foundation for policy and systemic changes to decrease ED overcrowding.

Ethics clearance:Institute Ethics Committee, PGI, Chandigarh; No. IEC/2014/557, dated 26 September, 2014.
Contributors: NM: analyzed the data and wrote the manuscript; JM: designed the study, analyzed the data, and edited and revised the manuscript; KN, SKA, AB: provided patients and reviewed the manuscript. All authors approved the final manuscript
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RESEARCH PAPER

Clinical Profile of Adolescent Onset Anorexia Nervosa at a Tertiary Care Center

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Objectives: To study the clinical profile and outcome of adolescent onset anorexia nervosa at a tertiary care center in Southern India. **Method**: Review of hospital records of adolescents diagnosed with anorexia nervosa. Outcome was assessed for those with a follow-up of atleast one year, by outpatient visit or by a telephonic interview. **Findings**: Data of 43 patients (28% males) with mean (SD) age at presentation of 13.4 (1.7) years were included. The mean (SD) BMI at presentation was 13.8 (3.2) kg/m^2, the lowest being 8.3 kg/m^2. 33 (76%) patients were hospitalized for nutritional rehabilitation. Of the 15 patients followed up 1-5 years later, one had died and 11 had achieved normal weight for age. **Conclusion**: As compared to other studies, this study showed a higher proportion of boys with anorexia nervosa. Further research is necessary to understand factors affecting long-term outcome.

Keywords: *Eating disorder, Management, Outcome.*

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Anorexia nervosa is an eating disorder characterized primarily by an altered perception of body image, resulting in significant weight loss and is influenced by bio-psychosocial factors. In India, anorexia nervosa is increasingly recognized as a cause of morbidity and mortality among adolescents. The reported lifetime prevalence of anorexia nervosa is 0.5-2%, with a peak age of onset around 13-18 years [1]. Literature reveals a changing epidemiology of this disorder, with increasing rates of eating disorder being diagnosed in younger children and in males [2-5]. Though the prevalence of eating disorders is higher in Western countries, there is an increasing trend of case reports from India [5]. With increasing incidence of anorexia nervosa in children, pediatricians become the first point of contact for many cases.

Our study aims to describe the clinical profile of adolescents admitted with anorexia nervosa in a tertiary care center in Southern India.

METHODS

This was a hospital record review of adolescents hospitalized with anorexia nervosa and their follow up after atleast one year of discharge. We reviewed the data of adolescents (aged 10 to 18 years) who were admitted either in the adolescent medicine facility or the child and adolescent psychiatry unit between May, 2006, and December, 2019, and details of patients with a diagnosis of eating disorder or anorexia nervosa were extracted. Adolescents who fulfilled the DSM-V criteria for anorexia nervosa or other specified eating and feeding disorders (OSFED) were included in this study. Those with diagnoses of bulimia nervosa, psychogenic vomiting or unspecified feeding disorders were excluded.

Data regarding the clinical profile and hospitalization was collected from the hospital database. Follow up of these patients was done after atleast one year of hospital discharge following initial hospitalization, either by an outpatient visit or a telephonic interview. Information regarding clinical symptoms, weight gain, and school performance was collected.

Data entry and analysis was done using Epidata software.

RESULTS

Over the 13 year 8 month period, 43 adolescents of whom 12 (27.9%) were males, were studied. Anorexia nervosa restricting type was the diagnosis in 23 (53.4%) adolescents, and 9 (20.9%) had binge-purge type. Other specified feeding and eating disorders (OSFED) were diagnosed in 11 (25.5%).

WHAT THIS STUDY ADDS?

- Profile of adolescents with anorexia nervosa at a tertiary center is described.

The mean (SD) age at presentation was 13.4 (1.7) years and the mean (SD) age at onset was 12.4(1.8) years. The youngest patient was 10 years old. 21 (48.8%) adolescents had a BMI below the 3rd centile, with one patient having a BMI of 8.3 Kg/m^2. Loss of appetite and abdominal pain were the two most common presenting symptoms seen in 30 (69.7%) and 20 (46.5%) patients, respectively (**Table I**). The mean (SD) calorie intake at presentation was 388 (247) calories per day. The most common triggers were peer pressure seen in 15 (34%) patients and family history of overt eating disorders or a significant adult who was reportedly health conscious in 8 (18.6%) patients. Menstrual irregularities were present in 19 (61.2%) adolescent girls, of whom 5 (26.3%) had primary amenorrhea and 10 (52.6%) had secondary amenorrhea. Co-morbid conditions such as obsessive compulsive disorder or depression were present in 11 (25.5%) patients. There was a family history of psychiatric illness in 9 (20.9%) patients.

Microcardia was present in 21 (48.8%) adolescents. The ECG changes seen in 6 (13.9%) adolescents included sinus bradycardia, QT prolongation and T wave changes. Echocardiography was done in five adolescents and was normal. Seven adolescents had MRI of the brain and abnormal findings were present in 5 (71.4%) of them. The abnormal findings included cerebral atrophy, white matter volume loss, periventricular hyperintensities and pituitary changes. Bone mineral density was done in 4 patients, 2 (50%) of whom had low mineral density.

Table I Clinical Profile of Adolescents With Anorexia Nervosa at Presentation (*N* = 43)

Characteristic	*Value*
Duration of symptoms (mo)[a]	12.2 (9.2)
BMI at presentation (kg/m^2)[a]	13.8 (3.2)
Weight loss	43 (100)
Loss of appetite	30 (69.7)
Vomiting	15 (34.8)
Abdominal pain	20 (46.5)
Bloating	11 (25.5)
Dizziness	14 (32.5)
Cold intolerance	6 (13.9)
Excessive exercise	12 (27.9)
Peculiar eating pattern	11 (25.5)
Fear of weight gain	20 (46.5)
Use of laxative / diet pills	2 (4.6)
Lanugo hair	4 (9.3)

Values are expressed as no. (%) or [a]mean (SD). BMI: body mass index.

Of the 43 adolescents, 33 (76.7%) were admitted for nutritional rehabilitation [mean (SD) stay, 13.7 (5.5) days]; the remaining 10 did not require hospitalization for medical treatment. Of the 33 admitted, 15 required initial feeding via nasogastric tube, while 1 patient required nasogastric feeds even at discharge. Hemodynamic instability was present in 12 (36.3%) of these patients, and refeeding syndrome was diagnosed in 10 (30.3%) of these patients. At discharge, the average daily calorie intake was 1935 calories and the average weekly weight gain was 1.1 kg.

Of the 43 patients, 10 (23.2%) were yet to complete a 1-year follow-up period, and 18 (41.8%) were lost to follow-up. One child died 18 months later with severe hemodynamic instability, and complications of electrolyte imbalance, coagulopathy and shock. Of the remaining 14 (32.5%) patients, 2 (14.2%) persisted to have symptoms, 1 (7.1%) patient had become overweight, and the remaining 11 (78.5%) had normal weight for age.

DISCUSSION

The proportion of males in the study was higher than that reported in other studies (9-15%) in adolescents [4,6]. Possible reasons include improved awareness and diagnoses, and the ease of families to attend an adolescent medicine clinic, thereby avoiding the stigma of referral to psychiatry. The age of presentation and onset was similar to data from Western studies [6,7], while the age of onset was lower than that reported in Asian studies [8,9]. The average BMI at presentation was similar to other studies [8,10]. Some adolescents who were overweight or obese prior to onset of symptoms, had a significant weight loss over a short period of time and their BMI at presentation was normal; the adolescents in this group were either the binge-purge type or the OSFED category.

Adolescents in the younger age group had a higher percentage of the binge-purge type of anorexia nervosa, while those in the older age group were of restrictive type. This finding is slightly different from previous studies, which show the younger age group to be more of the restrictive type [9]. The most common identified trigger factors were peer pressure and family influence, similar to data from other studies [9-11].

Mortality rates reported in adolescents [7,12,13] are lower compared to adults with anorexia [9,14,15]. Our small cohort size precludes comment on mortality, but further studies are required to better estimate mortality and outcome. The poor follow-up in our patients reflects the inability of the family to understand the severity of disease, stigma of a psychiatric illness and financial burden of treatment on the family.

Our data will assist pediatricians in identifying anorexia nervosa early, and lead to appropriate diagnosis and management to improve overall outcome.

Ethics clearance: Ethics Committee, Institutional Review Board, CMC, Vellore; No. 11511, dated September 3, 2018.
Contributors: MMB: concept and the study design was done; KEP, RJR, RYS, MMB: material preparation, data collection and analysis were performed; RJR, KEP: written first draft of the manuscript; MB, PM: revision of the manuscript; MB: final approval of the manuscript. All authors have read and approved the manuscript.
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RESEARCH PAPER

Etiology-Based Decision-Making Protocol for Pediatric Cholelithiasis

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Objective: We reviewed hospital records of pediatric cholelithiasis to develop an etiology-based decision-making protocol. **Method:** This retrospective study was conducted on consecutive pediatric cholelithiasis patients from July, 2014 to June, 2019 in a tertiary care center. Pediatric cholelithiasis was classified according to etiology, and the outcome of medical/surgical treatment was noted. **Result:** Data of 354 pediatric patients were analyzed. Commonest (56.2%) etiology was idiopathic; followed by ceftriaxone pseudolithiasis (26.8%). Pigment stones were associated with the highest rate of complications. Non-hemolytic stones had a lower complication rate and a high rate of resolution with medical therapy. **Conclusion:** Hemolytic and symptomatic stones warrant an early cholecystectomy, whereas asymptomatic idiopathic stones, ceftriaxone stones, and TPN-induced stones are candidates for medical therapy under close observation.

Keywords: *Ceftriaxone, Gall stone, Hemolytic anemia, Management, Outcome.*

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Pediatric cholelithiasis is increasingly being diagnosed nowadays because of the use of abdominal ultrasonography screening [1]. Its prevalence in the Indian population has been reported to be rare [2]. Common causes are idiopathic (30-54%), hemolytic disorders (20-30%), and non-hemolytic causes (20-30%) such as ceftriaxone therapy, total parenteral nutrition (TPN), obesity and cystic fibrosis [3]. The treatment of choice for pigment stones is surgery; however, guidelines and consensus are lacking for the management of other stones, developed a simple etiology-based decision-making protocol for pediatric cholelithiasis, after analyzing our institutional data.

METHODS

We received hospital records from July, 2014 to June, 2019 of consecutive patients <18 years of age with cholelithiasis/sludge in the pediatric surgical unit of a tertiary center. Case records with incomplete data, and those with a diagnosis of choledochal cyst were excluded.

Records were reviewed for demographic information, symptoms (non-specific abdominal pain, right hypochondriac pain or biliary colic, nausea,vomiting, jaundice), predisposing factors (body mass index, history of fast food eating habit, acute gastroenteritis, dehydration, hemolytic disorder, ceftriaxone injection, total parenteral nutrition) and complications (acute cholecystitis, chronic cholecystitis, choledocholithiasis, cholangitis, gall bladder perforation). Fast food eating habit was defined as frequent consumption of food containing a rich mix of refined sugars, salt, and fats, and low in fibers. The child was considered obese if BMI was >27 on IAP growth charts [4]. Ultrasonography (USG) findings included floating hyperechoic lesion in gall bladder with posterior acoustic shadow, echogenic sludge without posterior acoustic shadow, gall bladder wall thickening (>3 mm with 6 hours fasting), peri-cholecystic collection, common bile duct (CBD) stone, and CBD and intrahepatic biliary radical dilatation.

Cholelithiasis was classified according to etiology: pigment stones (due to hemolytic disorders; positive on hemoglobin electrophoresis), ceftriaxone pseudolithiasis (cholelithiasis noticed within 21 days of >3 days of intravenous ceftriaxone therapy with a normal pre-ceftriaxone ultrasound report), stone or sludge secondary to TPN (developing after TPN); all others were labeled idiopathic. Hemoglobin electrophoresis was done for all patients with cholelithiasis diagnosed on ultrasound, and all patients with hemolytic disorders underwent an ultrasound to document hemolytic stones.

Cholecystectomy (open or laparoscopic; depending on availability and patient condition) was performed for all pigment stones (on diagnosis or presentation with a complication). Medical therapy (Ursodeoxycholic acid (UDCA) 25 mg/kg/day for 6 months) was advised for all non-hemolytic stones. These patients were followed up with clinical examination and ultrasound every 3 months.

The stone resolution was defined as an anechoic gall bladder on two consecutive three-monthly ultrasounds. Patients developing complications were dealt with in an emergent manner. Choledocholithiasis was managed with endoscopic stone extraction and interval cholecystectomy. Treatment is given (routine/emergent/medical/cholecystectomy) and outcomes were recorded.

Data analysis: Data analyses were performed using an online Graphpad analyzer. All variables were analyzed descriptively, and chi-square test or *t*-test were used for statistical analysis. A value of $P<0.05$ was considered significant.

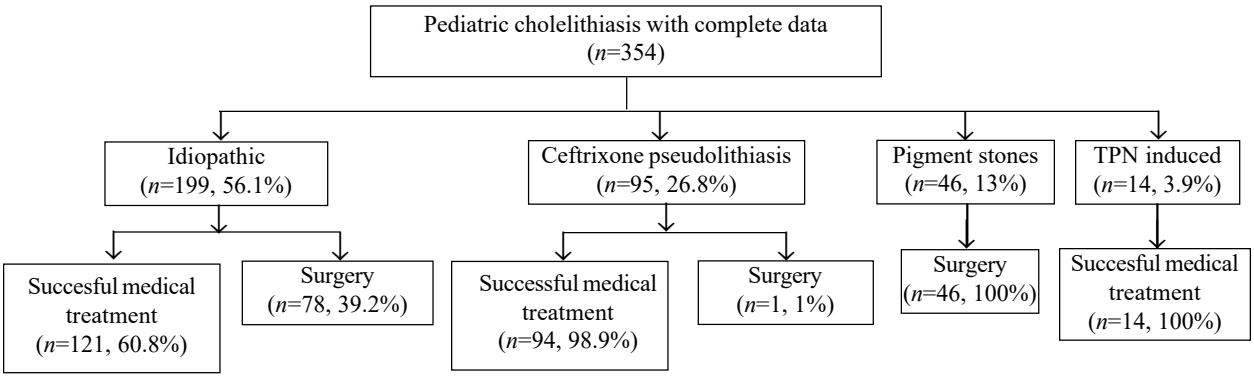
RESULTS

A total of 426 children (53.7% females) with cholelithiasis fulfilled inclusion criteria, and were included in the analysis (**Fig. 1**). Commonest (56.2%) etiology was idiopathic; followed by ceftriaxone pseudolithiasis (26.83%). The median (IQR) age was 6 (3-14) years. Incidence of obesity, fast food habits, and biliary colic was highest in idiopathic stones. Thirteen patients (13.7%) had a history of ceftriaxone administration after abdominal surgery, associated with a history of starvation for more than 48 hours. Most (45.8%) patients were asymptomatic; non-specific abdominal pain was the commonest (40.4%) symptom. The distribution of predisposing factors and symptoms among different etiologies is shown in **Table I**. On ultrasound, the largest stone size was 2.5 cm, and the mean size was not significantly different in all four etiological types of cholelithiasis. Solitary stone was the commonest (79.1%) presentation. Echogenic sludge without post acoustic shadow was found in all cases of TPN-associated cholelithiasis.

Pigment stones (n=46) were associated with the highest rate of complications (**Table I**) and underwent upfront elective cholecystectomy, planned cholecystectomy, and emergent cholecystectomy (for gangrenous cholecystitis) in 18, 22, and 6 patients, respectively. In idiopathic pediatric cholelethiasis (39.2%) had to undergo surgery because of biliary symptoms (37, 18.6%, elective cholecystectomy), complications (34, 17.1%, 5 emergent cholecystectomy), or failure of medical treatment in asymptomatic pediatric cholelethiasis (7, 3.5%). Complications viz. acute cholecystitis, chronic cholecystitis and choledocholithiasis were significantly higher in pigment stones as compared to idiopathic stones (P=<0.001, P=0.02 and P=0.02, respectively). Medical management with UDCA for 6 months was effective in 121 (60.8%) patients with idiopathic cholelithiasis and all TPN-associated cases. TPN stones were associated with the highest incidence of obstructive jaundice. One patient underwent emergent cholecystec-tomy for gall bladder perforation and biliary peritonitis. Gall-stone pancreatitis was not seen in any patient.

DISCUSSION

Increasing prevalence of reversible ceftriaxone-associated biliary pseudolithiasis is due to the common use of ceftriaxone in children for abdominal infections and peri-operatively in gastrointestinal surgery [5]. Ceftriaxone is excreted in bile where it gets 20-150 times concentrated and readily forms a reversible insoluble salt with calcium which precipitates into pseudo-stone formation [6]. Moreover, biliary stasis is known to occur in gastrointestinal infection, starvation, after abdominal surgery, and gram-negative sepsis [7]. History of admission for acute gastroenteritis/dehydration or previous abdominal surgery with the administration of ceftriaxone was common in our CP patients. Complications of cholelithiasis occur in 15-25% of pediatric patients; hence, guidelines for expectant/medical/



TPN: Total parenteral nutrition

Fig. 1 Treatment provided for various types of pediatric cholelithiasis.

Table I Characteristics of Children With Cholelithiasis (*N*=354)

Characteristics	*Total*	*Idiopathic (n=199)*	*Hemolytic (n=46)*	*Ceftriaxone pseudoi-thiasis (n=95)*	*TPN asso-ciated (n=14)*
BMI >27 kg/m^2 (obese)	49 (13.8)	42 (21.1)	5 (10.9)	2 (2.1)	-
Fast food habits	175 (49.4)	142 (71.4)	11 (23.9)	22 (23.2)	-
History of diarrhea/dehydration	102 (28.8)	11 (5.5)	9 (19.6)	82 (86.3)	-
Symptoms					
Asymptomatic	162 (45.8)	64 (32.2)	26 (56.5)	71 (74.7)	01 (7.1)
Non-specific abdominal pain	143 (40.4)	95 (47.7)	14 (30.4)	24 (25.3)	10 (71.4)
Biliary symptoms	47 (13.3)	40 (20.1)	6 (13.1)	1 (1.1)	-
Complications					
Obstructive jaundice	18 (5.1)	7 (3.5)	6 (13.1)	-	5 (35.7)
Choledocholithiasis	13 (3.7)	7 (3.5)	6 (13.1)	-	-
Acute cholecystitis	29 (8.2)	14 (7.1)	14 (30.4)	1 (1.1)	-
Chronic cholecystitis	20 (5.6)	12 (6.1)	8 (17.4)	-	-
Choledocholithiasis	13 (3.7)	7 (3.5)	6 (13.1)	-	-

Data in no. (%); BMI: body mass index, TPN: total parenteral nutrition; AGE: acute gastroenteritis; One child in idiopathic group had gall bladderperforation.

surgical treatment are needed for its management [8,9]. Hemolytic stones are advised cholecystectomy as the first line of treatment because they do not respond well to medical dissolution therapy and have a higher rate of complications due to impaction [8,10]. Ceftriaxone associated cholelithiasis and TPN induced pediatric cholelithiasis, on the other hand, are known for their reversible character and respond well to medical treatment [11]. UDCA is known to resolve PC in 19-37% of patients with non-hemolytic stones [8,10,12]. Higher (60.8%) stone dissolution with UDCA in the present study is unexplained.

Indications of cholecystectomy in idiopathic pediatric cholelithiasis are less clear and the decision is often based on clinical judgment, concerns for complications, and the surgeon's conviction (or lack of) in the efficacy of UDCA [13]. In our study, 40% of idiopathic cholelithiasis underwent surgery because of biliary symptoms or complications or failure of medical treatment in asymptomatic patients; supporting early surgery for symptomatic idiopathic stones. In the present study, the majority of idiopathic and asympto-matic idiopathic stones dissolved after medical treatment, suggesting a specific role of medical therapy in avoiding surgery. However, because of complications, medical treatment must be given under close observation. An unnecessary cholecystectomy entails needless risk and cost burden in a potentially dissolvable PC [14]. Also, pediatric cholecystectomy is more challenging for the surgeons because of its relative infrequency and the fact that surgical volume might not help lower complication rates [15]. The high incidence of ceftriaxone pseudo-cholelithiasis in our study raises concerns about the common use of ceftriaxone in pediatric practice. Awareness of this is important and consideration should be given to the use of equivalent antibiotic options.

Our algorithm (**Web Fig. 1**) allows a pre-emptive approach to avert complications, the best utilization of surgical options, and minimizes unnecessary surgery. Formulation of our protocol is based on the analysis of our data and lessons learned; it needs to be further tested in different settings and in a prospective design.

Our etiology-based treatment protocol developed with local data allows a judicious selection of pediatric cholelithiasis patients for surgery. Hemolytic and symptomatic stones warrant an early cholecystectomy. Asymptomatic idiopathic stones, ceftriaxone stones, and TPN-induced stones are candidates for medical therapy under close observation.

Ethics approval: Netaji Subhash Chandra Bose Medical College, Jabalpur, MP, India; No: IEC/NSCBMC/20/03, dated September 27, 2020.

Contributors: VA, AT: concept, design, definition of intellectual content, literature search, clinical studies, data acquisition, data analysis, manuscript preparation, editing and review; DS, RA: literature search, clinical studies, data acquisition, data analysis, manuscript preparation, editing, and review. All authors approved the final version of the manuscript, and are accountable for all aspects related to the study.

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WHAT THIS STUDY ADDS?

- An etiology-based decision-making protocol for pediatric cholelithiasis is proposed based on our experience.

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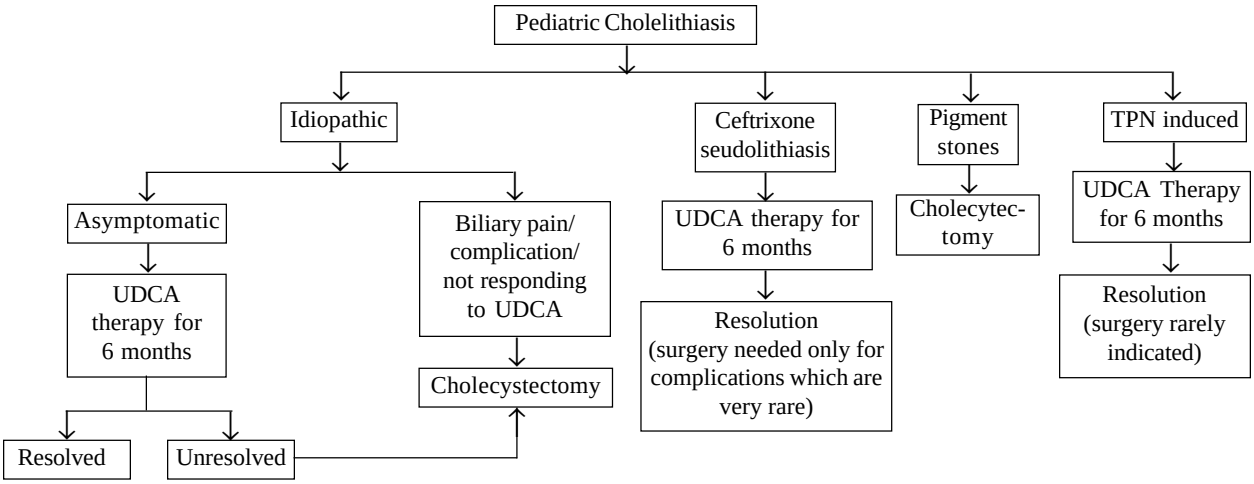

UDCA: Ursodeoxycholic acid; TPN: Total parenteral nutrition.

Web Fig. 1 Suggested etiology based decision-making algorithm for pediatric cholelithiasis.

RESEARCH PAPER

Developmentally Supportive Positioning Policy for Preterm Low Birth Weight Infants in a Tertiary Care Neonatal Unit: A Quality Improvement Initiative

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Objective: To improve developmentally supportive positioning practices by 50% in neonates weighing <1800 g, admitted in a neonatal intensive care unit over 6 months. **Methods:** Infant Position Assessment Tool (IPAT) scores were used for assessment of the ideal position. Proportion of neonates with IPAT score ≥8 and improvement of average IPAT score were the process and the outcome measures, respectively. At baseline, 16.6% of infants had optimum position. After root cause analysis, interventions were done in multiple Plan-Do-Study-Act (PDSA) cycles of educational sessions, positioning audits, use of low-cost nesting aids, and training of mothers. **Results:** Over 21 weeks, 74 neonates were observed at 714 opportunities. Over 6 months, mean (SD) IPAT score improved from 3.4 (1.4) to 9.2 (2.8). Optimum positioning was maintained in 83.3% neonates during sustenance phase. **Conclusions:** Low-cost interventions, awareness regarding standards of optimum positioning and involvement of primary caregiver can effectively improve infant positioning practices.

Keywords: *Conformational position, Infant Positioning Assessment Tool (IPAT), Nursing care, Posture, Outcome.*

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In developmental supportive care (DSC), positioning is the most important strategy that affects physiological stability and reduces stress [1]. Optimum positioning improves sleep, reduces pain, decreases apnea/desaturation episodes, improves thermal regulation, skin integrity and neurobehavioral organization [2,3]. Several studies have documented beneficial effects of supportive positioning interventions including reduction in musculoskeletal abnormalities and better neuromotor outcomes [4,5]. Frequent position changes with the use of 'nesting' or 'conformational positioner' have shown to improve the postural regulation with maintenance of optimum position [6,7].

The awareness of neonatal caregivers regarding infant positioning is scanty [8]. Improvement of practices were documented after educational training, positioning audits and policy formation [9]. This quality improvement (QI) initiative was undertaken to improve the develop-mentally supportive positioning practices in low birth weight neonates.

METHODS

Our neonatal intensive care unit (NICU) is a 24-bedded level-III unit with >2500 deliveries/year and >100 admission/month. The nurse: patient ratio is 1:1 for neonates on ventilator, 2:1 for sick neonates and 4:1 for relatively stable infants. The concept of DSC in our unit was limited to the routine use of a shoulder roll only. On a cross-sectional review of practice, over 80% admitted neonates at our center were found in unacceptable positions, supine with retracted shoulders/extremities (93.4%), undue neck flexion (70%) and excessive hip abduction due to oversized diaper (100%).

We aimed to improve developmentally supportive positioning practices by 50% in neonates weighing <1800 g admitted in the NICU over 6 months (April, 2019-September, 2019). This QI initiative was based on point-of-care quality initiative (POCQI) model [10]. Ethical clearance was obtained from Institute Ethics Committee.

Infant positioning assessment tool (IPAT; Philips Children's Medical Ventures), validated by several studies [11-14] was used to improve the positioning practices. Eligible neonates (birthweight <1800 g) were scored by a team of 'Positioning proponents (PP)', once in every shift and an average daily score was assigned to each baby. Proportion of neonates with average IPAT

score ≥8 was taken as the process measure. Improvement in mean IPAT scores was the outcome measure.

Baseline phase (4 weeks): In this phase, the applicability of IPAT score was validated in 6 neonates at 30 opportunities. A team of two doctors and six nurses identified as PP, were trained in developmentally supportive positioning practices and IPAT scoring. Mean (SD) IPAT score was 3.4 (1.4) and proportion of neonates with mean IPAT ≥8 was 16.6%. Potential causes of improper positioning identified through root cause analysis (**Fig. 1**) included lack of knowledge/skills, unavailability of positioning aids, high patient load, respiratory support, multiple infusions and non-availability of measurement tool.

Intervention phase: After baseline phase, Plan-Do-Study-Act (PDSA) cycles **(Web Fig. 1)** were used for interventions. IPAT was introduced and one-to-one teaching, hands-on demonstration-cum-practice session and assessment of nurses and residents were started. Data were recorded in excel sheet and plotted on run chart weekly. To prevent bias, average score was disclosed weekly.

PDSA-1: *Learning by doing (4 weeks)*: A schedule and the teaching material were distributed among team members. IPAT print-outs were made available at the bedside. Group teaching, informal bedside and one-to-one teaching, position demonstration on mannequin and baby were continued for next 2 weeks in every nursing shift. Nurses were assessed once weekly for IPAT scoring of five neonates with demonstration of ideal positioning. Additional review sessions were organized for newly-posted residents and nurses. Educational material was shared electronically. Finally, IPAT scoring was made a part of daily nursing care with scoring done in each shift and entered in nursing notes.

PDSA-2: *Customized boundary (4 weeks)*: Preparation of boundaries or 'nesting' with rolled linens was detected as one of the limiting factors due to non-availability of linens and reluctance of nurses for the labour-intensive process of preparing the nest. To resolve this, we prepared customized low-cost, washable and autoclavable, foam based reusable boundaries with covers by the hospital tailor (**Fig. 2**). This intervention decreased the require-ment of linen rolls and significantly reduced the time required for positioning of the neonates without impos-ing an additional burden to prepare nesting, boosting their enthusiasm for improving position. After this intervention, 50% of neonates had an IPAT score ≥8.

Two sick neonates were observed to developed occipital bedsore due to prolonged supine positioning. In order to maintain high IPAT score, nurses were maintaining supine posture with fixed boundaries without changing to lateral/prone positions. An amendment in positioning policy was made with inclusion of compulsory three-hourly position change.

PDSA-3: *Appraisal and improvement* (12 weeks): In this phase, we started selecting 'PP of the week' and appreciating them with a badge, which further improved the zeal of caregivers. Workshops on DSC with weekly refresher sessions were conducted. Now, positioning became a routine practice amongst the caregivers improving the proportion of neonates with IPAT ≥8 to 72.5%.

PDSA-4: Sizing the diapers (4 weeks): Following third PDSA cycle, it was analyzed that position of hips and legs could not be maintained because of oversized diapers. Since small diapers were not a part of hospital supply, we started making customized diapers for preterm with cotton and gauze (**Fig. 3**). After this, the number of

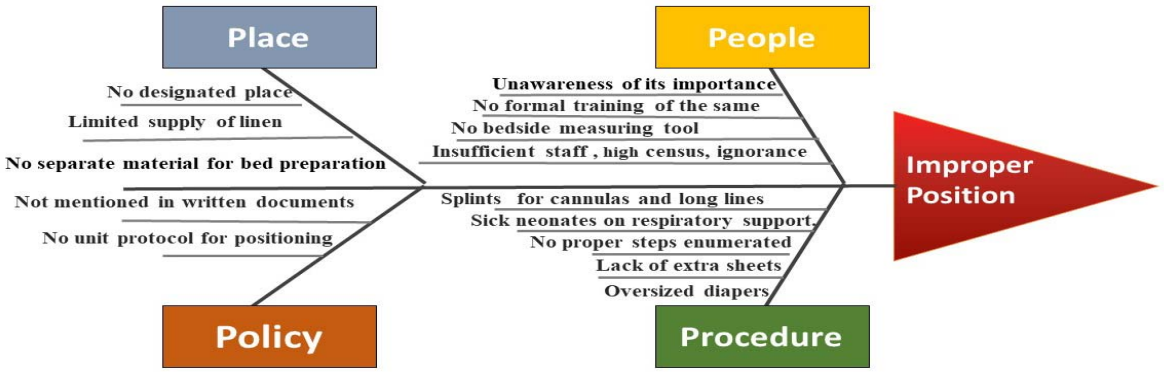

Fig. 1 Root cause analyses: Fish bone diagram for improper positioning.

Fig. 2 Positioning aid for optimum positioning.

neonates with oversized diapers reduced from 74.5-20%.

PDSA-5: Training of mothers (8 weeks): After fourth PDSA cycle there was a visible improvement in positioning practices of the unit. Team analyzed that most of the LBW neonates are shifted to step-down units with their mothers soon after clinical stabilization, and decided to train the mothers. Teaching materials and posters were prepared in the local language. Demonstrations were done using mannequins daily for one week. Every new mother was given a refresher tutorial. Training mainly focussed on preparation of boundaries using easily available homestuff such as towels and scarfs, preparation of shoulder rolls and its correct placement, difference between improper and proper positions and their long-term implications. 'Position expert' mothers were praised and asked to teach other mothers. Considerable acceptance was noted in their behavior.

RESULTS

Before starting interventions, each PP independently scored 6 neonates at 30 opportunities. Inter-rater reliability was analyzed with interclass correlation coefficient (95% CI) of 0.89 (0.83-0.94) using two-way mixed model, suggesting a strong level of agreement and high reliability of data-recording. For the position data, we calculated the baseline mean using the first 10 data-points and recalculated the mean whenever a shift in data was identified. In the baseline phase, 18 neonates with mean (SD) birthweight and gestational age of 1230 (265) g and 30.5 (2.6) weeks, respectively were observed over 4 weeks. Mean (SD) IPAT score was 3.4 (1.4) and 16.6% of eligible neonates had IPAT score ≥8.

Fig. 3 Customized diapers for extremely low birth weight babies.

Throughout this project, 74 neonates were observed at 714 opportunities. Forty-four nurses, 10 senior residents, 15 junior residents and 52 mothers were trained in standard positioning practices. Total 23 teaching sessions were conducted including 12 sessions of hands-on demonstration, 2 institutional workshop and 9 assessment sessions. For training of mothers, 12 teaching sessions and 20 demonstration-cum-hands-on training were conducted.

After first PDSA, mean (SD) IPAT score improved to 5.2 (1.6) with biggest limitation being lack of positioning aids. After third and fourth PDSA, introduction of nesting rolls and appropriate size diapers had significantly improved mean (SD) IPAT score to 9.2 (2.8) with sustenance for next 6 weeks**.** The chart showed less than expected number of runs signalling for an improved practice. In the last PDSA cycle, the baseline data was recorded again when the baby was shifted to step-down unit with the mother. Baseline mean (SD) IPAT scores of 3.5 (1.3) improved over next 5 weeks to 8.3 (0.2) (**Web Fig. 2**).

DISCUSSION

This short-term QI project aimed to improve the practice of infant positioning. A significant improvement was noted in the proportion of admitted neonates with IPAT score ≥8 from the baseline of 16.6-83.3%. In coherence with other projects on position improvement [12-14], nursing teaching and demonstration sessions were found to be most impactful. Inclusion of mothers in the loop was the most important factor for sustenance policy.

Compared to previous reports [12-14], the major difference in this project was the achievement of targeted

WHAT THIS STUDY ADDS?

- Compliance with developmental supportive positioning can be improved by standardizing positioning policy, staff education, low cost interventions to ease the process of positioning, and involvement of primary care giver to ensure long term benefits.

improvement within a short time-span. The quick response was attributed to multiple PDSA cycles, adopting the changes and policy formation in each step. Advent of customized nesting boundary was a cost-effective intervention with minimal consumption of linen. The biggest strength of this project was involvement of mothers as an addition to the concept of family-centred care. The major limitation was that we did not assess long-term developmental outcome.

To conclude, this QI project, using simple cost-effective interventions through multiple PDSA cycles and team effort, led to a considerable improvement in positioning practices of our unit. Involvement of mothers in the project was an important addition for better sustenance.

Ethics clearance: Institutional ethics committee, AIIMS, Rishikesh; AIIMS/IEC/19/698**;** dated April 12, 2019.
Contributors: JU, PS, SB: conceptualized and designed the study, coordinated and supervised data collection, drafted the initial manuscript; KCD, SS, RG: coordinated and supervised data collection, and reviewed and revised the manuscript. All authors approved the final manuscript.
Funding: None; *Competing interests*: None stated.

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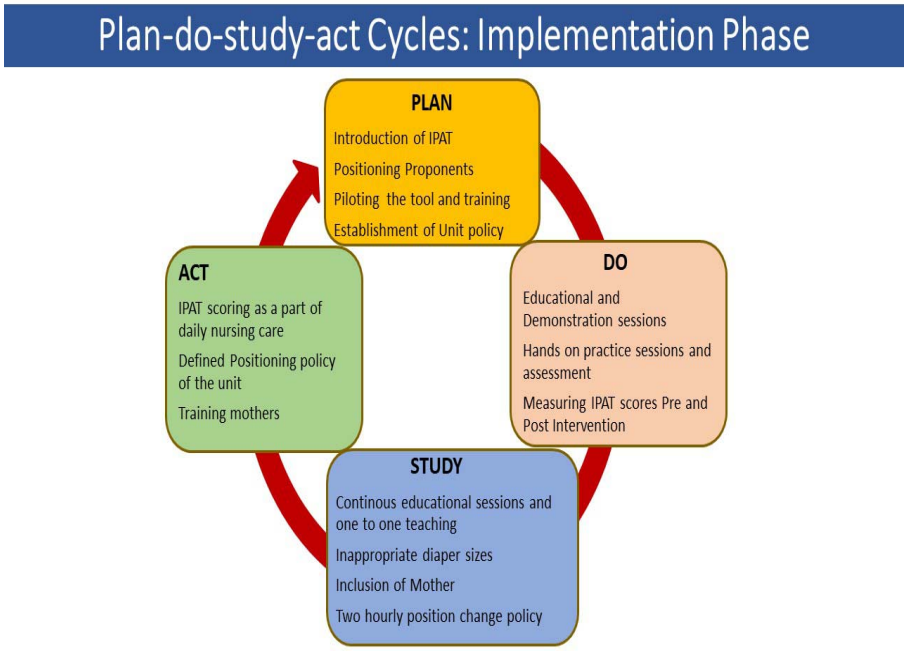
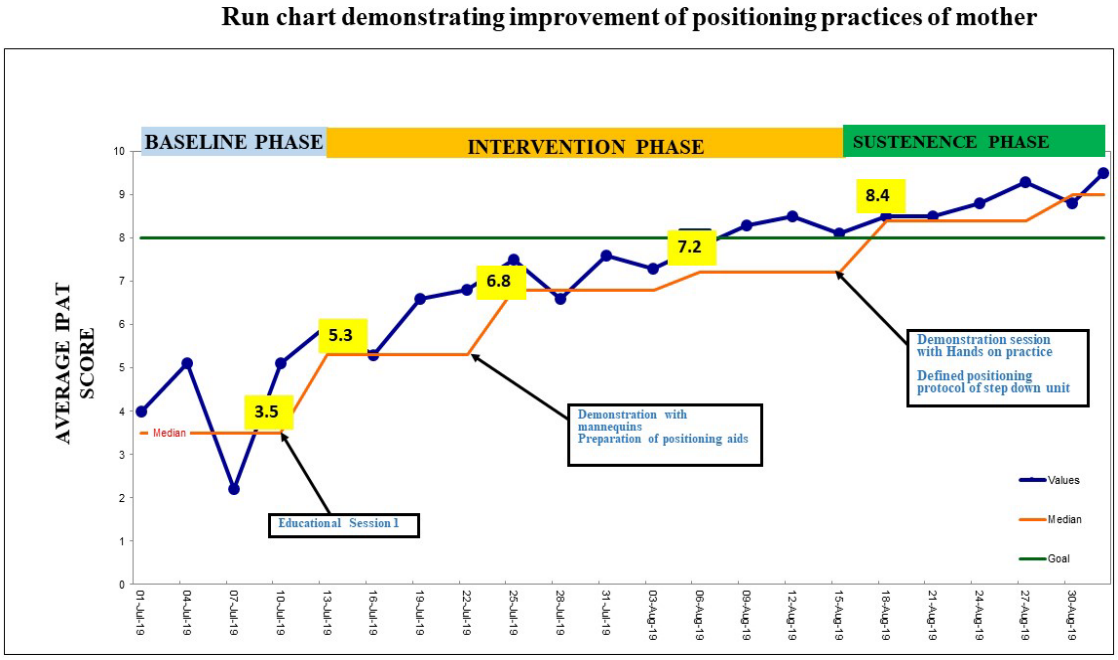


Web Fig. 1 Plan-Do-Study-Act cycles.



Web Fig. 2 Run chart demonstrating improvement in IPAT score of step-down LBW neonates with mothers.

RESEARCH PAPER

Clinical Profile and Outcome of Childhood Autoimmune Hemolytic Anemia: A Single Center Study

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Objective: To analyze clinical and laboratory parameters, and treatment outcomes of children with autoimmune hemolytic anemia (AIHA). **Methods**: Retrospective analysis of 50 children aged 0-18 years. Monospecific direct antiglobulin test (DAT) and investigations for secondary causes were performed. Disease status was categorized based on Cerevance criteria. **Results**: Median (range) age at diagnosis was 36 (1.5-204) months. AIHA was categorized as cold (IgM+,C3+/cold agglutinin+) (35%), warm (IgG+ with/without C3+) (28%), mixed (IgG+, IgM+, C3+) (15%) and paroxysmal cold hemoglobinuria (4%). Primary AIHA accounted for 64% cases. Treatment modalities included steroid (66%), intravenous immunoglobulin (IVIg) (4%), steroid+IVIg (4%), and steroid+rituximab (4%). Treatment duration was longer for secondary AIHA than primary (11 vs 6.6 months, P<0.02) and in patients needing polytherapy than steroids only (13.3 vs 7.5 months, P<0.006). During median (range) follow-up period of 73 (1-150) months, 29 (58%) remained in continuous complete remission, 16 (32%) remained in complete remission. **Conclusion:** Infants with AIHA have a more severe presentation. Monospecific DAT and a thorough search for an underlying cause help optimize therapy in most patients of AIHA.

Keywords: *Cerevance criteria, Direct antiglobulin test, Rituximab, Treatment.*

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Autoimmune hemolytic anemia (AIHA) is caused by the presence of auto-antibodies directed against antigens on the surface of red blood cells, leading to premature destruction [1,2]. AIHA is the main cause of acquired extra corpuscular hemolysis in children [1]. AIHA can be subdivided into primary (or idiopathic) and secondary. AIHA presenting with thrombocytopenia (Evans synd-rome) tends to have a more chronic and relapsing clinical course [3-5]. There is scarcity of data on Indian children with AIHA and their treatment outcome. We present data on children with AIHA from a single center in India.

METHODS

This is a retrospective analysis of data from January, 2007 to April, 2019 from our unit's database. Fifty children less than 18 years of age diagnosed with AIHA were enrolled in the study. AIHA was diagnosed based on the clinical presentation, positive direct anti globulin test (DAT) and at least one of the following: reticulocytosis, haptoglobin <10 mg/dL, and total bilirubin >1 mg/dL [7]. DAT test was done by gel card method (Bio-Rad). Infants were evaluated for TORCH profile. Based on clinical suspicion, hepatitis B, hepatitis C, HIV serology, Epstein-Barr virus (EBV) PCR, cytomegalovirus (CMV) PCR, *Mycoplasma pneumoniae* antibody test and antinuclear antibody (ANA) were done. For children with repeated infections, immunoglobulin profile, Lymphocyte subset analysis (CD3, CD4, CD8, CD19, CD16, CD56) was performed. Monospecific DAT test routinely was performed for all children with suspected AIHA after the year 2013. AIHA was categorized based on types of serological antibodies (IgG, IgM, IgA, C3, and/or combinations) found in monospecific DAT test, cold agglutinin test and Donath Landsteiner test. History of drug intake and recent blood transfusion was obtained for all patients.

Glucocorticoids were used as the first line therapy in both warm and cold AIHA. In cold AIHA, additionally, treatment of the underlying disease was prioritized. The patient was kept warm and in cases with very severe anemia, packed red cell transfusion was given with a heat generator inside the tubing. Intravenous methyl-prednisolone (2 mg/kg 8 hourly for 3 days followed by oral prednisolone, 2mg/kg/day for 4 weeks, then tapered gradually) was used for patients who were sick, unable to take orally or had very severe hemolysis.

If there was complete remission after 4 weeks, tapering of prednisolone was done by 10% with each dose change over a period of 6 months. If there was no remission/ steroid dependence with prednisolone dose of 0.2 mg/kg/day, second line treatment was used. Second-line therapy comprised of either intravenous immunoglobulin (IVIg), rituximab, cyclosporine, mycophenolate mofetil (MMF) or azathioprine. In steroid dependent cases, one of the immunosuppressants (cyclosporine, MMF, azathioprine) was used. In common variable immunodeficiency (CVID), IVIg was additionally used. Packed red blood cell transfusion as supportive therapy was given if the child had a hemoglobin value less than 3 g/dL or 3-6 g/dL with cardiac failure or respiratory distress and needing intensive care unit (ICU) care.All patients received folic acid and vitamin B12 during treatment to support hematopoiesis.

Patients were followed every month till complete remission and then 3-monthly till 1 year. Clinical and lab parameters at last follow-up were classified based on Cerevance criteria [6] into 4 categories: No remission (NR), partial remission (PR), complete remission (CR) and continuous complete remission (CCR).

Statistical analysis: Chi square test and Student *t* test (two tailed, unpaired) were used to compare variables between primary and secondary AIHA. *P* value less than 0.05 was considered significant. SPSS version 20.0 was used for the analyses.

RESULTS

Data of 50 children [median (range) age at diagnosis, 36 months (1.5 months-17 years)] were analyzed. Commonest clinical feature at diagnosis was pallor (100%) followed by fever (68%) and jaundice (60%). Hepatomegaly (90%) was seen more often than splenomegaly (38%).

Mean (SD) hemoglobin at presentation was 4.7 (1.6) g/dL. Out of 50 children, 72% children presented with very severe anemia (hemoglobin <3 g/dL, *n*=6) or severe anemia (hemoglobin 3-6g/dL *n*=33, 66%); only 1 child had mild anemia (>9g/dL). Admission to intensive care unit (ICU) was needed in 28% children. Leukocytosis (after correction for nucleated RBCs) was noted in 27 (54%) patients, and leucopenia in 4 (8%) patients. Only 3 children had thrombocytopenia at diagnosis. Reticulocytosis was seen in 37 cases (74%), whereas reticulocytopenia was seen in 13 cases (26%). Elevated lactate dehydrogenase (LDH) was seen in 86% children with median (range) LDH level of 521.5(163- 12858) U/L.

Direct anti-globulin test was 4+ positive in 26 children, 3+ positive in 11 children, 2+ positive in 5 children, and 1+ positive in 5 children.In three DAT-negative children, the diagnosis was based on clinicopathological suspicion after ruling out other causes of hemolytic anemia and on the basis of response to treatment. Two out of three DAT-negative patients were positive for Donath Landsteiner test. Monospecific DAT test was performed in 24 children after its availability from the year 2013; of which, IgG ± C3 was present in 10 (41%) children, IgM and C3 were present in 3 children (13%) and both IgG and IgM with C3 were present in 4 children (17%). Cold agglutinin testing was performed in 21 children and was positive in 13 children. Based on above results cold, warm, mixed AIHA and PCH was seen in 35%, 28%,15% and 4% children, respectively. In the other 18% children seen prior to 2010, AIHA was unclassified.

Secondary AIHA was identified in 36% cases with etiology being infection in 5 (10%) (*M.pneumonia,* 3; cytomegalovirus infection, 1; *Plasmodium vivax* malaria, 1), autoimmune diseases in 5 (10%) (autoimmune hepatitis, 2; systemic lupus erythematosus (SLE, 2; and giant cell hepatitis, 1). Other causes leading to secondary AIHA were Evans syndrome (6%); childhood malignancies (6%) (Hodgkin lymphoma, 2 and precursor B cell acute lymphoblastic leukemia, 1); CVID, 1(2%); and Wiskott Aldrich syndrome, 1 (2%).

Among infants, hemolysis was found to be much severe than those who developed AIHA after infancy (mean (SD) hemoglobin, 3.96 (1.18) vs. 5.13 (1.65) g/dL, *P*=0.01). In primary AIHA, the mechanism of hemolysis was more often IgM and combined antibody mediated than in children with secondary AIHA wherein it was mainly IgG-mediated hemolysis.

Steroids alone were used in 33 (66%) children; other medications used were IVIg in 2 children, steroid and IVIg in 2, steroid and rituximab in 2, steroid, rituximab and cyclosporine in 1, and steroid and other drugs (three or more) in 7 children. Other immunosuppressive medications used were mycophenolate mofetil and azathioprine. Among three patients of Evan syndrome, two patients responded to first line glucocorticoid therapy and one responded to second line therapy with IVIg followed by rituximab. One patient improved spontaneously and was not given any therapy.

Treatment duration was longer for children with secondary AIHA than primary (11 vs 6.6 months, *P*=0.02) and in patients needing polytherapy than those who improved with steroids only (13.3 vs 7.5 months, *P*=0.006). Median (range) follow up duration was 73 (1-150) months; 29 (58%) remained in CCR, and 16 (32%) in CR (**Fig.1)**. Despite relapse in 26% cases, 61.5% still showed good response to steroids. One patient with

WHAT THIS STUDY ADDS?

- Managing AIHA in infants and those with secondary AIHA is challenging, with almost one-third patients at our center needing additional agents to the steroid backbone.

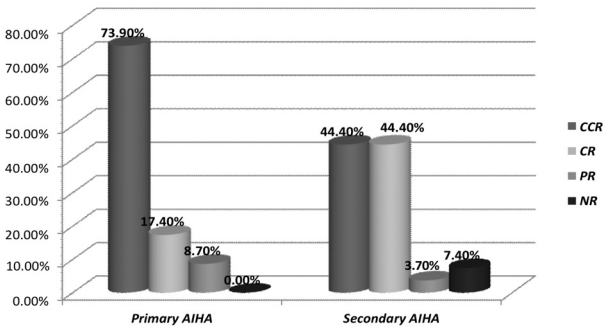
secondary AIHA and underlying Hodgkin lymphoma died due to fulminant fungal sepsis and hemophagocytosis.

DISCUSSION

We present our institutional data on pediatric AIHA and underscore the preponderance of AIHA in younger children; although, the median age at diagnosis in our study was higher than that in previous studies (10.8-16 months) [6,7]. Patients younger than one year required ICU care in view of severe anemia and hypoxia, similar to the report by Fan, et al. [7].

In our study, 94% cases had positive DAT, similar to another Indian study by Naithani, et al. [8]. Negative DAT in some patients may be due to low titer of IgG antibodies or IgA or IgM auto antibody mediated hemolysis. Reticulocytopenia seen in 26% cases was probably due to destruction of erythroid progenitors by autoantibodies [9]. A French national study [6] also observed a high incidence of reticulocytopenia (39%) indicating that although reticulocytosis is an important marker of hemolysis, its absence alone should not rule out AIHA.

We found that hemolysis was severe whenever combined or IgM coated antibody mediated hemolysis occurs. This observation was similar to previously published study by Sokol, et al. [10], which showed that compared to IgG mediated hemolysis alone, IgG along with IgM or IgA leads to more severe hemolysis. Secondary AIHA was due to infection in 10% whereas Fan, et al. [7] showed that infection accounted for 97.6%. In contrast to this, a French study [6] showed that secondary forms of AIHA were mainly due to immunological cause (53%) and infections contributed to a very small portion (10%). This observation may be due to increased burden of infection and early exposure of viruses like EBV in low or middle in-come countries.



AIHA: autoimmune hemolytic anemia; CCR: continuous complete remission; CR: complete remission; PR: partial remission; NR-no remission.

Fig. 1 Comparison of remission status among primary and secondary autoimmune hemolytic anemia (AIHA).

Aladjidi, et al. [6] showed 90% remission rates with 39% achieving CCR and 51% attained CR. This may be due to prolonged usage of steroids (median duration 8 months). Two patients with PCH had early disease remission. This may be due to the self-limiting nature of the condition; however, as per unit policy they were also treated with short course of steroids. If there was no remission/steroid dependence with prednisolone in a dose of 0.2 mg/kg/day, second line treatment was used [11]. We needed immunosuppressants as second line of treatment in 26% cases. Rituximab was used in the standard dose of 375 mg/m^2 per day [12].

Our study is limited by the fact that it is a retrospective study, comprises of a small cohort of patients and lacks protocol uniformity. Treating AIHA in children can be challenging and may need prolonged and complicated therapy, especially in secondary AIHA. We suggest that relapsed or refractory cases of AIHA should be cared by pediatric hematologists in a tertiary care center.

Ethics clearance: EC-SGRH; No.EC/09/20/1715, dated September 30, 2020.
Contributors: KBT: collection of data, analysis of data, writing of manuscript, revising it for important intellectual work; PS: collection of data, analysis of data, writing of manuscript; TP, DS: collection of data, analysis of data, writing of manuscript; AD, MK, AS: contributed patients, final editing of manuscript. All authors approved the final version of manuscript.
Funding: None; *Competing interest*: None stated.

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CLIPPINGS

Acute kidney injury in pediatric patients hospitalized with acute COVID-19 and multisystem inflammatory syndrome in children associated with COVID-19 (Kidney Int. 2021 Mar 3: S0085-2538(21)00268-4)

This retrospective study was conducted in patients 18 years of age or below admitted to study the incidence, clinical presentation and outcome of acute kidney injury (AKI) in children with COVID-19 and Multisystem Inflammatory Syndrome. Total 152 children were included, out of which 97 had acute COVID-19 and 55 had COVID -19 and MIS-C. AKI occurred in 11.8% of children with acute COVID-19 and MIS-C. They had increased WBC counts and lower serum albumin levels on admission and decreased intravascular volume and distributive/cardiogenic shock. In addition, pediatric COVID-19–related AKI was associated with poor outcomes, such as increased PICU and hospital length of stay. Their limitations were small sample size, retrospective study design, and most COVID-19 and MIS-C patients had a basic metabolic panel on presentation and during their hospital stay to assess for AKI. Utilization of serum creatinine without urine output and back-calculation of baseline creatinine values may have also underestimated the incidence of AKI. Therefore, further research in larger cohorts is needed to characterize AKI risk factors in children with acute COVID-19 and MISC

Long term renal survival of pediatric patients with lupus nephritis (Nephrol Dial Transplant. 2021 Apr 7: gfab152)

This retrospective study conducted from 2000 till 2020 at Hacettepe University, tried to find out the clinical presentation, treatment options and renal prognosis in children with lupus nephritis. Data collected from medical charts and electronic records of 53 lupus nephritis children who had kidney biopsy at diagnosis. Overall, 52% had lupus nephritis (LN); class IV LN (54.7%) was most common followed by class III LN (22.6%). Around 77.3% and 73% of children received complete and partial remission at 6 and 12 months, respectively. Five-and ten-year renal survival rates were 92% and 85.7%, respectively. This study demonstrated that male gender, failure to achieve remission within 1 year after induction treatment and requiring dialysis at the time of diagnosis were the best predictors of poor renal outcome. Limited sample sign, retrospective study design, and bias in starting CYC for patients with more severe disease were some of the limitations reported. Prompt recognition and aggressive management of paediatric LN are essential to achieve and maintain remission

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RESEARCH PAPER

Relationship of Maternal and Neonatal Variables With Breastmilk Sodium

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Objective: To determine breastmilk sodium changes in the first 72 hours after birth and to correlate maternal and neonatal variables with maternal breastmilk sodium. **Methods**: We enrolled 245 mothers and their exclusively breast-fed neonates in this prospective cohort study. Singleton, inborn babies of greater than 34 weeks gestation, who were exclusively breastfed for the first 72 hours were included. Babies who required neonatal intensive care unit (NICU) admission, top up feeds or discharged before 72 hours were excluded. Study outcomes were changes in breast milk sodium in the first 72 hours and association of high maternal breast milk sodium with various maternal and neonatal variables. **Results:** Mean (SD) breastmilk sodium steadily declined over the first 72 hours [53.5 (19.2), 38.5 (19.0) and 22.2 (10.6) mmol/L at 24, 48, 72 hours, respectively]. Breastfeeding ≤8 times per 24 hours in the first three days was the only factor significantly associated with high breastmilk sodium (*P*=0.008). Maternal age, gravida, mode of delivery, significant neonatal weight loss, hypernatremia, neonatal morbidities like fever, irritability, lethargy and poor suck had no significant correlation with high breastmilk sodium. **Conclusion:** Breastmilk sodium shows a steady decline in the first 72 hours after delivery. Feeding ≤8 times per day is associated high breastmilk sodium at 72 hours of age.

Keywords: *Breastfeeding, Frequency, Initiation, Lactation failure.*

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Breastmilk provides optimum nutrition for a neonate and reduces incidence of acute infections, chronic diseases and results in better neurodevelopmental outcomes. Adequacy of breastmilk is a consequence of several interrelated stages such as mammogenesis, lactogenesis, galactopoiesis and effective milk removal. Breast milk contains carbohydrates, lipids, proteins and minerals like sodium, potassium and calcium. In term babies, breast milk sodium is highest after delivery and falls by day 3 of life [1]. Persistent elevation of breast milk sodium is associated with inadequate lactogenesis [2] and decrease in the rate of infant feeding may be associated with a significant increase in sodium concentration of mother's milk [3]. High breast milk sodium levels have also been shown to be associated with neonatal malnutrition, dehydration and hypernatremia [4]. This prospective study was undertaken to study the changes in breast milk sodium in the first 72 hours of life in exclusively breast-fed infants and to explore the relationship between breastmilk sodium and maternal and neonatal variables.

METHODS

This observational study was carried out at the neonatal unit of the Pondicherry Institute of Medical Sciences from January, 2015 to March, 2016. We recruited mothers and their exclusively breastfed neonates consecutively. Inclusion criteria were all singleton inborn babies >34 weeks of gestation. Neonates admitted to neonatal intensive care unit (NICU), and babies discharged within 72 hours, were excluded. Assuming the neonatal morbidity among children with high maternal breast milk sodium to be 80%, absolute precision 5%, level of significance 5%, a sample size of 245 was calculated.

After getting written informed consent, neonates who met the inclusion criteria were recruited for the study. Maternal variables like age, parity, mode of delivery, timing of initiation of breast feeds, obstetric complications were documented in a pre-piloted proforma. Neonatal variables like gender, gestation, growth for gestational age, birth and daily weight, breast feeds, urine and stools per day were also documented.

All babies were assessed once a day and as required for percent loss of birthweight, fever, irritability, poor suck, lethargy and jaundice requiring phototherapy. Additionally, every time the mother complained of above-mentioned symptoms the baby was examined by the

principal investigator to confirm the findings. All babies were in a common postnatal ward. Babies were weighed naked on a calibrated infant weighing scale within 1 hour of birth and every day at 24, 48 and 72 hrs. of age, before feeds, and at the time of discharge. One infant weighing scale (Phoenix BWS, accuracy 5g) was calibrated and used for the study. Significant weight loss was defined as a weight loss of more than 10% of birthweight in the first 72 hours. If weight loss exceeded 10% of birthweight, serum electrolytes, blood urea and serum creatinine were measured and repeated as clinically indicated. Serum sodium >145 mmol/L was taken as hypernatremia.

Breastmilk sodium measurement: Mothers were asked to hand express about 2 mL breast milk at 24, 48 and 72 hours. One mL of milk sample was collected in a glass tube and centrifuged at 3000 rpm for 15 minutes for separation of the low-fat milk supernatant. Biochemical analysis of sodium in breast milk was performed immediately using a COBAS b 121 blood gas analyzer (Roche diagnostics) using ion selective electrode method. The breastmilk sodium concentrations were expressed as mmol/L. A value ≥21 mmol/L (median value) was considered as high breastmilk sodium at 72 hours after birth [1].

Statistical analyses: The data obtained was entered into Microsoft excel sheet and analyzed using SPSS Software version 20.0. Chi square test was used to find the association between two qualitative variables. Biserial point correlation was applied to see the correlation between breast milk sodium and categorized variables like weight loss and phototherapy. Spearman correlation was applied to see the correlation between breast milk sodium and continuous variables. A *P* value <0.05 was considered as significant.

RESULTS

Majority (94.3%) of the mothers were below 30 years of age and none were teenagers. Neonatal morbidity, described by mother, was seen in 107(43.6%) of babies studied. Poor feeding was the predominant complaint in 61 (57.0%), followed by lethargy in 29 (27.1%), fever in 13 (12.1%) and irritability in 4 (3.7%). Mean (SD) breastmilk sodium (BMS) level at 24 hours was 53.5 (19.2) mmol/L; at 48 hours it was 38.5 (19.0) mmol/L and at 72 hours it was 22.2 (10.6) mmol/L (**Fig. 1**). Maternal variables like age, gravida, morbidities and mode of delivery did not show any significant correlation with high breastmilk sodium (**Table I**). Among the neonatal variables, association of frequency of breastfeeding in the first 72 hours was significant with breastmilk sodium (**Table II**)

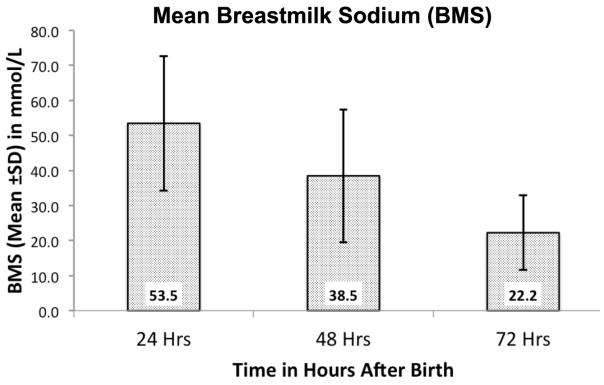

Fig. 1 Fall in breastmilk sodium in the first 72 hours.

DISCUSSION

This prospective cohort study of 245 exclusively breastfed neonates and their mothers found progressively decreasing breastmilk sodium during first 72 hours after birth.

Lactogenesis II starts around 30-40 hours of age in term babies and is complete by 72 hours characterized by copious milk secretion [5]. Authors have documented that the level of sodium drops precipitously by day 3 of

Table I Characteristics of Study Population and Breastmilk Sodium Levels at 72 Hours After Birth (*N*=245)

Variables	*No. (%)*	*Breast milk sodium (mmol/L)*	
		<21,n=107	*≥21, n=38*
Maternal age ≤30 y	231 (94.3)	101 (94.4)	130 (94.2)
Term gestation	224 (91.4)	96 (89.7)	128 (92.7)
Primigravida	161 (65.7)	71 (66.4)	90 (65.2)
Maternal morbidity	38 (15.5)	19 (17.7)	19 (13.7)
Mode of delivery [a]			
Normal vaginal	184 (75.1)	74 (69.1)	110 (79.7)
Instrumental	9 (3.7)	7 (6.5)	2 (1.4)
Cesarean	52 (21.2)	26 (24.4)	26 (18.9)
Breastfeeding started <1h	172 (70.2)	76 (71.0)	96 (69.6)
Birthweight, g			
1500-2499	25 (10.2)	11 (10.3)	14 (10.1)
2500-3499	203 (82.9)	88 (82.2)	115 (83.3)
≥3500	17 (6.9)	8 (7.5)	9 (6.6)
Growth characteristics[b]			
SGA	27 (11)	13 (12.1)	14 (10.1)
AGA	216 (88.2)	92 (86.0)	124 (89.9)
LGA	2 (0.8)	2 (1.9)	0
Male	137 (55.9)	56 (52.3)	81 (58.6)

All values in no. (%). P>0.05 for all comparisons except [a]*P=0.049. Intrauterine growth characterized as small (SGA), appropriate (AGA) and large (LGA) for gestational age.*

WHAT THIS STUDY ADDS?

- Frequency of breastfeeding ≤8 per day correlates significantly with high breastmilk sodium at 72 hours.

Table II Neonatal characteristics and Maternal Breastmilk Sodium at 72 Hours

Variables	*No. (%)*	*Breast milk sodium (mmol/L)*	
		<21 n=107	*≥21 n=138*
Breast feeds ≤8/d[a]	72 (29.4)	22 (20.5)	50 (36.2)
Passed urine <6 times in 24h	108 (44.1)	42 (39.2)	66 (47.8)
<4 stools in 24 h[b]	127 (51.8)	63 (58.8)	64 (46.4)
≥10% weight loss	77 (31.4)	38 (35.5)	39 (28.2)
Babies with hypernatremia	38 (15.5)	19 (17.7)	19 (13.7)
Received phototherapy	24 (9.8)	9 (8.4)	15 (10.8)
With one neonatal morbidity[c]	107 (43.7)	41 (38.3)	66 (47.8)

All values in no. (%). [a]P=0.008; [b]P=0.52; [c]either fever, poor feeding, lethargy or irritability.

life [2] and inadequate drop in breastmilk sodium levels may indicate failure of successful lactation. However, drop in sodium has not been conclusively proved as a useful surrogate marker for predicting successful lactation [6].

The decline in breast milk sodium over first 3 days postpartum is similar to previous studies [1,7,8]. Parturition brings about abrupt changes in plasma concentration of maternal hormones that trigger lactogenesis [9] and there is a serial fall in sodium and chloride in breastmilk [10]. Stress and delayed initiation [2], inadequate breast feeding evidenced as lactation failure is one of the most significant etiological factors for hypernatremic dehydration, which is a potentially devastating condition**.** Mothers who had drop in sodium levels to normal by day 3 had longer durations of exclusive breastfeeding, and their babies had higher per day weight gain in the first month of life [6]. Studies [4] showing that high breastmilk sodium is the cause for hypernatremic dehydration in exclusively breastfed babies have been disproved by others who concluded that it is direct breastmilk volume intake that is more important than actual levels of sodium in breastmilk [8].

Galipeau, et al. [11] studied maternal and neonatal factors responsible for delayed lactogenesis II as manifested by elevated breastmilk sodium levels. In this study, maternal age, gravida, antenatal morbidity, mode of delivery did not show significant correlation with high breastmilk sodium. This is in contrast to the findings of others who found that primiparity [12] and cesarean section [13] were associated with delayed lactation. We did not find similar results. The growth of the baby, birthweight, and time of initiation did not show significance in our study, but Narayan, et al. [12] have found mother's age <26 years and low birthweight to be associated with delayed lactogenesis. We found that high breastmilk sodium was more common in normal deliveries but that could be due to a large proportion of such deliveries in our study.

Average number of breastfeeds ≤8 per day in the first three days showed a very significant correlation with persistence of high breastmilk sodium. This has also been substantiated by others [8] that frequent feeding, at least more than 12 per 24 hours or frequent hand pumping [11] is necessary to initiate lactogenesis resulting in a drop of breastmilk sodium by 72 hours. Urine frequency <6 per day was seen more often in babies with high breastmilk sodium but it was not statistically significant. Also frequency of urine is variable in the first 72 hours and would not be a suitable predictor of adequate feeding, unlike after three or four days after birth [14]. In this study neonatal morbidities like fever, poor feeding, lethargy, irritability did not have any association with breastmilk sodium. Other workers [15] have studied neonatal morbidities in hypernatremic dehydration but none in relation to high breastmilk sodium.

The mean breastmilk sodium dropped steadily over the first 72 hours after birth. Breastfeeding frequency ≤8/ day correlated with high breastmilk sodium at 72 hours. Other maternal and neonatal variables did not have any correlation with high breastmilk sodium at 72 hours. The relationship of lactogenesis, breastmilk sodium and hypernatremic dehydration needs further evaluation to have a clinical application.

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CLIPPINGS

Prophylactic rituximab administration in children with complicated nephrotic syndrome (Pediatr Nephrol. 2021;36:611-19)

Rituximab has been successfully used for maintaining remission in complicated nephrotic syndrome patients. This study evaluated the efficacy of prophylactic rituximab therapy for maintaining remission after B cell recovery has been achieved. This retrospective study enrolled children with steroid dependant and frequently relapsing nephrotic syndrome who had SRNS in past. They received single dose of rituximab at a dose of 375 mg/m^2 in addition to immunosuppressive therapy. Once they received B cell remission, these children were divided in to two groups: one who received additional dose of rituximab as prophylaxis (n=16) and other group who did not receive any prophylaxis (n=45). Fifty-percent relapse-free survival after the last rituximab treatment was 667 days in the rituximab group and 335 days in the other group (P=0.001). Multivariate analysis showed that additional rituximab treatment was the only significant negative factor for early relapse, with a hazard ratio of 0.40 (P= 0.02). Fifty-percent relapse-free survival after B cell recovery was much longer in the rituximab prophylaxis group (954 vs 205.5 days, P=0.003). Absence of randomisation, patients using calcineurin inhibitors were no comparable between the 2 groups, small sample size and selection bias were some of the limitations seen. Therefore, a prospective randomized study with an adequate sample size needs to be performed to verify the clinical advantages of additional rituximab treatment

The impact of erythropoietin on short- and long-term kidney-related outcomes in neonates of extremely low gestational age. (J Pediatr. 2021;232:65-72.e7)

This multicentre, double-blind placebo controlled randomised controlled trial was conducted to evaluate the impact of erythropoietin on kidney related outcome in extremely low gestational age neonates (ELGANs) during hospitalization and at 22-26 months of corrected gestational age (CGA). Prevalence of severe (stage 2 or 3) AKI was 18.2 %. There was no statically significant difference in primary (severe AKI) or secondary renal outcomes (any AKI and serum creatinine/cystatin C values at days 0, 7, 9, and 14) between the groups during hospitalization. There were no significant differences in eGFR, albumin/creatinine ratio, rates of SBP >95th percentile, or DBP >90th or >95th percentiles at the 2-year follow-up visit between the two groups. Prevalence of AKI and renal complications were more in 22-26 months of CGA among ELGANs. Some limitations reported were that creatinine was not measured daily for some neonates, therefore, true AKI rate could be greater, the methods to capture kidney-related outcomes were not gold-standard methods, and a large number of patients did not have kidney-related metrics measured at the 2-year cGA time point.Therefore, studies using gold-standard measurements, studies that evaluate interventions to limit or prevent these outcomes, and evaluation of the most cost-effective methods for screening this high-risk population are greatly needed.

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RESEARCH PAPER

Diagnostic Reliability of Salivary C-Reactive Protein as an Alternative Noninvasive Biomarker of Neonatal Sepsis

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Objective: To assess if salivary C-reactive protein (CRP) can be detected in neonatal sepsis and correlate the levels of salivary and serum CRP. **Methods:** This analytical cross-sectional study included all neonates ≤28 days of life with suspected sepsis or with perinatal risk factors for sepsis. Saliva was collected using an absorbent swab and analyzed by enzyme-linked immunosorbent assay, along with serum CRP. **Results:** Salivary CRP was detectable in 135 subjects (99%). An increase was seen in median (IQR) levels from 0.25 (0.13,0.3) ng/mL in clinical sepsis group to 0.6 (0.3,1.4) ng/mL in screen positive/ blood culture negative group, and to 1.98 (0.54, 2.95) ng/mL in blood culture positive group. There was a moderate positive correlation between salivary and serum CRP (r=0.63, P value 0.01). On receiver-operator characteristics curve, the area under the curve of salivary CRP for predicting serum CRP ≥10 mg/L was 0.861 (95% CI, 0.78 to 0.94; P <0.001), with the optimal salivary CRP cut-off being 0.6 ng/mL. **Conclusion**: Salivary CRP could be used as an alternative biomarker of neonatal sepsis.

Keywords: *Detection, Elisa, Inflammation, Sensitivity.*

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Blood culture is currently the gold standard for diagnosing neonatal sepsis, but various biomarkers in serum are commonly used for rapid supportive diagnosis. Among them, serum C-reactive protein (CRP) is the most extensively studied biomarker in neonates and is found in various body fluids like serum and saliva [1,2]. Considering the difficulty with phlebotomy in neonates and the potential problems caused by blood sampling, alternative fluids like saliva could serve as an attractive non-invasive option for detection of CRP. Salivary diagnostic assays have now been well described in various systemic and oral diseases in adults [3,4]. However, translation into neonatal clinical practice is limited and is mostly restricted to cortisol assessment as a stress marker in term and preterm infants [5,6]. The present study was thus planned to investigate if CRP is detectable in saliva, and whether levels correlate with serum CRP, among neonates with sepsis.

METHODS

This was a cross-sectional study conducted in the neonatal intensive care unit of a tertiary care hospital in over a period of 18 months (September, 2015 to January, 2017). The study was approved by the Institute's research ethics committee.

Neonates (aged ≤28 days) of any gestational age, with clinical suspicion of neonatal sepsis or with perinatal risk factors of sepsis, were included in the study, after informed written consent. Neonates with major congenital malformations, oral infections and oral ulcers were excluded from the study. Demographic findings, including intrapartum maternal fever, presence of preterm and or prolonged rupture of membranes, chorio-amnionitis, gestational age, sex, antibiotic treatment, clinical features, and day of presentation were recorded. All subjects underwent a sepsis workup including serum CRP, complete blood counts, and blood culture, in addition to other investigations as deemed necessary by the treating physician. Serum CRP was measured using immuno-turbidimetric method using Roche Cobas C series analyzer (Roche Diagnostics). Neonates were then further classified into three groups: Group I – blood culture and sepsis-screen positive sepsis, Group II – blood culture negative but sepsis screen positive (serum CRP ≥10 mg/L and one additional parameter - neutropenia based on standardized age based charts or immature: total neutrophil ratios > 0.2), and Group III - only clinical features or perinatal risk factors.

Saliva was collected within 4 hours of collection of the serum sample. Antibiotics were started only after collection of the salivary sample. SalivaBio Infant's Swab

(SIS) (Salimetrics), was used for this purpose [7]. Salivary sampling was done 60 minutes before or after a feed or any oral procedure. The 90 mm SIS was placed between the cheeks and the lower gums after turning the infants head to one side. The swab was left in place for 3 minutes and then removed. The swab was then visually inspected and if found dry, re-introduced for an additional 3 minutes, up to a maximum of two attempts. Only clear saturated swabs were used. The swab was then introduced, saturated end first, into a plunger removed 5 mL syringe and then the re-introduced plunger was used to squeeze out saliva into cryovials. The process was repeated till 0.5 mL volume of saliva was obtained. If sample collected was inadequate, then the saliva collection was repeated with a longer oral stay period for the SIS. In case of any infant discomfort (gag, brow bulge, nasolabial furrow etc.) noticed during procedure, it was immediately discontinued and resumed after signs abated. After collection, labelled cryovials were frozen at –20ºC within 4 hours of collection. The saliva collection was done by the treating team members, after structured training. Each member was supervised during initial few sample collections, to ensure standardization.

On the day of the analysis, saliva samples were thawed and centrifuged. Samples were brought to room temperature before the dilutions. The Salimetrics salivary CRP enzyme immunoassay kit (Salimetrics), an indirect sandwich ELISA kit, was used as per the manufacturer's recommended protocol [8]. All samples were assayed in duplicate, and the average of the duplicates was used in the statistical analyses. Intra and inter-assay coefficients of variation were less than 10% and 15%, respectively. The salivary CRP was estimated in the entire cohort and also in the three groups of infants.

Assuming that salivary CRP has a minimum expected sensitivity and specificity of 75% to predict serum CRP ≥10 mg/L, and an expected prevalence of neonatal sepsis of 40% (as per departmental statistics for the previous year), with an absolute precision of 10% and 90% confidence level, the required sample size was 128 subjects (STATA IC, ver. 13). To account for 5% non-response rate, a total of 136 subjects were included in the final study.

Statistical analysis: Considering the skewed distribution of serum and salivary CRP levels, correlation was assessed using Spearman rank order (Spearman's rho) coefficient. The utility of salivary CRP in predicting elevated seum CRP (≥10 mg/L) was assessed by receiver operating characteristic (ROC) curve analysis and an appropriate salivary CRP cut off value was calculated. The sensitivity, specificity, predictive values and likelihood ratios of salivary CRP at the derived cut-off were calculated for predicting serum CRP ≥10 mg/L and positive blood culture. *P* value <0.05 was considered statistically significant. IBM SPSS version 22 (SPSS Inc.) was used for statistical analyses.

RESULTS

A total of 182 neonates satisfied the inclusion criteria. 32 neonates were excluded because of refusal of consent or already receiving antibiotics. Adequate salivary sample could not be obtained in 9 neonates and in 5, the samples were not analyzed because of contamination. Thus,136 neonates were included in the final analysis. The median (IQR) birthweight was 1.98 (1.34,2.57) kg and gestational age was 34.5 (32,37) weeks. Early onset sepsis (≤3 days) was seen in 88 (64.7%) of the population. Salivary CRP was detectable in 135 (99%) neonates and the levels increased significantly from Group III to Group I neonates (**Table I**).

There was a moderate and statistically significant positive correlation between salivary and serum CRP values in the entire study population (r=0.63; P =0.01) and in Group I (r=0.63; P =0.01) and Group II neonates (r =0.5; P =0.01).

The area under the ROC curve for salivary CRP to predict serum CRP ≥10 mg/L was 0.861 (95% CI, 0.78 - 0.94, P<0.01), indicating good predictive validity. Based on the co-ordinates of the ROC curve, the cut-off of 0.6 ng/mL was chosen as the optimal salivary CRP cut-off value for predicting serum CRP ≥10 mg/L. Salivary CRP ≥0.6 ng/mL had a 77% sensitivity, 94% specificity, 99% positive predictive value and 35% negative predictive value, for predicting a serum CRP level of ≥10 mg/L; and 75% sensitivity, 58% specificity, 44% positive predictive value and 85% negative predictive value, for predicting a positive blood culture.

DISCUSSION

We found that CRP can be detected in saliva of neonates with sepsis, and increases significantly in those with

Table I Serum and Salivary C-Reactive Protein Levels in Neonates (*N*=136)

Variables	*All neonates*	*Group I n=43*	*Group II n=77*	*Group III n=16*
Serum CRP (mg/L)	36.4 (19, 57.7)	63 (44.6, 83)	33 (19.4, 43.8)	8 (6.2,9)
Salivary CRP (ng/mL)	1.9 (0.4, 20.6)	1.98 (0.54, 2.95)	0.59 (0.33, 1.44)	0.25 (0.13,0.3)

All values in median (IQR). Group I-Blood culture +/ sepsis screen +; Group II-Blood culture/sepsis screen +; Group III-Clinical/perinatal risk factors only.

WHAT THIS STUDY ADDS?

- Salivary CRP could be used as an alternative biomarker for neonatal sepsis as it increases in septic neonates and positively correlates with serum CRP.

elevated serum CRP, compared to those with only suspicion of sepsis but with non-elevated biomarkers (group 3). We also found a moderate positive correlation of salivary CRP with serum CRP in these neonates.

There is limited previous data on the diagnostic utility of salivary CRP in neonatal inflammatory conditions and its comparison with serum CRP. Iyengar, et al. [9] showed that salivary CRP was detected in 97% of neonates with inflammatory states, especially post-operative. The levels also moderately correlated with serum CRP levels. Omran, et al. [10] found a significant difference between salivary CRP values in septic and healthy infants, with a moderate correlation between salivary and serum CRP. The levels of salivary CRP in our study was different from previous reports [9,10]. Some of the observed differences may be explained by variation in population characteristics. Our study population included both preterm and term infants, whereas others had included mainly post-operative neonates (only 12 with sepsis) [9], or had recruited only term infants [10]. Another reason could be the use of different assay methods and pre-processing techniques. We used a highly sensitive salivary CRP assay [8] and a validated saliva collection method [7]. A significant positive correlation between salivary and serum CRP was also seen by these authors [9,10]. Both adult and pediatric population studies have shown a good correlation between salivary CRP and serum CRP levels, in a variety of clinical conditions [11]. Although positive correlation is reassuring, this is insufficient to advocate salivary CRP as a replacement for serum CRP, considering the population characteristics of our study. More studies in neonates, across a variety of inflammatory conditions, showing similar correlation, are required, in order to change practice.

Proper collection of saliva is important for ensuring accuracy using salivary diagnostics and is even more challenging in neonates. Previous researchers [9,10] had used an improvised 1 mL syringe attached to low-wall suction, to collect saliva, a method previously described by Dietz, et al. [12]. We found this method difficult to use in very low birth weight infants as it frequently resulted in blood mixed saliva, related to mucosal trauma. Hence, we used the SIS for saliva collection, which has been previously validated for salivary analytes [7]. We found that by using a proper technique, maximal uncontami-nated saliva recovery was possible with less patient discomfort.

We found that infants with negative sepsis screen/ blood culture, with only clinical signs or perinatal risk factors of sepsis also had detectable salivary CRP levels (range of 0.11-1.39 ng/mL). This possibly indicates a normal physiological increase in salivary CRP levels to detectable range, in the initial days of life, similar to serum CRP. To our knowledge, there is no previous published normative data on salivary CRP levels in healthy infants. Extrapolation from adult studies could also be fallacious due to the differing population characteristics, and variation in salivary CRP levels among studies, with reported levels ranging from 0.03-24.2 ng/mL [13,14]. Further large studies are necessary to identify normative ranges of salivary CRP in healthy neonates of different gestational ages. The performance of salivary CRP during serial analysis, and also against the 'gold standard' blood culture, was not done in our study. Also, we did not normalize salivary CRP concentration for salivary flow rate and protein concentration, because previous data on the utility of this and the ideal normalization method in neonates was lacking. These areas should also be addressed in future trials.

Our study suggests that salivary CRP could be used as an alternative biomarker to serum CRP in neonatal sepsis. However, widespread usage in neonates will require further research into saliva collection methodology, standardization of assay procedure, establishing normative values and determining cost effectiveness. As of now, it appears a very useful diagnostic surrogate for blood sampling.

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Contributors: SD: conducted the study experiments, performed data collection and analysis; and wrote the final manuscript; SK: contributed to statistical analysis and critical evaluation of the final manuscript; GS: conceived and supervised the study, verified the statistical analysis, contributed to the critical evaluation of the final manuscript approved the final version of the manuscript.
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RESEARCH PAPER

Long term Immunogenicity of Single Dose of Live Attenuated Hepatitis A Vaccine in Indian Children - Results of 15-Year Follow-up

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Objectives: To measure anti-HAV antibodies 15 years after a single dose of live attenuated hepatitis A vaccine in Indian children. **Methods**: Of the 143 children vaccinated in 2004, 109 were evaluated in 2019, clinically and for anti-HAV antibodies. These children have been assessed clinically every year, and for anti-HAV antibodies in 2004, 2007, 2010 and 2014. **Results**: Of the 109 children who came for the present assessment, 11 had received additional doses of hepatitis A vaccine in 2004/2007 because of low anti-HAV titre (<20 mIU/mL). In the remaining 98 children, 94 (96%) had seroprotective levels with a geometric mean titre of 79.6 mIU/mL. Seroprotection rate in all 109 children was 86.2%. **Conclusions**: Single dose of live attenuated hepatitis A vaccine in Indian children demonstrated robust immunogenicity at 15 years post vaccination.

Keywords: *Hepatitis A vaccine, Anti-HAV anti bodies, Immune memory, Safety.*

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Live attenuated hepatitis A vaccine (H2 strain) has a long history of development and research (in China) for nearly three decades [1,2]. Promising immunogenicity, safety and protection have been reported, using a single dose of the vaccine [3]. In India, a live vaccine was licensed in 2005 and has been used extensively since then. Immuno-genicity studies of the single dose regimen in India have matched the Chinese reports [3,4]. Both, World Health Organization (WHO) and Indian Academy of Pediatrics (IAP) have endorsed the single dose schedule of live hepatitis A vaccine in the routine immunization of children aged one year or above [5,6].

In this study, we report the anti-HAV antibodies at 15 years from the first Indian study of single dose live HAV vaccine in children. Immunogenicity data from the same cohort at 2 months, 30 months and 10 years post-immunization has previously been reported [7-9].

METHODS

The study began in 2004 wherein 143 children were given a single dose of H2 strain of a live attenuated hepatitis A vaccine (Biovac-A, Wockhardt Ltd) and assessed for anti-HAV antibodies 2-months post vaccination [7]. These children were then called for follow-up every year, for clinical assessment and to record history of hepatitis, if any. They were assessed serially for anti-HAV antibodies in 2007, 2010 and 2014 [8,9]. Subjects with low anti-HAV antibody titres (<20 mIU/mL) were given additional doses of vaccine viz., in 2004 they were given two doses of the then licensed HAV vaccine (Havrix Jr GSK Biologicals) and in 2007 they received an additional dose of Biovac-A vaccine. No further vaccines were given in 2010 or 2014.

Contact details of the cohort were maintained by medical social workers. At the yearly visits, participants/parents were asked for history of hepatitis like illness (fever, anorexia, nausea, vomiting and jaundice). Clinical examination included noting for hepatomegaly or splenomegaly, if any. Parents were reminded to report complaints of hepatitis like illness immediately. No diary was given to participants for recording signs and symptoms. In the present study (2019) too, these children were clinically assessed for evidence of hepatitis (if any) and their anti-HAV antibodies were measured.

After routine clinical assessments, blood samples were collected and sent for total and IgM anti-HAV antibody analysis (Cobas anti-HAV electro-chemiluminescence immunoassay, ECLIA, Roche Diagnostics Deutschland GmbH) to an independent accredited laboratory (SRL Diagnostics). Seroprotection rate was defined as proportion of subjects with total anti-HAV antibody level ≥20 mIU/mL. Geometric mean titre (GMT) for anti-HAV antibodies was calculated as per standard method. Data was entered in predesigned paper case report forms (CRFs). All study documents were

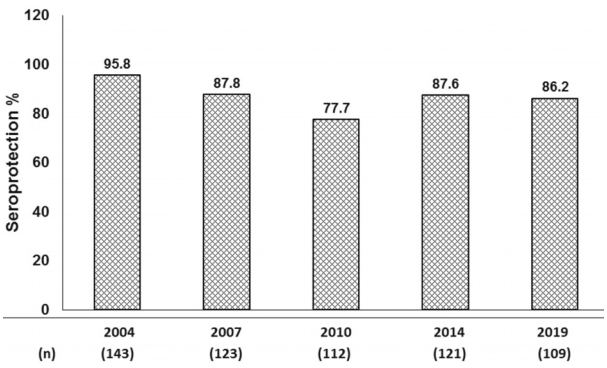


Fig. 1 Serial seroprotection rates over 15 years (%).

maintained in a dedicated study cupboard with restricted access. Data analysis was done using Stata 13.1 (StataCorp). All analysis was done using two-sided tests at alpha 0.05 (95% confidence level).

During the study of 15 years, institutional ethics committee approval and informed consent were obtained three times: Initial study (2004), second phase of study (2007-2015), and third phase of study (2016-2019). During the study, whenever a participant attained the age of 18 years, informed consent was obtained from him/her for continuing in the study.

RESULTS

Of the original 143 children who received a single dose of live attenuated hepatitis A vaccine in 2004, 109 subjects (72 males) came for the fifteen-year follow-up assessment in 2019. Mean age was 19.7 years (range 16-26.8 years). The number of subjects who came for follow-up since vaccination is as follows: 2.5 years (*n*=131), 6 years (*n*=126), 10 years (*n*=121) and 15 years (*n*=109). None of the parents/children retracted consent in writing. Clinical examination of the participants did not reveal any abnormal findings, and none gave any history of hepatitis like illness in the past.

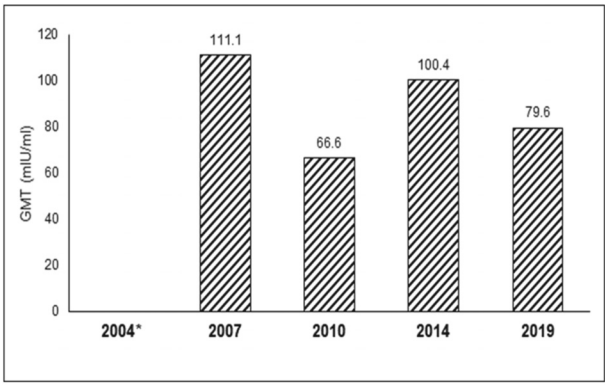
Of the 109 children who came for the present follow-up, 4 had received two doses of licensed inactivated HAV vaccine in 2004, and 7 others had received a second dose of live HAV vaccine in 2007, as their total anti-HAV antibody levels had dropped to <20mIU/mL.

Of the remaining 98 children, 4 had low anti-HAV titres (<20 mIU/mL) giving a seroprotection rate of 95.9%. If the 11 children who were given additional doses of HAV vaccine are also included, the seroprotection rate in all 109 children was 86.2%. The comparison of sero-protection rate in 2019 with previous assessment years is shown in **Fig. 1**.

All children were found to be negative for anti-HAV IgM. The total anti-HAV geometric mean titre (GMT) in 'seroprotected children' who received single dose of live attenuated vaccine (*n*=94) is 79.6 mIU/mL (95% CI 69.2-91.56). The comparison of GMT value at 15 years with previous assessment years is shown in **Fig. 2**.

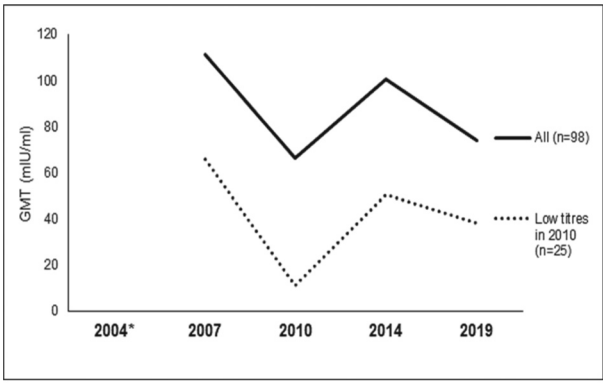
In 2010, there were 25 children with anti-HAV titres <20mIU/mL. They were not given any additional dose / doses of live/inactivated HAV vaccine. The serial anti-HAV GMTs of these 25 children as compared to all 98 with single dose of live HAV vaccine is shown in **Fig. 3**. In 2014 and 2019, 23 of these 25 regained seroprotective levels. In 2019, the anti-HAV antibody titre of two other children (who had seroprotective levels earlier) are now < 20 mIU/mL.

Children who received two additional doses of an inactivated HAV vaccine in 2004 (*n*=4), or an additional



**Anti - HAV tires > 100 MIU/mL were not quantified in 2004, Hence GMT of 2004 not represented in figure. GMT-geometric mean titers.*

Fig. 2 Serum anti-HAV antibody GMTs over 15 years.



**Anti - HAV tires > 100 MIU/mL were not quantified in 2004, Hence GMT of 2004 not represented in figure. GMT-geometric mean titers.*

Fig. 3 Serum anti-HAV antibody GMTs of children with low titers in 2010.

WHAT THIS STUDY ADDS?

- A single dose of live attenuated hepatitis A vaccine shows robust immunogenicity with seroprotection level of 86.2% at 15 years after vaccination in Indian children

dose of live HAV vaccine in 2007 (n=7) have continued to show seroprotective levels since additional vaccination.

DISCUSSION

This 15-year follow-up of a cohort of children vaccinated with a single dose of live attenuated HAV vaccine shows a seroprotective rate of 86.2% with anti HAV GMT value of 79.6 mIU/mL. The serial seroprotection rates of the cohort are 95.8% at 2 months, 87.8% at 30 months, 77.7% at 6 years, 87.6% at 10 years and 86.2% at 15 years. This evaluation at 15 years confirms the robust long-term immunogenicity of a single dose of live HAV vaccine and compares well with other Indian and Chinese studies [3,10-12]. The comparable long term Chinese seroprotection data (Zhuang, et al.) at 15 years is 81.3% (GMT 128 mIU/mL) [2,3]. The other Indian long term multicentric study reported an immunogenicity of 97.3% at 5 years with GMT of 127.1mIU/mL [11].

In our serial evaluations since 2004, we used two types of immunoassay test kits: Axsym HAVB ELISA (Abbott Labs), (2004, 2007 and 2010). These kits were not available in India in 2014. Hence, we used COBAS kits based on ECLIA technology (Roche Diagnostics) in 2014 and 2019.The higher antibody titres of 2014 and 2019 could be due to differences in kits as ECLIA based reports are known to be of a higher sensitivity as compared to ELISA [13]. Alternatively, higher titres could be due to 'booster like' response to exposure to naturally occurring antigens of HAV in the community [14]. As the child grows from a teenager to adulthood, the frequency of consuming food and water outside the home increases, thereby increasing exposure to hepatitis A.

Another limitation of our study was cohort contamination with additional doses of hepatitis A vaccine [9]. In 2004, 6 children were given two doses of inactivated HAV vaccine and in the next evaluation at 30 months, 9 others were given a second dose of the live HAV vaccine. At the 6 yrs follow-up in 2010, 25 children were found to be seronegative. These 25 children were not given any additional doses of vaccine. Interestingly, 23 of these 25 were in the seroprotected range in the present evaluation, further implying the probability of an anamnestic response to natural boosters. Chen, et al. [15] have recently demonstrated that anamnestic responses via memory B and memory T cells may provide long term protection after a single dose of live Hepatitis A vaccine, despite low levels of anti-HAV antibodies.

In conclusion, 15-year follow-up after a single dose of live hepatitis A vaccine (H2 strain) demonstrated robust immunogenicity in Indian children. The continued safety and immunogenicity profile of the vaccine reiterates its value in primary immunization of Indian children. As a policy decision, the single dose schedule cuts costs while providing definitive long-term protection.

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Ethics clearance: KEM Hospital Research Centre Ethics Committee; No. KEMHRC/LFG/ EC/778 dated June 6, 2016.

Contributors: SB, AS, AB: designed the study, recruited patients, analyzed results and wrote the manuscript; RJ, KD, VK: provided technical help needed for the study.

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Competing interests: SB, AS and AB: received investigator fee for conduct of the study; RJ, KD and VK are paid employees of Wockhardt Ltd.

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RESEARCH PAPER

Adverse Drug Reactions Following Propranolol in Infantile Hemangioma

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Accepted: January 06, 2021.

Objectives: To assess the adverse effects of propranolol therapy in infantile hemangioma. **Methods:** An ambispective study was conducted from August 2011 to December 2019. In retrospective arm all children managed for infantile hemangioma with propranolol were included and case records were assessed for adverse reactions. In prospective arm the adverse reactions were identified on the basis of predefined criteria. **Results:** A total of 514 patients (358 retrospective records) were included. A majority, 378 (73.5%) patients had an excellent response, 75 (14.5%) had partial response and 61 (11.8 %) had no response. A total of 82 (15.9%) patients experienced at least one adverse effect. Diarrhea with weight loss (27, 32.9%) and irritability with decreased sleep (21, 25.6%) were the most common adverse effects. The adverse effects in 22 (4.2%) cases lead to the discontinuation of propranolol. Younger age, low body weight and early onset were risk factors for development of severe adverse reactions. **Conclusion:** Young children with low body weight were at higher risk for adverse effects of propranolol.

Keywords: *Beta blocker, Diarrhea, Hypoglycemia, Severe ADR, Weight loss.*

Infantile hemangiomas are one of the most common vascular anomalies in children with an overall prevalence ranging from 5-10% [1]. These follow natural growth pattern of rapid proliferation and rapid involution [2]. Around 12-24% of infantile hemangioma can have one or more complications and need intervention [3]. Steroids have been the first line of treatment but their side effects prompted the use of different treatment modalities like lasers, vincristine, triamcinolone, bleomycin and other intralesional agents for sclerotherapy [4-6]. Few studies later followed with excellent results of propranolol in infantile hemangioma with minimal adverse effects [7,8]. The most feared complications of beta-blockers are hypoglycemia and cardiac toxicity, with few reports of rashes, irritability, gastroesophageal reflux [9]. Rarely the adverse effects lead to stoppage of treatment. We conducted this study to assess the adverse effects and their risk factors in our population.

METHODS

An ambispective study was conducted in the Department of Pediatric surgery in children with problematic infantile hemangioma, which were identified as those as those who caused functional problem, cosmetic problems or complications and needed treatment. Retrospective data were collected from August, 2011 to July, 2016. The prospective data collection period ranged from August, 2016 to December, 2019. Ethical approval was taken from the institutional review board.

The study included all the patients with problematic infantile hemangiomas treated with propranolol as monotherapy without any concurrent medication. Diagnosis of infantile hemangioma was made on the basis of clinical examination and spectral color doppler ultrasound. All children are given oral propranolol at a dose of 2 mg/kg per day in three divided doses as out-patients after informed consent from the guardians. Parents are informed about the possible side effects and danger signs like refusal to feed, lethargy, and advised to report at the earliest if any of these signs appear. The children are assessed for the response by using the clinical photograph taken at the beginning of treatment and at follow-up after three months and at six months. Clinical response was classified as complete response with apparently no residual disease and not requiring adjuvant treatment (>75% response), partial response with a residual disease requiring adjuvant treatment (25-75% response), and non-response with poor response (<25% response) or progressive increase in lesion size even after six months of treatment.

In the retrospective arm, records were used to document the gender, age and weight at the treatment initiation, hemangioma location, response to propranolol, type and duration of onset of the adverse drug reactions (ADR) following propranolol initiation, and discontinuation of propranolol whether transient or permanent (with or without re-challenge). Children on any other

medications like antiepileptics or those with inadequate record were excluded from the study. Propranolol was not used in children with acute respiratory illness and history of bronchial asthma. A careful patient history, clinical examination and electrocardiography (ECG) were performed in patients to ascertain risk factors or contraindications regarding the use of propranolol.

In the prospective arm, the adverse reactions were divided into mild, moderate and severe. Mild adverse reactions were defined where propranolol could be continued without any cessation. Moderate adverse reactions were when propranolol was restarted after discontinuing for 2 weeks without recurrence of adverse reactions. Severe adverse reactions were defined as those which required permanent discontinuation of propranolol as adverse reactions reappeared after restarting the drug after 2 weeks. In severe adverse reactions, second line of treatment like intralesional bleomycin/triamcinolone was started. Children who were not in follow up till completion of therapy or the start of second line (due to adverse reaction or non-responders to propranolol) were excluded.

Statistical analysis: Statistical analysis was conducted using SPSS 22.0 for Windows (SPSS Inc). Data were checked for normal/skewed distribution using Shapiro-Wilk test. Pearson chi-squared test and Fisher exact test were used to analyze categorical variables. Multivariate logistic regression analyses using stepwise forward logistic regression was performed to detect the independent risk factors for adverse reaction with adjusted odds ratios (OR) and 95% confidence intervals (CI). *P* value less than 0.05 was considered significant.

RESULTS

Retrospective data were available for 417 patients out of which 59 were excluded with incomplete records. In the prospective arm 156 children were included and 23 were excluded to enroll a total of 514 (312 boys) children. The median (IQR) age at the start of propranolol therapy was 7.0 [IQR] (5.0-12) months at a median (IQR) body weight of was 9.5 kg (7.0-10.5) kg. Only 56 (10.8%) patients has multifocal lesions and majority had unifocal disease. Head and neck (304, 59.1%) as the most common location followed by trunk (108, 21.0%), extremity (65, 12.6%), genitalia (9, 3.6%) and intraoral (18, 3.5%). Indications for treatment were cosmetic (151, 29.3%), functional (102, 19.8%), difficulty in handling (97, 18.8%), ulceration (92, 17.8%) or multiple of these indications (72, 14.0%). A total of 378 (73.5%) patients had excellent response, 75 (14.5%) had partial response and 61 (11.8 %) had no response.

Table I shows the distribution of mild (*n*=43), moderate (*n*=17) and severe adverse events (*n*=22). Propranolol was reinitiated following a gap of 2 weeks in eight out of ten cases in retrospective arm with severe adverse event. In remaining two cases re-challenge with propranolol was not performed as both had severe respiratory distress after propranolol therapy and intralesional sclerotherapy was used as second line. In all children with adverse reaction except those with severe bronchospasm, propranolol was restarted after a period ranging from 1-3 weeks.

Table II shows multivariate analysis for the risk factors for development of severe adverse effects for propranolol. On subgroup analysis for increased risk of severe adverse effects in children with adverse effects, age ≤7 months (OR 2.71, 95% CI 1.07-6.8) and weight less than 9 kg (OR 2.03, 95% CI 0.84-4.89) were found significantly associated.

DISCUSSION

Diarrhea, sleep disturbances, cold peripheries and agitation were the common ADRs with propranolol treatment in infantile hemangioma. Most ADRs were mild to moderate only, not requiring cessation of propranolol therapy or maximum withdrawal for 2 weeks. Diarrhea and weight loss emerged as severe ADR, which mandated permanent withdrawal of propranolol. Young age and low body weight predisposed for severe ADR.

We acknowledge the small sample size as limitation of the present study, although we tried to maximize the sample size by keeping ambispective study design. These adverse effects may even be affected by regions and social/cultural variation, which need to addressed.

Table I Adverse Reactions in Infants Treated With Propranolol

	Mild/moderate adverse reactions (n=60)[a]	*Severe adverse reactions (n=22)*
Weight loss/diarrhea	15 (25)	12 (54.5)
Irritability/cry	16 (26.6)	5 (22.7)
Respiratory	5 (8.3)	3 (13.6)
Hypoglycemia	4 (6.6)	2 (9)

Data expressed as no (%)[a] *Loss of appetite, Gastroesophageal reflux and Rash were seen in6,9 and 5 children.*

Table II Risk Factors for Severe Adverse Reactions

Variable	*Crude odds ratio (95% CI)*	*P value*
Age ≤7 mo	7.04 (2.39, 20.69)	<0.001
Male sex	1.2 (0.45, 3.19)	0.71
Duration (mo)[a]		
0-15	33.75 (3.78, 301.22)	<0.001
15-30	1.22 (0.12, 12.64)	
Weight ≤9 kg	11.87 (3.78, 37.28)	<0.001

[a]*Making 30 as reference category.*

WHAT THIS STUDY ADDS?

- Diarrhea with weight loss was the most common severe adverse reaction of propranolol therapy for infantile hemangioma. Younger children with low body weight were more susceptible.

Since the first report [8] of excellent results of oral propranolol in infantile hemangioma, it has been rapidly accepted as the first line of treatment [10,11]. The most commonly reported ADRs of propranolol are sleep disturbances, hypoglycemia, peripheral vasoconstriction and agitation. Propranolol can be continued safely in cases showing mild to moderate symptoms [12]. Nearly 2-3% of cases suffer from severe reactions requiring discontinuation of therapy. In the present study, permanent withdrawal of propranolol was needed in 4.2% cases only, which is slightly more than Western data [13-15]. Sleep disturbance is one of the most common ADR of propranolol due to high drug penetration across the blood brain barrier as it is lipid soluble [15]. In our series, sleep disturbance was second most common adverse reaction after diarrhea and weight loss. Diarrhea as an ADR is usually self-limiting and intractable diarrhea is extremely rare [16-18]. Most of the patients in present study belonged to economically poor background and had low weight for age. An acute episode of diarrhea thus made them prone for acute malnutrition. We recommend weighing the benefits and risk of weight loss with persistent diarrhea when considering propranolol continuation.

Propranolol is safe and effective in children with infantile hemangioma. Young children with low body weight are at higher risk for adverse effects of propranolol. Diarrhea and weight loss should be carefully monitored.

Ethics approval: IEC, IMS-BHU; No. Dean/2015-16/EC/75, dated 26 May, 2016.
Contributors: VP: conceptualized the paper, extracted the data, analyzed it and drafted the paper; PT: conceptualized the paper, was overall responsible for quality of data collection and maintenance, modified and finalized the draft; MI, AM, DK: clinical care, and data recording and analysis; SPS: patient care, quality maintenance and modification of draft of the paper. All authors approved the final manuscript.
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REVIEW ARTICLE

Headache in Children and Adolescents: A Focus on Uncommon Headache Disorders

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Migraine and tension-type headache are common in children and adolescents, but several other headache disorders may pose a great challenge in diagnosis and management to families and attending clinicians. In this review, we highlight several of these disorders, which need appropriate assessment to make the right diagnosis and appropriate investigations where necessary. Timely recognition and implementation of appropriate management strategies can improve the health of children with some disorders, and is vital in achieving improvement in the quality of life.

Keywords: *Migraine, Stabbing headache, Thunderclap headache, Trigeminal autonomic cephalalgia.*

Headache is common in children and adolescents. About 60% of children worldwide report at least 3 attacks of headache per year [1]. Tension-type headache (TTH) and migraine are the most common headache disorders in schoolchildren with a prevalence of 8 and 23%, respectively [2,3]. Despite the high prevalence of headache in children, it continues to be under-diagnosed and undertreated. Many children are managed at home with over-the-counter medications, some children are managed at primary care and only a small proportion of children, with difficult to treat headache disorders, are referred and managed at specialist pediatric services.

Migraine and, to a lesser degree, TTH are well-studied and described in the pediatric literature, but little is written on many others, especially rare primary headache disorders and the uncommon variants and complications of common headache disorders in children and adolescents. Except for migraine, the three editions of the International Classification of Headache Disorders (ICHD-1, ICHD-2 and ICHD-3), provided definitions and criteria for the diagnosis of all headache disorders derived from studies on and experience in adult patients [4-6]. It is conceivable that the clinical presentations of most, if not all, primary headache disorders in children can be different than those in adults [7]. The differences can be due to the inherent nature of the disease itself, neurodevelopmental factors, biopsychosocial influences of the disease, life-style and education, and also children's response to pharmacologic and non-pharmacologic therapies. Therefore, studies on uncommon headache disorders in children and adolescents are badly needed in order to better define the conditions and inform management decisions.

CLASSIFICATION

Headache disorders are classified on the basis of etiology into primary (no other underlying cause), secondary (when headache is a manifestation of another disorder) and undetermined etiology (**Box I**). Headaches are also sub-classified on the basis of frequency of attacks and duration of the headache disorder into episodic (less than 15 days per month) or chronic (attacks occur on at least 15 days per month over at least three consecutive months). Migraine is further sub-classified according to clinical features (different types of migraine) and trigeminal autonomic cephalalgias (TACs) are sub-classified on the basis of attack duration.

ASSESSMENT OF THE CHILD WITH HEADACHE

In the absence of diagnostic tests and biomarkers, the diagnoses of primary headache disorders are based on the clinical features and globally acceptable definitions and diagnostic criteria. A focused and detailed clinical history is essential in assessment of children with headache in order to make a positive diagnosis, and

Box I Classification of Most Common Headache Disorders in Children

Primary headaches
- Migraine
- Tension-type headache
- Trigeminal autonomic cephalalgias
- Others

Secondary headache
- Medication overuse headache
- Posttraumatic headache
- Brain tumors
- Idiopathic intracranial hypertension

Others
- Cranial neuropathies
- Facial neuropathies
- Others

appropriate classification. Making the right diagnosis allows explaining the condition to the child and the family, making a rational decision on investigations if needed, offering the most appropriate treatment options and helps in predicting prognosis. Essential elements of clinical history, general examination and neurological examination are summarized in **Table I** and management workup may follow the steps as shown in **Fig. 1**.

The severity of pain is best assessed by its effects on behavior and activities; severe headache stops all activities during attacks, moderate headache stops some but not all activities, and mild headache dose not interfere with normal daily activities. Absence of symptoms between attacks and complete return to normal self is an important feature of primary headache. Secondary headaches should be suspected and considered if red flags are detected on the clinical history, and physical and neurological examinations (**Box II**).

Table I Clinical Assessment of the Child with Headache

Clinical history	*Examination*
Duration of headache disorder	*Physical examination*
Frequency of attacks	Weight, height
Duration of attacks	Blood pressure
Site of maximum pain	General examination
Quality of pain	Ear, nose and throat
Severity of pain	Sinuses examination
Trigger factors	*Neurological examination*
Aura symptoms	Optic discs
Associated symptoms: loss of appetite, nausea, vomiting, light intolerance, noise intolerance, dizziness	Cranial nerves Motor system: muscle bulk, tone, power, coordination, reflexes
Relieving factors Symptoms between attacks	Cerebellar system: ataxia, nystagmus, intention tremors

The diagnosis of common primary headache disorders such as migraine and tension-type headache can be made confidently on clinical history, normal examination, absent red flags and on the application of the ICHD-3 criteria. Other less common headache disorders may cause difficulties in diagnosis and management and will be the subject for this review.

GENERAL MANAGEMENT STRATEGIES

Exploring and addressing the concerns of patients and their families are the first important steps in successful management. Advice on healthy life style – regular meals, sleep, exercise and rest may reduce impact and improve coping with headache.

For acute treatment, simple analgesics should be used in appropriate dosages and as early as possible after onset of headache. Children should avoid taking painkillers on more than three days per week in order to avoid medication overuse headache (MOH). Triptans can also be given in acute migraine attacks in accordance with local license and regulations and with similar precautions to avoid MOH.

Evidence-based recommendation in the prevention of migraine can be hard to find with conflicting evidence, however treatment with propranolol, topiramate, flunarizine and amitriptyline can be considered on individual basis. Specific treatment for rare headache disorders will be discussed separately.

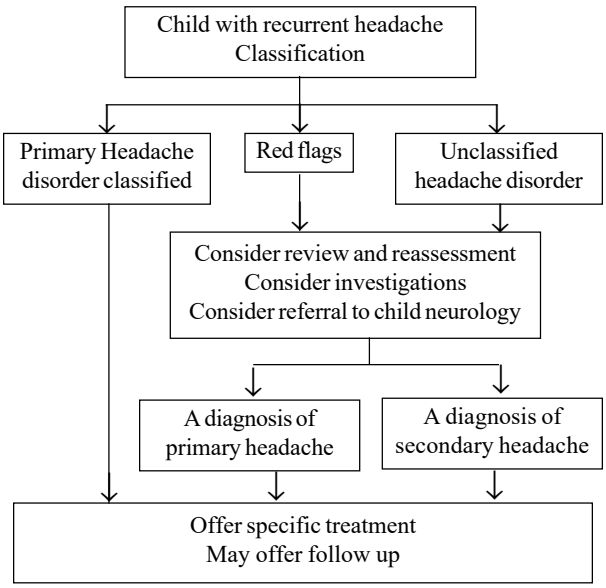

Fig. 1 Headache assessment and classification pathway.

Box II Red Flags and Indications for Investigations

Clinical history
Side locked headache
Acute progressive headache
Vomiting on waking up
Deteriorating vision
Seizures
Personality change
Persisting symptoms between attacks
Physical examination
Hypertension
Faltering growth
Delayed puberty
Neurological examination
Papilledema
New neurological deficit
Ataxia
Nystagmus
New squint

CHRONIC MIGRAINE

Migraine is a common disorder with a prevalence of around 10% in schoolchildren [1], and chronic migraine (CM) is a subtype of migraine. The diagnosis of CM is made when headache occurs on at least 15 days per month on at least 3 consecutive months, of which at least on 8 days per month the headaches are those of migraine [6].

Migraine may present as CM from the onset, but it can also evolve over a period of time in children with episodic migraine. It is estimated that about 2% of adolescents suffer from CM and at least half the patients overuse medication; simple analgesics or anti-migraine drugs such as sumatriptan [8]. CM is more common in girls than boys and more prevalent in adolescents than in younger children. CM has a significant impact on the child's quality of life, school attendance and educational attainment as compared to children with episodic migraine and control healthy children [8].

Management

The management of children with CM can be difficult and, ideally, needs a multidisciplinary approach and a positive contribution from parents and guardians, clinical psychology services, education and school teachers and also from school nurses. Investigations are not necessary except in presence of red flags or new abnormalities on neurological examination. An individual migraine management plan agreed upon by the child, the parents and members of the multi-disciplinary team (MDT) promotes better management of headache at home and at school and may reduce impact on education and quality of life.

Medical management starts with reducing the risk of medication overuse and treatment of MOH when present. Pain killers should, therefore be avoided as they have a limited role. Preventive drugs aim to reduce the frequency and the severity of migraine attacks and to improve the quality of life. Several medications are used but with sometime, a conflicting evidence for their effectiveness. Amitriptyline, with and without cognitive behavioral therapy (CBT), topiramate, flunarizine and botox are commonly offered to patients [9-11]. Greater occipital nerve block and botox injections are possible future management options. Calcitonin-gene related peptide (CGRP) monoclonal antibodies are shown to be successful in migraine prevention in adults, but still awaiting trials and licensing in children.

Prognosis: The prognosis and the long-term course of CM have not been well studied in children, but the impact on quality of life is a consistent feature and studies showed reduced educational attainment and earning power during adult life [12].

HEMIPLEGIC MIGRAINE

Hemiplegic migraine (HM) is a form of migraine with motor aura and depending on presence or absence of other affected family members; it is divided into familial hemiplegic migraine (FHM) or sporadic hemiplegic migraine (SHM). FHM is sub-classified according to the underlying genetic mutation; FHM1 is associated with a mutation on the *CACNA1A* gene, FHM2 on the *ATP1A2* gene, FHM3 on *SCN1A* and FHM (other loci) when no genetic mutation can be found despite the familial occurrence of the disease.

The prevalence of HM is not known, but is considered rare. About 2% of patient seen at a specialist children headache clinic have HM [13]. Girls are more commonly affected than boys and its peak incidence is during adolescence.

The clinical presentation of HM can be distressing to the child, frightening to the family and can pose a dilemma in diagnosis for clinicians. Attacks can be triggered by minor head trauma and followed by complex aura; a combination of visual, sensory, speech and motor symptoms. The headache that follows can be severe and is often associated with intense nausea and vomiting that may lead to confusion and dehydration.

A recent study on a cohort of 46 children with HM showed that children present with fewer non-motor auras than adults and the first attack may be preceded by transient neurological signs and symptoms especially in early childhood [14]. The attacks can last over 1-2 days and children will be, invariably, investigated with

neuroimaging to rule out space occupying lesions, intracranial bleeding or arterial ischemic strokes. Investigation will always be necessary, especially on the first presentation as the diagnosis, as defined by (ICHD-3), can only be made after at least two fully reversible attacks.

Distinguishing HM from stroke can be difficult on clinical features alone as they share many symptoms. Standard MRI of the brain may not be helpful and specialist cerebral perfusion studies using functional MRI and arterial spin labeling (ASL), only available at limited centers, may demonstrate areas of cerebral hypoperfusion soon after onset of symptoms and hyperperfusion 12-14 hours after onset. The changes in perfusion are not limited to the territories of the main cerebral arteries but they are evident across the boundaries suggesting neural rather than vascular basis of this phenomenon [15,16].

Management

Treatment of acute attacks once the diagnosis is established should aim at reassuring the child and parents, providing effective pain relief, hydration and monitoring of symptoms. Simple analgesics (paracetamol 10-20 mg/kg or ibuprofen 7.5-10 mg/kg) are the preferred options. The use of triptans is currently not recommended as all clinical trials excluded children and adults with HM and there is no evidence of their safety in children and adolescents. The exclusion from trials was based on the theoretical risk of triptans exacerbating cerebral vasoconstriction and causing cerebral infarction.

Care should be taken to prevent dehydration by encouraging oral fluids, but intravenous fluids may be necessary. Antiemetic medications such as metoclopramide or ondansetron may also be needed.

Preventative treatment may be necessary if attacks are prolonged, frequent or causes distress to child and parents. Topiramate, amitriptyline and flunarizine in particular are good treatment options.

Prognosis: Counseling of patients should take into account the known natural history of the disease, which is characterized by periods of remissions and relapses. The attacks of HM tend to be frequent and severe during adolescence and also in late adult life with periods of remission in between. A follow up of eight family members for a mean period of ten years showed the disease to be clinically stable [17]. Children with FHM1 due to associated *CACNA1A* gene mutation have an increased risk of progressive ataxia in late adult life and children with FHM3 due to *SCN1A* mutation are at a higher risk for epilepsy.

THUNDERCLAP HEADACHE

Thunderclap headache (TH) is a term given to describe sudden severe headache that reaches its peak intensity within seconds or minutes and persists for hours. TH may become recurrent over several weeks if untreated. TH is a secondary headache in most cases and a diagnosis of primary TH should only be made after full, appropriate and timely investigations including neuroimaging. The prevalence of TH in children and adolescents is not known and it is rare in pediatric clinical practice. Cases reported in children are mostly secondary to sinus venous thrombosis [18].

The clinical features of TH in children are probably similar to those in adults. The clinical picture is usually dominated by the abrupt onset of the headache and its severe intensity described by patients as the most severe headache they have ever experienced, prompting them to seek urgent medical advice and assessment. The headache attacks can be brief, but repetitive. Although the criteria for the diagnosis of TH in ICHD-3 (**Box III**) are that of primary TH, it is always necessary to exclude underlying intracranial vascular disease.

Secondary TH: TH may be the presenting symptoms in patients with several intracranial vascular disorders. The most common causes of TH in children and adolescents are intracerebral haemorrhage, cerebral venous thrombosis, subarachnoid haemorrhage with or without ruptured arterial aneurysms and reversible cerebral vasoconstriction syndrome (RCVS). Secondary TH may not be immediately distinguishable from primary TH and therefore full neurological examination and measurement of arterial blood pressure are mandatory. Prompt recognition and urgent appropriate assessment start at emergency department in order to avoid life-threatening complications [19].

Management

Investigations at presentation should include brain MRI and MR angiography. Other investigations should also include CSF opening pressure, CSF microscopy, culture, protein and glucose (paired with blood glucose) and examination for xanthchromia at appropriate time interval from presentation. MRA may need to be repeated if RCVS is highly suspected, as vasoconstriction may not be apparent in the early stages of the disease.

Box III Criteria for Diagnosis of Thunderclap Headache

A. Severe head pain fulfilling criteria B and C
B. Abrupt onset reaching maximum intensity in <1 min
C. Lasting for ≥5 min
D. Not better accounted for by another ICHD-3 diagnosis.

Treatment aims to relieve symptom while addressing the management requirements of the underlying condition.

CHIARI MALFORMATIONS HEADACHE

Chiari malformation is a congenital anomaly of the posterior cranial fossa characterized by crowding of its contents, a caudal displacement of the cerebellar tonsils and brainstem through the foramen magnum and an associated expansion of the CSF spaces in the cervical and possibly the thoracic spinal canal creating a static or progressive syrinx. Chiari malformation is classified into types 1-4 depending on the degree of malformation, the extent of cerebellar and brainstem herniation into the foramen magnum, cerebellar hypoplasia, syringomyelia, obstruction of CSF flow and presence of an encephalocele.

Chiari malformation type 1 (CM1) is the most common form and it is asymptomatic in the vast majority of cases. In CM1, there is mild to moderate crowding of the posterior fossa and a descent of cerebellar tonsils between 5-10 mm below the foramen magnum with no or a very small syrinx. It is commonly reported as an incidental finding in about 1% of children and young people undergoing brain and spine MRI [20].

Headache due to CM1 is described in the ICHD-3 as brief episodes lasting less than 5 minutes of occipital or suboccipital pain, precipitated by cough or Valsalva maneuver and it remits after successful treatment of CM1 [6]. It is important to keep in mind that children with CM1 may also complain of other types of headache such as migraine and tension-type headache, and they should not be confused with CM1 headache. On rare occasions, children with CM1 may present with other symptoms related to brainstem or cerebellar dysfunction including visual disturbances, dysphonia, dysphagia, sleep apnea, incoordination and sensory disturbances.

The diagnosis of CM1 is usually made on the sagittal MRI of the brain and the cervical spine. The management of children with CM1 is non-surgical in most patients after discussion with a neurosurgeon and a neuroradiologist. Medical management should include appropriate management of the pain symptoms and a follow up imaging over a period of time to confirm the non-progressive nature of the malformation and the size of the syrinx, if present, in particular [21].

Surgery is only recommended for patients with features suggesting brainstem or cerebellar compression, a large or progressive syringomyelia or with a poorly controlled, typical CM1 headache disorder. A single center experience in the surgical treatment of children with CM1 over 25 years showed that children with typical CM1 headache who were treated with foramen magnum decompression (FMD) plus duraplasty achieved a greater improvement in their headache than those treated with FMD alone [22].

PRIMARY STABBING HEADACHE

Primary stabbing headache (PSH) is an uncommon syndrome, also called ice-pick headache, characterized by very short attacks. While ICHD-2 states that pain is confined exclusively to the trigeminal territory, it has been accepted in ICHD-3 to cross beyond the boundaries of the trigeminal nerve and can be unilateral or bilateral in location (**Box IV**). The typical stab lasts a few seconds; however, attacks lasting up to 15 minutes in children and adolescents have been reported [23].

Although the exact prevalence of PHS in the pediatric population is unknown, 4-5% of children referred to headache centers have PSH [23-25]. In an Italian study, 12.4% of children with headache younger than 6 years had PSH [26]. Girls and boys are equally affected, though in adults PSH is more common in females [27]. Other primary headaches; migraine and TTH may coexist with PSH in children [28-30]. Associated symptoms, such as photophobia, phonophobia, nausea, and dizziness, have been described in 20-50% of patients. Absence of autonomic symptoms is the main feature that differentiates PSH from trigeminal autonomic cephalalgias.

The pathophysiology of PSH is unknown, but spontaneous firing of trigeminal fibers or abnormalities of the descending pain control have been suggested as possible mechanisms [27]. The relatively higher prevalence of PSH in younger children suggests that PHS may be a precursor of migraine/TTH [16].

Treatment of PSH in children is often not necessary, unless the stabs are very frequent and interfere with normal activities. Evidence for any medications in the treatment of PSH is lacking, but indomethacin may offer an excellent relief of pain when given in a dose of 25 mg three times per day for 6-8 weeks alongside omeprazole for gastric protection. Melatonin, amitriptyline, propanol, and COX2-inhibitors were effective in adults and may be used in children [27].

Box IV Criteria for the Diagnosis of Primary Stabbing Headache

A. Head pain occurring spontaneously as a single stab or series of stabs and fulfilling criteria B and C
B. Each stab lasts for up to a few seconds
C. Stabs recur with irregular frequency, from one to many per day
D. No cranial autonomic symptoms
E. Not better accounted for by another ICHD-3 diagnosis.

TRIGEMINAL AUTONOMIC CEPHALALGIAS

Trigeminal Autonomic Cephalalgias (TACs) are also uncommon, especially in pediatric age. However, they must be considered even in young children, in order to offer the appropriate treatment. They include cluster headache (CH), Paroxysmal hemicrania (PH), Hemicrania Continua (HC), and Short-lasting Unilateral Neuralgiform headache attacks with Conjunctival injection and Tearing (SUNCT) or with cranial autonomic symptoms (SUNA). **Table II** presents the characteristics and the differential diagnosis of these conditions.

Cluster Headache

The prevalence of cluster headache (CH) in the pediatric population is around 0.1% [31]. CH is characterized by severe and sometimes excruciating unilateral pain, mainly in the orbital, supraorbital, and/or temporal region, lasting 15-180 minutes and recurring up to 8 attacks per day. Attacks are often associated with restlessness and/or ipsilateral autonomic symptoms, such as conjunctival injection, lacrimation, nasal congestion, rhinorrhea, eyelid edema, forehead and facial sweating, miosis, and ptosis. The usual presentation of CH is that of recurrent bouts of headache, each bout consisting of several distinct headache attacks. In episodic CH, the bouts of headache last from 7 days to 1 year, and are separated by headache-free periods without treatment of at least 3 months. In chronic CH, the headache-free period between bouts is shorter than 3 months. It has been recently suggested that the attacks can be shorter and less frequent in children than in adults and restlessness can be more difficult to demonstrate [7,32].

CH is more common in children over 10 years of age, but it has been reported in children younger than 6 years of age [33,34]. Complex genetic factors are probably involved in CH etiology [35,36]. However, it was noted that there is a low prevalence (9%) of CH in the relatives of young patients [34]. Environmental factors such as high exposure to second hand smoking has been suggested as a risk factor for CH development [34,37].

Table II Characteristic Features of Trigeminal Autonomic Cephalalgias

	Cluster headache	*Paroxysmal hemicrania*	*SUNCT*
Female: Male	1:5	1:1	1:1.5
Frequency	1-8/d	1-40/d	Up to 200/d
Attack duration	15-180 min	2-30 min	5-240 sec
Agitations	90%	80%	65%
Autonomic features	Yes	Yes	Yes
Response to indomethacin	No	Yes	No

SUBCT: Short-lasting unilateral neuralgiform headache attacks with conjunctival injection and tearing.

Treatment: Treatment of acute attacks in children and adolescents consists of administering, as early as possible after onset, either sumatriptan 10 mg or zolmitriptan 5 mg (as nasal spray), which are the only licensed triptans for adolescents 12-18 years of age in Europe as well as high flow 100% oxygen at 12-15 L/min with a rebreathing mask [38].

For the prevention of CH, verapamil is the drug of first-choice in adults. It has been used at the dose of 3-10 mg/kg/d in children and adolescents [38]. However, verapamil can be difficult to manage in pediatric age because of its side effects (effect on length of the PR interval and the negative inotropic effect). Alternative treatments are melatonin (0.1-0.2 mg/kg/d) and topiramate (1-2 mg/kg/d). A short course of steroids like prednisone 2 mg/kg/day is reported to stop the cluster within 5 days [34]. Greater occipital nerve block was shown to be effective in three children [39].

Paroxysmal Hemicrania

Paroxysmal hemicrania (PH) is characterized by short lasting (2-30 min), multiple and unilateral pain attacks, with a typical attack frequency of more than 5 per day. Pain is commonly associated with ipsilateral cranial autonomic symptoms. PH is defined as episodic when attacks last from 7 days to 1 year and are separated by time intervals longer than 3 months. In chronic PH, the attack has to last more than 1 year without interruption or with pain-free intervals shorter than 3 months. In children, PH can be atypical with bilateral pain, attack duration longer than 30 minutes and attack frequency less than 5 per day making it difficult to differentiate from CH [7,40,41].

PH responds well to treatment with indomethacin, making it a good therapeutic first line option in all patients with unilateral short-lasting pain, associated with cranial autonomic symptoms.

Hemicrania Continua

Hemicrania continua (HC) is characterized by continuous unilateral headache with exacerbations of moderate or greater intensity for at least 3 consecutive months. As in PH, cranial autonomic symptoms ipsilateral to pain and/or sense of restlessness are needed for the diagnosis. The response to indomethacin should be complete, but can be variable in some children. Only a few pediatric cases of HC have been published with one patient responding to treatment with Botulinum toxin A [42,43].

Box V Learning Points for Uncommon Pediatric Headache Disorders

Chronic migraine

- Affects 1-2% of adolescents
- Management requires multidisciplinary approach in most patients and realistic targets
- Management aims to revert chromic migraine to episodic migraine
- Emphasis on life style factors and preventive treatment· Avoid medication overuse and address it if present

Hemiplegic migraine

- Diagnosis of HM can only be made after at least 2 fully reversible episodes
- Investigations and neuroimaging will be necessary at first presentation
- Triptans are not recommended for treatment of acute attacks
- Flunarizine may be a good option for the prevention of HM

Thunderclap headache

- TH presents with sudden onset severe headache that reaches its peak within minutes
- Always exclude underlying intracranial cause by appropriate investigations

Chiari malformation 1

- CM1 can be asymptomatic incidental finding in about 1% of people
- Headaches due to CM1 are short, occipital and triggered by Valsalva maneuver. Discuss with a neurologist, a neurosurgeon and a pediatric neuro-radiologist
- Surgical treatment only required in a small proportion of patients

Primary stabbing headache

- Headache due to PSH are very brief and repetitive
- No associated autonomic features
- Excellent response to indomethacin can be expected

Trigeminal autonomic cephalalgias

- Duration of attacks are important in diagnosis, but acknowledge some overlap
- At least one autonomic feature is present
- Agitation and distress are important features
- PH and HC respond to indomethacin in many patients
- Nasal sumatriptan and high flow oxygen are effective acute treatment for CH.

SUNCT and SUNA

These short-lasting neuralgiform attacks, lasting from 1 to 600 sec, involve the trigeminal territory unilaterally. The attacks can be isolated or can recur in series. Pain is associated with conjunctival injection and/or tearing in SUNCT or other cranial autonomic symptoms, such as nasal congestion and/or rhinorrhea, eyelid edema, forehead and facial sweating, miosis, and/or ptosis in SUNA. Only four cases of pediatric SUNCT (3 idiopathic and 1 symptomatic) have been described thus far [7].

CONCLUSIONS

Pediatricians are familiar with the diagnosis and treatment of common headache disorders in children and adolescents. Awareness of the different types of atypical or rare headache disorders allows better assessment and a more successful management. Learning points related to these disorders are detailed in **Box V**.

Diagnosis should be based on the recognized clinical criteria of the ICHD. Investigations to exclude serious underlying neurological disorders may be necessary, but should be interpreted with caution, in order to avoid over-diagnosis of incidental findings such as Chiari malformation 1. Chronic migraine can be associated with adverse impact on quality of life and can be difficult to manage. Hemiplegic migraine may pose diagnostic difficulties making investigations necessary including MR angiography, especially at first presentation. Trigeminal autonomic cephalalgias and stabbing headache are relatively rare in children, but with the correct use of diagnostic criteria, management plans with appropriate treatment options and realistic expectations, it is possible to address the patient needs.

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Impact of Air Pollution on Allergic Rhinitis and Asthma: Consensus Statement by Indian Academy of Pediatrics

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Justification: Rising air pollution is an ever-growing threat to many human diseases. Poor air quality has been directly correlated with respiratory allergies with a disproportionate affection among the pediatric age group. A clear understanding of common air pollutants and their potential contribution in allergic rhinitis and asthma is lacking. **Objective:** To formulate a consensus statement for appropriate understanding among pediatricians and general practitioners about the effects of air pollution on respiratory allergies and their prevention. **Process:** A group of experts (Pediatric pulmonologists and allergy specialists) from across India were appointed by the Indian Academy of Pediatrics (IAP) to formulate a consensus statement on 'Allergy and Air pollution'. A virtual meeting was conducted on 6th April 2020 to discuss in detail regarding various issues related to the subject and a writing committee was formed with broad consensus. After extensive literature review and multiple virtual sessions, the current document was prepared and circulated via email to the representatives from central IAP and IAP environment chapter. All the experts approved the consensus with minor modifications after a detailed discussion on 29th September 2020 on a virtual platform. **Recommendations:** Air pollution is the emerging contributor to respiratory allergies due to various mechanisms including oxidative stress and compromised mucociliary clearance. Children are more vulnerable to both outdoor and indoor pollution, due to their unique physiological characteristics. Knowledge about pollutant particle size and air quality index will help in demarcating level and extent of airway involvement. Relevant environmental history in difficult allergic rhinitis and asthma cases, along with conventional pharmacological measures, is warranted. Multipronged approach, targeted at community, physician and individual levels, needs to be emphasized to improve air quality and reduce economic and psychological burden of respiratory allergies.

Keywords: *Air quality index, Allergy, Asthma, Pollution, Rhinitis*.

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Increasing levels of air pollution and its impact on health has emerged as an area of immense concern across the world. Air pollution was found to be responsible for 16% of global deaths, of which 92% were in low- and middle-income countries (LMICs) [1]. There is ample evidence emerging on the role that poor air quality could adversely affect child health. Children remain a vulnerable group to the effects of air pollution due to their outdoor play activity, breathing higher concentration of pollutants, more mouth breathing behaviour, higher minute ventilation, an ineffective nasal filtering capacity, and an underdeveloped detoxification and antioxidant defence systems [2]. There is a simultaneous increase in the prevalence of respiratory allergies worldwide [3], with the prevalence of wheeze ranging from 7-20% and allergic rhinitis between 11-24% [4]. This increase correlated with demographic changes of the cities including urbanization, air pollution and environmental tobacco smoke [5]. Hence, a pediatrician needs to be aware about the impact of air pollution on allergic rhinitis and asthma, the two most common chronic conditions that one addresses in daily clinical practice. By understanding the interaction between air quality and allergy, patient management can be improved with targeted preventive and therapeutic measures.

PROCESS

A group of experts (pediatric pulmonologists and allergy specialists) from across India were appointed by the Indian Academy of Pediatrics (IAP) to formulate a consensus statement on 'Allergy and Air pollution'. A virtual meeting was conducted on 6 April, 2020 to discuss in detail regarding various issues related to the subject and a writing committee was formed with broad consensus. After

extensive literature review and multiple virtual sessions, the current document was prepared and circulated via email to the representatives from central IAP and IAP environment chapter. All the experts approved the consensus with minor modifications after a detailed discussion on 29 September, 2020 on a virtual platform.

Terminology

Commonly used terms, which a pediatrician needs to know, in the context of air pollution and allergy are:

Allergen: An allergen is a protein component which produces an immunologically potent reaction in which the immune system perceives a threat in susceptible individuals, which otherwise is harmless to a majority of people. An allergen is responsible for initiating an allergic reaction.

Pollutant: Pollutants contaminate the environment and render the natural resources toxic or unsuitable for use when it crosses permissible limits. Air pollutants have been known to augment the allergenicity of certain pollens and fungal spores, but are directly non-immunogenic.

Irritant: An irritant is a substance, mainly chemical, which may cause injury to mucosal tissues even after a single exposure. Prolonged exposure may result in airway diseases like asthma or skin disorders like contact dermatitis/eczema.

Trigger: Trigger is a stimulus, which when comes in contact with the immune system, initiates a physiological exaggerated response, which can be a manifestation of a disease. Allergens, pollutants and irritants can be triggers of an allergic reaction.

Inducers: Inducers are agents which enable the complex interaction of a pollutant and the immunological mechanism. These may be *i*) exogenous, either microbial (pathogen-associated molecular patterns, that function through dedicated receptors, and virulence factors) or non-microbial (allergens, irritants, toxic compounds), and *ii*) endogenous such as signals produced by stressed or damaged tissues [6].

Particulate matter (PM): It is a predominant air pollutant which is frequently used as a proxy indicator of air pollution. In urban areas, it includes dust, smoke, and liquid droplets emitted into the air mainly by vehicles, factories, and construction activities. Coarse particles are PM ≥2.5 microns (µm) to 10 µm (PM10) in aerodynamic diameter, while fine particles are <2.5 µm (PM2.5). Ultrafine particles have sizes up to 100 nanometers (nm) (PM0.1). Collectively, the PM less than 10 µm are called respirable PM. Coarse particles get deposited in the upper airway, whereas fine and ultrafine PM can reach up to the alveoli.

Air Quality Index (AQI): This index has been developed for disseminating easy information about complex parameters of air quality as a single number. Calculation of AQI is usually computed from the concentration of six major pollutants – ozone (O_3), PM, nitric oxide (NO_2), sulphur dioxide (SO_2), carbon monoxide (CO) and lead (Pb), Further data availability, averaging period, monitoring frequency and measurement methods are used. However, PM (PM10, PM2.5) used in AQI is the most commonly used marker of exposure to air pollution.

Sources of Air Pollution

Pollutants can be classified into household air pollution (HAP) or ambient air pollution (AAP), primary (if directly emitted into the atmosphere), or secondary (if these react or interact therein, e.g., ozone-O_3) based on their source and derivation. **Table 1** enumerates various sources of air pollutants. The AQI derived from these pollutants is categorized as per their ill effects on human health. The Indian standards for 24-hour air quality is; however, relaxed when compared to United States Environmental Protection Agency (USEPA) or WHO standards because of underlying higher background pollution (i.e. is dust and natural sources) which poses a challenge to achieve USEPA air quality standards in a very short time [7]. An updated knowledge of AQI and its impact on health can help a clinician to provide quality care to his patients.

Pathophysiology

Exposure to air pollutants enhances the airway responsiveness to aeroallergens via several mechanisms as shown in **Fig. 1**. The pathophysiological mechanisms [12] include: *i*) Traffic related pollutants and global warming triggered pollen allergens release, *ii*) enhancement of antigenic properties of biological aerosols (e.g. plant-derived components and pollens) by air pollutants after adhering to their surface, *iii*) increased penetration of allergens and subsequent airway sensitization by the compromised mucociliary clearance, *iv*) transport of free allergens to lower airways after binding to particulate pollutants like smoke, dust, and diesel exhaust particles (DEP), *v*) changes in the epithelial structure and micro-flora through oxidative stress and inflammatory reactions leading to allergic immune response, *vi*) co-localization of adjuvants and allergens on PM creating multivalent epitopes to cross-link several IgE receptors, and triggering an exaggerated IgE response, *vii*) chemical modification and oligomerization of allergens by reactive oxygen and nitrogen species (ROS/RNS), thus enhancing their immunogenicity with biological aging, and *viii*) epigenetic changes causing DNA methylation in the promoter region of immune effector genes by cigarette smoke.

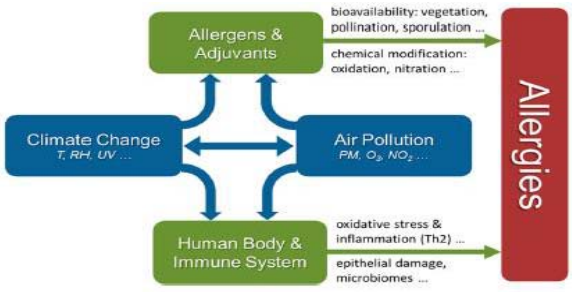
Table I Types of Air Pollution and Their Sources

Ambient air pollution (AAP)	
Pollutants	Sources
Particle matter (especially PM 2.5, PM 10, ultrafine PM)	Fuel combustion (vehicles, factories, residential heating, dust and construction)
Ground level ozone	Fuel combustion, chemical reaction between oxides of nitrogen and Volatile organic compounds (VOCs) emitted from natural sources and/or due to human activities
Carbon monoxide	Vehicles, combustion of wood, fossil fuels
Nitrogen oxides (NO, NO3)	Fuel or industrial combustion
Sulphur oxides	Industrial activity, coal burning, diesel
Lead and mercury	batteries, radiators, waste incinerators, metals, ore, industries, thermometers
Polycyclic aromatic hydrocarbons (PAC)	Forest fires, incineration, and engines, coal and tar residue
Household air pollution (HAP)	
PM, CO, NO2	Fuel combustion (cooking, incense, candle and mosquito repellent coil burning) and human bioactivity
Second hand tobacco smoke	Smokers
Pesticides, Solvents, Benzene and VOC	Paints, floor finishes, furniture, polyurethane foam
Allergens	Allergens from furred pets, dust mites, cockroaches, rodents and molds.
Building related (radon, asbestos)	Rock formation underneath buildings

Prepared using information from Sharma, et al.[8], Goldizen, et al. [9], Schwartz [10] and Gorai, et al. [11].

How does air pollution affect asthma and allergic rhinitis?

In more than 27 studies, it has been shown that an acute increase in air pollution plays a significant role in asthma flare-ups [13,14]. Nearly 15% of flare-ups in asthmatic children were found to be attributed to TRAP (Traffic-related air pollution) [15], especially in those whose homes were close to roadways with a heavy truck density [16]. Hence, children exposed to higher levels of NO_2, Ozone, PM 2.5 and PM 10 for a longer duration have lower lung function and lung growth [17]. With increased life expectancy, they might unmask asthma in the future and also have the potential of developing chronic obstructive pulmonary disease (COPD) [18]. Furthermore, prenatal exposure to NO_2, SO_2, PM 2.5, and PM 10 can affect the lung growth in a fetus, which can be associated with an increased risk of asthma in childhood.



Reproduced with permission [11]. UV-Ultraviolet, PM-Particulate matter, O3-Ozone, NO2-Nitrous oxide, Th2-Type 2 Helper cells, T-Temperature, RH-Relative Humidity.

Fig. 1 Pathophysiology of the interplay of air pollution and allergies

Other contributing factors include exposure to mosquito coil, incense sticks and environmental tobacco smoke (ETS). Burning of one mosquito coil produces as much PM 2.5 as 100 cigarettes and as much hydrocarbon as 50 cigarettes [19]. Incense sticks burning produces polyaromatic hydrocarbons, benzene, carbon monoxide and PM 2.5. In a study in Cardiff during Easter, the pollution inside a church due to incense burning showed a marked increase in ultrafine PM, PM2.5 and PM 10. The oxidative stress was 25-30 times higher than that of cigarette smoking [20]. Exposure to cigarette smoke can trigger asthma symptoms, can lead to a flare up and even affect a prenatal fetus. Prevalence of infant passive smoking is 10% in Sweden, 60% in Greece, 40% in USA and 50-70% in South East Asia. Second hand smoke (SHS) is as detrimental to health as active smoking. It contains more than 4000 chemicals, of which 250 are harmful. In addition, there is a strong correlation with childhood obesity, asthma and ambient air pollution [21]. Higher exposure to early life TRAP increased the rate of change of childhood BMI [22], a known co-morbidity of difficult to control asthma in children.

Many epidemiological and clinical trials reveal that

patients with allergic rhinitis, when exposed to pollutants, have worsening of their symptoms [23]. In a randomised controlled trial involving 253 adults with seasonal allergic rhinitis (SAR) to ragweed pollen, it was observed that controlled exposure to DEP and ragweed pollen in a special exposure unit, significantly increased SAR symptoms compared to ragweed exposure alone. This effect persisted beyond the end of the DEP + pollen exposure period [24].

CONSENSUS STATEMENT

Evaluation of Impact of Air Pollution in a Child

A pediatrician must take a detailed environmental history (**Box 1**) in every child with asthma or allergic rhinitis during the initial contact. Investigations are needed in select situations and currently documented in research settings only. Measures of inflammation like Fractional Excretion of Nitric Oxide (FENO) or functional assessment with spirometry or Impulse Oscillometry (IOS) should be monitored [25]. Airway FENO level is a surrogate marker of eosinophilic inflammation and corticosteroid sensitivity in bronchial asthma [26]. It was found that annual increase in PM2.5, PM 10 and NO2 level were associated with significantly higher FENO level [27]. The impact of air pollution on lung function testing needs more evidence to establish correlation. Measurements of metabolites of polycyclic aromatic hydrocarbons (PAH) and cotinine/ creatinine ratio in urine may guide to the amount of air pollution and second hand smoke exposure [28].

Box 1 Evaluation by Environmental History – Questions to be asked by a Pediatrician

Indoor Home Environment

- Does the home have a separate kitchen? What fuel is used for cooking?
- Is there any smoker at home?
- Do you use mosquito coil, agarbatti or insecticide spray?
- Was there any recent renovations at home, civil work, painting or wood work? What is the type of wood used?

Home Surroundings

- How far is the home located from main road?
- Is there any construction work near home?
- Any garbage dump, weeds or farm fields with post-harvest burning
- Are there any industries or mills close to home?
- Does the child have aggravation of symptoms during Diwali or Holi?

School related

- How does the child travel to school?
- How far is the school located from main road?
- Is there any construction work near school or any renovations in school?

Prevention and Counselling

A multipronged strategy should be applied at various levels of the healthcare infrastructure to prevent the effects of air pollution on children. Medical organisations need to make efforts in the field of research and publication to disseminate knowledge among healthcare professionals, colleagues, students and the community on air pollution and its effects. Pediatricians must counsel and advice caregivers about various measures which can be utilised to prevent the effects of air pollution on child health.

Measures to Reduce Outdoor Air Pollution and/or Its Effects

Face mask: For the community at large, wearing any mask is recommended when the AQI score exceeds 200 or at PM2.5 concentrations of 150 mcg/m^3 and above [36]. Although, N95 mask is most effective for filtering PM2.5 particles, a reusable 3-layer cotton cloth mask can suffice for many children.

Clean fuels for vehicles: Encourage use of newer and lesser polluting fuels, with less exposure to diesel fumes [29]. Electric vehicles can be a welcome initiative.

Restriction of outdoor activities: Advice to keep children indoors when AQI is poor or pollen count is high [30].

Measures to Reduce Indoor Air Pollution

Reduce molds, dust and dust mites concentration: This can be achieved by washing bedding on weekly intervals using hot water at 54^{O}C or by employing commercially available mite-proof bedding. Feather dusters disperse dust and allergens and thus should be avoided. Moist cloth should be preferred over dry ones for wiping dirt [31]. Vacuum cleaners should be sealed tightly to avoid a dust leak. Cleaning products with a high composition of volatile organic compounds (VOC), scents, or odours should be avoided.

Home ventilation and use of air purifiers: An air purifier can be used in cases where significantly poor air quality is documented. To help choose an air purifier, the patient can be advised to choose one with a Clear air delivery rate (CADR) of more than 600, and with a Minimum efficiency reporting value (MERV) of the High efficiency particulate air (HEPA) filter more than 14. One must; however, replace and clean filters periodically and install the machine away from walls or furniture.

Cooking fuels: Use of clean fuels for cooking like LPG should be encouraged at home. Biomass fuels and stoves should be avoided.

Cessation of smoking (both active and passive):

Exposure to ETS should be reduced in all forms. Public health measures like bans on smoking in public places and media education campaign on benefits of cessation are useful [32].

Cessation of use of mosquito coils, agarbatti and dhup sticks in households [33].

Furniture with pressed wood need to be avoided: These types of furniture can emit high levels of VOC, formaldehyde and carbamide (urea) [34]. Formaldehyde is commonly used to bond the adhesives in pressed wood products. It is a hazardous pollutant that can pose a serious threat to health if exposed for a long period of time. The rate at which formaldehyde is released into the air is accelerated by heat and humidity, so avoid placing new pieces of furniture over or near a heat source.

Renovation and painting of the house needs to be done during a holiday break when children are out of home.

Selecting a school or building a new house needs to be considered very carefully so that it is at least 100 meter away from the main road.

Measure to Build Lung Capacity

Yoga and relaxation therapy: These have been found to increase the peak expiratory flow rate in children as well as improve the lung function and quality of life in adults.

Exercise and other physical activities: Regular aerobic activity has the potential to improve lung capacity and reduce bronchial hyper responsiveness

Management of Asthma and Allergic Rhinitis in the Presence of Pollution

Although the principles of management in children with asthma and allergic rhinitis remain the same, focus needs to be laid on exposure to air pollution as a trigger of flare-ups and cause for poorly controlled symptoms. In addition to avoidance of exposure of pollutants on high pollution days, asthma flare-ups can be prevented by using maintenance dose of inhaled corticosteroid therapy (ICS) on a regular basis [35]. Studies suggest that this approach has shown to decrease an adverse response to pollutant exposure [36]. Some additional measures like use of reliever medication or stepping up of ICS for few days during poor air quality monitored by AQI and pollen calendar is advisable, although there are limited studies to support this. Stepping down of ICS and outdoor activities should be discouraged on days with a high AQI or high pollen count.

Management of allergic rhinitis with intranasal corticosteroids (INCS) and antihistamines should be based on the severity of symptoms and treatment should be continued on days of poor air quality as per the AQI. Despite recent discoveries on mechanistic biomarkers and signal pathways of cellular oxidative stress injury secondary to pollutant exposure, efficacy studies on pharmacological therapy of AR patients exposed to specific pollutants is currently lacking [37]. In adults, fexofenadine demonstrated efficacy and a well-tolerated safety profile in ragweed AR patients exposed to ragweed associated to Diesel exhaust particles (DEP) in an environmental exposure unit. There was improved nasal symptom scores following ragweed plus DEP exposure when pre-treated with fexofenadine compared to the placebo group [38,39]. However, paediatric focused clinical studies are needed to address the need of managing allergies caused or aggravated by air pollution. In children with poorly controlled asthma or allergic rhinitis despite high doses of conventional medication, the contribution of air pollution needs to be considered and evaluated in detail.

CONCLUSION

Air pollution has a significant impact on respiratory allergies in children through various mechanisms. Physicians managing children with allergic rhinitis and/or asthma regularly need to be well versed with the pathophysiology, evaluation and management, and be able to suggest targeted preventive measures. More research in pediatric patients is needed to enhance our knowledge and practices in this field.

Note: A detailed version is available at *www.iapindia.org*

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Understanding Exome Sequencing: Tips for the Pediatrician

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Exome sequencing is gaining popularity as a genomic test for the diagnosis of Mendelian disorders in children. It is essential for pediatricians to familiarize themselves with this technique and its interpretation. This brief review discusses some of the key components of a clinical or research report on exome sequencing for a practicing pediatrician, so as to enable them to utilize this test well and provide timely referrals to a clinical geneticist.

Keywords: *Next-generation sequencing, Sanger sequencing, Targeted gene panel, Monogenic*.

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Exome sequencing (ES) has emerged as one of the most powerful and widely available tools in the clinic for diagnosis of monogenic disorders. This has resulted in pediatricians encountering a large number of exome sequencing reports (clinical or research) in their practice. We intend to provide a brief guide to pediatricians, to interpret these reports to enable them determine the clinical relevance of the reported variants and thus help in post-test counseling. Readers may refer to earlier articles for a review of clinical indications, methodology and laboratory workflow of exome sequencing [1-4]. The relevant questions related to exome sequencing are listed here with explanations.

Was Exome Sequencing Ordered for an Appropriate Indication?

Currently, ES is indicated when a monogenic disorder (disease caused by alterations in a single gene) is suspected. It is not a test for diagnosis of aneuploidy (Trisomies 13, 18, 21), copy number variations (Di George syndrome due to 22q11.2 microdeletion), triplet repeat disorders (Fragile X syndrome) or multifactorial disorders (neural tube defects). There are easier, accurate and inexpensive tests for the diagnosis of beta thalassemia, spinal muscular atrophy, Duchenne muscular dystrophy, hemophilia A, achondroplasia, Apert syndrome, sickle cell disease and fragile X syndrome. In individuals with Duchenne muscular dystrophy, 65-80% of disease causing variants are large deletions or duplications of one or more exons in *DMD* gene [5]. Hence a test like multiplex ligation-dependent probe amplification (MLPA), which detects deletions and duplications of exons, is the investigation of choice. Variations in *HBB* gene (a small gene with only three exons), which cause sickle cell disease and beta thalassemia can be detected by Sanger sequencing of the entire gene. However, rarely ES may end the diagnostic odyssey by diagnosing or ruling out (with less confidence) a monogenic disorder.

Differences Between Trio and Singleton Exome Sequencing

Trio exome sequencing (ES of proband and parents) is the ideal approach for diagnosis of a monogenic disorder. Additionally, other affected or unaffected family members may be included for a comprehensive analysis. Trio (or more) ES can confirm the segregation of the variant, prioritize the rare disease-causing variants and confirm de novo origin of variants. However, to reduce the cost, often a singleton ES (only proband) is considered. In some autosomal dominant conditions with incomplete penetrance (like Treacher Collins syndrome, due to heterozygous variant in *TCOF1* gene), a heterozygous variant identified in the proband may be identified in one of the parents who could be clinically asymptomatic or have unrecognizable mild clinical features. In such instances, it is essential to evaluate the parents in detail before disregarding the variant as benign.

What Is the Relevance of Systematic Phenotyping and Providing Clinical History and Differential Diagnoses to the Laboratory

It is crucial to know that symptoms, signs, results of investigations (haematological, biochemical or radiological) and a three-generation pedigree are used extensively to interpret the exome data by the laboratory. The laboratory would also benefit from a list of differential diagnoses provided by the clinician. Clinical validity of a disease-causing variant is assessed by the information that

the clinician provides to assess whether an identified variant is truly responsible for the phenotype of a patient. A laboratory attempts to achieve this by searching the databases of known disease-causing variants and by comparing the phenotype of previously reported patients. A pedigree helps to infer the possible mode/s of inheritance as well.

Were the Genes in Question (Your Differential Diagnoses) 'Covered' Well by Exome Sequencing?

Coverage refers to the fraction or breadth of the target region that is actually sequenced [6]. Coverage may differ from one service provider to another and most laboratories provide the coverage of genes of interest in the ES report. If a clinical diagnosis of osteogenesis imperfecta is made, then the clinician checks whether all the 20 genes known to cause this condition are covered by ES. Coverage may depend on biases in DNA sample preparation, GC content of the target region and differences in the efficiency of capture kits [6]. The term depth, which is often used interchangeably, denotes the number of times (50x, 100x, etc) a region is sequenced. A sufficient read depth of at least 20x is required to ensure sensitivity and specificity of a variant call and assessing allelic balance (especially to determine heterozygous or mosaic nature of the variant) [3]. It is the responsibility of the laboratory to be confident of calling a variant accurately. If ES does not cover certain genes or regions causing a particular phenotype, then alternate methods like Sanger sequencing may be used to sequence those regions.

What Are the Different Levels of Evidence to Imply Disease Causation of a Variant?

At the variant level, segregation of the variant in diseased individuals in a family, inheritance consistent with the proposed mode of inheritance and mechanism of disease causation (haploinsufficiency, loss of function or gain of function) and a low frequency of the variant in the population may provide evidence for disease causation. The variant may be deleterious if the variant is present in an evolutionarily conserved site or alters the protein domain essential for the function of a protein. Experimental evidence for altered quantity or function of a protein by a variant can also be considered as an evidence for causation. The variant may be considered significant if the variant is present in a gene or a pathway already known to cause a disease phenotype [2].

Is the Interpretation of the Variant Correct?

The laboratory provides a classification of the variant identified based on the recommendations of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology [7]. The variants are classified as pathogenic, likely pathogenic, benign, likely benign and variants of uncertain significance (VUS). While the first two categories confirm the diagnosis, VUS poses a huge challenge. The following points may be considered to check whether the interpretation is correct:

Is the variant already reported to cause the phenotype? Databases like Human Gene Mutation Database *(http://www.hgmd. cf.ac.uk/ac/all.php)*, Leiden Open Variation Database (LOVD) (*https://www.lovd.nl/*) or ClinVar (*https://www.ncbi.nlm.nih.gov/clinvar/*) may be used for this purpose. There are some disease-specific databases like Infevers [variant information on genes related to autoinflammatory diseases (*https://infevers.umai-montpellier.fr/web/index.phpm*)] or Osteogenesis Imperfecta Variant Database (*https://oi.gene.le.ac.uk/home.php?select_db=COL1A1*). LOVD provides access to each of these disease-specific databases.

Does the phenotype of the patient match with the gene and disease identified? It is essential for a clinician to re-examine the patient to check for certain phenotypic features, relevant to the disease in question, which might have skipped attention earlier. Information regarding specific phenotypic features of a particular condition can be obtained from databases like Online Mendelian Inheritance in Man (OMIM; *https://www.omim.org/*).

Does the inheritance pattern of the reported variant and the mechanism of disease causation match with the diagnosis? Mechanism of disease is an essential aspect to consider while evaluating the clinical relevance of a variant. For example, *FGFR2* related craniosynostosis syndromes result from heterozygous gain of function variants in *FGFR2* gene. Hence identifying a loss of function variant in *FGFR2* gene may not be clinically relevant for craniosynostosis. Similarly inheritance pattern of a variant needs to be checked. For example, heterozygous variations in *FLNB* can result in Larsen syndrome (MIM#150250) which is inherited in an autosomal dominant manner. Homozygous or compound heterozygous variations in *FLNB* can cause Spondylocarpotarsal synostosis syndrome (MIM#272460), inherited in an autosomal recessive manner.

How do you proceed if a VUS is reported? In case of VUS, the clinician should ensure that adequate effort has been made by the laboratory in reviewing existing literature, disease, variant databases and segregation of variants in the family. It is recommended that VUS should be used with caution in clinical decision-making [7]. Clinical, laboratory (enzyme assay) or radiological re-evaluation of the patient may provide further evidence for reclassification of a VUS as pathogenic or benign. It is appropriate to refer a family to a clinical geneticist for

assessment before providing prenatal diagnosis based on VUS. Reassessment of patient and the variant at a later date might also help in solving the dilemma.

What Is Meant by 'Segregation of Disease-Causing Variants'?

Segregation of variants helps in assessing whether a specific variant segregates with the disease status or a particular phenotype in the family. Segregation of variants can be done by trio (or more) ES or by Sanger sequencing of the candidate variants identified in the proband in parents and other affected or unaffected family members. Hence, it is essential to provide samples of affected or unaffected family members for testing along with that of the patient.

Segregation of variants is less important in autosomal dominant conditions with recurrent or known disease causing variants (Achondroplasia and Apert syndrome). It is also less critical for conditions with well-established disease mechanisms (glycine substitutions in *COL1A1* and *COL1A2* in progressively deforming or perinatal lethal osteogenesis imperfecta). However, to prove or disprove whether a novel variant or a novel gene causes a disease phenotype, segregation is essential. If a disease-causing variant does not segregate in a family, consider reduced or incomplete penetrance, age dependent penetrance, mosaicism, subtle or mild clinical features, adoption, gamete donation or disputed paternity.

Is Sanger Sequencing Always Required to Validate the Variant Detected by Exome Sequencing?

Sanger sequencing is used by many laboratories to establish the analytical validity or accuracy of the ES. ES may result in false positive or false negative results on a few occasions. A laboratory considers parameters like depth and coverage, presence of repetitive sequences and pseudogenes before deciding to undertake analytical validation by Sanger sequencing. The laboratory should determine if the variant is called with sufficient confidence. Sanger sequencing may be done to check for segregation of variants in family members as explained above.

How to Proceed if Exome Sequencing Does Not Yield a Result?

The possible outcomes of ES are provided in **Box I**. It is necessary to check if the clinical indication was appropriate, sufficient clinical information was provided and the laboratory has considered this information while reporting the variants. A negative ES result may indicate the presence of a variant that is not usually detected like large deletions, deep intronic variants, epigenetic changes (methylation abnormalities), triplet repeat disorders, or variants in repetitive regions**.** Even though copy number variants involving exons may be identified in ES (requires a different analytical step), alternate methods like chromosomal microarray (CMA) or MLPA may be required to confirm them. It is pertinent to consider whether the disease in question is really a monogenic disorder. A negative ES report could also indicate a phenotype due to an environmental or teratogenic agent (Aicardi Goutières syndrome, a genetic disease, may resemble congenital cytomegalovirus infection) or multifactorial disease. A chromosomal disorder or microdeletion/duplication syndrome should be considered when there is global developmental delay, facial dysmorphism and major or minor malformations, and a chromosomal microarray or MLPA may be ordered. In some instances, whole genome sequencing may be required to detect variants in non-coding regions if a trio ES is non-diagnostic. Reassessing the phenotype of the patient later may be helpful to identify symptoms and signs, which may evolve with age. It is important to ensure timely referral to a clinical geneticist for expert opinion. Illustrative examples are provided in **Web Table I.**

Box I Possible Outcomes of Exome Sequencing

- Identification of a single disease causing variant.
- No disease-causing variants are identified.
- Identification of multiple plausible variants causing a phenotype (multiple genetic disorders causing a blended phenotype).
- Findings that are unrelated to the phenotype being evaluated. These are known as 'secondary findings' [8].
- Variants of uncertain significance.

CONCLUSION

Pediatricians have an ever-increasing responsibility to be updated with evolving technologies in clinical practice. It is essential for a pediatrician to understand the merits and limitations of widely used ES and make timely referral to a clinical geneticist to provide the best possible care to a family with a genetic disease.

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Web Table I: Illustrative examples

1. A nine-years-old male was evaluated for intellectual disability and epilepsy. His chromosomal microarray was normal. Exome sequencing did not identify any known disease causing variant in genes known to cause intellectual disability. This child was re-evaluated and molecular testing for Fragile X syndrome by Triplet Primed PCR (TP-PCR) was performed. This test detected an expansion in *FMR1* gene, confirming the diagnosis of Fragile X syndrome. Fragile X syndrome and other conditions caused due to expansion of triplet repeats will not be detected by exome sequencing. This illustrates the need for clinical evaluation and planning the right testing strategy.
2. A two-years-old female born to consanguineous parents was evaluated for failure to thrive, polyuria and recurrent vomiting. She had hyponatremia, hypokalemia, hypochloremia and metabolic alkalosis suggestive of Bartter syndrome. Exome sequencing was done as Bartter syndrome is a genetically heterogeneous condition. No disease causing variants were identified by the laboratory. Since the clinician was sure about the diagnosis, visualisation of all the genes causing Bartter syndrome was performed in Integrative Genome Viewer (IGV). This showed absence of reads in the genomic region encompassing *CLCNKB* gene. Copy Number Variation analysis of exome data detected a deletion in the region chr1:16361791-16388753, which comprises the *CLCNKB* gene. This deletion was not observed in population databases. Complete gene deletion of *CLCNKB* in a homozygous state is known to cause Bartter syndrome type 3 (MIM# 607364). This was later confirmed by Multiplex Ligation dependent Probe Amplification (MLPA), which detected a homozygous deletion of exons 1 to 19 in *CLCNKB* gene. This example illustrates how a laboratory can improve the diagnostic rate by taking additional steps in the light of a clinical diagnosis.
3. A seven-years-old boy born to non-consanguineous parents was evaluated for intellectual disability and seizures. He had a four-years-old brother who had the same phenotype. Targeted exome sequencing (also popularly known as 'clinical exome' in India or Mendeliome) report showed a heterozygous missense variant, c.4723 G>C (p.Gly1575Arg) reported as a variant of uncertain significance (VUS) in *CHD2* gene, causing epileptic encephalopathy childhood onset, in both the siblings. On segregation of this variant in family members, it was observed that the asymptomatic mother carried the variant in *CHD2* in heterozygous state. This proved that the variant in *CHD2* was not responsible for the phenotype in the siblings. Hence the siblings were re-evaluated. Testing for Fragile X syndrome and chromosomal microarray were normal. Whole exome sequencing was done for both the siblings and a novel frameshift deletion c.2992delC (p.Leu998TrpfsTer4) in hemizygous state was detected in *KDM5C*, causing mental retardation, X linked, syndromic, Claes-Jensen type. This variant was inherited from their mother who was asymptomatic, demonstrating the X linked recessive pattern of inheritance. This example illustrates the importance of segregation of variants in family members for evaluation of VUS. This also illustrates that sometimes, clinically relevant variants may be missed in exome sequencing data.
4. An eight-years-old boy, born to third degree consanguineous parents, was evaluated for global developmental delay, neuroregression and seizures. On examination, he had spasticity, myoclonic

jerks and no hepatosplenomegaly. His magnetic resonance imaging (MRI) of brain showed diffuse cortical atrophy. Suspecting an autosomal recessive disorder, singleton exome sequencing was done for him. This detected a novel homozygous missense variant [c.965T>C, p.(Ile322Thr)], causing Sandhoff disease. This variant was classified as variant of uncertain significance. The variant was seen in heterozygous state in his parents. To ascertain the clinical significance of this variant hexosaminidase A and B enzyme assay was advised. Fundus evaluation for cherry red spot was also advised. This example illustrates the importance of re-evaluation and utility of additional tests in ascertaining the clinical significance of a variant of uncertain significance.

5. A five-years-old boy was evaluated for developmental delay, oligodontia and facial dysmorphism. On examination, his tone, power and reflexes were normal. His MRI brain showed hypomyelination. Echocardiography showed patent ductus arteriosus. Exome sequencing identified a novel homozygous variant c.2423G>A (p.Arg808Gln) in *POLR3A* gene causing leukodystrophy, hypomyelinating, 7, with or without oligodontia and/ or hypogonadotropic hypogonadism. The variant was classified as VUS. The variant was present in heterozygous state in his parents.

   The following points were considered to check the relevance of this variant and confirming the clinical diagnosis in the proband:

   a. Was the variant already reported?

      No. The variant was absent in disease databases like HGMD, LOVD and ClinVar.

   b. Does the phenotype of the patient match with the identified gene?

      Yes. In this case, the phenotype of the patient was matching with leukodystrophy, hypomyelinating, 7, with or without oligodontia and/ or hypogonadotropic hypogonadism caused by *POLR3A* variant.

   c. Does the inheritance of the reported variant and the mechanism of disease causation match with the diagnosis?

      Yes. In this scenario, the inheritance pattern (autosomal recessive) and mechanism of disease causation (loss of function) were matching with the diagnosis.

   d. Is the variant altering protein function?

      Multiple *in silico* tools can be used to check whether the protein function is altered. In this case, the protein function was predicted to be altered by multiple tools.

   e. Literature search identified that homozygous or compound heterozygous variants in *POLR3A* gene was known to cause leukodystrophy, hypomyelinating, 7, with or without oligodontia and/ or hypogonadotropic hypogonadism.

This illustrates how a variant of uncertain significance can be approached to ascertain clinical significance.

Competency-Based Assessment in Pediatrics for the New Undergraduate Curriculum

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Medical Council of India (MCI) implemented competency-based curriculum for undergraduate medical education nationwide in 2019, with assessment of competencies being its integral component. The curriculum has outlined some broad principles and components of assessment, but the process and schedule for formative and summative assessment is to be decided by the universities and institutions. In this document, we summarize the recommendations for the subject of Pediatrics, and propose an assessment model for summative assessment that can be adapted/adopted by universities and institutions. Few basic principles of formative assessment have been shared, the implementation of which may be the main challenge for the institutions. It is important to develop the capacity of the faculty for conducting the assessment under the ambit of principles of competency-based curriculum.

Keywords: *CBME, Curriculum, Medical education, University examinations.*

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The Medical Council of India (MCI) has recently introduced competency-based curriculum for undergraduate medical education [1]. Robust assessment process is a vital part of its implementation as this curriculum is outcome oriented (acquisition of competencies) rather than process oriented. Competencies are defined as habitual and consistent use of knowledge, skills, attitudes and communication related to a clinical problem [2]. A perusal of this definition indicates that competency-based assessment cannot be a one-time process or a year-end examination; rather it has to span over a period of time to assess if the student is using them 'habitually and consistently.' The MCI guidelines have rightly emphasized the role of formative and internal assessment in acquisition and development of competencies. We present an overview of assessment guidelines for the subject of pediatrics by erstwhile MCI and propose a model for competency-based assessment. We have refrained from any critique of these guidelines. Both internal and summative assessments must be planned and implemented by respective universities and/or colleges, with few variations. The underpinning concepts of competency-based assessment have earlier been described in detail [3].

INTERNAL ASSESSMENT

Internal assessment, though vital, has been a bone of contention due to its 'subjective' nature. However, expert subjective judgments can be as reliable as highly objective and standardized assessments. A 'Quarter model' of internal assessment had been proposed [4] to ensure that no test, teacher, tool or context contributes to more than 25% to the total. MCI recommendations have also proposed a minimum of three tests for pre- and paraclinical subjects and minimum of two tests per professional year for the clinical subjects supplemented by unlimited opportunities for formative ongoing assessments [1,5]. It is important to involve all teachers in the department in the assessment process to dilute subjectivity, to expose students to different ways of thinking about a problem, and to train junior teachers in assessment. A good record keeping in the form of a logbook or e-portfolio (an electronic document that contains a log of learning activities as well as an evidence of learning from those) is important to provide developmental feedback to the students, especially for clinical skills [6].

A welcome change in the new curriculum is that internal assessment marks are shown separately in the final report and are not added to marks of the University summative examinations. This provides us better opportunities for using internal assessment as multiple low stake assessment to assess acquisition of most competencies. A satisfactory performance in internal assessment (50% combined in theory and practical, minimum 40% in each) is still required to be eligible for appearing for university examinations [1,5]. However, it is

simultaneously imperative to develop a feedback mechanism not only to help students acquire the competencies but also to revisit and modify teaching-learning methods and strategies.

There are 406 competencies listed in the subject of Pediatrics out of which 23 are competencies that require certification at 'Perform' level (**Table I**) [7]. As the competency-based system focuses on integration of all domains of learning (cognitive, psychomotor, affective) with communication skills, it is not possible to assess the acquisition of competencies just by internal or intermittent summative examinations. While it is relatively easy to implement the summative component of internal assessment, a structured mechanism for formative component with regular monitoring of students' behavior and assessment of patient records and logbooks remains a challenge. The feasibility-criticality matrix provided in the Skill module [8] of erstwhile MCI can be used as a guideline to classify what should be tested during final examinations and what can be left for internal part. As a general rule, all certifiable skills (**Table I**) should be included in internal assessment while others can be included in the formative part. Similarly, teacher to student ratio, and availability of patients and skill labs should be considered to decide whether to use real patients or manikins. A high level of motivation and coordination among the teachers is required so that assessment is not taken as a formality or burden but is utilized to monitor acquisition of competencies as well as the department's teaching-learning program. Up to 20% marks in internal assessment have been allocated to logbooks and this could be used as a handle to encourage formative assessments. Assessment module by MCI [5], serves as a guide for development of formative assessment which may be personalized by respective Universities and institutions using the available resources [9].

A major issue with internal assessment has been

Table I List of Competencies in Pediatrics That Require Certification (Perform Level)

Competency	*No. required*
Perform Anthropometric measurements, document in growth charts and interpret	3
Perform Developmental assessment and interpret	3
Observe the correct technique of breast feeding and distinguish right from wrong techniques	3
Calculate BMI, document in BMI chart and interpret	3
Assess patient for fitness for immunization and prescribe an age-appropriate immunization schedule	5
Perform NG tube insertion in a manikin	2
Perform IV cannulation in a model	2
Perform Intraosseous insertion in a model	2
Assess airway and breathing: recognize signs of severe respiratory distress. Check for cyanosis, severe chest indrawing, grunting	3
Assess airway and breathing. Demonstrate the method of positioning of an infant & child to open airway in a simulated environment	3
Assess airway and breathing: administer oxygen using correct technique and appropriate flow rate	3
Assess airway and breathing: perform assisted ventilation by bag and mask in a simulated environment	3
Check for signs of shock i.e. pulse, Blood pressure, CRT	3
Secure an IV access in a simulated environment	3
Choose the type of fluid and calculate the fluid requirement in shock	3
Assess level of consciousness and provide emergency treatment to a child with convulsions/coma	3
Assess for signs of severe dehydration	3
Provide BLS for children on a manikin	3
Perform and interpret Urine DipStick for Sugar	3
Identify deviations in growth and plan appropriate referral	2
Identify a BCG scar	3
Interpret a Mantoux test	3
Perform AFB staining	3

Compiled from National Medical Council. Competency Based Undergraduate Curriculum for the Indian Medical Graduate, 2018. Vol. II [7]

'subjectivity' and 'bias', which prevents us from making its full use. An earlier proposed quarter model [4] provides useful guidelines. Similarly, many components of programmatic assessment (PA) [10] can also be incorporated, like utility of assessment [11] rather than attributes of individual tool or assessment, and using every assessment to provide liberal feedback to the students. Feasibility and educational impact are important attributes for both formative as well as internal assessment. To obviate the issues of subjectivity and bias, it is better to have all teachers of the department involved in the process [4]. Since senior residents are also considered teachers as per MCI guidelines, they can be used for many formative events, especially those involving procedural skills.

UNIVERSITY EXAMINATIONS

University examinations may not be the ideal way to assess competencies due to logistic concerns. However, they are extremely useful tools to help in quality maintenance and conducting assessment. Graduate Medical Education Regulations (GMER) 2019 [1] and MCI Assessment module [5] lay down the principles and broad structure for the university examinations. **Table II** summarizes the guidelines from these modules for summative assessment in the subject of Pediatrics [1,5,7]. Different universities may adopt different combinations of the same. A model for summative examination for undergraduates in Pediatrics is proposed in **Box I**. The scheme is in consonance with the proposals made by MCI. A key feature of this process would be to assess higher levels of Miller pyramid [12], aligning assessment with the competencies, and analyzing the analytical, synthetic and problem-solving skills of the students. The MCI module on assessment [5] has given few examples, which can be built upon. Examiner orientation, framing scenario-based questions, and having model questions to address various levels of Bloom Taxonomy are some inputs which need to be provided.

THE WAY FORWARD

Though introduction of competency-based curriculum for undergraduate medical education is a desirable and long-due change, it comes packaged with a lot of challenges. Assessment and documentation of acquisition of competencies will require a high degree of dedication, motivation and input in terms of time and resources, especially in busy clinical departments with perpetual shortage of faculty. The capacity building of the existing as well as new medical teachers is also a huge task. MCI has mandated participation in Curriculum Implementation Support Program (CISP) for all medical teachers [5], which will only be a sensitization. Moreover, the SARS CoV-2 pandemic in the years 2020 and 2021 has posed unique challenges and has interrupted the suggested sequence of teaching-learning and/or assessment for at least two successive batches of undergraduate students. Academic societies can play a definite role in capacity building of faculty and in helping the departments to develop appropriate teaching-learning methods and assessment strategies. As the first step towards uniformity, Indian Academy of Pediatrics (IAP) has already released learning objectives based on MCI competencies [13]. These will be useful for implementation of the competency-based curriculum for the first batch of MBBS students admitted in 2019. The next challenge is to develop an assessment model linked with competencies, especially the formative component

Table II Assessment for Competency Based Curriculum in Pediatrics

	Phase 2/ II professional	*Phase 3/III professional*	
		Part 1	*Part 2*
Internal assessment			
Theory exams	EOP only[b]	Twice + EOP	Twice + EOP
Attendance[a]	75%	75%	75%
Practical exams	End of I clinical posting (2 wk)	End of II clinical posting (4 wk)	End of III clinical posting (4 wk)
Attendance[a]	80%	80%	80%
University examination	-	-	
Theory	-	-	1 paper: 100 marks
Practical/oral/clinical			100 marks
Pass criteria	None	None	50% separately in theory and practical

[a]Attendance required; [b]As the students are not taught pediatrics theory during this phase, the theory assessment should cover the 'knowledge' part of clinical and practical skills taught during this phase; EOP: End of posting.

Box I A Suggested Plan for Summative Assessment (University Examination) in Pediatrics

Target: Final Year MBBS Student; *Type:* Summative; *Timeline:* At the end of Phase 3 (Third Professional – Part II examination)

Theory: 100 marks/ duration 3 hours

1. There will be one theory paper with two PARTS A and B.
2. PART A will be only multiple choice questions (MCQ) and PART B will have two Sections I and II.
3. Questions to be framed as per guidelines given in Assessment Module of CISP

Part/time	Section	Type of questions	Questions	Syllabus/ content[a]	Marks allotted
A/30 min		MCQ	20	Entire syllabus	20
B/150 min	I	Structured essay/long answer question (LAQ)	1	*Topics 1-20 + 1 SAQ from AETCOM	15 +25 =40
		Short answer question (SAQ)	5		
	II	Structured essay/LAQ/SAQ	15	*Topics 21-35	15 +25 =40

Practical: 100 marks

4. To be conducted in batches of not more than 40 per day
5. The idea is to have multiple examinations by multiple examiners to eliminate bias. At the end of the day, the student should have been exposed to all the examiners available on that day.
6. Number of examiners will be according to the number of students in the batch. Up to 12 students in a day, 2 examiners; 13-25 students: 4 examiners: 26-40 students: 6 examiners. Of all examiners, 50% should be external examiners.
7. Each examiner to assess a different case rather than putting many examiners on one case. This allows more number of cases to be included and helps in building validity and reliability of assessment.
8. Broadly assessment will consist of 2 pediatric cases (22 marks each), 1 newborn case (16 marks), OSCE (4 stations of 5 marks each), and Viva-voce (4 stations of 5 marks each).

A. Pediatric cases (conventional case presentation)

Each student will get two cases each as follows:

Case 1: General Pediatrics (22 marks)
- Case examination (including direct observation) 20 minutes and interaction with examiner: 15 min

Case 2: Systemic Pediatrics (22 marks)
- Case examination (including direct observation) 20 minutes, and 20 minutes and interaction with examiner: 15 min

Both cases should be taken by different set of examiners. The examiners should ensure assessment on direct observations of various competencies including history taking, examination, and counseling. This will improve the validity of assessment.

B. Newborn case (10 minutes station) (extended OSCE cum directly observed Station)

This case will be conducted like an extended OSCE cum directly observed station. In effect, it means increasing the time for each station and using a part of that time observing the student taking history or performing the examination. It's assessment will include Directly observed history taking/examination of newborn/counseling of mother. Directly observed procedural skills on Neonatal resuscitation will also be assessed on manikin and related equipment.

C. OSCE (4 stations each of 5 min, Unobserved)

Station 1: *X*-rays/ECG/ABG/Instrument
Station 2: Clinical spotters/photographs with tasks or questions related to diagnosis, management or prevention
Station 3: Video of a Clinical Problem/outpatient case scenario/counseling
Station 4: Emergency case scenario/procedures

The OSCE stations should be designed to assess clinical competence and should not become an assessment of theory alone.

D. Viva-voce (4 stations each of 5 min)

- Viva/oral examination should assess approach to patient management, emergencies, attitudinal, ethical and professional values.
- Candidate's skill in interpretation of common investigative data, *X*-rays, identification of specimens, ECG, etc. may also be assessed in coordination with OSCE.

[a]*As outlined in CBME document [5]*

in form of its structure, tools (e.g., logbook/portfolio), settings and schedule.

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Publication Ethics

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Publications in the field of medical literature are a matter of prestige and fame for doctors. While genuine research contributes to the existing scientific knowledge, fraudulent data make publication unreliable, demeans the credibility of the author and reduces faith in science. Research misconduct includes the three cardinal sins fabrication, falsification and plagiarism. To promote highest standards in publication ethics, Committee on Publication Ethics provides advice and guidance to journals and publishers. Investigators should abide by ethical norms during the conduct of the research. Journals also maintain editorial standards and have well-defined policies for responding to misconduct. With an increase in medical publications over the years, it is important for all stakeholders to abide by publication ethics, in order to uphold the sanctity of research and credence in science.

Keywords: *Authorship, Conflict of interest, Misconduct, Peer-review, Plagiarism, Research.*

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Integrity in scientific research and publication is the foremost essential element to determine its credibility. Medical and research institutions should promote good clinical practices among investi-gators and establish an institutional ethics committee for supervision of research. Journals should have a policy for safeguarding research data submitted to them, detect research misconduct and ensure accuracy and reliability of whatever is published [1].

To promote highest standards in publication ethics, an international body named Committee on Publication Ethics (COPE) was established to provide advice, guidance for day-to-day practice and education modules for journals and publishers. The core practices laid down by COPE may be followed by journals, keeping in mind the specific national and international codes of conduct [2].

Research Integrity

Research integrity deals with Misconduct (fabrication, falsification, or plagiarism) and Self-plagiarism (duplicate/ redundant publication, text recycling, salami-slicing) [3].

Research misconduct: This is defined as "fabrication, falsification, or plagiarism in proposing, performing, or reviewing research, or in reporting research results" [3]. These three actions are considered as cardinal sins of research conduct. Fabrication refers to the making up or construction of data or observations that never existed. Alteration or manipulation of a value to show desirable change is also fabrication. Falsification refers to the alteration or manipulation of research data, protocols or results, in an attempt to give a false impression [4]. A systematic review [5], showed that in a total of 18 surveys, a pooled weighted average of 1.97% (95%CI: 0.86-4.45) of scientists had self-admitted to have fabri-cated, falsified or modified data at least once. Further, 14.12% (95% CI: 9.91-19.72) alleged falsification done by their colleagues. The authors concluded that, consider-ing the sensitive nature of these surveys, the true pre-valence of misconduct is expected to be higher [5].

Plagiarism has been described as the "appropriation of another person's ideas, processes, results, or words without giving appropriate credit" [6]. Plagiarism is one of the most common form of research misconduct, where someone else's work (idea, data, results, or text) is presented by an author as his/her own, without acknowledging or taking permission from the original person/source. The Council of Science Educators considers it a form of piracy, where there is a clear intent of claiming credit by the offending author [4]. Plagiarism is also defined as "an instance of someone using someone else's intellectual product (such as texts, ideas, or results), thereby implying that it is their own" [7]. There is a lack of consensus regarding what percentage of plagiarism is acceptable in a manuscript. Conventionally, 5% or less text similarity is acceptable, while most apex bodies/editors consider anything above 10-20% as objectionable. However, even in less percentage of similarity, if the matching text is copied en-block, it is liable to be considered as significant plagiarism. Plagiarism has been categorized by COPE in to three types: *i*) Clear plagiarism (unattributed use of large portions of text and/or data and represented as one's own original work), *ii*) Minor copying

of short phrases (e.g. part of a discussion of a research paper), *iii*) Redundancy (i.e., copying from author's previously published work or self-plagiarism) [8].

Self-plagiarism: This occurs is when an author copies text/ results from his own previous publications. Though, the originally published article was the author's own ingenuity, its copyright is transferred to the publisher, once the article is published. Any copying of the work, albeit, the author's own, is labelled as copyright infringement. Duplicate/ Redundant publication involves publication of whole articles or substantial sections more than once, without due notification of this fact or cross-referencing, thereby misleading the readers to believe that this is the primary work [8]. Text-recycling is a type of self-plagiarism where the author uses short passages of texts or some figures from his own previous work, in multiple instances [3]. The first full report of the primary outcomes of a research is considered a primary publication, while secondary publications are additional reports of results of secondary objectives, subgroup analyses, or post hoc analyses. Such additional publications should clearly mention that these are publications of secondary analysis/objectives and duly reference the primary publication. The primary article should always be accepted for publication before other reports of secondary endpoints. Such secondary publications should avoid duplication and unjustified splitting of results across several publications. Salami-slicing is another type of self-plagiarism, where the same research or set of experiments is published in parts as different papers, with an intent to increase the number of publications. Few forms of prior publication which are not labelled as self-plagiarism are listed in **Box 1** [3].

Few online softwares can check for plagiarism of the whole or a part of the document subject to whether the software is paid or free. All softwares may not have complete access to entire published literature or to grey literature (content that is beyond academic or commercial publishing) which may miss detection of plagiarism at some places.

Responding to Research Misconduct

Journals should have well-defined policies to handle research misconduct. Editors may need to consult the journal owner (e.g. a scholarly body/society) and the publisher for legal advice.

Box 1 What is Not Self-Plagiarism

- Abstracts and posters presented during conferences.
- Results presented at meetings.
- Results kept in databases and clinical trials registries (data without interpretation, discussion, or conclusions).
- Dissertations/theses in university archives.

Most of the operational guidelines, provided by COPE [8] suggest that the journal should initially contact the corresponding author in writing, ideally enclosing the signed authorship statement, stating the concern regarding the identified research misconduct. If the reply from the corresponding author is unsatisfactory, or he admits guilt, the submission is to be rejected with information sent to all the authors and the institution. There should be a confidential two-way communication between research institutions and journals. In most instances, investigation into this matter is carried out by the research institutions, employers, funding body, or the relevant national statutory body rather than the journal themselves [9].

Following investigations, if an article is proven to be fraudulent, journals may publish retractions or expressions of concern. However, responsibility for disciplining the investigators and ensuring responsible conduct of research lies with the institution [9]. In case of plagiarism involving minor copying of text phrases, the review process may continue, but the corresponding author may be apprised of the disconcerting fact in neutral terms, while asking for reframing the copied phrases or citing appropriately with references [8].

Research Ethics in Journal Articles

Ethics approval: Journals should ensure that authors provide a statement mentioning approval obtained from a registered ethics committee and that the study conforms to recognized standard guidelines (Declaration of Helsinki/ ICMR). Adherence to such guidelines certifies responsible conduct of research, taking care of the autonomy, confidentiality and justice to the subjects [10,11]. Few research protocols may be exempted from ethics review like when there is no likely or possible harm to the study participants or where already available information is being analyzed. However, these studies should seek exemption from respective ethics boards before the study begins. Case reports per se do not need any ethics approval but need consent from the patients and/ or parents/guardians before publication.

Ensuring anonymity: Identifying information of any subject should not appear in an article. Authors should mention whether written consent was obtained. CARE Guidelines may be followed for ensuring adequacy and transparency while publishing case reports [12]. The International Committee of Medical Journal Editors (ICMJE) guidance states that "Informed consent should be obtained if there is any doubt that anonymity can be maintained". For example, masking the eye region in photographs of patients is inadequate protection of anonymity [12].When publishing family genograms,

journals should require consent from family members [14].

Registration of trials: Publication of clinical trials requires a prospective registration of the trial in national/ international registries, which should be included in the text of the main manuscript.

Reporting standards: Authors are required to report their study in a manner conforming to the relevant reporting standards, e.g., Consolidated Standards of Reporting Trials (CONSORT) for clinical trials, Standards of Reporting Observational Studies in Epidemiology (STROBE) for observational studies [15].

EDITORIAL STANDARDS

Authorship criteria and dispute: Authorship depicts contribution of the person in the research published, and has far-reaching academic and social implications, being linked with promotions, recognition, credit and accountability. It is different from contributor ship which may only signify one's participation in the study without any authorship [16]. ICMJE recommends fulfilment of all of the following criteria (**Box 2**) to be eligible as an author. Those who do not satisfy the authorship criteria but may have helped in data collection or supervision of the study, may be named in the acknowledgement section.

The names and the order of authorship order are confirmed by the authors cannot be modified or changed after submission without the permission of the editors. It is recommended to decide the authorship before starting the study to avoid confusion and unpleasantness during manuscript submission. Sometimes, the name of a large collaborative group may be used in authorship where individual members may also be recognized by names for due credit. The corresponding author is the person responsible for submission and communication with the journal [17].

A dispute regarding authorship may occur when an author's contribution is not highlighted or is falsely credited. Unethical authorship practices are usually driven by the pressure to publish [18,19]. A common authorship misconduct is guest authorship where peers and colleagues, are added a co-authors on mutual agreement without having fulfilled the criteria for authorship. Authorship may also be unjustifiably gifted to co-authors as a sign of gratitude and for shared responsibility for work, though not fully qualifying authorship. This is sometimes done to acknowledge supervisors or those involved in financing. An honorary authorship is one which is granted to a senior with administrative/hierarchical powers, even without having contributed significantly to the development of the manuscript, to facilitate publication, appease authorities at work (coercive authorship) or improve credibility of the manuscript among readers [19]. The most serious form of misconduct is sold authorship where authorship is obtained in lieu of money. Ghost authorship is the reverse of the above forms of authorship, where there is a wrongful exclusion of a contributor's name. This may happen when a hired professional author is recruited for publication purpose, or when the professional alliance or insufficient experience of a peer may endanger the reputation of the publication. Use of scientometric methods like tracking publication profile and biblio-graphic data via online platforms can help detect likely suspicious activity [20]. Around one-third of 1246 authors, majority of whom had published in journals with impact factor between 2 and 5, reported chief reasons for gifted authorship as complimentary and to avoid conflict at work, or increase the article acceptance rate. Articles from Europe and Asia, especially case reports/series and those with higher number of authors, were more likely to receive honorary authorship [21]. A significant decline in ghost-authorship has also been recorded with professional medical writers now receiving due credit [22].

Box 2 ICMJE Criteria for Authorship [13]

- Substantial contributions to the conception or design of the work; or the acquisition, analysis, or interpretation of data for the work; AND
- Drafting the work or revising it critically for important intellectual content; AND
- Final approval of the version to be published; AND
- Agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Source: ICMJE Recommendations for the conduct, reporting, editing, and publication of scholarly work in medical journals [13].

Contributor role taxonomy (CRediT) has been recently introduced as a more structured format of declaring author contributions. It shows the credit for being in lead, equal or supportive roles for different aspects of a manuscript development, namely, concep-tualization, methodology, software, validation, formal analysis, data curation, investigations, resources, writing of original draft, writing-editing and review, visualization, supervision, project administration and funding [23]. Such systematic and structured declaration of contribution increases transparency in authorship and helps to identify individual authors, thus being more advantageous in collaborative research [24]. The Consortia for Advancing Standards in Research Administration Information (CASRAI) is a non-profit, Canada based organization, which manages and supports CRediT taxonomy. The Contributor Role Ontology (CRO) is an open community resource which credits author contributions as an exten-sion of CRediT [16]. Creation of a persistent identifier to track a person's

name, affiliation and research work can help construct a scholarly graph for the particular person, comprehensively displaying the research credentials. Options for creating persistent identifiers are mentioned in **Web Table I,** which may have open or guarded display.

Pre-publication: Pre-publication of a manuscript or its part may be done by the authors on informal platforms other than the journal. Pre-publication does not undergo peer-review or text formatting as per the journal's instructions. It is thus quicker and easier but the credibility and validity of the content in pre-publication may be questionable. The details of pre-publication of an article should be communicated to the journal during submission.

Funding: Complete details of funder, the recipient, grant number and date of approval for a project should be declared in the manuscript, in order to acknowledge the role of funding agencies and to maintain transparency in research.

Conflict of interest: Conflict of interest (CoI) is a relationship or acquaintance like employment, stock ownership, partnership, honoraria, patents, etc., which may involve the author directly or through immediate family member. This may be perceived to introduce bias while publishing the results of a study or during the peer-review process, even when the judgment may not have been influenced. The declaration of such competing interests is entirely the responsibility of the authors in order to maintain transparency. Authors may best avoid getting into agreements with study sponsors for the rights of study data analysis and publication. In addition to authors, editors and reviewers should also disclose any CoI which may introduce bias in their decisions. A disclosure statement of the editorial staff may be declared by the journal from time to time [25].

Peer review: Peer review is a process of independent assessment of the submitted manuscript by a reviewer, applicable for all categories of articles, including invited reviews. However, subjecting a manuscript to peer-review process is not mandatory if the editorial board decides to reject it at the very outset, on the grounds of inappropriate quality or content as per the mandate of the journal. Peer reviewers are selected by invitation and are usually anonymized to ensure transparency. In a single-blind review, the identity of the reviewer is blinded from the authors, while the identity of authors is known to the reviewer. In a double-blind review, the identities of both reviewers and authors are blinded to each other. The final editorial decision may not strictly abide by the reviewers' comments, but comments of all reviewers and final editorial decision should be shared with the reviewers of the paper for improving learning. Reviewers should also maintain confidentiality and sanctity of the review process, without infringement of the intellectual content of the paper. Traditionally peer-review means commen-ting on an article before it is accepted for publication. However, with an increase in online journals where manuscript processing is fast-tracked, a peer-review may be done after the publication of the article. An informal post-publication review could be submitted at blogs or newsfeeds. Recently, few third-party websites provide access to the reviewers and authors to interact like PubPeer and PubMed Commons. The post-publication review thus increases the opportunity of discussion with more experts on the research, though the comments may get overwhelming and may need to be moderated. Journals usually acknowledge the contribution of peer reviewers [25]. Persistent identifiers can be created to credit the reviewers for their quality reviews acknowledging their contribution for further promotion and recognition [16].

Appeals: Authors can make an appeal against an editorial decision or editorial handling process. Editors usually acknowledge the appeal, though they may or may not revert their decision. Appeals should however, be made only when there is a genuine concern like technical errors or conflict of interest of peer-reviewers involved in the review process.

Corrections/erratum: Journals may sometimes need to publish corrections or corrigendum for previously published information, which may include correction in authors' names (not addition or deletion of an author), typographical errors in results or any modification in a reported fact in the paper which inadvertently changed the interpretation or meaning of the statement. The corrections in the results should not alter the conclusions drawn earlier. It can be reported by the author or a reader and needs to be confirmed by the authors before incorporation. An update of a previously published guideline or recommendation is not a correction, and should be published anew as a fresh manuscript. The corrected manuscript published in the journal, should also be displayed with the previous version of the article. The most recent version of the article should be cited for reference [26].

Retractions: A manuscript is retracted or removed from the journal if a serious degree of publication misconduct or a gross error in reporting results is identified, after publication of the paper. Common instances where papers have been retracted include plagiarism, falsification of data, misclassification or miscalculation leading to communication of wrong conclusions, or objection by third party for fraudulent work [26]. Retracted papers can be searched at *http://retractiondatabase.org/* or *http://retractionwatch.com/* which provides the date, journal, authors and country, as well as the reasons for retraction.

The announcement of the retracted paper should be displayed along with the abstract and full text of the paper at all places.

Withdrawal of articles: This pertains to removal of an already submitted article before it has been published, usually in view of ethical misconduct, or rarely due to author's personal reasons.

Copyright and intellectual property: All journals demand a written agreement by the authors for transfer of copyright of the article, including all its contents, to the publisher, after publication of the article. Thus, a manuscript submitted to a journal, with a signed copy-right transfer agreement, becomes the copyright of that journal and the authors forfeit all claims or intellectual right over the published work. Subsequently, the information in the article may only be used by the authors for honest and non-malafide interests, with due permission of the editor-in-chief.

CONCLUSIONS

Publication of medical research has significant implications for influencing public awareness, health policies, guidelines, vaccine development, drug licensing, etc. It also determines the credibility and honour of an author and his institution. As authors and reviewers, fabrication, falsification, or plagiarism should be strictly avoided. Authors should fulfill all the ICMJE authorship criteria and disclose any potential conflicts of interest or funding. It is our responsibility as researchers to uphold the standard and reliability of scientific reporting by following ethical practices in publishing.

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Web Table I Persistent Identifiers for Authors and Reviewers [16]

Open display and access • Cross Ref. *https://www.crossref.org* • Open Citations. *https://opencitations.net* • ORCID. *https://orcid.org* • Research Organization Registry (ROR). *https://ror.org/about* • Semantic Scholar. *https://www.semanticscholar.org* • VIAF. *http://viaf.org* • VIVO. *https://duraspace.org/vivo* • Wikidata Scholia. *https://www.wikidata.org/wiki/Wikidata:Scholia*
Guarded display • Dimensions. *https://www.digital-science.com/products/dimensions* • Google Scholar. *https://scholar.google.com/* • Microsoft Academic. *https://academic.microsoft.com* • Publons. *https://publons.com* • Scopus. *https://www.elsevier.com/solutions/scopus* • Symplectic Elements. *https://www.symplectic.co.uk* • Web of Science. *https://clarivate.com/webofsciencegroup/solutions/webof-science/*
Limited access • Academia.edu. *https://www.academia.edu* • Research Gate. *https://www.researchgate.net*

Cross-sectional Study to Identify the Range of Hemoglobin Levels in Normal Infants, Children, and Adolescents in India

Source Citation: *Sachdev HS, Porwal A, Acharya R, et al. Haemoglobin thresholds to define anaemia in a national sample of healthy children and adolescents aged 1-19 years in India: a population-based study. Lancet Glob Health. 2021; 9: e822-31.*

SUMMARY

In this population-based study, the authors constructed age-specific and sex-specific hemoglobin percentiles from values reported for a defined healthy population in the Comprehensive National Nutrition Survey (CNNS). Age-specific and sex-specific 5th percentiles of hemoglobin derived for this healthy population was used as the study cut-off to define anemia. These were compared with the existing WHO cut-offs to assess significant differences between them at each year of age and sex for quantifying the prevalence of anemia in the entire CNNS sample. From the CNNS survey 41210 participants had a hemoglobin value, 8087 of whom were included in this study and comprised the primary analytical sample. Compared with existing WHO cut-offs, the study cut-offs for hemoglobin were lower at all ages, usually by 1-2 g/dL, but more so in children of both sexes aged 1-2 years and in girls aged 10 years or older. Anemia prevalence with the study cut-offs was 19·2 percentage points lower than with WHO cut-offs in the entire CNNS sample with valid hemoglobin values across all ages and sexes (10·8% with study cut-offs vs 30·0% with WHO cut-offs). The authors concluded that these findings support the re-examination of WHO hemoglobin cut-offs to define anemia.

COMMENTARIES

Evidence-based Medicine Viewpoint

Relevance: This analysis [1] was designed to identify the range of hemoglobin levels in normal infants, children, and adolescents in India, and thereby derive age and gender appropriate cut-off thresholds to define anemia. The justification was that currently used operational definitions of anemia are based on thresholds set by the World Health Organization over five decades back. The evidence-base for defining these thresholds had several lacunae, meriting a definitive study with robust methodology to address the issue [1]. Further, data from the National Family Health Survey-4 was insufficient to resolve the issue on account of missing information across the age spectrum.

Study methods: The analysis [1] was designed as a cross-sectional examination of hemoglobin level among a representative nation-wide sample of infants, children, and adolescents deemed to be, as near normal as feasible. Although defining an individual as normal or healthy (as opposed to asymptomatic) is extremely difficult, the investigators did this by systematically excluding participants (from the analysis), who could have a clinical condition (determined by measurement of biomarkers) that affected erythropoiesis and/or hemoglobin levels.

The sample population was derived from the Comprehensive National Nutrition Survey (CNNS), designed to analyze the nutritional status, prevalence of specific micronutrient deficiencies, and associated risk factors in a nationally representative cross-section of infants, children, adolescents, and their households [2,3]. CNNS was conducted in a systematic manner across all 30 Indian states [3], using a multi-stage sampling design method, in order to include a represen-tative sample of households and participants from birth through adolescence. Over 112000 such participants were included, and biological specimens, including blood samples, were obtained from more than 49000 participants older than 1 year [3].

For this analysis [1], there were 41210 participants >1year whose hemoglobin level was available from the CNNS. Among them, those with laboratory evidence of any condition that could affect hemoglobin content adversely, were systematically excluded to achieve a filtered group of 'normal' participants. The first step of filtering excluded participants across all ages with iron deficiency (measured by serum ferritin and transferrin receptor levels), folate or vitamin B12 deficiency (measured by the respective levels in serum), inflammation (defined by C-reactive protein), vitamin A deficiency, and two abnormal hemoglobin variants viz.

HbS and HbA2. Those with hemoglobin value exceeding 5 standard deviations were also excluded, although the reasons were not given. The resultant pool of participants was used for the primary analysis. Among those older than 5 year, additional exclusions were made on the basis of biomarkers suggesting abnormal renal function (serum creatinine), dyslipidemia (serum cholesterol), and abnormal glycemic control (HbA1c). Three additional sub-groups were created by excluding those with *i*) low or missing serum albumin values (across all ages), *ii*) low or missing zinc levels (among those >5 year), and *iii*) evidence of stool parasitic infestation, or unavailable data for this (among those >5 year). The investigators then determined the range of hemoglobin values at age intervals of 1 year, and constructed percentile curves. The 5th percentile value was chosen as the threshold to define anemia. Data from participants in subgroups other than the primary analysis group were used for sensitivity analyses. Lastly, the investigators compared the age-wise prevalence of anemia by their definition against the thresholds prescribed by the World Health Organization.

Critical appraisal: Critical appraisal of the study methodology using criteria from tools designed for the purpose [4-7], is summarized in **Box 1**. Additional issues are highlighted below.

The authors reported that 8058 of 49486 (16.3%) blood samples obtained could not be analyzed because of insufficient volume or sample spoilage [1]. In a study with robust training of personnel, stringent sampling methods, meticulous storage and handling [3], loss of 1 in 6 samples appears to be disproportionately high.

It is unclear why the investigators chose the 5th percentile as the lower limit of the normal range. In Gaussian distributions, values below the 2.5th percentile mark (or two standard deviations) are generally considered abnormal. The authors mentioned that using this threshold would have resulted in a lower prevalence of anemia among the normal population, and a greater divergence from the WHO prevalence [1]. But this should not be a deterrent, considering the methodological limitations and biases in the studies from which the WHO thresholds were derived.

Although, the investigators did not present the age-wise range of hemoglobin values of the normal children and adolescents in the analysis, this can be indirectly inferred from the smoothed percentile curves in the publication [1]. Hemoglobin values in normal boys range from 9.0-13.5 g/dL at 1year, rising to 13.0-17.0 g/dL by 19 year. In normal girls, it ranges from 9.0-14.0 g/dL at 1y, with negligible increase beyond 5 year, remaining almost static in the range of 10.0-14.0 g/dL throughout childhood and adolescence. It appears that the 50th percentile value in boys rises steadily from about 11 g/dL at 1 year, to approximately 12.0 g/dL at 4 years, 12.5 g/dL at 10 years, and approximately 13.5 g/dL by 19 year of age. In contrast, the 50th percentile in girls, is around 11 g/dL at 1year, rises very slightly to around 12.0 g/dL by 6 year of age, and thereafter hovers around this level all through childhood and adolescence. The 5th percentile values paralleled the 50th percentile values. In boys, it was approximately 9.0 g/dL at 1 year, 10.0 g/dL at 5 year, 11.0 g/dL at 10 year, and just below 12.0 g/dL at 19 year. In girls, it was around 9.5 g/dL at 1 year, rising to just above 10.0 g/dL throughout childhood and adolescence. In both sexes, the values were 1.0 to 2.0 g/dL below the WHO thresholds used to define anemia.

Based on these thresholds, it will be interesting to see the hemoglobin values in children and adolescent with different types of anemia. This data is already available in the CNNS; one publication reported the prevalence of different types of anemia, but did not reveal the actual range of hemoglobin values [8]. It is essential to study the overlap in hemoglobin values between normal children, and those with different types of anemia.

The analysis [1] also has some additional interesting findings, not highlighted by the authors. For example, 13499 of 21586 children had to be excluded from the primary analysis on account of having laboratory markers of known causes of anemia. This translates to 62.5% of children across all age groups, suggesting that the prevalence of anemia (defined by robust laboratory biomarkers) is extremely high. Similarly, among those older than 5 years, 13407 of 19803 (67.7%) participants had to be excluded because of laboratory parameters confirming different types of anemia, or chronic conditions impacting hemoglobin. This suggest that two-thirds of children and adolescents have clinical conditions reducing hemoglobin. Likewise, 1203 of 5657 (21.3%) children >5 year old had lab confirmed zinc deficiency, and 767/4687 (16.4%) had stool infestation with parasites.

In the CNNS, 70% participants from 1-19 year had no anemia based on the WHO thresholds [1,8]. However, more than 60% participants had one or more laboratory markers of anemia (due to various causes), necessitating their exclusion from this analysis [1]. How to reconcile this difference? Three explanations are possible. One is that laboratory markers of anemia in children and adolescents, somehow do not go hand in hand with hemoglobin values, i.e., the markers could be far more sensitive than hemoglobin. The second is that the criteria for excluding >60% participants were 'any abnormality' in

Box I Critical Appraisal of the Study

A. *Clarity of aims and objectives.* The objective of this analysis was to determine the hemoglobin levels in normal infants, children, and adolescents in India, by age and gender, and thereby identify the lower limit of normal values to define anemia.

B. *Appropriateness of study design for the stated aims.* The cross-sectional study design provides a point estimate of the hemoglobin level in different age groups of participants, and hence a point prevalence of anemia. The cohort study design wherein a birth cohort could follow throughout childhood and adolescence to determine the actual trend of hemoglobin, is cumbersome and fraught with a different set of potential biases.

C. *Sample size.* The CNNS was designed to include at least 20350 children across the country in each of three groups, for measurement of laboratory biomarkers [3]. The sampling framework was adjusted to include participants from rural and urban locations, as well as residence in slums and other settings.

D. *Representativeness of included participants.* Efforts were made in the CNNS to ensure nationally representative participants across 30 states, representation of rural and urban areas, slum and non-slum residence, socio-economic status, etc [3]. However, in this analysis [1], there were unexpected deviations that could impact representativeness. For example, two-third of participants belonged to the two highest wealth quintiles, there were more males than females, and participants from rural areas outnumbered urban participants. The CNNS did not include blood sampling of infants younger than 1 year old. Similarly, the systemic diseases associated with anemia could be evaluated (and excluded) by laboratory tests only in children older than 5 year. The investigators rightly included only one participant in each age bracket, from a single household.

E. *Criteria for inclusion in the study*. In this analysis [1], the investigators included a group of children that can be considered 'normal'. This was achieved by systematically excluding participants from the CNNS who had laboratory evidence of clinical conditions that could affect hemoglobin level adversely.

F. *Description of study setting and participants.* Although not described in this report [1], the CNNS documents [3] describe the overall survey participants, including their demographic characteristics, social background, type of residence, family wealth status, maternal empowerment status, etc.

G. *Criteria for measurement of the condition/outcome of interest*. The CNNS used the current gold standard method for automated estimation of hemoglobin, adding several precautions and refinements for sample collection, transportation, and storage [3]. The CNNS also ensured quality control measures in laboratory methods, validation of laboratory procedures, and sufficient training for all personnel involved [3]. The authors did not have to undertake any additional measures for this analysis [1].

H. *Identification and handling of confounding factors*. The nature of this analysis was to exclude participants with any factor(s) that could confound the designation of participants as 'normal'. This was achieved very well, although strictly speaking, there is no limit to the exclusions that could be made. For example, it is unclear whether menstruating girls were excluded from the analysis. If not, the proportion of such participants and impact on the hemoglobin levels of the group, need to be evaluated. Unfortunately, the exclusion of all laboratory abnormalities (to obtain a completely normal cohort) could not be done because it reduced the sample size to very low levels. However, the authors did not disclose the actual number.

I. *Validity and reliability of outcome measurement.* In this analysis [1], the main outcome (anemia), was simply defined as hemoglobin less than the 5th percentile for that age and sex. Presumably the exact age of children was used for calculation (and not the nearest whole number), although it was not specified [1].

J. *Information about non-responders and those with missing data*. The authors reported that participants excluded from the analysis [1] were not very different from those included, in terms of age, growth parameters, and hemoglobin level. However, there were differences in household wealth and some other undisclosed factors.

K. *Statistical methods.* Appropriate statistical methods were used and described. In the CNNS, an elaborate weighting method was used to manage variations in the probability of inclusion. Analysis in the CNNS [3] and this study [1] were done in weighted samples. Additional sensitivity analyses were undertaken to deal with the problem of not being able to include children having only completely normal laboratory parameters.

L. *Funding information*. The CNNS itself was supported by the Mittal Foundation [3], however, this analysis of the data therefrom [1] was not funded. However, the authors additionally reported that the funder did not have any role in any aspect of this work [1].

seven laboratory parameters, whereas the criteria for defining types of anemia used a combination of two parameters to define iron-deficiency anemia [8]. The third possibility could be that participants with abnormal vitamin A levels and abnormal hemoglobin variants were also excluded, whereas these two causes were not counted in the proportion with anemia. However, it seems unlikely that non-inclusion of these two causes could reduce the anemia prevalence by half.

In this analysis [1], the mean *z*-scores for anthropometric parameters were lower than 0, in fact closer to -1.0 [1]. Although this fits within the broad range of normal anthropometry, it suggests that the participants were thinner/smaller than expected. This raises the question whether the low(er) hemoglobin recorded in them could be a cause.

Interpretations and implications: Although the functional implications of low(er) hemoglobin levels in normal children and adolescents were not explored in this analysis [1], it necessitates re-thinking the clinical as well

as public health consequences of anemia. If haemoglobin as low as 9.0 g/dL can be considered normal in infants, could it have any impact on growth, development, physical performance and cognition? Thresholds for initiating prophylactic and therapeutic micro-nutrient supplements in individual children would need revision. Blood transfusion thresholds in acute and chronic conditions may need to be re-examined. There could be public health implications in terms of modifications in focus, resource allocation, etc. One indirect silver lining could be that the thriving micronutrient supplement industry and the associated irrational prescription (and self-administration) of these products, may decline.

Conclusion: This analysis of data [1] from the CNNS [3] suggests that normal Indian infants, children, and adolescents have a wide range of hemoglobin across all ages. The lower limit in normal participants appears to be much lower than expected, calling for a re-look at the thresholds to define (and manage) anemia.

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Public Health Viewpoint

Anemia has continued to be a public health problem in India since many decades despite various interventions to reduce it at the national level. The prevalence of anemia among children 1-4 year, 5-19 year and among adolescents were reported as 40.5%, 23.4%, and 28.4% respectively. [1] The future scenario regarding anemia prevalence is also bleak in India and the neighboring South Asian Countries as the projected anemia prevalence among women in 2030 are 48%, 25%, and 32%. This means that India would still be falling short of anemia related nutrition targets of Sustainable Development Goals. [2]

The authors have diligently used the Comprehensive National Nutrition Survey data 2019 to answer a nagging public health nutrition question since many decades [3]. This study reports that that the newer cut offs suggested by this study lowers the prevalence of anemia among the entire CNNS sample by around 20 percentage points when compared to that with WHO cut offs. It also lowered the prevalence of anemia by 25·1 percentage points in ages 1-4 years, 15·3 percentage points in ages 5-9 years, 15·6 percentage points in ages 10-14 years, and 22·3 percentage points in ages 15-19 years. Even a slight change in cut-off will lead to a large impact on the absolute numbers for a populous country like ours. The indirect effect of such a change will also be on the possibility of increased resource allocation to various other public health problems. This study coerces the global health organizations as well as the pediatric associations across the world, by providing evidence from a large-scale study to rethink about hemoglobin cut offs, in India and for other countries [4]. Another study has also found similar results for anemia cut off among adult Indian population [5]. We need more studies primarily designed to find out hemoglobin cut offs for anemia in South Asian countries, including India. The public health impact of this study goes much beyond anemia. It also questions the need to re-examine the cut offs and the prevalence of other micro- and macro-nutrient deficiencies.

However, it would be pre-emptive to be celebrate the reduction in anemia using the lower cut offs in the study being discussed. We could be careful that the sustained efforts towards anemia reduction such as Anemia Mukt Bharat Abhiyaan could be slowed down with the present

findings. Despite the lower prevalence of anemia, as per the new emerging cut offs from this study, we still have a long way to go to eliminate anemia among children, and adolescents in our country.

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Contemporary Researcher's Viewpoint

Anemia continues to be a severe public health problem in India despite multipronged efforts through government programs for anemia control being in place for over five decades [1]. Like many developing countries, the World Health Organization (WHO) hemoglobin (Hb) cut offs are used in India to define anemia. These cut offs were proposed over half a century back in 1968. The cut offs were based on the studies performed predominantly on white populations in Europe and North America, which were reviewed by a group comprising clinical and public health experts [2]. Data from other ethnic groups/races/countries was not available for review. Recently, the validity of these cut offs is being questioned, as they appear to be higher for population groups from low- and middle-income countries. In fact, these widely used cut offs are under review by WHO itself in view of genetic and racial variations and other emerging evidences [3, 4].

Sachdeva, et al. [4] have recently published age and sex-specific Hb percentiles, which are based on values reported for healthy population in the CNNS 2019. From the whole survey population, authors have excluded children with low serum ferritin, folate, vitamin B12, and retinol levels. Also excluded were children with evidence of inflammation; variant Hb and history of smoking. They considered age and sex-specific 5th percentiles of Hb derived for this stringently defined healthy population as the study cutoff to define anemia. The authors then compared these thresholds with existing WHO cutoffs for children at each year of age and sex for quantifying the prevalence of anemia in the whole CNNS sample [4].

Compared with existing WHO cut-offs, the study cut-offs for Hb were lower at all ages. Anemia prevalence with these cutoffs was 19·2 percentage points lower than with WHO cutoffs in the entire CNNS sample [4]. In a similar study, comparing Hb in adults of different racial/ethnic descent, the Hb cut-off for mild anemia in Asians was lower at 11.22 g/dL. Using the Hb cut-off derived in this study by Verghese, et al. [5] in place of the WHO cut-off of 12 g/dL results in a 17.9% point decrease in the prevalence of anemia in India.

If the cut offs proposed by these two studies are accepted, prevalence of anemia in India will be under 40% which is the cut off for defining anemia as a major public health problem! A good feeling indeed. However, it may lead to complacency in government efforts for anemia control as the figure for overall anemia prevalence will still be very high.

Firstly, as pointed out by Verghese et al. [5], this will result in decrease of prevalence of mild anemia. However, the grades of severity of anemia based on Hb will not change [6]. We have to be cognizant of the fact that micronutrients' deficiencies may cause harm to developing brain and body in young infants even before anemia develops [7].

Most significant impact of this lowered cut off for defining anemia (and also labeling individuals with normal Hb) will be a change in Hb target while treating hematological conditions where therapy is aimed at keeping Hb normal for age. Most patients with transfusion dependent thalassemia (TDT) are managed on hyper transfusion regimen which aims to maintain a baseline hematocrit 'as nearly normal as practicable' [8]. With the lowered cut offs for defining normal or low Hb, a lowered cut off will be used for transfusion in TDT. For some conditions such as immune hemolytic anemia, an arbitrary target Hb of 10 gm/dl is advisable when tapering of therapy begins [9]. This may remain unchanged.

In conditions such as aplastic anemia and nutritional anemia also, transfusion practices may remain unchanged. Transfusion guidelines for anemia in children

with severe malnutrition recommend transfusions at Hb of 4gm/dl or 4-6 gm/dL if patients have respiratory distress [10,11]. Similarly, most patients with aplastic anemia receive transfusions between Hb 6-8 gm/dL [12]. However, as aim of the therapy while treating deficiency anemia is to bring Hb to normal (and continuing therapy further for replenishing the store), the target Hb will be set lower.

Secondly, the hematology consultations are likely to decrease. Maximum hematology consultations-over a quarter- are for anemia which takes away a significant time of the hematologists [13,14]. However, the decrease in consultations may not be as pronounced as the decline in anemia prevalence may indicate as most consultations for anemia are for moderate or severe anemia [14]. Anemia is the commonest cause of donor deferral in our country. Various studies have shown that anemia accounts for 6.5% of all donor deferrals and over 50% of all deferrals [15-17]. This is because the Hb cut off for donor suitability is 12.5 gm% for both male and female donors [17]. This cut off is likely to change in light of the lowered Hb cut off for defining anemia. This would result in more donor availability.

At the national level, the intervention required for alleviating anemia may become less intensive, require fewer financial inputs and will be more target-oriented due to lower prevalence.

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Pediatric Hematologist's Viewpoint

Anemia is defined as hemoglobin level more than two standard deviations below the mean for the age and sex of the child. It results in decrease in the oxygen carrying capacity of the blood leading to several physiological changes. It affects not only the growth and development of the child but may also lead to several soft neurological signs. Although India has made considerable progress in terms of health indicators such as neonatal and infant mortality rate, maternal mortality etc, anemia has remained a significant health problem in children and adolescents [1]. The National Family Health Survey (NFHS-3) revealed that approximately 70% of Indian children had anemia. The scenario was only slightly better in National Family Health Survey (NFHS-4) with almost 60% children suffering from anemia [2]. The cut-offs used for defining anemia in Indian children has been based on the studies by World Health Organization (WHO) which were carried out almost 50 years ago on predominantly white population [3]. Previous analyses of data from National Health and Nutrition Examination Survey (NHANES) have shown that hemoglobin concentrations among healthy Asian, Hispanic and Black population were lower

compared to the White population [4]. This variability has also been seen among different racial groups within the same country. These findings indicate that the cut-off for anemia needs evaluation in specific population groups [5]. This has significant implication for India where hemoglobin cut-offs determined in the local population could reduce the estimated prevalence of anemia. The present population-based study, which is representative of the healthy population of children and adults in India, supports the re-examination of WHO cut-offs to define anemia and seems suitable for national use [6].

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ERRATUM

Please note following corrections in the article titled "Effect of a Home Safety Supervisory Program on Occurrence of Childhood Injuries: A Cluster Randomized Controlled Trial" published in Indian Pediatr. 2020;58:548-52.
In Table I, sixth row, second column; the percentage should be '80%' in place of '83%.'
In figure 2, lowermost box; the text should be '…..caregiver supervisory attitude assessed' in place of '…caregiver supervisory attribute assessed.'
Appropriate corrections have already been done in the web version at *http://www. indianpediatrics.net/June2021/548.pdf*

Large Left Ventricular Thrombus in a Neonate - A Rare Complication of Severe Persistent Pulmonary Hypertension of the Neonate

A girl weighing 3.6 kg was born at term via spontaneous delivery through thick meconium after an uneventful pregnancy to a healthy primigravida mother. Apgar scores were 4 and 7 at 1 and 5 minutes, respectively, and cord blood pH was 7.27 with base excess of –5. She presented with respiratory distress and was diagnosed with meconium aspiration syndrome requiring chest drain, and mechanical ventilation. At 18 hours, she was diagnosed on echocardiography with persistent pulmonary hypertension of the neonate (PPHN), and inhaled nitric oxide (iNO) was added. She improved initially, did not require inotropes, and was off iNO, but she deteriorated suddenly on day 8 with pallor, hypertension and tachycardia – there were no concurrent central line or sepsis. Urgent echocardiography revealed severely reduced biventricular function and a large, 11×10 mm left ventricular thrombus, which resolved with enoxaparin and milrinone. Cardiac function remains normal at the age of 2.5 years, without any personal or familial underlying cardiomyopathy or prothrombotic condition.

Treatment of PPHN consists of ventilation, iNO, and inotropic support. Thromboembolic events, of which 16% are intracardiac, occur with risk factors such as central access, extreme prematurity, and inflammation. Intracardiac thrombi in the absence of congenital heart disease, cardiomyopathy, or post cardiac surgery are rare. Symptoms for central arterial thrombosis may be absent, or subtle such as lethargy, thrombocytopenia, or mimic coarctation. Therapeutic recommendations depend on thrombus location, etiology, and must be weighed against bleeding risk.

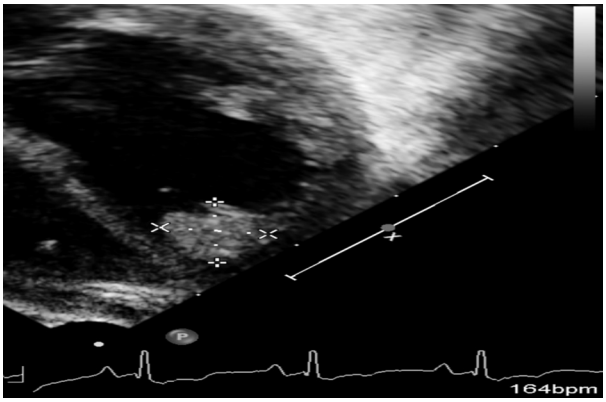

Fig. 1 Four chamber apical trans-thoracic echocardiographic image with left ventricular thrombus (with measurements).

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Language Barrier in Anganwadis in Eranakulam, Kerala

The utilization of Integrated Child Development Services in Kerala was found to be less than optimum in our previous study in Njarackal, Eranakulam, Kerala [1]. We conducted a qualitative study in the same area between March and May, 2018, to determine the reasons for underutilization of services. The method consisted of a focus group discussion with 11 mothers with young children, and in-depth interviews with two anganwadi workers and a Child development program officer. The recorded interviews were transcribed, themes were identified and triangulated.

Most mothers and functionaries desired better physical infrastructure at the anganwadis, where the children could run around and play. The mothers felt that most anganwadis had very limited infrastructure, so children had to sit and sleep in a small room throughout the day. Most mothers were satisfied with the cleanliness, food and attention given by the staff at anganwadis. Despite many shortcomings, most mothers were willing to send their children to the anganwadi. Some mothers commented "*children eat more when they are at anganwadi than at home.*"

The main reason for the parents not sending children beyond 4 years to the anganwadi was absence of English education. The medium of instruction in anganwadis is Malayalam, the local language and mother tongue of most children. English is not taught as part of nonformal education, except for the alphabet. Most parents conveyed their desire to admit their children into English medium schools in class I, where a major eligibility criterion is basic knowledge of English. Those students who attend pre-school at English-medium kindergartens have significant advantage over those who attend Malayalam-medium anganwadis. Hence, parents opined that they withdraw their children from the anganwadis and send them to private English medium kindergartens. Some mothers

were worried that "*children don't learn English, computer and Hindi at anganwadi*."

Parents are unlikely to change their preference for English-medium schools, and the government also has valid reasons for the language policy [2]. So, there does not appear to be a clear solution to the demand noted in this study for more English teaching in anganwadis. As there is a demand for English education in many parts of India, as is evident from news reports [3,4], a revision of curriculum of anganwadis needs to be considered, which may possibly improve acceptability of anganwadi services [3,4].

Ethics clearance: Institutional Ethics Committee, Amrita Institute of Medical Sciences, Kochi; No. IEC-AIMS-2016-COMM-182, dated December 31, 2017.

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Hemolytic Disease of Newborn: Beyond Rh-D and ABO Incompatibility

A full term female baby, with a birth weight of 2.24 kg presented with yellowish discoloration of eyes and skin at 18 hours of life. Antenatally, there was no evidence of fetal anemia or hydrops fetalis. On examination, baby had icterus till palms and soles but mental status, cry and muscle tone were normal. Baby was started on intensive phototherapy. Blood group was O positive in both the mother and the baby. Laboratory investigations showed total serum bilirubin (TSB) of 24 mg/dL with direct bilirubin (DB) of 2.72 mg/dL, reticulocyte count of 24%, direct Coomb test (DCT) of 3+, and hemoglobin of 8.2 g/dL. Peripheral smear was suggestive of hemolysis with macrocytes, microcytes, spherocytes and polychromasia with nucleated RBC.

In view of strongly positive DCT and no Rh negative or ABO setting and minor blood group incompatibility was done. The results of antibody testing for screening tests revealed the presence of multiple alloantibodies (anti-Rh17) in maternal serum. The Rh-kell antigenic phenotyping of the family members were as follows: Mother (blood group O, D+, C-, c-, E-, e-, K- (i.e D–), index case (blood group O, D+, C+ c-, E-, e+, K-), father (D+, C+ c-, E-, e+, K-) and sibling (D+, C+, c-, E+, e-, K+).

Baby was treated with intensive phototherapy and 1 g/kg of intravenous immunoglobulin (IVIG). Due to this rare variety of blood group, cross matching was not compatible with the available blood in various blood banks despite extensive search. By 30 hours of age, serum bilirubin reduced to 13 mg/dL and was out of exchange transfusion zone. Baby had rebound hyperbilirubinemia, probably due to mild hemolysis by persisting antibodies, and required intensive phototherapy on day 15 of life, and was discharged on day 19 of life. At discharge, parents were advised for regular follow-up for jaundice and anemia, and also counselled regarding the need of anti-Rh 17 antibody titers in next pregnancy, and periodic monitoring for fetal anemia and possible need of intrauterine transfusion.

The Rh blood group system is inherited as group of *Rh D* and *Rh CE* genes located on chromosome 1 [1,2]. Based on the expression of the major D antigen on their RBCs, typing as Rh positive or negative is done. RBCs also express C or c, E or e antigens. Rh deletion of C/c and E/e loci can rarely occur leading to D– blood group phenotype [4].

The mother's RBC Rh-kell phenotyping was (D–). Individuals with D– phenotype produce multiple Rh alloantibodies known as anti-Rh17 or Anti-Hro antibodies against C,c,E,e antigens if there is a history of sensitization such as childbirth (fetomaternal hemorrhage) or transfusion with red blood cells that express C/c or E/e antigens [3]. Anti-Rh 17 antibody is an antibody against Hro (Rh17) antigen and is produced in persons lacking any Rh group antigens except D antigen [4].

The Rh-kell phenotype of the index case suggested that the possible antibodies leading to hemolysis were anti-C and anti-e. The management of anti Rh-17 isoimmunization is similar to the management of Rh D isoimmunization including antenatal monitoring, intrauterine transfusion, use of IVIG and exchange transfusion, with a specification that blood used for fetal or neonatal transfusion should be Rh17 antigen negative blood [3]. However, it may be extremely difficult to get compatible or antigen negative red cells for transfusion. A recent review of case reports showed that most of the cases were managed by exchange transfusion, with or without intra uterine transfusion,

with very few cases managed by phototherapy alone [5]. They also recommend the first choice of blood that needs to be used in these neonates is washed and irradiated mothers' RBCs. If this is not available, then blood of relatives of pregnant woman, usually siblings, can be used. Other options include storage of woman's blood by freezing the RBCs before pregnancy or use of blood from blood banks where this blood is stored.

This case highlights the importance of considering isoimmunization due to other Rh antigens as an important cause of hemolytic disease of newborn.

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The "immunity debt" of the COVID19 pandemic

Unprecedented declines in common childhood infections were observed during the COVID 19 pandemic. These were mainly viral respiratory tract infections, gastroenteritis and some serious bacterial infections due to S. Pnemoniae, N. meningitides and H. Influenza. Even chicken pox fell by almost 40% in France during 2020. All this is largely attributed to the phenomenal implementation of non-pharmacological measures like masking, social distancing and hand hygiene. The fact that in the same period urinary tract infections did not decline gives credence to the idea that lower respiratory tract infections were not merely due to mere underreporting. This was important because it freed up valuable healthcare resources during the pandemic.

However this may add to the population of susceptible individuals resulting in larger epidemics in times to come which has been labelled the "immunity debt". For instance in Australia, no RSV infections were documented in their regular season in the first half of 2020 but was followed by a larger surge in the latter half of 202 which is not the usual season in the Southern hemisphere.

Seminal work done in the 1980's by Gray has documented that children develop repeated asymptomatic infections with various strains of S. Pnemoniae in the first 2 years of life which help in developing a robust immune response to both S. Pnemoniae as well as other viral invaders by blocking various entry points. The pandemic has interrupted the usual circulation routes of these bacteria due to the various interventions like school closures and lock downs. This may finally culminate in larger epidemics in subsequent times.

Besides the "immunity debt" which our children may have to repay at higher cost at a later debt, the worrisome fact is the decline in vaccinations rates all over the world. This may result in a surge of vaccine preventable infections in common times.

Interventions which pediatricians can undertake are emphasizing catch up vaccination of all regular vaccines and encourage other optional vaccine such as influenza, chicken pox, Rota virus and pneumococcus.
(Infectious Diseases Now, 2021)

Guidelines for evaluating febrile infants below 2 months

The American Academy of Pediatrics has just released clinical practice guidelines for the evaluation of well looking febrile infants below 2 months. This is an important problem for pediatricians.

The guidelines are based on data collected from Pediatric Research in Office Practice Network in the US. When a pediatrician sees a febrile infant, the key question we wrestle over is "Does this infant have an invasive bacterial infection or not?"

In the current guidelines, all infants below 21 days need to be admitted. Blood, urine and CSF cultures need to be taken. Empiric intravenous antibiotics must be started till reports are available. Samples for urine culture must be obtained either by catheterization or supra-pubic aspiration.

For infants between 22-28 days, while a urine and blood culture is mandatory, CSF culture may be deferred if procalcitonin levels are below 0.5 ng/mL. Babies between 29-60 days are considered low risk, and merely doing a urine routine examination is enough. Only if it is abnormal, urine cultures may be performed. However, blood cultures and monitoring in hospital with empiric antibiotics till culture reports are available is appropriate. If CSF is required, based on elevated inflammatory markers, evaluation for enterovirus is advised.In India, testing for malaria may also be appropriate in many parts of the country.
(Pediatrics August, 2021)

Opening schools safely in COVID-times

A lot of discussion is going on in the media regarding the impending third wave of coronavirus disease-19 (COVID-19), and the status of children during it. Reopening of schools is getting delayed meanwhile, thereby affecting the children's studies and social interactions. This study analyzed incidental infection data from a reopened private school located in a high prevalence area in Brooklyn, NY and corresponding local and regional community-based testing data (September, 2020 to April, 2021).

For safety purposes, the local health authorities had set up a program for weekly COVID-19 screening in all schools to monitor infection rates. Prevalence data were compared from testing done in school to community prevalence estimates determined from statistical models. SARS-CoV-2 prevalence in schools was lower than prevalence in the corresponding local or general community for all months.

The study, school might not be representative of other schools in different areas, and also school-specific strict COVID-19 protocols and guidelines in this school might have contributed to preventing further cases. Moreover, the student density and safety protocols in Indian schools may also vary from those in the reported school. As there are conflicting guidelines in various states for school reopening, we need robust multi-centric studies to look at the risks involved with school reopening, and take an informed decision regarding this important issue.
(Acta Paediatrica June 23, 2021)

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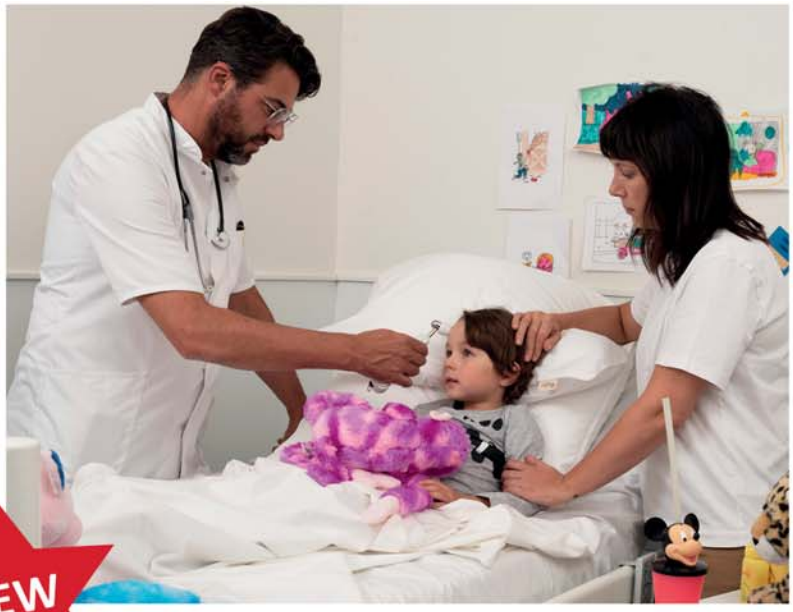

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