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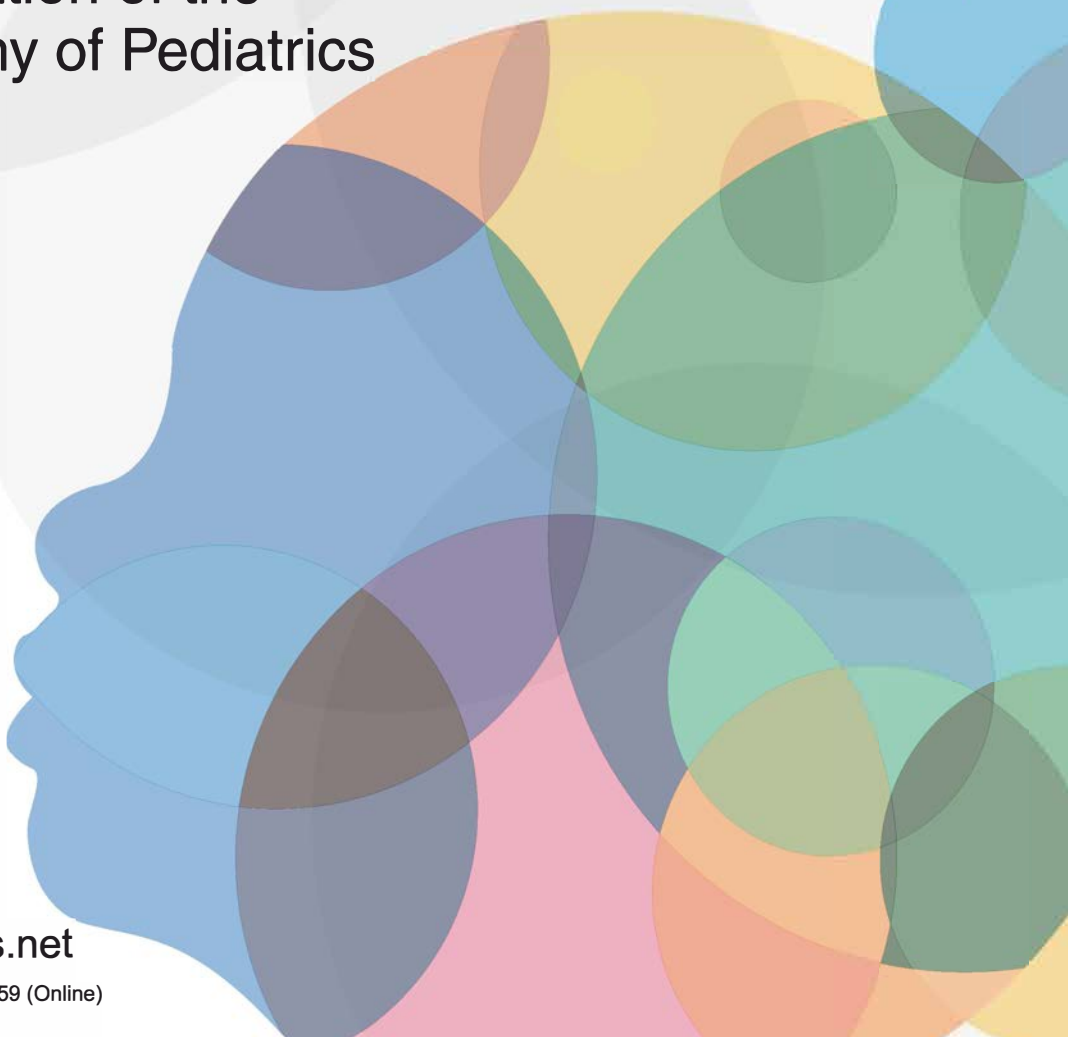

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Address for ordinary letters: **The Editor-in-Chief, Indian Pediatrics, P. Box No. 3889, New Delhi-110 049.**
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Academic Leadership for the Next Decade

BAKUL JAYANT PAREKH
President, Indian Academy of Pediatrics 2020
bakulparekh55@gmail.com

Dear IAPians, It gives me great pleasure to write to you about one of the most critical academic initiatives embarked upon by your Academy.

As the COVID-19 crisis has made us painfully aware, without connectivity and technology, it is impossible to function effectively in a locked-down world.

Even without this wake-up call, the whole world would agree that the Academy, just like all other organizations anywhere in the world, will transform into a Digital Academy. Organizations that do not have an underlying digital infrastructure with cutting edge capabilities will become increasingly ineffective and irrelevant, and fail to survive in the future.

IAPs own highly successful digital education efforts in the recent past, have made us aware of the immense possibilities and vastly enhanced capabilities that technology offers to an academic body like IAP.

No longer do we need to have any barriers of distance or cost to creating and disseminating education. We can also enhance the scope of our education to cover text material, lectures, panel discussions, workshops, case study discussions, and online clinics for doctors. We can conduct academic training from anywhere and access it from anywhere.

All our academic administration shall be made easy by our technology, whether it be registrations, payments, member services, local chapter websites, activities announcements, event calendars, and more.

Under the dIAP program, IAP has focused on building the end-to-end digital capabilities that a large scale teaching institute would require. We are now making the services available as a Digital Center of Excellence (DCOE), accessible on dIAP. The DCOE already includes online certificate courses with testing, an online interactive education platform, and an online reference library of text material and videos. DCOE is available to all IAP members and students of pediatrics. You may access the education services on *www.diapindia.org* on your computer or using your mobile phone browser.

In this context, it gives me great pleasure to announce that IAP shall soon make the Indian College of Pediatrics (ICP) a reality. The Indian College of Pediatrics, an initiative by our Past President Dr CP Bansal in 2013, shall come alive next year and transform our academic activities. ICP will be a leading global university for pediatrics, spanning online, offline and hybrid education. I am confident that ICP will accelerate its launch and growth by leveraging the capabilities built by dIAP.

MORE ABOUT DCOE

The online certificate courses and testing services of the DCOE already have over 150 professionally recorded and edited lectures by IAP experts, and an additional 200 are under production. The lectures will cover all pediatrics areas and organized into over a hundred courses/ modules. The best part about these modules is that they can not only be used for online education, but also by all our local chapters as standardized education material for local IAP experts to conduct physical classroom sessions and hybrid sessions. The recording, professional editing, and publishing are done free of cost by dIAP for all subspecialty chapters.

The online interactive education platform of the DCOE has already conducted over 250 national events in the last three months. This capability is now being made available to all regional, state, and local chapters of IAP to hold online events for their members. For all education events, dIAP enables industry sponsorships. For those events where dIAP is unable to obtain grants or sponsors, dIAP is giving a full subsidy and conducting the events free of cost for the chapters.

The online reference library of DCOE has a searchable archive of over 400 educational videos. And many shall include expert advice forums for IAP members.

INDIAN COLLEGE OF PEDIATRICS

The ICP is being revived, architected, and built as a significant initiative for launch in 2021. The ICP shall be an integral part of the Indian Academy of Pediatrics and serve as the highest academic wing of IAP.

DCOE capabilities shall accelerate the production and publishing of academic content for ICP. ICP will also acquire the administration and teaching capabilities of a full-fledged online university at no additional expense.

The DCOE shall itself be under the academic governance of the Indian College of Pediatrics. The Indian College of Pediatrics shall surely be the most comprehensive and large scale pediatrics university anywhere. Your Academy now has the capability to make this a reality, well before December, 2021.

Jai Hind!
Jai IAP!

NOTICE

Research papers are invited in the following categories of Awards for the year 2021

Award rules may be obtained from the Central Office on request and are also available at *www.iapindia.org/pdf/8297-IAP-AWARD-RULES-2020.pdf*

(The papers not submitted as per award rules will be rejected.)

Dr. James Flett Endowment Award (Two Prizes) for the best papers on "Social & Preventive Pediatrics".

Dr. ST Achar Endowment Award (One Prize) for the best paper on "Pediatrics".

Dr. SS Manchanda Neonatology Research Award (One Prize) for the best paper on "Neonatology".

Dr. V Balagopal Raju Endowment Award (Two Prizes) for the best papers on "Child Health".

Instructions:

1. The hard copy of the ABSTRACT as well as the FULL paper in 4 (FOUR COPIES) should be submitted to Hon. Secretary General, Indian Academy of Pediatrics, Kamdhenu Business Bay, 5th Floor, Plot No. 51, Sector 1, Juinagar (Near Juinagar Railway Station), Nerul, Navi Mumbai – 400706, India, along with the declaration certificate as prescribed in the award rules.
2. The ABSTRACT should not be more than 300 words.
3. The FULL award paper should be in the style of Research Papers published in *Indian Pediatrics*. See instructions for authors at *www.indianpediatrics.net* for details.
4. The title of the paper should be brief but adequately descriptive.
5. The text of the Abstract should be structured as per author instructions of *Indian Pediatrics*.
6. The papers not accepted for award competition will not be presented in any other category.

The last date for submission of award papers at the Central IAP Office (Hard Copy) is 30 September, 2020.

Presentation of papers:

Authors of the papers that have been accepted for presentation will be asked to present them at a suitable platform in early 2021. Authors will be informed of the venue and modality for the same.

Email: *centraloffice@iapindia.org* Website: *www.iapindia.org*

EDITORIAL COMMENTARY

Higher Physical Activity Levels in Children Have Wide Ranging Benefits: Towards Multisectoral Action

SHIFALIKA GOENKA[1,2*] AND RAJI DEVARAJAN[1]

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In this issue of *Indian Pediatrics*, Mehreen, *et al*. [1] elicit the validity and reliability of a tool for self-reported physical activity measurement in Indian children and adolescents 10-17 years of age. The correlation coefficients (95% CI) for sedentary behaviour and moderate to vigorous physical activity for Modified Physical Activity Questionnaire for Children, [MPAQ(c)], against accelerometer were 0.52 (0.36, 0.64) and 0.41 (0.23, 0.55), respectively, indicating moderate correlation. This is typical of self-reported physical activity tools globally. Self-reported physical activity remains the main stay for population-based studies, due to its low cost and practicality.

The benefits of physical activity are wide-ranging, enormous and lifelong. Also, behaviors etched in childhood set the foundation for adult behaviors. Strong scientific evidence demonstrates that higher amounts of physical activity are associated with increased cardio-respiratory and muscular fitness, improved bone health, cardiometabolic health, academic outcomes, better cognitive development (executive function, attention, memory, crystalized intelligence, processing speed), fewer symptoms of depression, and reduced risk of adiposity in children aged 3 to 17 years [2-5].

The World Health Organisation (WHO) and the Centres for Disease Control and Prevention (CDC) recommend that children and adolescents should indulge in 60 minutes of moderate to vigorous aerobic activity like running or swimming for a minimum of an hour each day. In addition, bone and muscle strengthening activities should be indulged in atleast three days a week. Bone strengthening activities are those which create an impact with gravity signalling the brain towards greater bone strength. Examples are: skipping, jumping, basketball, etc. Muscle strengthening activities include climbing stairs, climbing trees, dancing etc. Every day, several hours of active play is recommended. In addition, it is recommended that children be encouraged towards active transport to school and several hours of play after school hours. Screen time needs to be minimal, if at all, and at the most limited to 2 hours a day [2-5].

Globally, more than 80% (85% girls and 78% boys) of school-going adolescents, aged 11-17 years, do not meet the current recommendations of at least one hour of physical activity per day. In India, 73.9% (71.8% boys and 76 % girls) of the school going children did not meet the minimum recommended moderate or vigorous physical activity of 60 minutes per day [6]. Inactivity in these school-going adolescents was jeopardizing their present and future health.

"How can we get more children to be more physically active?" is the key public health question. Physical activity should be the most convenient, desirable, safe activity which should be performed during school hours, at home for recreation, and even during daily commute. In the context of the Covid-19 pandemic, open active transport and recreation has taken further precedence. In Europe, huge financial resources have been allocated to further widen the already wide side-walks, and cycling lanes, narrowing motor carriageways to half. Physical activity should be part of an essential foundation of growing up. According to the WHO's Global Action Plan on Physical Activity 2018-2030, we need to create active societies, active environments, active people and active systems [7]. Creating 'active people' requires access to prospects, across myriad settings, to help people of all ages to indulge in regular physical activity as individuals, families and communities. Creating 'active systems' requires implementation of effective synchronized national and subnational action to increase physical activity and reduce sedentary behaviour [7]. Effective governance, leadership, and multisectoral partnerships and advocacy, across all pertinent sectors is the key. Creating 'active environments' requires the establishment and protection of surroundings that promote physical activity in all forms and safeguard the dignity and rights of all people, of all ages, and backgrounds [7,8].

Urban forests/large green lush public parks within

0.4-0.5 km radius (>0.5 Ha) of every family, significantly increases physical activity (68-89 minutes or more) [9]. Wide sidewalks as wide as the roads are recommended and so are limiting motor carriageways. Ability to walk safely and pleasurably to school and parks are critical and so are play grounds, and other sporting facilities in schools. Air-pollution should not act as a deterrent to physical activity as the benefits from physical activity outweigh the risks of air pollution [10]. Lush green trees on either side of the road, in schools and in parks not only reduces the ambient temperature, but they also reduce the particulate matter and air-pollution [11]. A 'dashboard' of monitoring and accountability indicators, applicable to the Indian context, pertaining to safety, the environmental factors, accessibility and comfort of physical activity during daily living is required [12].

The Draft National Education Policy (2019) has given an emphasis on incorporating physical education and sports into the curriculum, starting in early school. The National Multi-sectoral Action Plan (NMAP) for prevention and control of common NCDs (2017-2022) in India has placed high importance for promoting physical activity [13]. Khelo India program has been launched by the Ministry of Sports [14]. The 'Fit India' movement was also launched in August, 2019. Dr. Fiona Bull, leading the Division of Physical activity at WHO says "*Young people have the right to play and should be provided with the opportunities to realise their right to physical and mental health and well being*."

To conclude, 'active children' require active schools, active roads, active policies, active communities, active urban design and active recreation. These will lead to a progressive, equitable, environmentally sensitive and active India.

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RESEARCH PAPER

Reliability and Validity of a Physical Activity Questionnaire for Indian Children and Adolescents

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Submitted: April 08, 2019; Initial review: July 29, 2019; Accepted: March 11, 2020.

Background: In low- and middle-income countries, sedentary behavior is widely prevalent in the young. Reliable and valid instruments are essential for evaluating sedentary behavior and physical activity in children and adolescents.

Objective: To evaluate the reliability and validity of an easy to use physical activity questionnaire for children and adolescents from India.

Study design: Evaluation of a questionnaire tool**.**

Participants: 104 children and adolescents belonging to the age group of 10-17 years were selected using a purposive sampling technique.

Methods: The Madras Diabetes Research Foundation - Physical Activity Questionnaire for Children and Adolescents [MPAQ(c)] was used to assess the various dimensions of physical activity. Physical activity was also objectively assessed using accelerometer worn around the waist for five complete days. The baseline administration of MPAQ(c) was done between November and December, 2017. Reliability of MPAQ was assessed by repeat administration after 2 weeks for upto a month later. Validity of MPAQ(c) was measured against accelerometer using Spearman's correlation and Bland and Altman agreements.

Results: Test-retest reliability of the questionnaire revealed good agreement (ICC: 0.77 min/wk). Correlation coefficients (95% CI) for sedentary behavior and moderate to vigorous physical activity for MPAQ(c) against accelerometer were 0.52 (0.36, 0.64) and 0.41 (0.23, 0.55), respectively indicating moderate correlation. Good agreement was present between MPAQ(c) and accelerometer for sedentary behavior [mean bias = -4.9 (±2SD -197.1 to 187.3) min/d].

Conclusion: MPAQ(c) is a valid and reliable instrument for evaluating physical activity in Indian children aged 10-17 years.

Keywords: *Accelerometry, Assessment, Obesity, Sedentary, Self-reported*.

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Physical activity (PA) is defined as any bodily movement produced by skeletal muscles that results in energy expenditure [1]. Approximately 38% of children in India between the ages of 13 to 15 years meet the recommended PA levels [2]. Reliable and valid instruments for evaluating PA in children are essential for monitoring and surveillance of PA levels in the population [3]. Objective tools like accelerometers quantify total PA well and are easy to use [4]. However, the use of accelerometers in large surveillance studies may be limited due to time and cost considerations [5,6].

A questionnaire as an assessment tool is acceptable, easy, practical and feasible for analyzing PA in children and adolescents in a developing country like India. PA questionnaires help gather qualitative information about the type, location and circumstances of activity that the individual engages in [7]. Hence, we developed a PA questionnaire called the Madras Diabetes Research Foundation – Physical Activity Questionnaire for Children and Adolescents [MPAQ(c)], which would be acceptable and easy to use for surveillance studies on children and adolescents aged 10 to 17 years in a developing country like India. The objective of research reported in this paper was to evaluate the reliability and validity of this questionnaire against objectively collected accelerometer data.

Accompanying Commentary: Pages 705-06.

METHODS

Children and adolescents belonging to the age group of 10-17 years from Chennai, Tamil Nadu, India were recruited. Participants were selected from 74 areas across the 15 zones of urban and rural areas of Chennai. Heterogeneity of the sampling framework was maintained throughout the re-

cruitment procedure by randomly recruiting participants from schools, and known households in the selected areas by door-to-door recruitment. A purposive sampling technique was used to select equal number of boys and girls across two age groups (10 to 14 and 15 to 17 years). For all participants, written informed consent from parents with the assent from the child were obtained before the start of the study. The Institutional Ethics Committee at Madras Diabetes Research Foundation approved the study protocol.

Anthropometric measurements and blood pressure were recorded using standard techniques. Height was measured using a stadiometer (SECA Model 213, Seca Gmbh Co, Hamburg, Germany) to the nearest 0.1cm. Weight was measured using a digital weighing scale (Tanita BC – 601, Tanita Corp., Japan) and recorded to the nearest 0.1 kg. Body mass index (BMI) was calculated as per standard formula. Waist circumference was measured in centimetres using a non-stretchable fiber measuring tape. Blood pressure and pulse was recorded in a rested sitting position in the right arm using a digital machine (Omron Corp., Tokyo, Japan) and rounded off to the nearest 2 mm Hg.

Madras Diabetes Research Foundation – Physical Activity Questionnaire for Children and Adolescents [*MPAQ(c)*]: This questionnaire has been developed from a PA questionnaire called the Madras Diabetes Research Foundation - Physical Activity Questionnaire [Adult version, MPAQ(a)], which was developed to assess PA levels in Asian Indian adults [9]. The questionnaire captures various dimensions of PA based on habitual and culturally relevant activities for upto a year.

The physical activity of children and adolescents can be generally divided into two main categories – school related activities and non-school related PA. The MPAQ(c) questionnaire was developed and validated in the English language. The questionnaire consists of 74 multiple choice questions presented in a ten-page survey form (***Web Appendix* I**). Participants were asked to recall information about activities undertaken in the following domains: at school/college, transport, activities of daily living, leisure and vacation/holiday time activity. For each activity, the average amount of time spent on the activity and frequency (daily/week/month/year) were docu-mented. Thus intensity, duration and frequency data were collected and weekday *versus* weekend analysis were made possible. The results from MPAQ(c) were tabulated based on the type of activity *viz*, sedentary and moderate-to-vigorous physical activity (MVPA).

Accelerometry: Participants had the accelerometer worn on a belt around their waist for five complete days (4 weekdays and 1 weekend) during waking hours; however, the device was allowed to be removed while bathing, swimming and sleeping. Moderate to vigorous physical activity was objectively assessed using the Actigraph (Actilife 5) GT3X+ Triaxial Accelerometer (Actigraph, Pensacola, Florida, USA) [10,11]. The device was worn on the hip of the dominant side (right in most cases). The accelerometers were initialized to monitor and record data in 60-second 'epochs' as 'activity counts' and sample frequency at 100 Hz. While initializing, each device was given a unique number denoting the individual participant with their age, gender, height, weight, date of birth and race. The GT3X+ device collects data from all three axis of movement regardless of the configuration, with Axis 1 collecting the vertical axis acceleration activity data, Axis 2 the horizontal axis data and Axis 3 the perpendicular axis data.

The baseline administration of MPAQ(c) was done between November and December, 2017. This was followed by a repeat administration after 2 weeks (average of 2-4 weeks) for upto a month later for assessing reliability.

For assessing relative validity, the MPAQ(c) was administered in a random order by trained researchers. The sample was chosen to get individuals across a wide age range, both genders and all categories of activity. The duration (minutes per day) spent in different intensity activities was calculated based on the coding scheme provided by Compendium of Physical Activities that describes the energy costs in terms of METs for various activities in children and adolescents aged 6 to 17.9 years [12,13]. The MPAQ(c) was administered anytime during the period the participant was wearing the accelerometer. Data from the MPAQ(c) was computed for a typical day, and then converted to minutes/day to make comparisons with the accelerometer data more realistic.

For content validity, the MPAQ(c) was evaluated by expert committee members at Madras Diabetes Research Foundation (MDRF). At first, the questions from MPAQ(a) were modified to suit the age group of children and adolescents. For instance, the work domain in adult questionnaire was replaced with school domain, and seasonal activity in adults was substituted with vacation for children and adolescents. Questions concerning sport activities in school and during weekends, lunch and snack break timings were found to be highly relevant. The experts evaluated the items in the questionnaire based on the content validity index (CVI), such that 1 was unsatisfactory and 4 was very satisfactory. The mean score of MPAQ(c) was 3.67 with a CVI of 0.92. The MPAQ (c) was considered to be suitable to be used by researchers to assess physical activity and sedentary behavior in the age group of 10 to 17 years.

Being an interviewer-administered questionnaire, inter-

rater reliability was measured to assess the agreement between the interviewers. One interviewer administered the questionnaire to the participant while the other interviewer passively observed and rated participant's response independently. This procedure was completed for a total of 30 participants by two interviewers who collected the questionnaires. A kappa value of 0.82 indicated good agreement among the interviewers.

Statistical analyses: Statistical analyses were performed using SAS (Statistical Analysis System) statistical package version 9.0 (SAS Institute Inc., Cary, NC). Shapiro-Wilks test was used to determine the normality of data. Mann-Whitney U test was used for those variables which deviated from normal distribution. Reliability of the MPAQ(c) was examined by calculating the intra-class correlation (ICC) of the activities reported by age and gender. ICC values of <0.40 were considered as poor agreement, 0.40-0.59 as fair, 0.60-0.74 as good and 0.75-1.00 as excellent agreement [14]. For assessing criterion validity, the MPAQ(c) was compared next to the triaxial accelerometer as a criterion. Spearman correlation coefficients and 95% CI were used for comparisons. Total duration (min/d) of time spent in sedentary and moderate-vigorous PA as estimated from the MPAQ(c) were compared against those recorded by the accelerometer using recognized cut-points [15]. As the accelerometer measured data was computed for an 8-hour valid day criterion, the data obtained from the MPAQ(c) was also calculated for a day so as to make it comparable. Bland and Altman plots were used to assess the agreement between data obtained using the MPAQ(c) and accelerometer (within the 95% limits). A *P* value <0.05 was considered as significant for all statistical measures.

RESULTS

A total of 110 participants responded to the MPAQ(c) on two occasions for the reliability study. Children and adolescents with incomplete MPAQ(c) data (*n*=2) or technical errors in the accelerometer instrument (*n*=4) were excluded from analysis. A final sample of 104 (53 between 10-14 y) participants were included in the study, of whom 43 and 61 participants completed the second round of questionnaire within 3 weeks and in the fourth week of the initial administration, respectively. Baseline characteristics of the participants are shown in ***Table* I**.

The test re-test reliability of the questionnaire on study participants as per gender and age-group is shown in ***Table* II**. The maximum time was spent in the sleep domain followed by school and recreation domains. The agreement between first and second round of MPAQ(c) for boys (*n*=49) was 0.81 and for girls, was 0.74. The ICC was 0.81 in the age group of 15-17 years which was higher than 0.73 in the age group of 10-14 years. Overall, ICC of total MET minutes per week between the two rounds of MPAQ(c) was 0.77.

Correlation coefficients (95% CI) for sedentary behavior and moderate-vigorous PA for MPAQ(c) against the accelerometer were 0.52 (0.36, 0.64) and 0.41 (0.23, 0.55), respectively.

A good agreement [mean (SD) bias =-4.9 (96.1) min/d] between MPAQ(c) and accelerometer for sedentary behavior of older and younger children was present. For moderate-vigorous PA, good agreement was observed [mean (SD) bias = 0.01 (0.44) min/d].

DISCUSSION

Our study showed good reliability of MPAQ(c) in both genders across the age range of 10 to 17 years. MPAQ(c) showed moderate correlation against objective accelerometer measurement.

The values of internal consistency obtained in this study were higher compared to another study with similar characteristics done in the Netherlands [16]. The reliability of MPAQ(c) seen in this study is similar to that reported in a systematic analysis by Chinapaw, *et al.* [17]. The authors in this systematic analysis summarized and appraised 61 questionnaires from 54 studies for measuring PA in children, adolescents and youth. Their results showed that the most reliable PA questionnaire in children aged 8 to 10 years, the Girls Health Enrichment Multisite Study Activity Questionnaire had an ICC of 0.82 (0.75 for boys and 0.82 for girls).

Using the triaxial accelerometer as criterion, validity has been done in several studies. In a study conducted at Toronto, among girls aged 8-9 years, correlation between moderate-vigorous data collected using Habitual Activity Estimation Scale and accelerometer was shown to be 0.24 [18]. Validity correlations for total PA in children and adolescents with congenital heart disease aged 9 to 18 years was 0.51 indicating moderate correlation [19] which is similar to our finding in the normal population.

Table I Baseline Characteristics of the Study Participants (*N*=104)

Characteristics	*Overall*	*Boys (n=49)*
Age (y)	14.4 (1.5)	14.5 (1.3)
BMI (kg/m^2)	20.6 (5.3)	20.0 (5.0)
Waist (cm)	69.8 (12.4)	72.0 (13.0)
*Systolic BP	112 (12.0)	114 (14.0)
Diastolic BP	70 (10.0)	70 (10.0)
*Pulse (bpm)	84.5 (12.0)	77.9 (9.9)

*All values in mean (SD); *P=0.01 for difference between boys and girls; BP: Blood pressure.*

Table II Test-retest Reliability of Madras Diabetes Research Foundation Physical Activity Questionnaire [MPAQ(c)]

Variables	*MPAQ(c) scores*			
	Boys (n=49)	*Girls (n=55)*	*10-14 y (n=53)*	*15-17 y* (*n*=51)
School	446.3 (51.3)	445.6 (49.9)	447.0 (51.4)	444.8 (49.7)
Physical training	11.3 (3.7)[#]	10.5 (3.0)[‡]	11.0 (2.8)[^]	10.8 (3.8)[^]
School sitting	338.7 (60.3)	329.8 (58.2)	337.2 (62.0)	330.7 (56.4)
Transport	47.7 (34.2)[#]	43.7 (34.4)[#]	43.1 (30.0)[^]	48.1 (38.2)[#]
Commuting by walk	23.2 (15.5)[^]	20.9 (13.1)[#]	19.3 (13.1)[‡]	24.8 (14.7)[#]
Commuting by bus	50.0 (36.0)	44.4 (38.6)	43.4 (32.0)	51.8 (42.5)
General[^]	100.8 (33.6)	118.1 (43.3)*	104.4 (31.9)	115.7 (46.3)
Personal care -brushing, toilet, dressing etc.	46.8 (17.0)[^]	58.1 (16.9)[‡]*	52.3 (16.7)[^]	53.3 (19.1)[^]
Eating (includes all meals, snacks and drinks) except that reported in the school section	39.9 (21.9)	38.3 (16.3)	37.3 (18.7)	40.8 (19.4)
Recreation	374.9 (111.2)[^]	394.7 (123.9)[^]	384.3 (125.1)[#]	386.4 (111.3)[^]
Recreational MVPA	56.6 (71.5)	46.0 (78.4)	67.6 (89.0)	33.8 (52.7)*
Cycling (*n*=46)	22.7 (23.1)[#]	13.8 (18.1)[‡]	20.6 (22.4)[‡]	15.2 (19.1)[^]
Football, basketball, tennis, volley ball (*n*=40)	24.6 (35.4)[^]	23.9 (44.3)[#]	18.2 (29.3)[^]	32.8 (51.0)[#]
Recreational sedentary behavior[$]	318.2 (99.0)[^]	348.6 (91.4)[‡]	316.7 (92.0)[‡]	352.6 (97.2)[^]
Watching TV	113.0 (50.8)[^]	93.3 (50.3)[‡]	110.3 (51.7)[^]	94.5 (49.9)[^]
Sleeping	541.7 (75.6)[^]	538.6 (74.0)[‡]	544.5 (78.0)[‡]	535.5 (70.9)[^]
Total MET, min/wk	12948.1 (2887.0)[#]	12894.0 (2946.6)[^]	12676.7 (2941.2)[^]	13171.9 (2873.4)[#]

*MPAQ(c) scores in mean (SD).[#]ICC values of ≥0.75-0.92, [^]ICC values of ≥0.62-0.74, [‡]ICC values of ≥0.50-0.59; *P<0.05 compared to boys; **P<0.05 compared to 10-14 years children; [$]Doing homework/tuition (including reading, writing or using the computer), sitting in a car, bus, etc, playing sedentary games (carom or chess) or computer/video games (like Nintendo or Xbox or PSP), watching TV/videos/ DVDs, watching movies/shows/concerts, using the internet, emailing or other electronic media for leisure, chatting, reading, listening to music etc; MVPA-Moderate-to-vigorous physical activity: Brisk walking as an exercise, cricket, jogging/slow running, dancing/aerobics/ yoga(asanas), cycling including exercise cycling/bike, conditioning exercise, running/sprinting, football, basketball, tennis, volleyball etc.*

According to the MPAQ(c) data analyzed against the accelerometer reading, both boys and girls over-reported sedentary behavior and MVPA. This over-reporting was also reported with adult women in Southern India [20]. A systematic review of 83 studies, which evaluated PA in the pediatric population, found that about 72% of MVPAs evaluated using a questionnaire were over-reported by children and adolescents when compared to the accelerometer [21]. Such inaccuracies are one of the main limitations of the study. The reason for such inaccurate responses by children can be attributed to several social and psychological factors. Another limitation is that accelerometers can underestimate the intensity of effort associated with walking, running and cycling and in activities that require the device to be removed such as swimming and sleep [23]. As the data collection was interview-based, there were high possibilities of data variance between the different interviewers, which could be another limitation, even though in our experience with proper training and practice this error can be negated. Though the validated questionnaire could be used for assessment in any part of the country, it was tested only with one particular group of children and adolescents in only one large metropolitan city. The high compliance and completion rate by the participants is the main strength of the study. The ethnic-specific questionnaire used has information about the type and schedule of PA while the movement sensors in accelerometer provide information on the actual quantity of PA, which will permit a better understanding for the validation of subjective instruments [24,25].

The present study has shown that the MPAQ(c) for children and adolescents has good test-retest reliability among the 10-17 years age group. It is an instrument that can be used to assess the levels of PA in children and adolescents in low and middle-income countries like India due to its good psychometric properties.

Ethical clearance: Institutional ethics committee of Madras Diabetes Research foundation; No. IRB00002640, December, 2014.

Contributors: TSM: involved in conduct of the study, writing the first draft of manuscript and carrying out consecutive revisions; HR: co-ordinated the study, helped in data analysis and revisions of the manuscript; CA: analyzed the data and helped in data interpretation; NJ: collected the data and gave inputs to the manuscript; MP,VM: contributed to critical revisions for

WHAT IS ALREADY KNOWN?

- Physical activity is positively associated with lowering the risk of obesity and its related complications in children and adolescents.

WHAT THIS STUDY ADDS?

- MPAQ (c) is country-specific questionnaire from India to capture physical activity levels among children and adolescents, and has good test-retest reliability in the 10-17 year age-group.

intellectual content of the manuscript; RMA: conceptualized the study, contributed inputs to data analysis and revisions of the manuscript.

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RESEARCH PAPER

Intake of Ultra-processed Foods Among Adolescents From Low- and Middle-Income Families in Delhi

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Objective: To assess the contribution of ultra-processed foods to the macronutrient intake of adolescents from low- and middle-income families in Delhi.

Method: Adolescents (*n*=1030) aged 12-16 years from four private and four government schools of Delhi were interviewed using 24-hour recall (repeated on three days), and a food frequency questionnaire.

Results: The mean energy intake from ultra-processed foods was 371 kcal (16.2%) of the total energy intake. The mean intake of macronutrients from ultra-processed foods was 7.1 g (16.3%) fat, 78.9 g (18.6%) carbohydrate and 4.8 g (10.9%) protein. Children from middle-income families consumed significantly higher (P<0.05) amounts of macronutrients coming from ultra-processed foods, as compared to those from low-income families.

Conclusion: Adolescents reported regular consumption of variety of ultra-processed foods, and measures to reduce this consumption and encouraging healthy food choices are urgently needed.

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

A new classification of foods based on the extent and purpose of their processing has been developed as per a tool known as NOVA [1]. Foods are classified as minimally processed foods like pasteurized milk, packaged grains *etc*, processed culinary ingredients like flours, sugar *etc*; processed foods like butter; and ultra-processed foods, which are "extraction of substances from whole foods followed by their subsequent assembling with lots of additives and processing aids enabling the manufacture of products with long shelf-life, improved palatability *etc*. like breads, cookies, biscuits and ready to serve beverages [1]. Adolescents in India face a triple burden of malnutrition, overweight and micronutrient deficiency [2]. Excessive intake of energy from foods high in fat, sugar and salt (HFSS) leads to obesity and associated co-morbidities [3,4]. Ultra-processed foods tend to be high in fat and sugar and increase the energy density of the diet [5,6].

Indian studies on the consumption of ultra-processed foods are few and are focused on limited foods. The present study assesses the contribution of ultra-processed foods to the macronutrient intake in diets of adolescents (aged 12-16 year) from low- and middle-income families in Delhi.

METHODS

The study was conducted in one purposively selected private and government/government-aided school each from North, South, East and West zones of Delhi between July, 2014 and July, 2016. Children from government schools belonged to low-income group and children from private schools belonged to middle-income group, which was verified using Kuppuswamy scale of socio-economic status (SES) classification [7]. Adolescents aged 12-16 years were enrolled by random selection of one section each from 7-11 grades in the respective schools.

A diet survey was carried out by using a pre-tested food frequency questionnaire and a 24-hour food record. The respondents were asked to record their frequency of consumption in the questionnaire, and actual consumption of foods and beverages for three days *i.e.* two working days and one holiday, in a food record. Intake of a food at least three times or more per week was considered as frequent consumption. The amount of food products consumed was assessed by using three and two-dimensional food models of standardized plates, glasses, spoons, ladles and bowls. For foods like chips, ready-to-serve beverages and confectionery, pack sizes were noted in order to assess the child's dietary intake for that food product. Ethical clearance was taken from the institutional ethics committee of Lady Irwin college. Written consent was taken from parents and assent from the school children.

Nutrient intake was calculated by using dietary assessment software, Diet Cal Version 5 (Profound Tech

Solutions Pvt. Ltd., Delhi, India) utilizing the nutrient composition data given by National Institute of Nutrition [8]. The contribution of all ultra-processed foods to the macronutrient intake in day's diet was assessed and compared across the income groups using an independent *t*-test or Mann-Whitney U test. P values less than 0.05 were considered as statistically significant.

RESULTS

Out of 1200 children recruited, 1030 (86%) adolescents (46% from private schools) were present on the day of collection of forms and provided completed forms.

The majority of adolescents (92%) consumed ultra-processed food items frequently. The mean daily intake of ultra-processed foods across income groups is shown in ***Web Table* I**. It was higher in middle-income group than low-income group. The contribution for macronutrients from ultra-processed foods across income groups was higher in middle-income group as compared to low-income group ***Table* I**. About 11-19% of daily macronutrient intake was from ultra-processed foods. The maximum contribution to energy intake from ultra-processed foods was by bakery products, followed by beverage concentrates.

DISCUSSION

The present study shows high intake of ultra-processed foods in the majority of adolescents, which was higher in middle-income group than low-income group. Intake of few ultra-processed food products has been reported in literature [2,5,9-13]. The mean consumption of carbonated beverages, fruit juices, ice creams and ready to serve fruit beverages was higher in a study [10] from developed countries than the present study. Previous studies [5,12] have reported lower mean intake of carbonated beverages than the present study. Data show a higher consumption of aerated drinks and chips regularly in school children in Delhi than the present study. This may be due to the difference in sample, age and income group studied.

Data from Comprehensive National Nutritional Survey [2] and National Nutrition Monitoring Bureau [13] reported that the intake for macronutrients and micronutrients was less than the recommended dietary intake (RDA) (10-19 year old children). Similar findings were reported in a study done in Delhi [9]. The present study did not look at the nutritional status of participants. However, a high consumption of ultra-processed foods, most of which are high in fat, salt and sugar and lacking in micronutrients, is disturbing. The fact that half the sample size was from low socio-economic groups shows that income is not a limiting factor and ultra-processed foods have penetrated all segments of society.

A limitation of the study was the difficulty in assessing portion sizes when respondents did not eat a full packet of the packaged product (*e.g.* chips) or a full portion size (*e.g.* piece of cake rather than a defined slice). Assessing the nutritional status of the respondents would have added more information.

Many reasons have been proposed for the high consumption of ultra-processed foods by children [2,5,13-15]. Consumption of these foods in excess could increase the risk of obesity and associated co-morbidities at a younger age. Attention needs to be given to availability of energy dense and HFSS ultra-processed foods in the home and school food-environment so that these foods do not replace fresh home cooked meals. Early introduction to the concept of healthy food choices in schools could help in ensuring better eating habits among growing children. The food industry also needs to pitch in by making available healthier food and beverage options which improve the nutritional quality of the diets of children of this country.

Contributors: AJ: design of study, data collection, data analysis and interpretation, writing paper; PM: design of study, data interpretation, writing and review of paper.
Ethics clearance: Institutional Ethics Committee of Lady Irwin College; ECR/12/INDT/DL/2014 dated May 06, 2014.

Table I Contribution of Ultra-processed Foods to Total Macronutrient Intake of Adolescents

Macronutrients	*Middle-income family (n=475)*		*Low-income family (n=555)*		*All adolescents (n=1030)*	
	Intake, mean (SD)	*% Contribution*	*Intake, mean (SD)*	*% Contribution*	*Intake, mean (SD)*	*% Contribution*
Energy (kcal)	*433 (245)	19.1	*315 (220)	13.5	371 (239)	16.2
Total fat (g)	*8.4 (6.9)	16.0	*6.0 (4.1)	16.7	7.1 (4.9)	16.3
Carbohydrate (g)	*91.3 (53.4)	21.4	*67.9 (48.4)	16.2	78.9 (51.7)	18.6
Protein (g)	*5.4 (5.0)	12.2	*4.2 (3.9)	9.8	4.8 (4.5)	10.9

*Percent (%) contribution = nutrient intake from ultra-processed foods / Total nutrient intake; *Significant difference in mean intake between middle- and low-income adolescents (P<0.001).*

WHAT THIS STUDY ADDS?

- Adolescents from middle-income families consumed significantly higher amount of energy, fat, carbohydrate and protein coming from ultra-processed foods, as compared to those from low-income families.

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Web Table I Intake of Ultra-processed Foods in AdolescentsFrom Low- and Middle-income Families

Food Items	*Middle-Income group (n=475)*		*Low-Income group (n=555)*		*P value*
	Frequent consumers, n (%)	*Intake, g or mL*	*Frequent consumers n (%)*	*Intake, g ormL*	
Preserves and accompaniment[*]					
Jam/Marmalade	190 (40)	*20.7 (10-33.3)	212 (38)	*20.4 (12-33.3)	0.88
Sauce/c*hutney*	114 (24)	8.6 (2.4)	90 (16)	7.2 (3.3)	<0.01
Confectioneries					
Candies	67 (14)	*4.6 (1.3-4.6)	54 (10)	*4.2 (1.3-8)	0.02
Chocolate	18 (4)	16.7 (9.2)	12 (2)	15.5 (3.9)	0.68
Bakery products					
Biscuit	314 (66)	32.7 (13.4)	305 (55)	28.3 (15.1)	<0.01
Cake/pastries	40 (8)	23.5 (2.3)	0	0	-
Breads	252 (53)	56.2 (14.1)	216 (39)	55.1 (16.7)	0.54
Beverage concentrate					
Syrup/s*herbet*	185 (39)	49.9 (25.5)	164 (30)	45.3 (20.7)	0.07
Squash	49 (10)	75 (0)	0	-	-
RTS beverages					
Carbonated beverage	229 (48)	224.8 (85.9)	207 (37)	175.9 (51.8)	<0.01
Non-carbonated fruit beverage	66 (14)	193.9 (61.4)	48 (9)	127.7 (54.3)	<0.01
Fruit juice	17 (4)	169.8 (83.3)	0	0	-
Milk based beverage	5 (1)	60 (0)	0	0	-
Miscellaneous food items					
Breakfast cereals	77 (16)	42.1 (20.1)	28 (5)	30.9 (2.9)	<0.01
Sweetmeats	40 (8)	36.5 (12.1)	8 (1)	25.6 (8.3)	<0.01
Ice cream	59 (12)	37.5 (16.2)	109 (20)	16.7 (5.7)	<0.01
Savories (chips/*namkeens*)	35 (7)	18.7 (5)	30 (5)	17.5 (6)	0.43
Noodles/pasta	15 (3)	30.8 (12.9)	20 (4)	22.2 (11.5)	0.01

Intake in mean (SD) except [*]median (IQR); Intake of solid and semi-solid foods in grams (g) and beverage concentrates and RTS beverages in milliliters (mL); RTS: Ready to serve beverages; frozen vegetarian snacks and packaged meat products were consumed by 5 children each in only in middle income group and the median (IQR) intake was 10 (9,10) and 7 (6,7).

RESEARCH PAPER

Serum Presepsin, Proadrenomedullin andTriggering Receptor Expressed on Myeloid Cells-1 (TREM-1) as Biomarkers for the Diagnosis of Acute Pyelonephritis

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Objective: To investigate the diagnostic values of serum presepsin, proadrenomedullin (proADM) and triggering receptor expressed on myeloid cells-1 (TREM-1) levels in children with acute pyelonephritis and lower urinary tract infection.

Methods: Peripheral venous blood and urine samples were obtained before starting antibiotic therapy at the time of admission in all patients. Serum TREM-1, presepsin and proADM concentrations were determined by the enzyme-linked immunosorbent assay method.

Results: 82 children (38 acute pyelonephritis, 24 lower urinary tract infection, 20 controls) were enrolled. Serum proADM and TREM-1 levels were higher in patients with acute pyelonephritis than those of lower urinary tract infection and controls (P=0.001 and P<0.001, respectively). Both serum proADM and TREM-1 levels had predictive value for diagnosis of acute pyelonephritis (P=0.006 and P<0.001, respectively). ROC analysis showed that proADM and TREM-1 had positive predictive values for diagnosis of acute pyelonephritis (AUC=0.830, P=0.003; and AUC=0.843, P <0.001, respectively).

Conclusion: Serum proADM and TREM-1 levels could serve as early biomarkers for the diagnosis of acute pyelonephritis in children.

Keywords: *Fever, Prediction, Prognosis, Urinary tract infection.*

Acute pyelonephritis is a common problem in infants and children. The available biomarkers are not sufficiently accurate for the prediction of acute pyelonephritis in children [2]. One possible alternative biomarker is CD14, a receptor for bacterial lipopolysaccharides (LPSs) and peptidoglycans that is expressed mainly by macrophages and neutrophils. After the binding of LPS to CD14 through the LPS-binding protein, the soluble form of CD14, presepsin, is released in the circulation [3]. Serum presepsin levels increase within two hours of inflammation onset, which is even earlier than the time required to observe increases in procalcitonin (PCT) or C-reactive protein (CRP) [4]. Another possible diagnostic biomarker is adreno-medullin, a vasoactive peptide that is expressed and secreted by a variety of tissues [5]. Proadrenomedullin (proADM), a precursor of the active peptide adrenomedullin, is produced by renal tissues in stressful situations. Several researchers have reported on proadrenomedullin in adult patients with urinary tract infection [6]. Another potential biomarker is the triggering receptor expressed on myeloid cells-1 (TREM-1). This is a member of the immunoglobulin super family, and its expression in neutrophils and monocytes/macrophages is stimulated by bacterial products [7]. Elevated TREM-1 levels lead to the release of proinflammatory cytokines [8].

The identification of new biomarkers for the early diagnosis and treatment of acute pyelonephritis is important for preventing its long-term complications. The aim of the present study was to investigate the values of serum presepsin, proADM, and TREM-1 levels for the diagnosis of acute pyelonephritis in children.

METHODS

This study was a single-center diagnostic accuracy study, initiated after institutional ethics committee approval among children with urinary tract infection who were followed up in our pediatric nephrology department between November, 2017 and December, 2018. The included patients were selected using convenience sampling. Written informed consent was obtained from parents of all children. The children were aged 2 to 18 years and had presented with symptoms of urinary tract infection like fever (body temperature ≥38°C), dysuria, vomiting, and malodorous urine. Patients with known urinary tract anomalies, comorbid bacterial or viral infections, antibiotic use in the previous week, and

glomerular filtration rate <90 mL/min/1.73 m^2 were excluded from the study. Sex- and age-matched healthy controls were also included in this study. ***Fig.* 1** shows the flow of participant selection. Index tests in our study consisted of serum presepsin, proADM, and TREM-1 levels. Criteria for acute pyelonephritis were accepted as reference standard.

The diagnosis of a urinary tract infection was made based on the presence of at least 100,000 colony-forming units/mL of a single uropathogen cultured from the urine specimen and on evidence of pyuria (WBC count ≥5, as measured with a high-power field on a microscopy urinalysis). Acute pyelonephritis was defined as the presence of pyuria and a positive urine culture, plus the presence of a level ≥2 for the following criteria: fever, high CRP (>0.8 mg/dL), and/or ESR (>20 mm/h), as well as neutrophil counts above normal values for the age.

After admission and before starting antibiotic therapy, peripheral venous blood (for a complete blood cell count, erythrocyte sedimentation rate, CRP, and PCT levels) and urine samples were obtained for all patients. Blood samples were taken within the first 24 hours of fever in the patients with acute pyelonephritis. The operators performing the tests were blinded to the clinical data.

For the measurement of serum presepsin, proADM, and TREM-1 levels, serum was separated and stored at –80°C until further use. Enzyme-linked immunosorbent assay (ELISA) method was used utilizing commercially available TREM-1 (R&D Systems, Minneapolis, USA), presepsin (MyBioSource, San Diego, USA), and proADM (MyBioSource, San Diego, USA) ELISA kits [9,11]. The intra-assay and inter-assay coefficients of variation of all the kits were <12%. The absorbance readings and calculations were made using an ELx808 microplate reader (Biotek, Bad Friedrichshall, Germany). The TREM-1 and proADM levels were expressed as pg/mL; the presepsin levels were expressed as ng/mL.

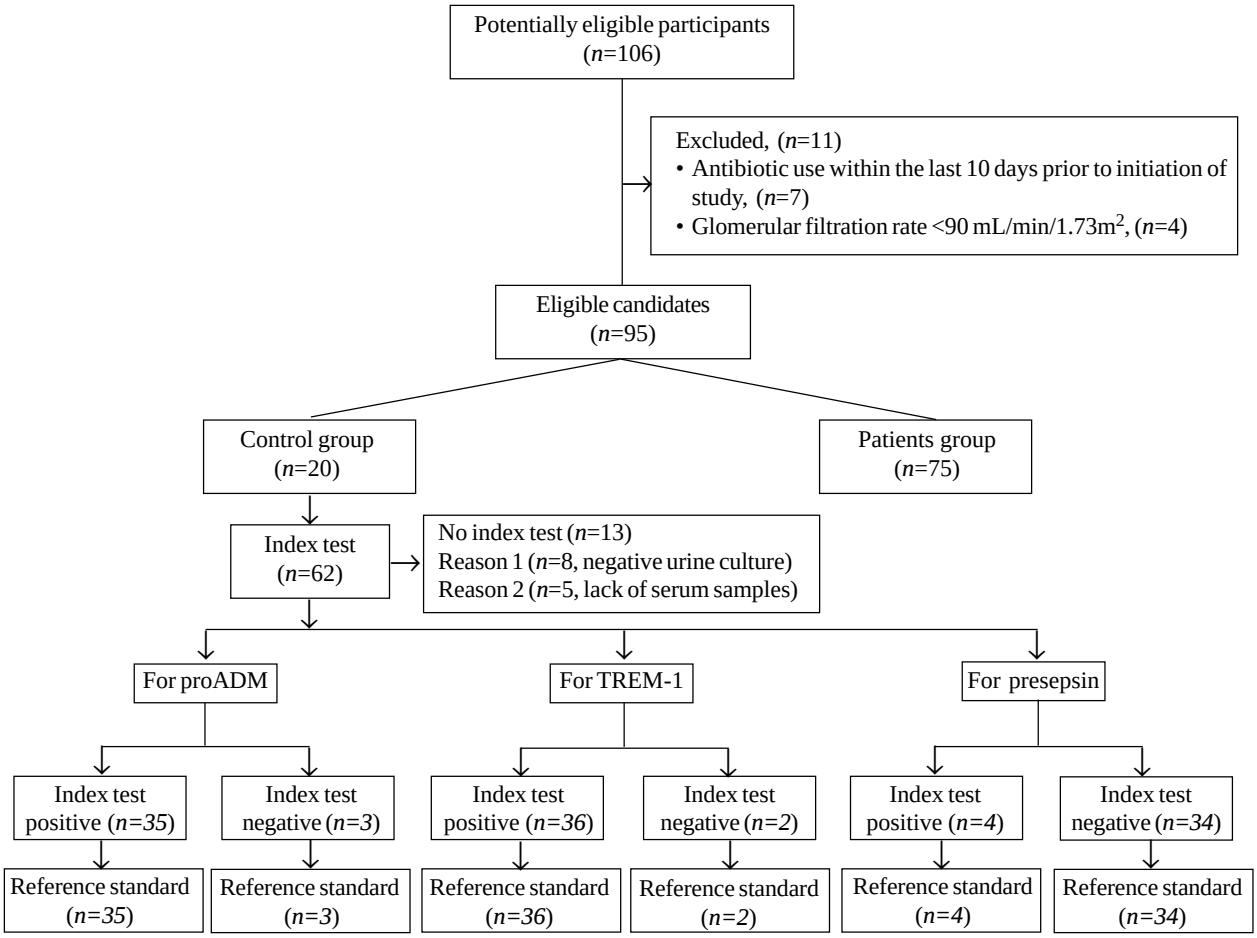

Fig. 1 *Participant flow in the study.*

Statistical analyses: Statistical analyses were performed using SPSS 11.0 (SPSS Inc, Chicago, IL). The Kolmogorov Smirnov test was used to determine normality of data. A receiver operating characteristic (ROC) analysis was used to determine the cut-off values and the sensitivity/specificity of the proADM and TREM-1 levels. The true and false positive/negative values for the proADM and TREM-1 levels were used for determination of acute pyelonephritis. A *P* value <0.05 was considered significant.

RESULTS

In total, 62 patients (38 with acute pyelonephritis, 24 with lower urinary tract infection) and 20 healthy controls were included in this study. The patients with acute pyelonephritis had higher PCT and neutrophil counts (*P*=0.005, *P*=0.031, respectively). The serum proADM and TREM-1 levels were higher in patients with acute pyelonephritis than in those with lower urinary tract infection or in the controls (*P* <0.001 for proADM, *P*=0.001 for TREM-1, respectively). The proADM, and TREM-1 levels were similar between the patients with lower urinary tract infection and the controls (***Table* I**).

The ROC curve analysis showed a cut-off value for proADM of 63.86 pg/mL for the prediction of acute pyelonephritis in patients with urinary tract infection [Area under the curve (standard error) 0.83 (0.064), *P*=0.003]. The serum TREM-1 levels showed a sensitivity of 97.3% and specificity of 84% for acute pyelonephritis prediction, with a cut-off of 127.37 pg/mL [AUC (SE) 0.84 (0.005), *P*<0.001]. Serum proADM and TREM-1 were above the cut-off values in 35 and 36 patients, respecctively with acute pyetonephrites, and 21 and 20 subjects, respectively without acute pyclonephrites. Positive and negative predictive values for proADM were 92.1% and 87.5%, respectively. Positive and negative predictive values of TREM-1 were found to be 94.7% and 83.3%, respectively.

DISCUSSION

The serum presepsin levels have been reported to increase in the early stage of infection. The findings from a study on adults revealed higher serum presepsin levels in patients with acute pyelonephritis than in healthy controls [12]. However, in agreement with our results, the serum presepsin levels were not useful for the prediction of acute pyelonephritis, as presepsin was detected in healthy individuals as well as in patients with bacterial infection, and may reflect the intensity of the innate immune response [13].

A few studies have evaluated the predictive value of serum proADM levels in adult patients with urinary tract infection [6]. Stalenhoef, *et al*. [14] showed that the serum proADM levels were significantly higher in patients with febrile urinary tract infection requiring hospitalization than in patients completing treatment at home. Our results also showed that proADM could be a useful biomarker for the early diagnosis of acute pyelonephritis in children.

Ehsanipour, *et al*. [19] investigated serum TREM-1 levels in children with urinary tract infection and found

Table I Baseline Characteristics of the Study Subjects

Parameters	*Acute pyelonephritis (n=38)*	*Lower urinary tract infection (n=24)*	*Control (n=20)*
Female, no.(%)	8 (47.4)	11 (45.8)	8 (40)
Age^, y	8.1 (4.14)	7.9 (3.54)	9.3 (4.25)
BUN^, mg/dL	10.5 (2.44)	9.8 (4.12)	9.6 (3.61)
Creatinine^,mg/dL	0.4 (0.15)	0.3 (0.13)	0.3 (0.15)
WBC^, mm^3	12548.5 (2796.12)	9037.5 (1381.76)	9411.1 (2482.36)
Neutrophils,‡ per mm^3	7050 (5100-12950)	3212.5 (2754-5850)	2400 (2250- 6100)
ESR, mm/h	16 (11-24)	10 (7-19)	8 (6-15)
CRP, mg/dL	3.41 (0.96-5.12)	1.8 (0.76-2.54)	1.23 (0.98-3.11)
Procalcitonin*, ng/mL	0.09 (0.04-0.45)	0.04 (0. 02- 0.07)	0.03 (0.02-0.05)
Presepsin, ng/mL	0.06 (0.04-0.18)	0.05 (0.04-0.08)	0.04 (0.05-0.09)
Pro-ADM*#, pg/mL	356.8 (210.48)	200.3 (110.91)	176.4 (94.82)
TREM-1*$, pg/mL	249.03 (181.6-373.11)	139.5 (114.85-202.27)	158.4 (119.7-14.61)

*Data shown as ^mean (SD) or median (inter-quartile range). BUN; Blood urea nitrogen; WBC; White blood cells; ESR; Erythrocyte sedimentation rate; CRP: C-reactive protein; Pro-ADM: Proadrenomedullin; TREM-1: Triggering receptor expressed on myeloid cells-1; *P=0.005; $P=0.001, #P=<0.001, ‡P<0.05.*

WHAT THIS STUDY ADDS?

- Serum proadrenomedullin and Triggering receptor expressed on myeloid cells-1 (TREM-1) levels could serve as biomarker for early diagnosis of acute pyelonephritis in children.

no significant difference between patients with upper and lower urinary tract infection [19]. However, the TREM -1 levels were significantly higher in the children with urinary tract infection than in the controls [15]. In our study; however, the serum TREM-1 levels had a predictive value for diagnosing acute pyelonephritis.

One limitation of the study was the small size of our study population. Another was that we could not evaluate the associations between the biomarkers and the findings of dimercaptosuccinic acid (DMSA) scintigraphy, because we could not perform scintigraphy within the first two weeks of diagnosis of urinary tract infection in our patients. Despite these limitations, our results may be useful as a guide for future studies.

In conclusion, our results revealed that the serum proADM and TREM-1 levels could be useful for the early diagnosis of acute pyelonephritis. Further studies are needed to validate the diagnostic values of these biomarkers in children with urinary tract infection.

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Contributors: NC: concept, design, definition of intellectual content, protocol development, literature search, clinical studies, data acquisition, drafting the work, manuscript preparation, ZKK: design, definition of intellectual content, data acquisition, data analysis, drafting the work, AG: literature search, data acquisition, clinical studies, drafting the work. All authors approved the final manuscript submitted.
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RESEARCH PAPER

Assessment of Autonomic Nervous System in Children with Celiac Disease: A Heart Rate Variability Study

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Objective: We evaluated the activity of autonomic nervous system in children with celiac disease by using heart rate variability (HRV) analysis. **Methods:** HRV parameters of 37 children with celiac disease were compared to 36 age- and sex-matched healthy controls. None of the participants had a systemic, central or peripheral neurological disease. **Results:** Statistically significant differences were present in two parameters; standard deviation of all RR intervals (SDNN) and standard deviation of 5-minute RR interval means (SDANN). Age was negatively correlated with mean, minimum and maximum heart rate. Duration of disease was positively correlated with low frequency power-high frequency power ratio. No correlation was found between anti-tissue transglutaminase IgA level and HRV parameters. **Conclusion:** Celiac disease may affect autonomic nervous function in children even if there are no symptoms of dysautonomia.

Keywords: *Complications, Comorbidity, Extra-intestinal, Neurological involvement.*

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Celiac disease, an autoimmune inflammatory enteropathy, has manifestations beyond the gastrointestinal system with neurological manifestations such as cerebellar ataxia and peripheral neuropathy present in up to 10% of the patients [1]. The data about the frequency of involvement of autonomic nervous system (ANS) in celiac disease are still insufficient, in particular for the pediatric age group. In adult studies, it was shown that the frequency of associated ANS disorder in patients with celiac disease was up to 45% [2]. Some of these patients were asymptomatic or existing symptoms were ascribed to some other cause.

Sympathetic and parasympathetic components of ANS can be tested by heart rate variability (HRV) analysis, which is a reliable and selective method [3]. In this study, we evaluated the activity of ANS in children with celiac disease by using 24-hour rhythm Holter monitoring and compared the results with healthy controls.

METHODS

We included patients with celiac disease under follow up at the Pediatric gastroenterology department of Kecioren Training and Research hospital, between January, 2018 and January, 2019. Written informed consent was obtained from parents of all participants. The control group was selected from attendees of general outpatients clinic who were considered to be healthy based on history, examination and routine laboratory tests. Children with congenital and/or acquired heart disease, arrhythmia, with a history of either gastrointestinal and neurological disorders or other chronic illnesses, and taking daily medications were excluded from the study. None of the participants reported syncope, presyncope, light-headedness, headache, and dizziness. The local ethics committee cleared the protocol.

All the patients in the study had compatible diagnostic features of celiac disease including histologic findings on duodenal biopsy and positive anti-tTG IgA levels. Complete blood count, blood glucose, lipid profile, electrolytes, thyroid stimulating hormone, coagulation parameters, liver function tests and serum vitamin levels were also evaluated in children with celiac disease.

A standard transthoracic echocardiographic imaging including two-dimensional and colour-Doppler examination was performed to all participants. Cardiac functions, cardiac structures and additional cardiac abnormalities were evaluated by the same pediatric cardiologist. 24-hour ambulatory electrocardiogram recordings were used for HRV analysis in all cases. Recordings taken with DMS 400-3A solid-state recorder were evaluated *via* computer by using the Cardioscan II series (DM Software, USA) software. Details of analysis

of HRV are provided in ***Web Box* I**.

Statistical analyses: The Statistical package for social sciences program version 21 (SPSS Inc., Chicago, IL, USA) was used for statistical analyses of data. Shapiro - Wilk test was used to assess the normal distribution of variables. According to distribution, Student t test or Mann Whitney U test were used to compare groups. The differences in median values between more than two independent groups were analyzed with the Kruskal–Wallis test. If the result of the Kruskal–Wallis test proved to be significant, the condition causing this difference was determined by Conover non-parametric multiple comparison tests. Correlation between variables was assessed by using Pearson or Spearman correlation based on normality of distribution. Statistical significance level was considered as a *P* value of under 0.05.

RESULTS

Forty-four patients of celiac disease were initially recruited, but only 37 patients and 35 controls completed the study. The mean age of participants was 12.4 years (IQR: 9-16 years). Although all celiac patients were recommended gluten free diet at the time of diagnosis, there were only 20 patients in the study group adhering to gluten free diet. Baseline characteristics of all participants are summarized in ***Table* I**. The groups were comparable for all parameters except, body mass index was lower in the study group. Mean disease duration in the patients was 2.05 years (IQR: 2.6 years, 2.5 months - 2.8 years), and the mean serum level of anti-tTG was 75.9 mg/dL (IQR:8.8-157 mg/dL). Histological examination of the duodenal biopsy specimens were compatible with Marsh type 3 in all patients. A total of 5 cases were diagnosed as type 3a, 17 cases were diagnosed as type 3b, and 15 cases as type 3c according to modified Marsh classification. All participants were euthyroid and had low C-reactive protein concentration. Anemia and microcytosis was documented in only one case.

Table I Baseline Characteristics of Children With Celiac Disease and Unaffected Controls

Characteristic	*Study group (n = 37)*	*Control group (n = 35)*
*Age, y	13 (3,18)	14 (5,18)
Female	22	18
‡Body surface area, kg/m^2	17.3 (3.02)	19.8 (3.2)
Systolic BP, mmHg	108.3 (9)	109.5 (8.5)
Diastolic BP, mmHg	63.7 (8.5)	63.6 (7.7)
Disease duration, y	2 (0-10)	-

*All values in mean (SD) except *median (IQR); BP: Blood pressure; ‡P=0.001.*

Echocardiographic evaluation of all participants was normal. Though systolic functions were within normal limits in all participants, both mean (SD) ventricular ejection fraction and shortening fraction were significantly higher in the control group [72.3 (5.1)% *vs* 75.2 (5.3)%; *P*=0.02] and [40.7 (4.4)% *vs* 44.8 (5.0)%; *P*=0.005], respectively.

During 24-hour rhythm Holter monitoring evaluation, one patient had rare extra supraventricular beats in the study group; whereas in the control group, three participants (one with rare extra ventricular beats and the other two with rare extra supraventricular beats) were found. HRV parameters including both time-domain and frequency-domain components are summarized in ***Table* II**. Statistically significant differences were found in only two parameters *viz* Standard deviation of all the RR intervals (SDNN) and Standard deviation of 5 minute RR interval means (SDANN).

There was a statistically significant negative correlation between age and minimum HR, maximum HR, average HR (r=-0.474; -0.358; and -0.553, respectively; all *P*=0.003, *P*=0.03, *P*<0.001). In addition, a positive

Table II Time-domain and Frequency-domain Variables in Children With Celiac Disease and Unaffected Controls

	Celiac disease (n = 37)	*Control group (n = 35)*
Heart rate, bpm		
Minimum	52.7 (7.92)	49.5 (7.28)
Maximum	163.8 (16.59)	163.7 (23.52)
Average	88.5 (13.12)	86.3 (16.41)
*SDNN, ms	133.9 (41.2)	163.0 (46.43)
SDNNi, ms	69.4 (26.99)	72.5 (19.83)
*SDANN, ms	115.9 (36.84)	144.0 (45.45)
RMSSD, ms	44.8 (17.13)	45.9 (14.51)
pNN50, %	19.2 (11.48)	20.9 (10.490
Power, ms		
Total	4507.5 (2781.06)	5330.0 (2634.35)
VLF	2777.9 (2027.12)	3318.4 (2022.19)
HF	636.1 (382.03)	657.9 (315.19)
LF	1026.7 (536.86)	1186.5 (493.53)
LF/HF	1.93 (0.85)	1.96 (0.75)

*All values in mean (SD); SDNN; standard deviation of all the RR intervals, SDANN; standard deviation of 5 minute RR interval means, SDNNi; the mean of the 5 minute RR interval standard deviations, RMSSD; the square root of the mean of the squared differences of two consecutive RR intervals, pNN50; the percentage of the beats with consecutive RR interval difference of more than 50 ms, VLF; very low-frequency power, LF; low-frequency power, HF; high-frequency power; *P<0.01.*

correlation was duration of diagnosis found between age and SDNN (r=0.339; *P*=0.04). Ratio of LF/HF was positively correlated with duration of diagnosis (r =0.516; *P*=0.001). No correlation was found between serum anti-tTG IgA levels and HRV parameters. When participants were divided into three groups as patients with celiac disease adhering to gluten-free diet, not adhering to gluten-free diet, and healthy controls, statistically significant difference between controls and celiac patients adhering to gluten- free diet were present in only time domain parameters of SDNN and SDANN (*P*=0.01).

DISCUSSION

We studied HRV parameters among 37 children with celiac disease and 36 unaffected controls, and found two parameters (SDNN and SDANN) to be abnormal in the children with celiac disease.

In adult studies, the frequency of neurologic involvement in celiac disease is reported to be 22-45% [2,8]. On the other hand, despite limited information, neurologic and psychiatric symptoms are lower and observed in 13-33% of the pediatric patients with celiac disease [9,10].

Autonomic neuropathy is considered to be a complication of celiac disease, developing without any neurological symptoms [2]. HRV parameters calculated on the basis of the mean of the RR change, such as, RMSSD, p NN50 are less influenced by the cardiac circadian rhythm and these parameters are considered to be the most sensitive to parasympathetic drive. However, SDNN and SDANN are the best known of time-dependent variables and have been widely used to provide information in the evaluation of HRV [3,4]. We carried out HRV analysis as it is the most feasible, reliable and easiest method for studying autonomic involvement in the pediatric population [4,6]. HRV parameters have been reported to be significantly lower in CD patients than in healthy people [11,12]. The results of our research are compatible with previous reports.

Although, it is difficult to define which component of autonomic nervous system is affected mainly in children with celiac disease, we estimate that they have asymptomatic dysautonomia and sympathetic-parasympathetic imbalance. The increase in high frequency power (HF) reflects parasympathetic activity and the increase in low frequency power (LF) reflects mainly sympathetic activity. LF/ HF ratio is an index of sympathovagal balance and total power (TP) is used to evaluate the entire action of ANS [3-6]. All parameters of these two methods have a strong correlation. Our results are consistent with the data published by Felus, *et al.* [11], reported about 20% of the patients had parasympathetic dominance whereas 36% had sympathetic over-activity. We found a positive correlation between disease duration and ratio of LF/HF. LF/HF ratio is known as a measure sympathovagal balance [3,4]. This positive correlation can possibly be attributed to the duration of the gluten-free diet; through the numbers were small to confirm this premise.

The possible mechanisms leading to neurologic complications include immune interaction and changes secondary to malabsorption of vitamins and micro-elements [13,14]. In adult studies, some markers of celiac disease like anti ganglioside antibody titers or IL-10 have been reported to have a predictor role for autonomic nervous system impairment [15]. Our findings showed that there was no relationship between anti-tTG IgA levels and HRV parameters. We think this may be due to the fact that pathogenic antibodies (anti-gliadin, anti-tTG or others) need time to penetrate the nervous system and produce permanent damage [7].

The limitations of this study are that the sample size is small, and the study was performed in a heterogeneous patient population in terms of the period of gluten-free diet and disease follow-up. In addition, studies comparing children with celiac disease and children with other gastrointestinal disorders in terms of HRV will provide more information about the mechanism of impaired autonomic nervous system activity. Other limitations originate from the method of 24-hour rhythm Holter monitoring itself, which can be affected from daily physical activity and posture of individuals. However, this study is important in that it focuses attention on autonomic involvement in children with celiac disease, which is usually not appreciated by clinicians.

In conclusion, celiac disease causes disturbances of the autonomic activity in pediatric population, which is lower than adults, but may have implications for management and outcome.

Ethical clearance: Local ethics committee of Health Sciences University, Kecioren Training and Research Hospital; No. 15/ 1864 dated March 03, 2019.
Contributions: SK: idea, concept, design, control, supervision and critical review; SK,SS: data Collection and/or Processing, analysis and/ or interpretation; literature review: writing the article, materials and references and finding.
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Web Box I Details of Heart Rate Variability Analysis

Heart rate variability analysis was done using time domain and frequency domain methods based on the measurement of changes in consecutive RR intervals on 24 hour rhythm Holter recordings.

Analysis of time domain: Done from the variation of heart rate during a standard time interval based on RR distances between two consecutive sinus beats. By this analysis, parameters including; the standard deviation of all RR intervals (SDNN), the standard deviation of 5 minute RR interval means (SDANN), the mean of the 5 minute RR interval standard deviations (SDNNi), the square root of the mean of the squared differences of two consecutive RR intervals (RMSSD), the percentage of the beats with consecutive RR interval difference of more than 50 ms (pNN50) were calculated.

Frequency domain analysis: This was done from periodic signals, an average of 500 sequential R-R intervals divided into various bands of frequency response. Total power (the area under the spectral curve from 0.01 to 1.0 Hz,TP), very low-frequency power (the area under the spectral curve from 0.0033 to 0.04 Hz, VLF), low-frequency (the area under the spectral curve from 0.04 to 0.15 Hz, LF), and high frequency band power (the area under the spectral curve from 0.15 to 0.40 Hz, HF) were examined by this analysis and LF / HF ratio was calculated.

Guidelines on Diagnosis and Management of Cow's Milk Protein Allergy

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Justification: Cow's milk protein allergy (CMPA) is increasingly being diagnosed in the West, while there is scant data on the subject from India. There is low awareness among pediatricians about its diagnosis and management; leading to improper diagnosis. **Process:** A group of experts from the pediatric gastroenterology sub-specialty chapter of Indian Academy of Pediatrics (Indian Society of Pediatric Gastroenterology, Hepatology and Nutrition) met at Mumbai on 26 October, 2018 and discussed various issues relating to the subject. A broad consensus was reached and a writing committee was formed. They met again on 11 August, 2019 at Chennai for a detailed discussion. The statement was sent to the entire group by e-mail and their approval obtained. **Objective:** To formulate a consensus statement enable proper diagnosis and management of Cow's milk protein allergy. **Recommendations:** Cow's milk protein allergy is most common in the first year of life. Gastrointestinal manifestations are usually non-IgE mediated and therefore skin prick test and specific IgE levels are not useful in diagnosis. Clinical response to elimination diet followed by a positive oral food challenge is diagnostic. In patients with only gastrointestinal manifestations, sigmoidoscopy and rectal biopsy may be considered as an alternative. Management involves strict avoidance of all forms of bovine milk protein. For infants who are artificially fed, an extensively hydrolyzed formula is the first choice. Soy formula is an alternative in those above six months of age. Since most infants outgrow the allergy, elimination diet is only for a limited period and re-evaluation should be done periodically.

Keywords: *Extensively hydrolyzed formula, Food allergy, Non-IgE mediated, Oral food challenge, Rectal biopsy*.

Food allergy is an adverse immunological response to proteins in food and must be differentiated from food intolerance, which is a general non-specific term for any adverse reactions to particular constituents of food. Cow's milk protein allergy (CMPA) is an immune- mediated reaction to various proteins in cow's milk. It is the most common food protein allergy in infants and children [1]. The reaction may be IgE-mediated, non-IgE mediated or mixed. CMPA may have cutaneous, respiratory and/or gastrointestinal manifestations.

In India, awareness among pediatricians is low leading to misdiagnosis or concurrence with parents that the child has allergy. This results in wrong dietary advice and unnecessary use of expensive formulas. The prevalence of CMPA peaks in infancy (1.5 -3 %) and falls to less than 1% at 6 years of age [2]. About 10 to 15% of children who have CMPA are also allergic to soy and the risk of cross- allergy is higher if symptoms begin below 6 months of age [3]. There are no epidemiologic studies on the prevalence of food allergy including CMPA in Indian children. Among hospital-based studies, CMPA was reported as a cause of malabsorption syndrome in 6% children of all ages and 13% of children below 2 years with chronic diarrhea [4]. CMPA was the cause in 35% children below 3 years of age presenting with chronic diarrhea in another study [5].

PROCESS

The pediatric gastroenterology sub-specialty chapter of Indian Academy of Pediatrics (Indian Society of Pediatric Gastroenterology, Hepatology & Nutrition) organized a meeting of select members of the group at Mumbai on 26 October, 2018. Brief presentations on various aspects of the topic were followed by a detailed discussion. A broad consensus was reached and a ten member writing committee was formed. This committee met again on 11 August, 2019 at Chennai for a detailed discussion. The statement was finalized and sent to the entire group by e-mail. Suggestions were incorporated and consent was obtained from all the members .

RECOMMENDATIONS

Diagnostic Modalities

A good reliable history and clinical examination are the cornerstones of diagnosis. Common differential diagnosis like infective colitis, celiac disease, gastro-esophageal reflux disease, eosinophilic esophagitis, immune deficiency and persistent diarrhea should be kept in mind. Empirical exclusion therapy without confirmation of diagnosis is unscientific and best avoided.

Diagnostic Elimination Trial

Current European Society of Pediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) practice guidelines suggest that the initial diagnosis of CMPA should be made on the basis of diagnostic elimination of cow's milk proteins from the diet and then it is to be confirmed by an oral challenge with CMP if there is a response to the elimination diet [6]. Elimination should be total, and particular attention should be paid to hidden sources of the antigen (*e.g*. avoiding biscuits or cake). If symptoms do not improve with strict elimination, the diagnosis of CMPA is unlikely.

Oral Food Challenge

A double blind placebo controlled food challenge is the gold standard for diagnosing CMPA, though it has the disadvantages of requiring a longer time to perform, needing patient and parents co-operation and being expensive [6]. Hence, in most instances (except in those with uncertain or questionable response to the initial oral challenge), an open food challenge is done, wherein the child is continued on a normal milk containing diet. If the patient remains without symptoms for two weeks, then CMPA is ruled out. However, if symptoms recur, then the diagnosis of CMPA is confirmed. Oral food challenge (OFC) is the most specific test for diagnosing food allergy and reliably distinguishes sensitization from clinical allergy. They are more standardized in IgE- mediated reactions, and should be done under medical supervision. However, in cases of severe anaphylaxis, the patient should be on a therapeutic elimination diet straightaway [6]. This test is required before re- introduction of the allergen after therapeutic elimination period is completed to confirm development of tolerance.

Endoscopy and Biopsy

An Indian study done on CMPA noted that sigmoidoscopy (82%) and rectal biopsy (97%) gave best information in patients with gastrointestinal manifestations of CMPA [7]. Histological changes are similar and non-specific in all food allergies and therefore should be interpreted only in the context of appropriate clinical setting. The most frequently seen endoscopic findings are focal erythema, erosions and nodular lymphoid hyperplasia in 40–90% of cases [8]. The presence of more than 60 eosinophils in six high power fields and/or more than 15–20 eosinophils per high power field is highly suggestive for CMPA .

Other Methods

Scoring system for screening: The Cow's milk-related symptom score (CoMiSS) has most commonly been used [9]. However, there is no agreement on cut-off values and it has poor sensitivity and specificity [10]. Until more studies are available from developing countries, CoMiSS cannot be recommended as a screening tool in our setting.

Specific IgE antibodies to cow milk: Specific IgE antibodies detect the presence of circulating antibodies against CMP. However, positive IgE neither confirms allergy nor differentiates between sensitization and clinical allergy [26]. Specific IgE tests are not useful in the diagnosis of non-IgE mediated CMPA .

Skin prick test: Skin prick tests are used to detect the presence of IgE tissue bound antibodies. It can be considered in IgE-mediated disease, but a positive test does not confirm allergy. Wheal size of ≥5 mm (≥2 mm in an infant <2 year) is associated with a higher specificity. A negative skin test rules out IgE-mediated reactions, with negative predictive values of 95%. The wheal size is significantly larger in children with persistent disease compared to those who outgrow CMPA and therefore is useful as a prognostic indicator [11]. Infants are generally less responsive to skin prick tests. It is not validated in non-IgE mediated CMPA and may result in false positive or false negative diagnosis [12].

Approach to Diagnosis

CMPA is a clinical diagnosis, and there is no single test or biomarker that is pathognomonic of the condition. The ESPGHAN criteria of 2012 have done away with routine intestinal biopsy [6]. Allergen elimination followed by oral food challenge, has been advocated as the cornerstone of diagnosis.

A structured approach is needed for accurate diagnosis and should start with an allergy focused history (including family history) and physical examination. If the clinical features discussed earlier are present in an infant or young child, CMPA should be considered in the differential diagnosis. Clinical pointers that suggest IgE-mediated disease are the involvement of two or more systems, commonly the skin, gastrointestinal and respiratory tract. On the contrary, non-IgE mediated

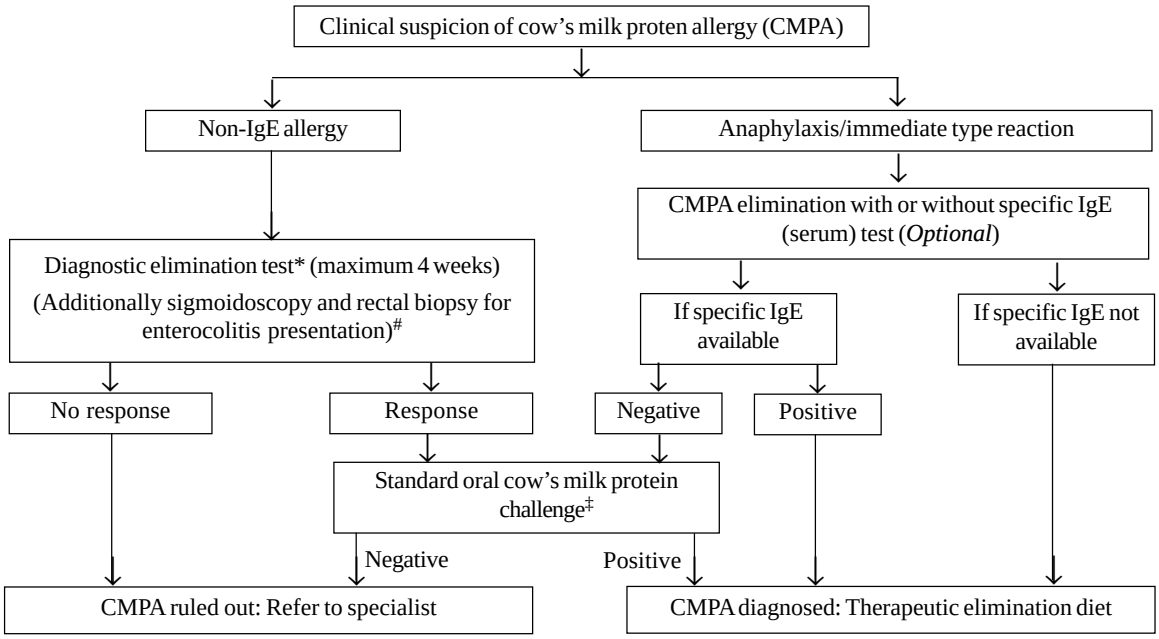
disease (which is more common in India) may manifest with only gastrointestinal symptoms. The expert group does not advocate testing for cow's milk protein (CMP) - specific IgE in serum as a routine, considering the high cost and as it indicates only sensitization (food elimination and challenge needs to be done for diagnosis). However, It may be useful with acute/ life threatening symptoms such as stridor, wheeze, angioedema and anaphylaxis. Here, the food challenge may be delayed by a year if CMP- specific IgE is positive and there is symptom resolution with an elimination diet. Approach to a child with suspected CMPA is given in ***Fig*.1**.

The initial diagnosis of CMPA should be made on the basis of a diagnostic elimination test. Response to CMP withdrawal is noticed within 3-5 days for those with immediate manifestations, 1-2 weeks for those with delayed clinical manifestations, and 2-4 weeks for those with chronic diarrhea/ failure to thrive [13]. If the child does not show any improvement during this time period, a diagnosis of CMPA is ruled out on most occasions. A few exceptions are: some children have associated soy protein allergy or allergy to other components of the extensively hydrolyzed formula (eHF) that has been used during milk restriction; some sick infants may also have multiple food protein allergies (such as egg, wheat, soy, nuts, sea fish). In both these situations, an amino-acid based formulation (AAF) should be used during the allergen elimination, and if there is no improvement in symptoms on this too, then CMPA is ruled out as a cause for the child's symptoms [6].

For those on exclusive breastfeeding, elimination requires excluding milk and milk products from the mother's diet (while she continues to breast feed the infant). Care must be taken to remove sources of CMP from the breast-fed infants being given supplementary feeds in addition. For non-breast fed patients, all sources of milk protein should be stopped and infants should be started on an extensively hydrolyzed formula. Soy formula may be used beyond 6 months of age. For older children, all forms of milk and milk products should be stopped as part of the elimination [6].

Patients who show an improvement of symptoms with allergen elimination (as above) should be subjected to an oral milk challenge after 2-4 weeks of a CMP free diet in the asymptomatic period to confirm the diagnosis [13].

Procedure for oral food challenge: CMP either as formula or pasteurized milk (in <12 months age) or pasteurized milk (in >12 months age) is administered cautiously in the following manner: 1 mL, 3 mL, 10 mL, 30



*#Subset of patients with enterocolitis: sigmoidoscopy and rectal biopsy is useful; *Exclusively Breast Feeding (EBF) infant: Eliminate all Cow's Milk Protein containing food in mother; Mixed/ Formula fed: Eliminate all Cow's Milk Protein food/ formula in mother & infant. eHF/ soy trial; Symptoms with first CMP feeds: return to EBF (maternal restriction of milk protein not required) (Elimination duration: 1-2 weeks for most, 2-4 weeks for chronic symptoms); ‡Exclusively Breast Fed: Mother returns to normal CMP diet; Mixed/ Formula Fed: Home challenge with CMP formula/ milk*

Fig. 1 *Approach to a child with suspected cow's milk protein allergy.*

mL, 100 mL (given every 30 minutes), which can be done on an out-patient basis [14]. The child should be observed for two hours, and then sent home with an instruction to continue at least 200 mL of milk/day and to stop if there is recurrence of symptoms. The child should be reviewed after two weeks to decide whether to continue milk or to stop milk again depending on the clinical response to milk introduction. For those with severe reactions on initial presentation (IgE-type), the milk challenge is administered in an even more graded fashion (0.1 mL, 0.3 mL, 1 mL, 3 mL, 10 mL, 30 mL, 100 mL: given every 30 minutes) as an in-patient with all resuscitation facilities including injection adrenaline to manage anaphylaxis. A positive reaction to milk introduction confirms the diagnosis of CMPA. If no reactions occur, 200 mL/day of milk is continued for two weeks to look for any delayed manifestations.

Since non-IgE mediated gastrointestinal symptoms appear to be the commonest manifestation of CMPA in India, sigmoidoscopy and rectal biopsy can also be considered for confirmation of diagnosis in children whose parents do not give consent for oral food challenge. In children with no response to diagnostic elimination diet or those in whom alternative diagnosis is strongly considered, further investigations are necessary.

Management

The safest strategy for the management of CMPA is the strict avoidance of CMP for a defined period [6]. A delay in diagnosis may result in failure to thrive, anemia, and hypoproteinemia; however, there is ample evidence that over-diagnosis or wrong diagnosis results in unnecessary dietary restrictions, increased risk of rickets, decreased bone mineralization and great economic burden [15]. The choice of an appropriate substitute to fulfill the nutritional requirements during the time of CMP avoidance is crucial. There are some variables which should be considered before recommending alternatives to milk feeds **(*Box* II**).

Exclusively Breast-fed Infants

CMPA in an exclusive breast-fed infant is usually mild and majority of these infants do not have anemia or failure to thrive. Breastfeeding is continued till at least 6 months of age and the mother is advised to avoid bovine milk and all dairy products (cheese, yogurt, paneer, butter, ghee) as well as milk containing foods in her diet. It may take up to 72 hours for the antigens to disappear from breast milk and for clinical response after withdrawal of milk and milk products [16]. The maternal elimination diet is maintained for 3 to 6 days in those with IgE-mediated allergy, while in non-IgE mediated it is two weeks in those without atopy, and 4 weeks in those with atopic dermatitis or allergic

Box I Clinical Manifestations of Cow's Milk Protein Allergy

IgE mediated syndromes (Onset- immediate to <1 h)

Immediate food hypersensitivity, perioral utricaria/ erythema, angioedema/ anaphylaxisGeneralized rash, vomiting, wheezing, cough

***Non-IgE mediated* (Onset - late >24 h, usually after 5-7 d)**

Proctocolitis: Fresh bleeding per rectum, constipation

Enteropathy: Watery diarrhea, failure to thrive, protein losing enteropathy, occult gastrointestinal bleeding

Enterocolitis: Bloody diarrhea, anemia/hypo-proteinemia

Esophagitis: Reflux like symptoms, vomiting/feed refusal, dysphagia

Gastritis/Gastro-duodenitis: hematemesis, occult gastrointestinal bleed

Atopic dermatitis

Mixed (Onset-intermediate, <24 h)

Food Protein Induced Enterocolitis syndrome (FPIES): Vomiting/diarrhea/colitis, shock like symptoms with severe vomiting, diarrhea, neutrophilic leukocytosis and metabolic acidosis

Table I Differentiation Between Cow's Milk Protein Allergy and Lactose Intolerance

	Cow's milk protein allergy	*Lactose intolerance*
Types	IgE and Non-IgE mediated	Congenital, primary (age-dependent decline in lactase enzyme) and secondary (mucosal damage after severe gastroenteritis or other causes)
Mechanism	All or none phenomenon, is an immune reaction to milk protein	Quantity-dependent, due to deficient lactase enzyme in brush border
Symptoms	Multisystem (gastro-intestinal/skin/ respiratory	Only gastrointestinal (diarrhea, flatulence, pain)
Natural history	Recovers by 4-5 y of age in majority	Recovers in days-weeks in secondary, permanent in congenital and primary

colitis [6]. If symptoms persist even after this period, other allergens or a different etiology should be considered. If the symptoms improve or disappear, CMP may be reintroduced as a challenge in the maternal diet. If symptoms recur then CMP should be avoided as long as she is breast-feeding. Calcium supplementation (1000 mg per day in divided doses) is essential for the mother during the period of elimination. Very strict elimination diets that exclude not only milk but also fish, soy, wheat and gluten products may cause unnecessary nutritional imbalance in the mother and are best avoided.

Infants on Mixed Feeds

CMP is completely withdrawn along with all unmodified animal (goat/sheep/buffalo/camel) milk proteins. However, breastfeeding should be continued without any elimination in the maternal diet [6]. In infants less than 6 months of age with mild to moderate reaction, extensively hydrolyzed formula (eHF) with proven efficacy is recommended [17]. There is safety concern regarding use of soy in infants less than 6 months of age and cross-allergy to soy is seen in in 10-15 % of infants with CMPA. Soy is however cheaper and more palatable than eHF and these factors should be weighed when alternate formula is recommended. Children with IgE-mediated CMPA tolerate soy protein better than non-IgE mediated CMPA. In infants more than 6 months of age with mild to moderate reaction, soy protein formula can be used instead of eHF if there are financial constraints [18]. If the diagnosis is reasonably certain, but there is no improvement within 2 weeks of eHF, then amino acid formula (AAF) should be tried before CMPA is ruled out. In infants who are sick or have severe or life threatening symptoms AAF should be the first choice rather than eHF [6].

Exclusive Formula Fed Infants

Breast feeds must be started if they were stopped only recently. Rest of the management protocol is the same as in group II.

Box II Factors to Consider When Deciding Alternatives to Bovine Milk

Age: Whether older than or younger than 6 months

Feeding pattern: Exclusive breastfeeding, mixed feeds (breastfeed and formula) or exclusive formula feeds.

Type of allergy: IgE-mediated or non-IgE mediated.

Severity of reaction: Severe or mild to moderate

Clinical manifestations: Gastrointestinal, respiratory or skin.

Financial considerations: Affordability

A simple algorithm for management of infants with non-IgE mediated CMPA is shown in ***Fig.* 2**.

Management of IgE-mediated CMPA

When an infant with CMPA presents with classical symptoms of IgE-mediated allergy such as angioedema, urticaria or anaphylaxis, emergency care should be provided as for any allergy, and all forms of CMP should be immediately withdrawn. In mild to moderate allergy, eHF is the first choice. Only those who do not respond to the above measures should be switched to AAF. Those with severe allergy require hospitalization and should be given only AAF. OFC should be done with caution only in a hospital setting between 12 to 18 months. In those with severe IgE mediated allergy, OFC should be done only with eHF [16].

The elimination diet should be continued for atleast one year and re-evaluation done every 6 months subsequently [40]. The prognosis of infants and children with CMPA is good as 50% will tolerate CMP by 1 year, >75% by 3 years and >90% by 6 years of age [19]. Only 5 % would continue into adulthood. High total IgE and specific IgE levels correlate with a higher age of acquiring tolerance [20].

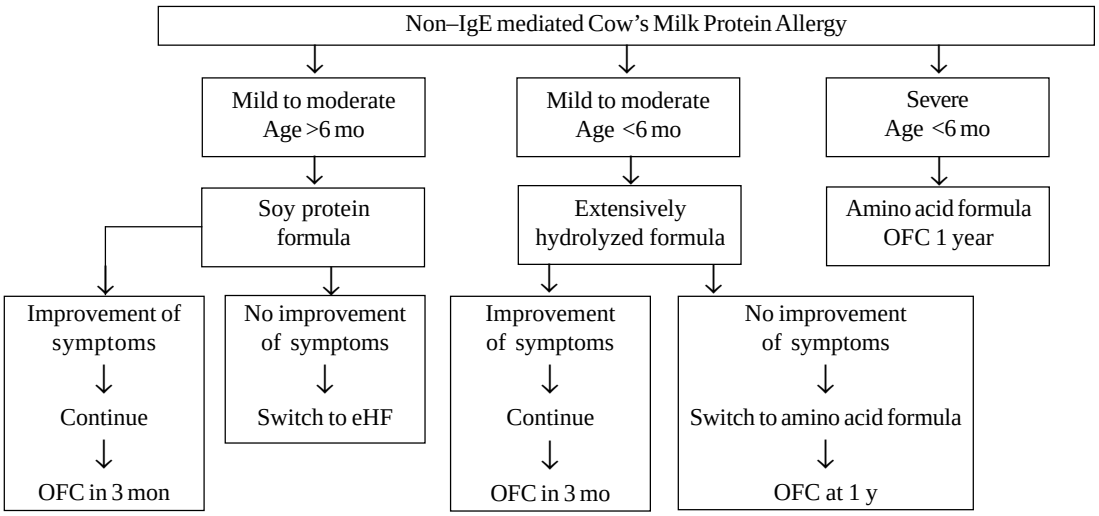
Prevention

Primary prevention aims to delay the first exposure of infants to cow's milk protein, while secondary prevention involves avoiding antigen exposure in high-risk atopic infants. Tertiary prevention is when clinicians advise cow's milk avoidance as a means of treatment after confirmation of diagnosis.

Exclusively Breast-fed Infants

The best way to prevent CMPA is exclusive breast-feeding for 4-6 months (17-27 weeks)[21]. The incidence of CMPA is lower (0.5%) in exclusively breast-fed infants compared to formula-fed or mixed-fed infants. The reproducible clinical reactions to CMP are mild to moderate in the majority. A plausible explanation is that the level of CMP in breast milk is 100, 000 times lower than that in cow's milk and is in the form of peptides and not as intact protein [22]. Breast milk also contains proteases a digesting protein. The other protective factors in breast milk are maternal antibodies and chemokines which reduce the development of allergy, hormones and growth factors which potentiate maturation of gut associated lymphoid tissue (GALT), polyunsaturated fatty acids (PUFAs), glycoproteins, oligosaccharides and micro RNAs which exhibit immune function.

There is no evidence that modification of maternal diet during pregnancy or lactation has any protective effect



OFC: Oral food challenge; eHF: Extensively hydrolyzed formula.

Fig. 2 *Management of infants with non-IgE mediated cow's milk protein allergy.*

against allergy in at-risk infants. Moreover an exclusion diet may cause nutritional deficiencies in the lactating mother and infant [23]. Allergen avoidance should be advised only when the breast-fed infant has proven CMPA. There is no evidence to suggest that delaying introduction of solid foods, or even potentially allergenic foods, beyond age 4-6 months offers any protective effect. Supplementary foods should be introduced one at a time in small quantities, preferably while the mother is still breastfeeding but not before the infant is at least 17 weeks of age to prevent other allergies [24].

Infants Not Exclusively Breast-fed

There is no role for milk formula with intact protein from other animals or soy protein in the prevention of allergy. The role of hydrolyzed formulae (both partial and extensively) in prevention of CMPA is still debated. If there is a family history of allergic disease in both parents, there may be some justification in using partially hydrolyzed formula with whey protein as a starter formula, if exclusive breastfeeding is not possible. However, a recent meta-analysis concluded that there is no benefit of using hydrolyzed formula to prevent CMPA [25].

CONCLUSION

CMPA is primarily a disease of infancy with increasing incidence. While manifestations of IgE- mediated disease is immediate with multi system involvement, non-IgE disease is delayed with symptoms related to the GI tract. Clinical response to an elimination diet followed by an oral food challenge is the cornerstone of diagnosis. In children with persistent diarrhea and colitis, sigmoidoscopy and biopsy are useful in diagnosis. Breastfeeding should be continued and all cow's milk protein should be stopped. Most children will outgrow the allergy between 12 and 18 months of age.

Contributors: JM: coordinated and edited the paper; SKY: verified the scientific content; ASr and RM: authored the segment on prevalence and clinical features; AS and YW: the segment on diagnostic modalities; UP,GR: the segment on approach to diagnosis, MS,GS: the segment on management and prevention. All authors participated in finalizing the paper.
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RECOMMENDATIONS

- In non-IgE mediated cows' milk protein allergy (CMPA), milk specific-IgE and skin prick tests are not useful in diagnosis.
- Oral food challenge following a clinical response to elimination diet is mandatory in diagnosis. In those with only gastrointestinal manifestations, sigmoidoscopy and rectal biopsy should be considered
- Duration of diagnostic milk protein elimination needed to observe clinical response varies from 3-5 days in IgE-mediated allergy and 2-4 weeks in others.
- Unmodified mammalian milk (cow, buffalo, donkey, goat or camel) should not be used in infants with proven CMPA.
- In artificially-fed infants with CMPA, extensively hydrolyzed formula is the first choice. Soy formula may be considered above 6 months of age. Amino acid formulas are needed only in a small subset of infants.
- Delaying introduction of solid foods, or even potentially allergenic foods, beyond 4-6 months of age has no protective effect in high risk infants.

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Ensuring Exclusive Human Milk Diet for All Babies in COVID-19 Times

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The coronavirus disease (COVID-19) pandemic has ramifications for the delivery of newborn nutrition and care services. World Health Organization recommends continuation of breastfeeding in these difficult times, with due precautions. If direct breastfeeding is not possible, milk expression should be explored. Pasteurized donor human milk from milk banks may be fed if mother's own milk is not available. To universalize access to human milk, the Indian government has proposed the establishment of comprehensive lactation management centers/milk banks, lactation management units, and lactation support units at all levels of the public health system. Due to COVID-19, these centers are encountering additional challenges cutting across interventions of rooming in, breastfeeding, milk expression, and provision of donor milk and kangaroo mother care. We discuss issues faced and alleviation measures taken by these centres in relation to provision of an exclusive human milk diet for infants during the pandemic.

Keywords: *Breastfeeding, Donor Human Milk, Kangaroo Mother Care, Pandemic.*

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The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pandemic has brought forth unprecedented global and local challenges, more so in developing countries like India. While on one hand, the country has launched measures including a nationwide lockdown to curb the spread of the disease, on the other, efforts are on to minimize disruptions to the delivery of essential services such as maternal and newborn health and nutrition [1]. Estimates suggest that lower coverage of interventions for six months due to the pandemic will result in 17% (1.15 million) more newborn and child deaths globally [2].

Breastfeeding and a human milk diet has a key role in preventing life-threatening infections in infants during the COVID-19 pandemic thus reducing the stress on the health system. Considering that, India has a high newborn mortality rate and a third of all preterm births, in the world [3], ensuring adequate availability of breastmilk becomes even more pertinent. Of the 25 million births, nearly 43% (11 Million) are not breastfed within the first hour [4] and nearly 30-50% preterm/sick babies in neonatal intensive care units (NICU) in India lack access to breastmilk [5].

INFANT FEEDING AND COVID-19

So far, SARS-CoV-2 has not been detected in the breastmilk of mothers with COVID-19 [6]. Preliminary data indicates a strong immunoglobulin A dominant SARS-CoV-2 immune response in breast milk of COVID-19 infected mothers [7]. In February 2020, the World Health Organization (WHO) advised early and exclusive breastfeeding for COVID-19 suspected and confirmed mothers, while encouraging them to take adequate precautions. Mothers who are too ill to breastfeed are advised to feed their expressed breastmilk [8]. Guidelines by Indian Council of Medical Research (ICMR) and other professional bodies propagate similar advice [9-11].

In absence of the above, pasteurized donor human milk (PDHM) from a human milk bank (HMB) is recommended over formula milk. Once mothers recover, they should be supported for relactation [8,11]. PDHM compared to formula milk reduces the risk of sepsis, necrotizing enterocolitis, diarrhea and feeding intolerance, and the length of stay in NICU [12]. Feeding supplementary PDHM is associated with increased exclusive breastfeeding at six months of life [13].

As per the National Guidelines for Lactation Management in Public Health Facilities [14], it is proposed to establish facility-based lactation management centers at all levels of the public health system. Comprehensive lactation management centers (CLMCs) or integrated milk banks at tertiary centers, lactation management units at secondary centers, and

lactation support units at primary care centers [14]. Currently, 83 CLMCs exist in India, yet the number is insufficient to meet the large demand for human milk, despite 1,000,35 babies having benefitted from PDHM in the year 2019 (data not shown).

ENSURING ACCESS TO HUMAN MILK

The COVID-19 pandemic has posed several challenges to the provision of newborn nutrition and care interventions including maternal support, breastfeeding, kangaroo mother care (KMC), family participatory care, and human milk bank operations and supply of PDHM. A comprehensive newborn nutrition and care response comprising of optimal lactation support and accessibility to PDHM is needed to improve neonatal health outcomes. Globally, efforts are on to address such challenges. Recently, a virtual group consisting of members from 37 countries has been formed to facilitate the sharing of information and best practices and discussion of evidence to prepare human milk banks to deal with COVID-19 challenges [15].

We herein highlight the issues related to access to newborn nutrition and care and how facilities in the country are addressing them during and beyond COVID times.

Access to Mothers' Own Milk

Suspension of outpatient services and restrictions on travel, in addition to staff shortage (absence and irregularity due to travel restrictions in lockdown), made it difficult for mothers and families to get access to feeding support. Mothers are being discharged sooner and if their babies are in neonatal intensive care units, they get little opportunity to learn to breastfeed or express and feed mothers' milk to their babies due to which, they are resorting to formula feeding at homes. Due to infrastructure constraints and mandate to transfer COVID-19 positive mothers to separate COVID-19 wards, many facilities are separating COVID-19 positive mothers from their babies, making breastfeeding a challenge. Few facilities that can isolate, place mother and baby together, in such cases, breastfeeding is practiced following strict hygiene measures. Early discharge of the baby along with a healthy attendant is encouraged at some places, which makes breastfeeding difficult. However, some hospitals prefer not to discharge babies, owing to fears that family may have COVID-19 cases. There may be hesitation in allowing mothers of outborn neonates to breastfeed, express milk, or conduct KMC (as they are not sure of their background) as an infection control measure. Some facilities end up feeding PDHM or formula owing to a shortage of staff to handle the collection and transfer of expressed mother's milk. Mothers and families, and health care workers have fear and confusion around COVID-19 and breastfeeding, along with a sense of heightened stigma about the disease which is impacting access to breastmilk.

Guidance suggests hospitals should provide more information, psychosocial, and technical support to lactating mothers and their families to build their confidence and establish and sustain milk supply. Providers or family members (with personal protection equipment) should provide support in breastfeeding and caring for newborns [11]. Facilities have reported adopting various methods to improve lactation in mothers, including introducing music and religious book reading sessions to make mothers feel relaxed; allocating rooms in NICUs for mothers along with regular thermal screening and adherence to strict hygiene measures; arranging for additional breast pumps for all mothers; and information dissemination platforms through calls, live chats, and dedicated helplines. Some centers are using these to provide support and guidance on the continuum of breastfeeding.

Rooming-in and KMC

WHO and the Italian Society for Neonatology recommends that infants and mothers with suspected or confirmed COVID-19 should be allowed to remain together and practice rooming-in throughout the day and night [16,17]. Some facilities temporarily discontinued the practice of KMC for all eligible neonates as a precaution but resumed it when babies showed signs of distress (personal communication – Dr Kumutha J, Chennai). Hospitals, in general, do not recommend KMC by COVID-19 positive mothers, and recommend engaging healthy family members to conduct KMC. Many staff and mothers are not comfortable in practicing KMC for fear of transmission of infection *via* close contact. Rooming-in is difficult as COVID-19 positive mothers are shifted to COVID-19 wards/ facilities while their babies remain in the neonatal units.

Guidance by professional bodies indicates that facilities where isolation of COVID-19 suspected/ confirmed mother and her baby is possible, rooming-in with direct breastfeeding should take place. Where isolation is not possible, mother should be temporarily separated from baby until she is confirmed negative [11]. Some facilities have created separate COVID postnatal wards wherein babies are bedded in with mothers. Lactation counsellors use microphone to conduct group counselling maintaining adequate distance.

Donor Human Milk

PDHM is a lifesaving resource for babies in neonatal intensive care units who cannot receive their mother's milk

[18]. Various studies have shown thermal inactivation (specifically heat treatment of 60 °C for 30 minutes of donor human milk) of respiratory viruses particularly the MERS corona virus [19,20]. A recent study regarding heat stability of SARS-CoV-2 suggests that it is killed at 56 °C centigrade within 30 minutes [21]. Chambers, *et al.* [22] also demonstrated that when control breastmilk samples spiked with replication competent SARS-CoV-2 virus were treated by Holder pasteurization, no replication-competent virus or viral RNA was detectable. Another recent study conducted on five different SARS-CoV-2 isolates from Germany, France and the Netherlands in five individual breast milk samples showed that human breast milk containing infectious SARS-CoV-2 can be efficiently inactivated using standard holder pasteurization [23]. Holder pasteurization used in HMBs exposes the milk to 62.5 °C for 30 minutes.

Many centers have temporarily suspended human milk banking services for reasons such as absenteeism and irregularity of staff due to imposed lockdown. Number of facility-based milk donors has reduced because of earlier discharges and discontinuation of outpatient services. Amid lockdown, home collection of donor milk, and community milk collection has almost stopped. Some mothers may be reluctant to donate fearing donation as a point of exposure. Few hospitals are finding it hard to devote time to milk culture and outsourcing has become difficult for others due to transportation restrictions. To make up for the reduced supply, some units are rationing PDHM to the most vulnerable, small, and sick babies, which may lead to increase in formula feeding for babies without access to maternal milk.

PDHM provided during any COVID-19 related separation, should be coupled with optimal lactation support to ensure maternal milk supply at the earliest. This reduces the excess demand for PDHM which can be given to those who need it the most [15]. Guidelines by professional bodies suggest greater vigilance and modification of donor screening procedures [11]. CLMC staff are asking additional questions related to symptoms, travel and contact history, and excluding symptomatic / at-risk donors. Strict hygiene procedures are followed while supporting expression, transportation, and handling of milk. Despite restrictions related to external lab facilities, few CLMCs are collecting and storing donor milk so that it can be tested once lab facilities become available. One facility has created a WhatsApp group of lactating mothers to motivate them to donate milk and is picking up donated milk from homes, which has led to an increase in donation and number of babies benefitting from donor milk [Personal communication: Dr Senthil Kumar, Coimbatore].

CONCLUSIONS

Strengthening of lactation management systems to universalize access to human milk is a key strategy to reduce newborn morbidity and mortality during and beyond the pandemic. We, therefore, call on partners and stakeholders to highlight importance of neonatal nutrition during the pandemic; fund research on breastmilk and COVID-19; facilitate innovations across CLMC processes to improve access to maternal milk and PDHM; equip zonal reference centres to support facilities to adequately prepare for future emergencies; and support the consolidation of the countrywide Human Milk Bank Association of India to facilitate communication, sharing of data and best practices, and evidence generation among milk banks. Now is the time for all stakeholders to jointly mount a comprehensive response against the virus - one that encompasses effective newborn nutrition and care practices to protect the future citizens of our country.

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Liver Biopsy in Children

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Liver biopsy is the cornerstone of medical decision-making for a wide range of hepatic diseases in children. The indications for liver biopsy vary greatly depending on the ease of diagnosis with non-invasive tests, the need to stage of disease, and the role of histological evaluation in management of liver disease. Multiple methods of liver biopsy are available to the clinician and are utilized based on clinical circumstances, cost, and consideration of contraindications. Collaboration between the clinician and pathologist is important in order to handle the tissue sample appropriately and interpret the histology. The purpose of this paper is to provide a broad overview of liver biopsy indications, techniques, pre- and post-biopsy care and complications, interpretations, contraindications, recent advancements, and pitfalls that occur with liver biopsies.

Keywords: *Chronic liver disease, Diagnosis, Histopathology, Management.*

Acute and chronic liver diseases in children are multifactorial. Liver biopsy is considered the gold standard for diagnosis, prognosis, and assessment of treatment response for liver diseases. With the availability of ultrasound guidance and effective and safe sedation in children, it has become a routine procedure in many parts of the world. The purpose of this article is to provide an overview of the role of liver biopsy in children which includes its indications, overview of the most common techniques used, complications that may arise, handling of the tissue properly, interpretation of results, contraindications, recent advancements, and the pitfalls of liver biopsy.

INDICATIONS FOR LIVER BIOPSY

Evaluation of liver tissue is critical in the management of various diseases of children as listed in ***Box* I**.

Diagnosis

Detailed history and thorough clinical examination are the first steps in evaluation of children suspected to have liver disease or hepatic involvement with systemic diseases. Non-invasive tests of blood, urine, and stool combined with appropriate imaging of the liver are deployed first and liver biopsy is reserved for further information. Over the last few decades, improvements in imaging techniques can help assess fibrosis in liver, provide high resolution images to assess the biliary tree, and highlight the nature of liver masses. Commercially available genetic and enzyme testing kits can provide confirmation of many liver diseases without liver biopsy. Newborn screening of all babies born in USA is helpful in capturing common but not all genetic and metabolic diseases.

Neonatal cholestasis is caused by a wide range of etiologies and, some causes need urgent intervention before irreversible damage occurs. Biliary atresia needs urgent diagnosis as Kasai procedure, earlier in the neonatal period has a higher success rate [1]. A timely diagnosis of many metabolic disorders can be corrected by removal of the offending agent or providing corrective medication. Initial laboratory evaluation of neonatal cholestasis consists of a comprehensive metabolic panel, complete blood count with differential, gamma-glutamyl transferase (GGT), prothrombin time (PTT), international normalized ratio (INR), direct and total bilirubin [2]. Abdominal ultrasound and hepatobiliary scintigraphy are useful adjuncts in diagnosis. However, liver biopsy remains the diagnostic gold standard for diagnosis of neonatal cholestasis [3], especially biliary atresia, as imaging of the anatomical abnormalities of biliary passages is difficult to assess at a young age [4]. Liver biopsy has the highest sensitivity (100%) in detecting biliary atresia with a specificity of 94.3% and an accuracy rate of 96.9% compared to other diagnostic modalities like magnetic resonance cholangiography (MRCP), ultrasonography, hepatobiliary scintigraphy or single-photon emission computer tomography [5].

Obesity in children has become a global problem with prevalence of over 19% in some populations including in India [6]. Liver ultrasound has previously been used to diagnose nonalcoholic fatty liver disease (NAFLD) based on the degree of echogenicity. However, echogenicity

does not correlate well with steatosis or fibrosis and cannot distinguish between the two. Ultrasound quality also varies with technician's skill level [7]. Transient elastography has been shown to accurately assess the degree of fibrosis by measuring liver stiffness but has not been fully adapted or validated for pediatric use [8]. Liver biopsy provides a reliable assessment of liver steatosis and fibrosis [7]; although, its use for diagnosis of NAFLD is debatable [9,10].

Prognosis

Liver biopsy can be used as a prognostic tool to assess disease severity and staging of fibrosis. The degree of fibrosis or types of cells found in the tissue sample facilitates risk stratification of morbidity and mortality [11]. Staging of fibrosis on biopsy can help determine the progression of NAFLD [12]. Additional screening modalities such as upper endoscopy to monitor for varices and hepatocellular carcinoma screening should be done when progression to cirrhosis is observed [13].

Hepatitis C infection can be diagnosed with laboratory values alone, yet LB is still regarded as the gold standard in staging and monitoring disease severity as determined by degree of fibrosis in adults [1,14]. Recent studies in children with Hepatitis C to evaluate direct-acting antiviral therapy did not require liver biopsy [15].

LB must be performed to properly stage and provide a prognosis in those with autoimmune hepatitis (AIH) [16].

Hepatoblastoma is the most common malignant tumor of the liver in children. Liver biopsy is performed to determine the classification of the hepatoblastoma into epithelial type or mixed epithelial/mesenchymal type. The epithelial type is further classified into the subtypes: fetal, embryonal, and small cell undifferentiated. The small cell undifferentiated hepatoblastoma subtype has a poor response to chemotherapy and a worse prognosis [17].

Managing Therapy

Liver biopsy can guide and cater management approaches. Specific treatment can be initiated or withheld based on the histological findings.

Measuring port-based plasma cell infiltrate can help predict a greater than 90% chance of relapse when monitoring treatment of AIH [18]. Withdrawing immunosuppression therapy for patients with AIH is predicated on LB results. After 1 to 2 years of normal biomarkers, liver biopsy is necessary to prove resolution of inflammation [16].

Prior to treatment, liver biopsy is recommended for children with Hepatitis B to assess necro-inflammation and stage of fibrosis [19]. Pediatric patients with chronic Hepatitis B who have moderate to severe necro-inflammation on liver biopsy are likely to benefit from antivirals or interferon treatment [20]. However, benefit is not established for children with mild necro-inflammation or fibrosis. Degree of fibrosis or cirrhosis on liver biopsy can also help modify treatment regimen, as interferon can cause further decompensation of liver function [21].

Pediatric acute liver failure is a clinical syndrome in which previously healthy patients develop rapid hepatic dysfunction. There is often a reluctance to perform liver biopsy in this setting due to safety concerns such as bleeding risks and an elevated INR. However, a recent single-center retrospective study demonstrated that liver biopsy was performed safely in most children [22]. LB assisted in the diagnosis of 62% of patients and aided in treatment decisions in 9 of 26 cases [22].

PRE-BIOPSY WORKUP

Preliminary laboratory evaluation prior to liver biopsy includes a complete blood count, prothrombin time/ international normalized ratio, partial thromboplastin time, and bleeding time [7]. Some institutions recommend blood typing in case of procedural bleeding [11]. In order to minimize complications, imaging should be reviewed by experts prior to liver biopsy to evaluate for presence of biliary dilatation and concerning focal lesions such as a hemangioma [11]. Depending on the institution, liver biopsy may be done in an operating room or endoscopy suit. Appropriate sedation should be administered.

BIOPSY TECHNIQUES

Percutaneous Liver Biopsy

The percutaneous liver biopsy is the most common modality as it is less invasive and less expensive than other approaches. It can be performed by physical examination or with ultrasound guidance.

The percussion-guided or blind method is the classic liver biopsy technique. It is performed by percussing over the right upper quadrant of the abdomen, at the mid-axillary region, to find the biopsy site. This method can be used for diffuse liver pathology and is the least expensive method of liver biopsy.

The image-guided method typically involves the use of ultrasound to mark the site of biopsy and can be useful when targeting a lesion within the liver. This method can provide a better tissue yield and decrease complications associated with liver biopsy [23]. While ultrasound may have an additional upfront cost, ultrasound guided liver biopsy may be cost effective when considering higher likelihood of complications that may occur with blind liver

biopsy [23]. In a study of 102 pediatric patients who underwent an ultrasound guided liver biopsy or a blind liver biopsy, serious complications such as intrahepatic hematoma were higher in the blind liver biopsy group [24]. Kader, *et al*. [25], showed that ultrasound guidance prompted more optimal biopsy site selection in 25% of cases compared to previously determined locations with percussion.

The aspiration or suction-type needle uses steady suction with a syringe connected to the needle (***Web fig.* 1**), which is advanced into the liver to aspirate tissue. For children, there is often a penetration limiter to monitor the depth that the needle can advance. This suction technique can lead to more tissue fragmentation.

The Tru-Cut needle is the most commonly used cutting-type needle. This device consists of an inner obturator with a wedge to entrap tissue fragment and an outer surrounding cutting sheath. The obturator is advanced into the liver and then the sheath is advanced to cut the liver and remove the specimen. These types of needles have been shown to provide better specimens in patients with advanced fibrosis or cirrhosis since they limit fragmentation or shattering of the sample [26].

Spring-loaded cutting needles trigger a rapid firing of the inner obturator needle and outer cutting cannula via semi-automatic or fully automated mechanism (***Web fig.* 2**) [27]. These needles allow the user to perform the procedure without the 'jabbing motion' practiced in the aspiration and cutting-type methods. A study of 836 adult patients, showed that the spring-loaded automatic biopsy needles obtained larger specimen size compared to the Tru-Cut needle (1.7 mm vs 1.5 mm, P <0.05); however, the larger size was not clinically important [28].

Box I Common Indications for Liver Biopsy in Children

- Etiology and severity of neonatal cholestasis
- Abnormal liver function tests and/or hepatomegaly
- Etiology and severity of acute liver failure
- Cryptogenic cirrhosis or portal hypertension of unknown etiology
- Staging of chronic hepatitis of infectious, autoimmune, or other causes
- Etiology and level of duct involvement in sclerosing cholangitis
- Subtyping of metabolic and genetic diseases of the liver
- Drug-induced liver disease
- Hepatic masses
- Systemic disorders that may present with either fever of unknown origin or multi- organ infiltration or involvement
- Post liver transplantation rejection
- Veno-occlusive or graft *versus* host disease in non-liver transplants
- Obtain tissue to identify and culture infectious agents
- Effectiveness of medications and treatment interventions for chronic liver disease
- Screening of relatives of patients with familial diseases in subclinical presentation

Modified from reference 2,11,19,31,33.

Trans-jugular Liver Biopsy

Trans-jugular approach to liver biopsy is typically reserved for patients with severe liver disease with coagulopathies or ascites and in hematological conditions, where percutaneous liver biopsy is contraindicated due to the high risk of bleeding [29]. The biopsy specimen obtained by trans-jugular route is often regarded as smaller and more fragmented than percutaneous samples. Cholongitas, *et al*. [30] showed that percutaneous and trans-jugular modalities had similar yields for histological diagnosis. However, a recent study in India showed poorer tissue yield with trans-jugular than the percutaneous liver biopsy [31]. The technical successes and complications were similar between the two approaches, but overall had a lesser outcome for length of specimen, number of complete portal tracts, adequacy for reporting, and fragmentation [31].

Surgical or Laparoscopic Liver Biopsy

Wedge liver biopsy allows for direct visualization of the liver surface but traditionally was thought to have an increased risk profile. The laparoscopic method allows for larger specimen size, immediate control of procedure-associated bleeding, and ability to visualize tissue prior to biopsy [11,32]. According to the European Society for Pediatric Gastroenterology Hepatology and Nutrition (ESPGHAN) position paper on liver biopsies, laparoscopic liver biopsy should be considered if increased risk of bleeding, ascites of unknown etiology, evaluation of abdominal mass, failure of previous percutaneous liver biopsy, and the requirement for a large biopsy sample for enzymatic analysis [33].

The advantages and disadvantages of different types of LB techniques have been summarized in ***Table* I**.

POST-BIOPSY CARE AND COMPLICATIONS

After the procedure, vital signs (blood pressure, heart

Table I Advantages and Disadvantages of Different Methods of Liver Biopsy

Method of Liver biopsy	*Advantages*	*Disadvantages*
Blind Percutaneous	• Least expensive method • High tissue yields and less fragmentation reported • Can be a bed-side procedure	• Inability to sample focal lesions or avoid risky areas • Higher rates of complications over image-guided liver biopsy
Image-guided	• More optimal biopsy site and target focal lesions	• Lesser complication reducing cost over blind liver biopsy method • Need additional resources - Imaging machine and trained personnel
Transjugular	• Safer in patients with low platelet, coagulopathies, ascites • Also measure pressure readings	• Technically challenging • Smaller size and fragmentation of tissue reported • Age and size restrictions
Surgical or laparoscopic	• Ability to biopsy focal lesions or get large sample size • Useful with patients with abnormal anatomy, ascites. • Immediate control of procedure-associated bleeding	• Most invasive method • Longest post-procedure hospital stays • Most expensive method

rate, respiratory rate, oxygen saturation) should be followed and patients should be observed for any complications [33]. According to ESPGHAN [33], "minor" complications of LB in children include pain, subcapsular bleeding that does not require transfusion or prolonged hospitalization, infection, minor bile leak or hemobilia, and arteriovenous fistula. "Major" complications include bleeding, including hemobilia, that requires transfusion, surgery, or intensive care management; pneumothorax or hemothorax; and death.

Matos, *et al*. [34] observed patients for at least six hours after liver biopsy and discharged them if no complications occurred. Another study found that same-day observation for less than eight hours is well tolerated in low-risk children undergoing liver biopsy in outpatient settings [35]. Contact sports should be avoided for one week after biopsy [33].

Bleeding: If bleeding is suspected, a complete blood count and abdominal ultrasound should be obtained to assess for hematoma. However, if bleeding is thought to be life threatening, surgical intervention should never be delayed. In a study of 179 pediatric liver biopsy, 6% had a decrease in hemoglobin of more than 20g/L after biopsy, but none required transfusion [36]. In the same study, 4% developed a small hematoma at the biopsy site immediately after the procedure that was controlled with local compression. In a larger study of 469 pediatric liver biopsies, bleeding was reported in 2.8% of patients which increased with malignancies or post bone marrow transplant [37]. Liver-transplanted children are often treated with aspirin to prevent thrombosis of the hepatic artery. The bleeding incidence or complication rate did not increase with low-dose aspirin; however, low-molecular-weight heparin therapy and focal lesions were identified as risk factors [38]. Image-guided percutaneous liver biopsy with gelatin sponge embolization of the biopsy tract has recently been suggested as a strategy to minimize bleeding complications in high risk patients. The associated complications are minimal, and this can be considered as a first-line approach in the setting of coagulopathy [39].

Pain: Pain was reported in 59% of pediatric patients post-biopsy, which responded to acetaminophen [36]. Transient pain was reported in 20% of patients in another study, most often localized to the liver biopsy site and/or to the right shoulder [40]. Prolonged pain warrants further examination to rule out intraabdominal complications.

Infection: Infection is a risk that can occur at the liver biopsy site or systemically. In a study of pediatric outpatient liver biopsy, only one patient became septic (0.2%) [41].

Arteriovenous fistula: Arteriovenous (AV) fistula after liver biopsy is a rare complication. One (0.2%) case of AV fistula was seen in outpatient liver biopsy setting (patient also had sepsis), and it resolved spontaneously [41].

Pneumothorax: This is a rare complication of liver biopsy. Only one pediatric case was reported in a study of 469 patients (0.2%) [37].

Bile leak: The rate of bile leaks was 0.6% in one study [37] after pediatric liver biopsy and was reported in only one (0.8%) patient who had bilious drainage from the biopsy site in another study [39].

Death: In a study of 469 pediatric liver biopsies, 3 (0.6%) deaths occurred; all of which had a history of malignancy or hematological disease [37]. In two other studies with 626 and 223 patients, no deaths were recorded [36,41].

SPECIMEN HANDLING AND INTERPRETATION OF RESULTS

The liver biopsy should represent the parenchyma and portal tracts. The amount needed for diagnosis depends on the type of disease because of the patchy, heterogeneous nature of some liver diseases. Focal disease and/or small specimen samples can misrepresent the histological picture. At least 11 portal tracts are needed for accurate specimen interpretation in adults [42], which is proportional to biopsy size. Larger length of biopsy can be attained with multiple passes, but at the cost of higher rate of complications [43]. The exact core length needed to diagnose liver disease is debatable, but currently a core 20mm in length and 1.8mm in diameter is recommended by ESPGHAN Hepatology Committee [33].

The clinical and histological findings help dictate how best to assess the specimen. Often, the tissue sample is fixed in formalin to allow for histochemical (hematoxylin and eosin or Masson trichrome) and immunohistochemical staining for examination under light microscopy. The specimen can be frozen in liquid nitrogen at -80°C for PCR studies, genetic analysis, biochemical analysis, or molecular analysis. The specimen can be fixed in glutaraldehyde and processed for electron microscopy for certain metabolic storage disorders or progressive familial intrahepatic cholestasis. Culture of a portion of the specimen may be indicated if infection is suspected. The tissue may also need quantitative copper analysis for Wilson disease. The tissue sample can also be stained for iron if an iron overload disease is suspected.

It is vital that the pathologist investigating the tissue sample be experienced with liver diseases. The diagnosis should be determined in collaboration with the hepatologist/gastroenterologist and pathologist. If the diagnosis is uncertain, a second opinion from a liver pathologist should be obtained [44].

CONTRAINDICATIONS

There are no established laboratory cutoff values for patients at risk for bleeding. However, INR >1.5 and platelet count <60,000/mL indicate an increased risk for bleeding and may require transfusion prior to liver biopsy [33,40]. In patients with ascites, percutaneous liver biopsy should be avoided due to risk of bleeding and/or biopsy site leakage of fluid, potentially leading to peritonitis [33]. A transjugular liver biopsy is recommended in these circumstances. Other contraindications include vascular tumors, echinococcal cysts, morbid obesity, extrahepatic biliary obstruction, and bacterial cholangitis [40]. The management of different contraindications of percutaneous liver biopsy are mentioned in ***Table* II**.

PITFALLS OF LIVER BIOPSY

The sample size from the biopsy must be enough for accurate interpretation. LB is associated with sampling variability because disease can be focal and heterogenous and tissue may not always be extracted from the areas of

Table II Contraindications to Percutaneous Liver Biopsy

Potential contraindications	*Solutions recommended*
Prolonged PT or INR >1.5	FFP or factor concentrate; transjugular approach in older children; tract embolization.
Platelets <60,000/mL	Platelet transfusion before liver biopsy; trial of propranolol; transjugular approach if still concerns after transfusion.
Prolonged bleeding time	Blood products
Hemodynamically unstable	Critical care to stabilize
Vascular lesions within liver	Real-time image guidance liver biopsy
Infection of hepatic bed/cholangitis	Appropriate antibiotics
Uncooperative patient	General anesthesia
Ascites	Drainage of fluid or transjugular approach
Intrahepatic biliary dilation	Plugging of the tract
Peliosis	
Hemangioma	Real-time image guidance liver biopsy
Morbid obesity	Transjugular approach
Hydatid disease	Surgical resection of cyst

KEY MESSAGES

- Liver biopsy, although invasive, can assist in diagnosis, prognosis, and direct therapeutic management of numerous pediatric liver diseases.
- Complications of liver biopsy are rare and most commonly consist of localized pain or bleeding.

highest yield. The standard specimen from a LB typically only represents 1/50000th of the liver; thus, sampling error can be significant and approach 20-30% [40].

RECENT ADVANCES

Thrombocytopenia is a contraindication to percutaneous liver biopsy and thus can delay diagnosis and staging of disease. However, newer advances have recently been made discovered to aid in this problem. An Indian study showed that propranolol corrected thrombocytopenia in 62.7% of children who previously were not able to undergo liver biopsy due to hypersplenism-related thrombocytopenia [45]. The US-FDA approved Doptelet (avatrombopag) to treat thrombocytopenia in adults with chronic liver disease who are planned to undergo a medical procedure [46]. Advances in imaging with Fibroscan/ US elastography to stage fibrosis, MRCP to evaluate bile ducts, MRI with EVOST (a gadolinium-based contrast agent) to assess nature of liver tumors, and MRI derived proton density fat fraction (PDFF) to quantitate fat in liver are some of the tests gaining popularity. Wide availability of these modalities may reduce the dependence on LB in the near future.

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Duchenne Muscular Dystrophy: Journey from Histochemistry to Molecular Diagnosis

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The August, 1970 issue of *Indian Pediatrics* reported an article describing the histopathological and enzyme histochemical features in the muscle biopsy of children with Duchenne muscular dystrophy (DMD) [1]. This article provides an opportunity to introspect on the evolution of diagnosis of children with Duchenne muscular dystrophy. In the present era of molecular genetics, the article provides an insight into the importance of muscle biopsy, which has gradually lost its way in this tale of last 50 years in diagnosis of children with DMD.

THE PAST

Naryananan, *et al*. [1] reported a descriptive study on clinical, histopathological and enzyme histochemical features of 23 children with Duchenne muscular dystrophy. Among the 23 patients, 5 children had an onset before 3 years of age while the remaining 18 children had onset between 4 to 10 years of age. Two girls were included in those 23 children with rest being boys. Histopathological features described by authors include marked variation in muscle fibre size with hyaline degeneration of muscle, alteration in sarcoplasmic nuclei with central nuclei and clumps of atrophic nuclei, and increased endomysial connective tissue. Enzyme histochemical analysis revealed increased lactate dehydrogenase activity in the dystrophic muscle fibre suggestive of increased anaerobic metabolism along with decrease in aerobic metabolism in terms of decrease in succinic dehydrogenase. Increased ATPase activity observed in the muscle was postulated by authors to decrease ATP content with consequent impairment of muscular activity. Authors have emphasized on correlation of clinical symptoms to enzyme histochemical features like muscle weakness to increase in ATPase activity in the muscle fiber and increase in LDH activity in connective tissue to increased fat deposition.

Historical Background

In late 1860s, Duchenne performed muscle biopsy on a patient with a myopathy. Till 1970s, pathologists largely relied on histopathological features based on routine hematoxylin and eosin staining. Enzyme histochemistry was introduced in the early 1970s. The present article is one of the first few articles that describe the enzyme histochemical features in children with Duchenne muscular dystrophy. Electron microscopy had limited application for muscular dystrophies, but played a major role in the pathological diagnosis of congenital myopathies in the early 1980s [2]. Immunohistochemistry was introduced in 1980s that described absence of dystrophin protein in children with DMD. Western blot also provided qualitative information on expression of proteins in muscular dystrophy [3]. In late 1980s, with the advent of molecular diagnostics, polymerase chain reaction (PCR) was available worldwide and gradually replaced muscle biopsy as the first line investigation for diagnosis of DMD. This technique detects large deletions in 60-65% of patients with DMD. Subsequently, by the year 2003, multiplex ligation probe analysis (MLPA) was introduced for detecting mutations in DMD gene. MLPA is a quantitative method to detect deletion and duplication in all the 79 exons of *dystrophin* gene, and is also useful in carrier testing.

THE PRESENT

DMD is an X-linked recessive disorder resulting from deletion or duplication in the DMD gene. Indian studies

have revealed a yield of 73% for detecting mutations (deletion and duplications) in DMD gene by MLPA [4]. Conventionally, patients with suspected DMD/Becker muscular dystrophy (BMD) who test negative for DMD mutation by MLPA were subjected to immunohistochemistry on muscle biopsy. Studies have revealed that 36% of MLPA negative patients were detected to have DMD by immunohistochemistry [5]. With the advent of next generation sequencing (NGS), single nucleotide variations, small deletions, insertions and splice site changes in the DMD gene could be further screened among MLPA negative patients.

In the study by Tallapaka, *et al.* [4], 10 of the 14 MLPA negative patients were detected to have sequence variants in DMD gene. Kohli, *et al.* [6] have reported 97% yield of NGS among patients with DMD. As NGS is good at detecting large deletion and duplications, it might become the investigation of choice in years to come. Although, there has been gradual decline in the cost of NGS, still, for many in India, cost remains a major constraint in adopting the same as the first line investigation.

In this genetic era, when we look back at the study by Narayanan,*et al.* [1] the diagnosis of DMD was made purely on the basis of clinical phenotype, high creatine phosphokinase (CPK) levels and muscle histopathology. There is an overlap in the clinical and histological features of DMD with limb girdle muscular dystrophy (LGMD 2I) resulting from mutation in Fukutin related protein (FKRP) [7].Dubowitz, *et al.* [8], way back in the year 1965, have considered LGMD and late onset spinal muscular atrophy to mimic DMD and have beautifully outlined how their histological features differ.

THE FUTURE

There is a paradigm shift from histopathological diagnosis to genetic diagnosis that has far more implications beyond establishing a diagnosis. Accurate genetic diagnosis is an essential tool for designing personalized treatment of DMD [9]. Detection of point mutation in exon 53 and exon 51 provides an avenue for recently approved treatment strategies for DMD including exon 51 skipping (Eteplirsen) and exon 53 skipping therapies [10,11]. Similarly, non-sense mutation in DMD provides opportunity to enrol the patient in the research for drug Ataluren (Stop codon read through). There is emerging interest in CRISPR-Cas9 mediated genome editing as a potential therapy for DMD, which obviously will require identification of point mutation [12]. Apart from clinical benefits, improvement in the dystrophin expression as determined by semiquantitative immunohistochemistry and Western blot are often considered as an outcome measure in clinical trials among children with DMD [10]. Apart from treatment implications, genetic diagnosis will have implications for estimating the reproductive risk, enabling carrier testing and prenatal diagnosis.

The rapid advances in genetic diagnosis have bypassed burdensome workup including invasive muscle biopsy, and prevent uncertainty in the diagnosis. However, parents must be always involved in decision-making, and the cost, utility, validity, and limitations of genetic testing must be clearly explained. Muscle biopsy remains the investigation of choice among MLPA negative patients who are either unable to afford NGS or whose NGS have revealed variants of unknown significance (VUS). In this era of molecular diagnosis, histopathological features and enzyme histochemistry might have limited historical role.

Despite rapid genetic advances, few patients remain undiagnosed. Recent studies have demonstrated that non-coding mutations could be an important source of unresolved genetic disease that could be detected by analyzing DMD transcripts in muscle biopsy using mRNA [13]. Hence, muscle biopsy is useful not only for establishing the diagnosis but may be useful for genetic counselling of patients who remain undiagnosed on MLPA and NGS. There is a lot of emerging interest in proteomics of DMD for better understanding of pathogenesis and possible avenues for treatment [14]. Hence, a tale that started 50 years back on advances in improving the diagnosis of DMD by muscle biopsy, has implications even in this advanced molecular era.

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Guidelines on Hemolytic Uremic Syndrome by Indian Society of Pediatric Nephrology: Key Messages

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Hemolytic uremic syndrome is an important cause of acute kidney injury that requires dialysis in children. The diagnosis and management is difficult due to limited diagnostic facilities and non-availability of specific complement inhibitors. We describe salient features of the recent Indian Society of Pediatric Nephrology consensus guidelines on hemolytic uremic syndrome.

Keywords: *Factor H antibodies, Plasma exchange, Thrombotic microangiopathy*.

Hemolytic uremic syndrome (HUS) is an important cause of acute kidney injury (AKI) requiring renal replacement therapy. Rapid diagnosis and management is necessary to limit irreversible renal damage. Although Shiga toxin-associated HUS constitutes the chief form of the disease worldwide, burden of this illness in India is not clear. School-going children show high prevalence of anti-factor H (FH) antibody-associated HUS. While International guidelines emphasize comprehensive diagnostic evaluation and complement blockade with eculizumab, access to these facilities is limited in India. Given the difference in epidemiology and challenges in management, guidelines for treatment of HUS were recently published by the Indian Society of Pediatric Nephrology (ISPN) [1]. This article highlights key messages from these guidelines.

DIAGNOSIS

Importance of Demonstrating Schistocytes and Thrombocytopenia

The diagnosis of HUS requires all of the following: *(i)* microangiopathic hemolysis characterized by anemia (hemoglobin <10 g/dL), fragmented red cells on peripheral smear (schistocytes ≥2%) and either high lactate dehydrogenase >450 IU/l or undetectable haptoglobin; (*ii*) thrombocytopenia (platelets <150,000/µL), and (*iii*) AKI (rise in creatinine by 50% over baseline). Guidelines for identifying schistocytes on peripheral smear are available [2]. Rarely, HUS may have an indolent presentation with AKI and systemic hypertension without thrombocytopenia or microangiopathic hemolysis. Renal biopsy is usually not required.

Rule-out Infections

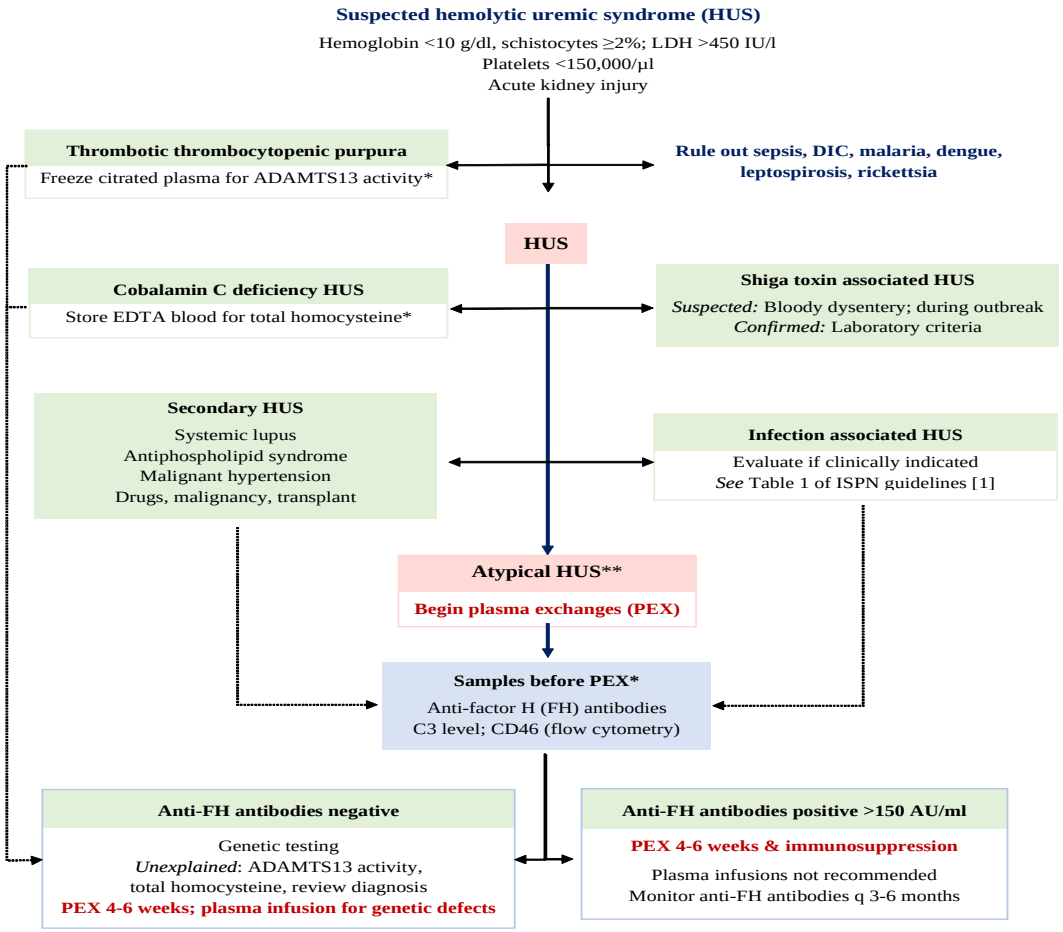
Disseminated intravascular coagulation (DIC) and thrombotic thrombocytopenic purpura (TTP) should be ruled out in patients with suspected HUS. Infections that mimic/trigger HUS, *e.g.*, malaria, leptospirosis, dengue, rickettsia and H1N1 infection should be excluded, if clinically suspected.

DIC is characterized by prolonged prothrombin time or activated partial thromboplastin time, low fibrinogen, elevated D-dimer and soluble fibrin monomers. TTP is rare in childhood; persistent thrombocytopenia (<30,000/µL) and mild/no AKI is suggestive. Blood samples should be stored and later processed for ADAMTS13 activity, if etiology of microangiopathic anemia is unclear.

Evaluation

ISPN guidelines endorse the etiology-based classification of HUS (***Fig.*1**) [3]. Epidemiology of HUS in India differs from that in developed countries. Worldwide, the chief cause of HUS is gastrointestinal infection with Shiga toxin producing *E. coli* (STEC-HUS), which is seen in 80% patients. In India, infection with *S. dysenteriae* has declined significantly and prevalence of patients with STEC-HUS is also low. STEC-HUS is suspected if occurring within 2 weeks of bloody diarrhea – infection is diagnosed by stool culture and demonstration of virulence genes, fecal Shiga toxin or IgM antibodies to serogroup specific lipopoly-saccharide.

Cobalamin deficiency accounts for ~6-8% patients with HUS. Feeding difficulties, seizures, abnormal



*ADAMTS13, A disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13; CD46, membrane co-factor protein; DIC, disseminated intravascular coagulation; LDH, lactate dehydrogenase; *See Box I for evaluating patients with HUS, including storing and processing of samples; **Also consider atypical HUS if positive non-synchronous family history or recurrent disease.*
Reprinted by permission from Springer Nature: Pediatric Nephrology (Bagga A, Khandelwal P, Mishra K, Thergaonkar R, Vasudevan A, Sharma J, et al. Hemolytic uremic syndrome in a developing country: Consensus guidelines. Pediatr Nephrol. 2019;34:1465-82.).

Fig. 1 *Approach to patients with hemolytic uremic syndrome (HUS).*

muscle tone, developmental delay and megaloblastic anemia are common; one-third lack extra-renal features. High blood levels of total homocysteine (>100 µM/L) followed by genetic screening is confirmatory [4]. Samples may be stored and processed later (***Box* I**). Specific therapy includes parenteral hydroxycobalamin, oral betaine and folate [4]. Secondary causes of HUS include systemic lupus, malignant hypertension and antiphospholipid antibody syndrome.

In a significant proportion of patients, HUS is associated with uncontrolled activation of the alternate complement pathway, termed atypical HUS (aHUS). A diagnosis of aHUS is made when infection, cobalamin-associated and secondary forms of HUS are excluded (***Fig.* 1**). These patients need detailed evaluation to determine the underlying cause. However, access to microbiological and complement assays is limited in India. Given the implications of accurate diagnosis, physicians taking care of these patients must be aware regarding appropriate screening. Units lacking facilities for assays must store samples for later analyses.

Screen for anti-FH Antibodies

Unlike European cohorts, anti-FH antibody-associated illness accounts for ~50% pediatric atypical HUS (aHUS) in India, chiefly affecting children aged between 5 and 15 years. Given therapeutic implications of this diagnosis, experts recommend prompt screening for anti-FH antibodies prior to instituting plasma exchange (PEX) therapy. ELISA test is available at multiple centers, with

BOX I Evaluation of Patients With Hemolytic Uremic Syndrome

Diagnosis

Complete blood counts; peripheral smear for schistocytes; reticulocyte count[a]

Lactate dehydrogenase, haptoglobin[a], direct Coombs test[b]

Blood: creatinine, electrolytes, transaminases, bilirubin, complement C3[a]

Urinalysis

Rapid test for malaria, dengue; IgM antibodies for dengue, leptospirosis (if suspected)

Coagulation profile[a](suspected systemic sepsis)

Ultrasound abdomen

If clinical features present: Echocardiogram, neuroimaging, amylase, troponin T

Determining cause of HUS

Essential

- Investigate for infection associated or secondary HUS, if clinically suspected
- Anti-factor H antibodies[a,d]; antinuclear antibodies
- CD46 expression on neutrophils (flow cytometry)[a,d]
- Store blood for ADAMTS13 activity[a,c,e]; total homocysteine[a,c]

Selected patients

- Suspected Shiga toxin associated HUS: Stool culture; PCR for *stx*1, *stx2* genes[f]
- Suspected pneumococcal HUS: Culture, PCR, ELISA; peanut lectin agglutination assay
- Gene sequencing: *CFH*, *CFI*, *CFB*, *C3*, *CD46*, *DGKE, THBD, MMACHC*
- Multiplex ligation-dependent probe amplification: Copy number variations *CFHR1*-5

ADAMTS13 disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13; CD46 membrane cofactor protein; CFHR complement factor H related; ELISA enzyme linked immunosorbent assay; PCR polymerase chain reaction; stx Shiga toxin; [a]Blood samples should be drawn before plasma exchanges or infusion; [b]Positive with pneumococcal infection, lupus; must be tested prior to administering blood products; [c]Plasma to be separated from fresh citrated blood (ADAMTS13) and EDTA blood (homocysteine) within 1-hr of collection and frozen at -20 to -70°C; [d]Division of Nephrology, Department of Pediatrics, All India Institute of Medical Sciences, New Delhi; [e]Department of Hematology, Christian Medical College, Vellore; [f]Postgraduate Institute of Medical Education and Research, Chandigarh.

turn-around of 7-14 days [5]. Commercial ELISA kits may show false positive results and underestimate antibody titers, limiting their role on follow-up; a positive threshold for these kits is also not defined [6].

Pathogenic variants in genes encoding proteins of complement and coagulation pathways: *CFH*, *CFI*, *CFB*, *C3*, *CD46*, *THBD* and *DGKE* are associated with aHUS in 30-40% cases. Patients without anti-FH antibodies require sequencing of these and other genes by next-generation sequencing (NGS) and *CFHR1-5* copy number variations by multiplex ligation-dependent probe amplification (MLPA). These studies are useful for guiding management, prognosis, risk of relapses and allograft recurrence, and allow genetic counseling. NGS allows rapid, simultaneous sequencing of multiple genes; declining costs have made studies more accessible. Patients with anti-FH antibody-associated HUS do not require genetic screening, except if: (*i*) onset before 4 years of age, (*ii*) relapsing course, (*iii*) family history of HUS, (*iv*) illness that is refractory to PEX, and (*v*) prior to kidney transplantation.

MANAGEMENT

Atypical HUS: Across the developed world, complement blockade with eculizumab, the C5 monoclonal antibody, is the standard of care for patients with aHUS. However, eculizumab is expensive and not available in India and developing countries. In absence of anti-complement therapies, intensive PEX is less than ideal, but the only alternative. For our country, timely institution of PEX (60-75 mL/kg; fresh frozen plasma as exchange fluid) is most appropriate for patients with suspected aHUS.

PEX by filtration or centrifugation method, must be done at centers with expertise [7]. PEX is administered daily until hematological remission (platelets >100,000/μL, schistocytes <2%, LDH less than upper limit of normal for 2 consecutive days) and tapered over 4-6 weeks. Maintenance therapy with plasma infusions is

advised every 10-14 days for patients with mutations in complement genes, especially *CFH* and *CFI.* There is limited benefit of PEX in patients with: (*i*) microbiologically confirmed STEC-HUS, without cardiac or neurological involvement, (*ii*) infection-associated HUS or, (*iii*) pathogenic variants in *CD46* and *DGKE.*

Anti-FH antibody associated aHUS: Since aim of therapy for patients with anti-FH associated HUS is reduction of titers, PEX are most appropriate to achieve this goal. Plasma infusions do not remove antibodies and are not a substitute for PEX. Findings from a nationwide database on 436 patients with anti-FH disease show that high antibody titers ≥8000 AU/mL at onset, delayed PEX and short duration PEX (<14 days) predict adverse outcomes [8]. Combination of PEX and immunosuppression was most useful [1,8].

Immunosuppression must not be used without confirming presence of anti-FH antibodies. Therapy is initiated with prednisolone 1 mg/kg/day for 4 weeks, then alternate-day followed by tapering over 10-12 months. Therapy includes cyclophosphamide (500 mg/m^2 intravenously once in 4-weeks) for 5 doses. About 15-30% patients relapse; high anti-FH titers (>1300 AU/mL) during remission predict early relapses [8,9]. Antibody titers should be sequentially measured, especially in the first 12-24 months of follow up. Maintenance therapy with mycophenolate mofetil or azathioprine for 18-24 months, and tapering prednisolone further reduces the risk of relapses.

CONCLUSIONS

Given limited diagnostic capabilities and lack of access to eculizumab, international guidelines on aHUS are not likely to be implemented in developing countries in the near future. The present guidelines provide a systematic and algorithmic approach to management of patients with HUS, tailored to the distinct epidemiology and available repertoire of investigations and therapy. The guidelines underscore the importance of appropriate supportive care, and need for regular and prolonged follow-up. Capacity building for diagnosis and therapy of HUS and other complement related disorders is also required.

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IN THE DARKER VEIN: BEING A PEDIATRIC ONCOLOGIST

One pediatric specialty which can use some light in the darkness is pediatric oncology. There is also the fact that pediatric oncologists deal a lot more with veins. This makes it appropriate that I represent the community, writing about the perils (and of course the rewards) of being a pediatric oncologist in a light yet darker vein.

Despite being a different species in the broad genus of pediatricians, the role of pediatric oncologists is little understood. On a personal note, try explaining that to your relatives. "Pediatric Oncology? Do kids get cancer?" -A middle aged uncle who was a casual visitor to my outpatient clinic asked (despite the board outside clearly stating the fact that I am a pediatric oncologist)."They do. But the outcomes are far better compared to adults", I replied. "Must be very depressing to see kids dying of cancer". "Yes it is. But eight out of ten kids survive cancer". And the conversation goes on a loop until one of us gets tired. I chose the road less travelled by and it has made all the difference in my life - the difference of thinking zebra when you hear hoof beats (Yes, I borrowed that from the Immunodeficiency Foundation).

Amongst our fellow pediatricians who are considered benign, we are looked upon as malignant. In our defense, I like to think we are malignant only to those who are malignant (now, that sounds like a punch dialogue in the mass hero movies). We are looked upon as vampires by the kids (not the cute vampires of the Twilight series, but an evil Dracula), but console ourselves saying that we return the blood we draw by the way of repeated transfusions.

The transformation from a pediatrician to a pediatric oncologist is complete when you start to think like this (I refer to these as the Scott criteria* after my mentor Dr.Julius Scott, a great pediatric oncologist): starting piperacillin for febrile neutropenia in a child with dengue and leukopenia, thinking of bone pains when you see a child with growing pains, thinking of neutropenic typhilitis in a child with viral diarrhea, a child with enlarged lymph nodes makes you think of Hodgkin lymphoma as the first differential diagnosis and a child with seborrhea, Langerhans Cell Histiocytosis (LCH).

On occasion, our extra vigilant attitude strikes gold-we become saviors. These include instances like spotting an innocent looking ear discharge in an infant with seborrhea and working up for LCH, or picking up a sinister Non Hodgkin Lymphoma of the bone masquerading as osteomyelitis. That redeems the lost pride until we encounter the next set of "D" questions.

We are not the only breed of professionals dealing with oncology who undergo this transformation. The pathologist becomes the hero when he detects the malignant cells in the seemingly innocuous 'pus' of a psoas abscess which is actually a lymphoma. Still, we live in constant dread of the words 'inadequate'. Like the infamous duck analogy (for those who don't know- please google 'five doctors on a duck hunt") where the pathologist says that the specimen was inadequate even when we think we provided the entire duck. Unlike the clash of egos between pediatricians and pediatric surgeons, pediatric surgeons and pediatric oncologists make a happy couple and they know what we want to keep us happy. 'The case of the missing node' is an extremely rare incident when the draining lymphnodes required to be sampled for staging are missed!

I wish the phrase *Primum non nocere* (first, to do no harm) applied to parents as well. The guilt of not being in control of their kids' illness makes parents take extreme measures like pouring gallons of papaya leaf extract juices for thrombocytopenia down the throats of unwilling kids with leukemia. Then there are parents who display an ostrich attitude. I recall a case of a child with febrile neutropenia brought to us leisurely on the third day of fever (when actually it is a medical emergency). On being questioned, the parent replied, "You asked us to bring him if he had 'persistent fever', but his fever subsides with every dose of paracetamol". I still cannot fathom whether it was a case of denial or that I needed to improve my communication skills.

At the end of the day, kudos to the pediatric oncologists and pediatricians out there who inspired me to join 'the order of the phoenix', the kids who rise from the ashes after being through chemotherapy, the parents who have the 'never ever give up' attitude and all the others who help them. Blood is thicker than water and to us- the pediatric oncologists, blood is thicker than normal saline, and colloids. And the bond we share with the family is sacred.

*(*Not real scientific criteria, but representation of the author's perspective on the thought process of pediatric oncologists.)*

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Online Survey of Vitamin D Supplementation Practices in Children and Adolescents

We collected information regarding vitamin D supplementation practices of 230 pediatricians through an online survey. Routine supplementation was being practiced during infancy, 1-5 y, 6-10 y, and >10-19 y age by 187 (81.3%), 60 (26.1%), 34 (14.8%) and 41 (17.8%) respondents, respectively. 182 (79%) participants promoted sunlight exposure, and 171 (74.3%) did not measure serum 25-hydroxy vitamin D levels before supplementation. The survey highlights that majority of pediatricians prescribe routine vitamin D supplementation during infancy, but not beyond.

Keywords: *Deficiency, Management, Sunlight.*

Vitamin D deficiency is considered as a significant public health problem in Indian children, which can be prevented by supplementation, fortification and dietary recommendations [1,2]. The Global Consensus for Nutritional Rickets recommends 400 IU vitamin D supplementation daily during infancy [3]. Supplementation in older children is recommended only if symptomatic vitamin D deficiency or high-risk conditions are present. The Indian Academy of Pediatrics (IAP) recommends daily supplementation of vitamin D in healthy Indian children of all ages (newborns till adolescents) to meet the recommended daily allowance of vitamin D [4]. There are no recommendations yet by the Government of India for routine vitamin D supplementation. In view of varying recommendations, we conducted this survey among pediatricians in India to assess vitamin D supplementation practices for infants and children.

The data were collected as an online survey through a Google form by phone (Whatsapp) or e-mail. Participants were selected from personal contacts with no geographical restrictions. Participant's consent was incorporated within the online form. The information was collected in an anonymous format. Practice parameters were collected separately for age groups less than 1 year, 1-5 year, 6-10 year and 10-19 year. Approval of the institutional ethics committee was obtained prior to the conduct of the study.

The survey consisted of 11 close-ended questions to cover professional experience in years, knowledge about any vitamin D supplementation guidelines, practice of vitamin D supplementation across different ages, duration (infrequent if less than 6-12 months/year) and dose of vitamin D supplementation if practiced, practice of measuring serum 25-hydroxy vitamin D (25OH-D) before supplementation, and practice of promoting sunlight exposure. Assuming a prevalence of vitamin D supplementation as 15% among healthy adolescents, a sample size of 196 respondents was needed with an absolute precision of 5% and 95% level of confidence.

A total of 230 (140 from private sector) responses were collected over two weeks. The majority (56%) of respondents had more than ten-year experience and only 10% were pursuing pediatric residency. Most (204, 79%) respondents were aware of the national or international guidelines for vitamin D supplementation. ***Table* I** shows the vitamin D supplementation practices of these respondents across different age-groups. Eight (3.5%) reported use of a monthly 60,000 IU dose across different age groups. Overall, 182 (79%) promoted sunlight exposure and 171 (74.3%) did not measure serum 25OH-D levels before supplementation.

The present study highlights that the practice of routine vitamin D supplementation prescription was mostly limited to infancy, with a minority practicing routine vitamin D supplementation beyond infancy in the recommended doses [4]. The recent Comprehensive National Nutrition Survey [5] reported low prevalence (14-24%) of vitamin D deficiency (serum 25OH-D <12ng/mL) in Indian children beyond infancy, raising concerns over need of routine vitamin D supplementation in apparently healthy children in this age. A cohort study on annual vitamin D prescriptions of over two million children from UK showed 26-fold rise in prescribing trend between 2008-2016 with maximum rise in adolescent age group (higher for girls than boys) [6]. There were 24 different dosing regimens used with pharmacological doses (>1000 IU/day) prescribed in 42.8% children with insufficient vitamin D levels, suggestive of poor compliance to national guidelines [6].

The meager supplementation rates in older children in the present study probably were related to absence of felt-need for vitamin D supplementation in this age-group. The limitation of this study was that the information was self-reported, and no physical verification of prescriptions was done. Moreover, the data were collected from only opt-in respondents who could be contacted personally by the study team, which may have introduced selection bias. Information on geographical location of participants and the seasonal preferences was not collected.

To conclude, the present survey highlights the varied vitamin D supplementation practices among pediatricians with majority prescribing supplementation during infancy but not for older children and adolescents.

Ethical clearance: Institutional ethics committee of Maulana Azad Medical College, New Delhi; 73/01/2020/41, dated March 13, 2020.
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Table I Practice of Pediatricians Regarding Vitamin D Supplementation for Different Age-groups (*N*=230)

Practice	*Infants*	*1-5 y*	*6-10 y*	*>10-19 y*
Routine supplementation	187 (81.3)	60 (26.1)	34 (14.8)	41 (17.8)
Infrequent dosing (<6-12 mo/y)	26 (13.9)	16 (26.7%)	26 (76.5)	30 (73.2)
Dose appropriate*	161 (86.1)	44 (73.3)	21 (61.8)	28 (68.3)

*Data presented as n(%), where n=responses/those practicing routine supplementation; *Dose 400 IU in infants and 600 IU beyond infancy [4].*

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Celiac Disease in Indian Children and Adolescents with Type 1 Diabetes

To estimate the time trend and prevalence of celiac disease in 208 children with type 1 diabetes by retrospective case review. Tissue transglutaminase (TTG IgA) levels were done within the first six months of diagnosis and annually on follow-up. Celiac disease was diagnosed in 35 (16.8%; 3 before diagnosis, 18 at initial screening and 14 on follow-up). 14 subjects with negative TTG serology at presentation, developed celiac disease after 3.9 (2.9) years (range 1.4 - 12.6 years, 85.7% within 5 years). Celiac disease is common in Indian children and adolescents with type I diabetes, developing in most within five years of diagnosis.

Keywords: *Complications, Glycosylated hemoglobin, Screening, Tissue transglutaminase antibody.*

Celiac disease is an important association of type 1 diabetes identified in 2-25% subjects [1]. The disease has significant impact on growth, bone density and glycemic control [2], emphasizing the need for timely detection. Non-specific features frequently result in delayed and missed diagnosis highlighting the need for screening [1]. The current guidelines differ in terms of initial and follow-up screening protocol for the condition [3-5]. Further, there is no information regarding time trend of celiac disease in Indian children and adolescents with type 1 diabetes. We, therefore, evaluated the time trend of celiac disease in Indian children and adolescents with type 1 diabetes with special emphasis on its predictors.

Case records of children and adolescents with type 1 diabetes presenting to our pediatric endocrinology clinic from August, 2008 to July, 2019 were reviewed after approval from the institutional ethics committee. All patients at our centre are advised IgA tissue transglutaminase antibody levels (TTG) at presentation and annually. IgA TTG is performed using Euro immune anti TTG IgA ELISA kit. Subjects without record of baseline TTG level within the first six months of diagnosis and those with incomplete records were excluded. Duodenal biopsy was advised when TTG levels were more than 20 RU/L and graded using modified Marsh criteria [6]. Celiac disease was diagnosed as per ESPGHAN 2012 recommendations [4]. TTG levels were repeated after 3, 6 and 12 months and annually in those not willing for biopsy, and celiac disease was diagnosed in subjects with TTG levels persistently above 200 RU/L**.** Information about age at diagnosis of type 1 diabetes, duration of follow-up, anthropometry, celiac disease status, thyroid profile, gastrointestinal symptoms and glycosylated hemoglobin (HBA1C) levels was recorded.

Data were entered and analyzed using IBM Statistical Package for Social Sciences (SPSS version 25.0, SPSS, Inc., Chicago, IL, USA) for Macintosh. Independent sample *t* test and Chi square test were used to compare continuous and categorical variables respectively, to identify predictors of celiac disease at presentation and on follow-up. *P* value less than 0.05 was considered significant.

Out of 262 children and adolescents with type 1diabetes (TID) presenting during the study period, 40 without baseline TTG levels and 14 with incomplete records were excluded from the study. The remaining 208 subjects (79.4%, 106 boys) included in the study were diagnosed at the median (IQR) age of 9.2 (6.4) years and followed up for median (IQR) 2.4 (4.4) years. Celiac disease was identified in 35 (16.8%).

Three patients had celiac disease 8.3 (8.1) months before the onset of diabetes, and TTG was positive in 21 (10.2%) at presentation. Celiac disease was histologically confirmed in 15 of these. Of the remaining six, three (2 boys) were diagnosed as celiac disease in view of persistently elevated IgA TTG levels above 200 RU/L. IgA TTG levels normalized over a median period of 4 months (3-12 months) in the other three. Biopsy proven celiac disease was diagnosed in 14 (7.5%) with negative initial screening after a median (IQR) 2.9 (2.7) years of diabetes onset. New onset celiac disease was identified in 12 (85.7%) within five years of diabetes diagnosis. Gastrointestinal symptoms were absent in all diagnosed within 10 years of diabetes but present in a boy identified after 12.6 years.

Those with celiac disease at baseline had significantly compromised weight and BMI, and worse HBA1C at diagnosis of T1D, as compared to both those with new onset celiac disease and those without celiac disease. No difference was observed in mean age at diagnosis of T1D, height SDS and insulin requirement. The difference in the gender and thyroid dysfunction between the three groups was not statistically significant (***Table* I**).

Findings of our study indicate high prevalence of celiac disease (16.8%) in Indian children with type 1 diabetes. This is substantially higher than Western reports and may reflect difference in the distribution of HLA genotypes [7].

Table I Comparison of Subjects with Celiac Disease at Presentation, Incident Disease on Follow-up and those without the disease

Parameter	*Celiac disease*		
	At diagnosis (n = 21)	*At follow-up (n = 14)*	*None (n = 173)*
Age, y	8.9 (4.6)	7.5 (5.1)	9.1 (4.3)
[^]Weight	-1.5 (1.4)	-0.8 (1.2)	-0.3 (1.2)
Height	-1.3 (2.2)	-0.6 (1.2)	-0.4 (1.3)
[*]BMI	-0.9 (0.7)	-0.6 (1.1)	-0.2 (1.0)
[#]HbA1c, %	13.7 (2.6)	11.8 (3.7)	9.8 (2.3)
[$]Insulin, U/kg	1.0 (0.3)	1.1 (0.4)	1.0 (0.3)

Age: age at type I diabetes diagnosis; BMI: Body mass index; Weight, height and BMI in standard deviation scores; HbA1c: Glycosylated hemoglobin; [$]current insulin requirement; [^]P=0.02; [*]P=0.01; [#]P<0.001.

Incident celiac disease contributed to 40% of subjects of our study resulting in higher prevalence than cross-sectional Indian studies (3.8-13.5%), despite similar baseline levels [1]. This is commensurate with a multi-centric follow-up study of 4322 Italian subjects [8], and a seroconversion rate of 2.8% over 3.6 years in an Australian study [9]. Gastrointestinal symptoms were absent in most diagnosed on follow-up, highlighting the need for periodic TTG levels over follow-up as suggested by previous studies [10]. The fact that 85% of these were identified within five years of diagnosis suggests annual screening till this period in line with ISPAD 2018 and ADA 2018 guidelines [3,5]. Symptom-based screening may be considered beyond this period.

Age at diagnosis, anthropometry, hemoglobin A1C level, thyroid dysfunction and gender did not predict celiac disease at presentation in contrast to higher risk in younger children and female gender in the multi-centric trial in Italy [8]. Compromised weight and glycemic control in subjects with new onset celiac disease indicates the potential adverse effect of the disease.

Our study is limited by its retrospective nature; but uniform implementation of institutional protocol with high concordance of TTG IgA testing and availability of detailed growth and glycemic control data provided real life information about time trend of celiac status. Importantly, over 80% of subjects on active follow-up underwent TTG levels at each time point. Further, small sample size and no data on HLA subtypes add to the limitations of our study.

Our study confirms the high prevalence of celiac disease in Indian children and adolescents with type 1 diabetes. The identification of the disease in asymptomatic children with negative initial serology highlights the need for annual screening till at least five years of diagnosis.

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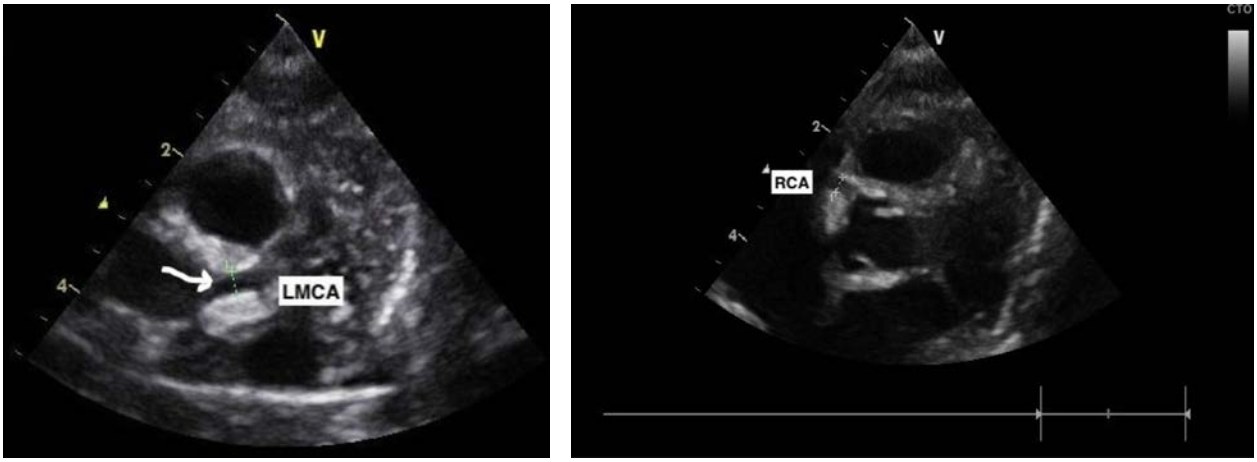
Novel Coronavirus Mimicking Kawasaki Disease in an Infant

Coronavirus disease (COVID-19) caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) uncommonly causes severe disease in children [1]. Over the last two months; however, a new hyper inflammatory condition manifesting as Kawasaki disease or Kawasaki-like shock syndrome has been described in children above 4 years of age, across Europe and USA [2-4], with increased risk of mortality. One case from India has also been reported [5]. Here we report an infant presenting with fever and clinical manifestations of Kawasaki disease and subsequently screening positive for COVID-19.

A 4-month-old healthy baby, weighing 5.6 kg, and born to non-consanguineous parents, presented with high-grade fever for 4 days. Fever was spiking 6-8 hourly reaching 39 ^{0}C. He developed an erythematous macular rash over the trunk, palm and sole on second day. On admission the child was hemodynamically stable and was breastfeeding normally. He was very irritable with red lips, congested throat and small cervical lymphadenopathy without any cough or nasal congestion. He had clear chest, normal regular heart sounds and a saturation of 97-98% in air. Investigations revealed a hemoglobin of 9.9 g/dL, total leucocyte count of 14770/mm^3 with 50% neutrophils, platelet count 4.25×10^9/L, C-reactive protein (CRP) of 115.6 mg/L, normal liver enzymes with albumin 30 g/L and globulin of 22 g/L, and a normal chest *X*-ray. His nasopharyngeal swab was sent for SARS-CoV-2 RT-PCR and other viral PCR tests. Treatment was started with meropenem and vancomycin after sending blood and urine culture, but fever continued till the third day of admission, when he developed non-purulent conjunctivitis with left subconjunctival hemorrhage. Repeat CRP showed a higher value of 178.2 mg/L. With evolving clinical signs simulating Kawasaki disease, an echocardiography was performed. It showed normal left ventricular function, perivascular brightness and diffuse ectasia of coronary arteries with left middle coronary artery (LMCA) of 2.7 mm (*Z* score +2.6) (***Fig.* 1a**), left anterior descending artery of 2 mm (*Z* score +2.9) and proximal right coronary artery (RCA) of 2.4 mm (*Z* score +3.6) (***Fig.* 1b**). Oral aspirin (80 mg/kg) and intravenous immune-globulin (IVIG) (2 g/kg) therapy was started. He stayed stable clinically and did not need intensive care. Fever subsided after 24 hours of finishing IVIG infusion, and the child became playful. Subsequently, SARS-CoV-2 reverse transcriptase polymerase chain reaction (RT-PCR) showed a positive result and he was shifted to a COVID-designated hospital. All cultures were negative till 7 days. Repeat blood test revealed a downward trend of CRP (148 mg/L). Swab for other viruses was negative. To date the baby is stable, afebrile, and is kept under observation in the pediatric ward. His mother was also subsequently found positive for SARS-CoV-2.

Children of all ages can acquire COVID-19, although they appear to be affected less commonly than adults [1,6,7]. The most common symptoms in pediatric SARS-CoV2 infection are fever and cough [1,6]. This infant also presented with fever but his extreme irritability was unusual. In a previous series, approximately 11% of infants had severe or critical disease [1]. This infant was never critical throughout the period of hospitalization.

During this pandemic, Jones, *et al.* [2] published the first case of a 6-month-old female admitted and diagnosed with classic Kawasaki disease, which tested positive for COVID-19. This



(a) *(b)*

Fig. 1 *Ectasia of (a) left main coronary artery (LMCA) and (b) right coronary artery (RCA).*

was followed by more similar reports of children with COVID-19 and clinical features that are similar to those of toxic shock syndrome and atypical Kawasaki disease and laboratory findings associated with increased inflammation [3-5]. Royal College of Paediatrics and Child Health (RCPCH) labeled this new inflammatory entity as Paediatric multisystem inflam-matory syndrome temporally associated with COVID-19 [8]. Case definitions include persistent fever, inflammation and evidence of single or multi-organ dysfunction after exclusion of other microbial causes. The case mentioned showed a rise of CRP without any neutrophilia, lymphopenia or organ dysfunction.

Our case was very similar to that described by Jones, *et al.* [2] but that girl had persistent tachycardia and most of the clinical features of KD with normal echocardiography. Riphagen, *et al*. [3] reported a case series of 8 children (only 3 tested COVID-19 positive) needing intensive care support with a hyper-inflammatory shock. One child died after a massive cerebral infarction. All of them had features mentioned in RCPCH guidelines with minimal respiratory symptoms [3]. These children and the two infants with Kawasaki disease most likely had a similar pathogenesis with varied consequences, which needs further research to define it.

India is still in the early stage of this pandemic and has not yet had many children with severe COVID-19. This 4-month-old child presenting as typical Kawasaki disease represents a novel presentation among the very young population with COVID-19.

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Portal Hypertension in a Case of Klippel Trenaunay Syndrome

Klippel Trenaunay syndrome (KTS) is a sporadic disorder that belongs to PIK3CA-related overgrowth spectrum of disorders. The diagnostic criteria for KTS comprise of presence of capillary malformation, venous with or without lymphatic malformation and limb overgrowth. Only 63% patients have all three clinical manifestations [1]. Here we describe a case of KTS presenting as mixed venolymphatic malformation with complication in the form of portal hypertension due to dysplastic portomesentric veins.

We report an 11-year-old girl who presented with swelling of the right gluteal region noticed since birth. This swelling slowly progressed to involve the whole of the right lower limb accompanied by dilated veins over lateral aspect of the ankle. At six years of age, she developed clusters of small vesicles with warty appearance in the affected lower limb which ruptured spontaneously discharging serous fluid. Baseline hemogram, kidney and liver function tests were normal. Skin wedge biopsy performed was consistent with lymphangioma circumscriptum. Ultrasound doppler of gluteal region revealed dilated anechoic tortuous channels showing no flow within suggestive of lymphangioma. Ultrasonography of abdomen was normal. MR angiography of limb revealed extensive soft tissue hypertrophy involving right gluteal, thigh and upper leg

with dilated vascular channels in posterior compartment of leg, suggestive of venolymphatic malformation.

At eight years of age, she developed severe pallor with anasarca and bleeding per rectum. On examination, ascites and splenomegaly were evident. Investigations revealed hemoglobin of 3.9 g/dL, total leucocyte count of 4200/mm^3 and platelet count of 50000/µL. She was transfused with packed RBC and platelets. Liver and kidney function tests were normal. Ultrasound of the liver unveiled portal vein thrombosis with periportal collaterals and massive splenomegaly. Upper gastrointestinal endoscopy was normal. External hemorrhoids was identified by proctoscopy. CT angiography of the abdomen revealed dilated main portal vein with fusiform aneurysmal dilatation of left branch of portal vein and superior mesenteric vein with foci of thrombus within. Multiple collaterals were seen at porta **(*Web Fig*.1a**), pericholecystic region, head and body of pancreas with dilatation of left gonadal vein and splenomegaly. There were abnormal soft tissue and vascular channels within the subcutaneous and intramuscular plane of right gluteal region with grossly dilated right internal iliac veins and presence of abnormal draining vein arising from soft tissue (***Web Fig***. **1b**)

At 11 years of age, she again presented with pancytopenia. On examination, there was autoamputation of terminal phalanx of 4th toe of the affected limb with surrounding skin necrosis. Limb deformity was also present with previous existing features (***Web Fig* 1c**). The patient was transfused and was put on prophylaxis for portal hypertension and referred to vascular surgery department for further intervention.

KTS is a low flow vascular malformation in an overgrown limb. Somatic heterozygous gain of function mutations in a mosaic pattern in the *PIK3CA* gene was recently identified in patients with KTS [2]. These somatic mosaic mutations affect only a portion of the body and since these mutations occur as a post zygotic event, they are not transmitted to progeny.

Absence of central nervous findings, truncal fatty-vascular growth, paraspinal fast-flow lesions and skeletal abnormalities distinguishes KTS from other *PIK3CA* related disorders. Amongst other overgrowth disorders, absence of arterial involvement distinguishes it from Parker Weber syndrome. Lack of nevi differentiates it from Proteus syndrome. Our patient exhibited two of the cardinal features of KTS. Portal hypertension could have resulted from development of thrombosis in ectatic portomesentric veins. External haemorrhoids and splenomegaly could be a complication of the primary disease or portal hypertension.

Patients with large and complex vascular malformations in KTS generally tend to have a higher risk for thromboembolic disease. Visceral involvement, as a consequence can result in significant morbidity and mortality [3]. Lymphatic malformations are also prone to rupture and recurrent infections. In KTS patients, magnetic resonance imaging of the abdomen, pelvis and lower extremities should therefore be performed in the early infantile period or at the time of initial presentation. Colour and spectral Doppler ultrasound can also be used to image the vascular malformations when available. In contrast to other *PIK3CA* disorders, occurrence of malignancies are less likely.

Symptom specific management includes surgical debulking for complex lymphatic malformations, sclerotherapy for minor vascular malformations, pulsed dye laser for capillary lesions and orthopedic interventions for deformities. Sirolimus has been found to have a promising role in complex slow flow vascular malformations [4]. PI3K and mTOR inhibitors are also being actively investigated [5]. Prophylactic anticoagulant therapy can be considered in patients with complex vascular malformations prior to radiological or surgical procedure.

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Acute Transient Pancreatitis Associated With Milk Allergy in an Infant

Acute pancreatitis is uncommon in children, and is usually due to biliary obstruction, medications, and systemic or idiopathic diseases [1]. Acute pancreatitis associated with food allergy in children is rare, and the mechanism is unclear [2]. We report an infant with acute transient pancreatitis associated with milk (casein) allergy.

A 1-year-old boy presented with a history of urticaria and vomiting after his first exposure to formula milk at the age of 6 months. At that time, his cow milk-specific immunoglobulin E (IgE) was positive, so milk was avoided. After that, he was continued on a milk-free diet. However, he had eaten meat such as beef, chicken, and pork. On the day of presentation, around 1 hour after eating pork, he vomited and had a rash on his face. His parents gave him an antihistamine preparation and brought him to our hospital. The rash had developed to involve the whole body, but there were no further digestive symptoms or wheezing. He was diagnosed as allergic reaction probably caused by milk, and treated with antihistamines and hydrocortisone, according to the Japanese guideline for food allergy [3]. Later, it was found that the pork contained casein as a meat-softener. As the family had eaten the roasted pork at a restaurant, there was no labeling for casein use as softener. In addition, blood examinations revealed elevations of pancreatic enzymes (total amylase 427 U/L; pancreatic amylase, 423 U/L (normal, 21-64 U/L); elastase1, 2480 ng/dL (normal, 300 ng/dL); lipase, 4840 U/L (normal 17-57 U/L); and phospholipase A_2, 5390 ng/dL (normal, 130-400 ng/dL)). Venous base excess was – 4.2, and inflammatory markers and other hepatobiliary enzymes were normal. With a diagnosis of acute pancreatitis, he was treated with fasting, an antibiotic (piperacillin), and a histamine-2 blocker (famotidine). Twelve hours later, the serum pancreatic amylase decreased to 75 U/L, and the urticaria improved. Ultrasonography and computed tomography of the abdomen did not show pancreatic swelling or dilatation of the pancreatic duct. There were no abnormalities of the liver and gastrointestinal tract, and gallbladder. Trial of oral feeds did not lead to abdominal symptoms. At 36 hours after the ingestion, the serum pancreatic amylase level was normalized (9 U/L), and he was discharged. After one month, all pancreatic enzymes were normal. He was able to eat meat such as beef, chicken, and pork after this event.

Food allergy commonly cause digestive symptoms such as abdominal pain and vomiting associated with food allergy [3]. Pancreatitis associated with food allergy has been widely reported in adults though less common in children [2,4-6]. The prognosis and mechanism are unclear. Previous reports reported no association with sex, type of food that caused the allergy, and the severity of food allergy. However, these reported cases are characterized by having gastrointestinal symptoms (abdominal pain and/or vomiting), absence of abnormalities of hepatobiliary enzymes other than pancreatic enzymes, and a good clinical course. The disorder of the pancreas is expected to be transient. Our case of acute transient pancreatitis associated with milk (casein) allergy also had these characteristics.

Several cases of pancreatitis with food allergen-induced eosinophilic gastroenteritis have been reported. The causes of pancreatitis were assumed to be pancreatic eosinophilic inflammation and local duodenal inflammation [4]. In food-induced allergic responses, mast cells are involved in digestive symptoms *via* IgE. Mast cells also have important roles in acute and chronic pancreatitis and multiple organ failure. In the present case, no endoscopic examination was done, so it was not possible to confirm the presence of inflammatory cells in the digestive tract and subsequent pancreatitis. Inamura, *et al*. [6] proposed that edematous swelling at the ampulla of Vater, associated with mast cell inflammation, caused occlusion of the pancreatic duct and stagnation of pancreatic juice, resulting in pancreatitis. This hypothesis implies that the pancreatitis is a secondary disease following gastrointestinal changes due to allergy. Considering the rapid improvement of pancreatic enzyme levels and the good prognosis, this is the most likely theory.

In conclusion, the present case appeared to have symptoms of milk (casein) allergy, and acute pancreatitis was diagnosed based on biochemical abnormalities. It is necessary to take transient pancreatitis into account as a diagnosis when a child with food allergy shows digestive symptoms.

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Duodenal Web with Trichobezoar: An Unusual Presentation

Duodenal atresia and duodenal web cause upper gastrointestinal obstruction and usually present in neonatal age soon after birth [1]. However, delayed presentation has been documented in literature in first few months or years of life related to partial obstruction. [2]. Here we report duodenal web presenting in the third year of life associated with trichobezoar in the duodenum.

A 2-year-old girl with Down syndrome presented with complaints of recurrent vomiting of 2-3 months. The vomiting was non-bilious in nature and it contained 'cherry seeds' eaten about 3-4 months before. The child had no history of abdominal distension, blood in vomitus or bowel complaints. The child was well hydrated, afebrile, and with no previous complaints. Abdominal examination showed non-distended abdomen. No definite lump or tenderness was palpable. There was no free fluid and bowel sounds were normal. *X*-ray of abdomen revealed 'double bubble' with paucity of distal gas. A contrast study was done using water-soluble contrast agent, which showed hugely distended stomach with delayed drainage and normal small bowel. She was explored through supra-umbilical transverse incision. A hugely dilated stomach was identified. The second part of duodenum had a windsock deformity. Duodenotomy revealed a pre-ampullary web with trichobezoar obstructing the lumen. The gastric outlet was normal. The child was managed by duodenoduodenostomy. She remained well in post-operative period and is well on follow-up after 3 months.

Duodenal atresia or duodenal web may be identified on antenatal ultrasound or usually presents in first week of life with recurrent vomiting. The condition can be picked up on plain x-ray of abdomen showing 'double-bubble' appearance. Duodenal web; however, may present late due to partial obstruction [1]. Duodenal atresia and duodenal web are caused by abnormal duodenal development at 6-8 weeks of gestation, and are known to be associated with Down syndrome [1].

Trichobezoar is a condition where a collection of hairs form a mass that does not pass into the intestine and causes obstruction. Usually the trichobezoar occurs in the stomach, and it may extend into the intestine as a tail causing Rapunzel syndrome [3]. The trichobezoar occurs more commonly in persons with psychiatric diseases with trichotillomania. Although no behavior of eating hair was noted by parents, the same may be present/ have happened accidentally due to intellectual deficit or inadequate supervision by parents. The cherry seeds reported in history may have precipitated the obstruction either by themselves or by acting as a nidus for the trichobezoar. Trichobezoar is usually diagnosed on ultrasound or CT scan of abdomen, and managed by retrieval through laparotomy or laparoscopy [4].

A similar case has been reported in the French literature [5]. The association of duodenal atresia and Down syndrome helped us in suspecting the duodenal atresia. Although phytobezoars have been reported in early life, the incidence of trichobezoar in third year of life is rare. For a child with Down syndrome and recurrent vomiting, the differential for duodenal atresia should be high on the list and needs to be evaluated and managed promptly.

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CASPR2-Mediated Autoimmune Encephalitis in a Toddler

Autoimmune limbic encephalitis commonly presents in adults, frequently associated with Leucine-rich glioma inactivated protein-1 (LGI1) and Contactin associated protein-2 (CASPR2) antibodies, and is uncommon in children. Here we report a case of CASPR2 mediated autoimmune limbic encephalitis in a toddler.

A 19-month-old girl, born to consanguineously married parents, who was developing typically, presented with excessive irritabilityand decreased sleep for the past 40-50 days.Child had received diphtheria-pertussis-tetanus(DPT) booster dose 50 days back. Initially the symptoms were attributed to vaccination, but irritability kept gradually increasing. Child gradually regressed in language and cognition. She was not able to speak and on admission was able to only coo.There was behavioural change in the child in the form of new onset head banging, throwing of previously favourite toys and was avoiding going to the mother.Child was not mingling well

with others, did not play like before, was not showing interest in surroundings and there was cognitive decline in the form of not showing body parts and not recognizing mother. However, child was able to walk without support, and there was no motor regression. There was no history of seizures, fever, vision disturbance, involuntary movements, abnormal eye movements, and ataxia. Child had an uneventful birth history and family history. On examination, child was extremely irritable and crying inconsolably. Child had pallor and vitals were stable. There was no lymphadenopathy and organomegaly. There were dried crusted rashes with excoriation over trunk and limbs, with hyperpigmentation. Neurological examination did not show any cranial nerve involvement or motor weakness. There was no muscle twitching/fasciculation/neuromyotonia with spasticity, the best elicited power was 4/5 in all limbs,with brisk deep tendon reflexes with ankle clonus with bilateral extensor plantar response. Sensory and cerebellar examination was normal. Meningeal signs were absent.

Possibility of autoimmune encephalitis, connective tissue disorder and malignancy, possible lymphoma with paraneoplastic manifestations were considered as differential diagnosis. Peripheral smear examination, erythrocyte sedimentation rate and C-reactive protein were within normal units, and serum antinuclear antibody was negative. Magnetic resonance imaging of brain and nerve conduction study were normal. CSF analysis revealed one lymphocyte, normal protein and sugar, and positive oligoclonal bands. Electroencephalogram revealed diffuse slowing. Autoimmune encephalitis panel done by indirect immunofluorescence technique in transfected cells was positive for CASPR2 antibody in the serum and negative in the CSF.

Chest *X*-ray, ultrasound abdomen, computed tomography (CT) chest, CT abdomen, and bone marrow aspiration done to rule out the possibility of malignancy were non-contributory. Child was treated with intravenous methylprednisolone for 5 days with intravenous immunoglobulin 2g/kg, and showed clinical improvement within 72 hours. The child's irritability decreased and gradually child started sleeping well, started showing interest in surroundings, regained previous vocabulary of nearly 100 words, speaking two word phrases and was interacting well. The skin lesions resolved within 10 days after starting immunosuppressive therapy. After administration of intravenous methylprednisolone for five days, child was treated with oral prednisolone 2 mg/kg bodyweight per day for 6 months and was gradually tapered and stopped as child was completely asymptomatic and was gaining new milestones. Child has been on follow-up for the last one year. At age of 29 months, the child has achieved age-appropriate developmental milestones.

CASPR2–mediated limbic encephalitis is characterized by the onset of cognitive deficits, psychiatric disturbances, seizures, peripheral nerve hyper-excitability, neuropathic pain and insomnia in association with detection of Caspr2 antibodies in serum or cerebrospinal fluid, with or without underlying malignancies. There is strong male predominance with risk of malignancy in adults [3].

CASPR2 is a membrane protein expressed in the central and peripheral nervous system, which is essential for proper localization of voltage-gated potassium channels (VGKC) [4]. The most common presenting symptoms in adults are cognitive disturbance (26%), seizures (24%), peripheral nerve hyperexcitability (21%) and neuropathic pain (18%) [3]. Fewer than 10 pediatric cases have been reported with CASPR2 autoimmunity, so the phenotypes and immunotherapy responsiveness is less well-defined in children [4]; though, encephalopathy, seizures, neuropsychiatric symptoms, neuropathic pain and cramps are described as clinical features in children, with a median age of onset of 13 years [5,6]

Children with CASPR2 autoimmunity are under-recognized because neuropathic pain and symptoms of peripheral nerve hyperexcitability are difficult to characterize in children [5]. Peripheral nerve hyperexcitability has been documented in patients with CASPR2 autoimmunity[1]. One of the limitations of our case report is that we could not document the presence of neuromyotonia or evidence of peripheral nerve hyperexcitability in this child by electrophysiology.

The sub acute onset of behavioral disturbance, cognitive and speech regression should be considered as clinical clues to suspect autoimmune encephalitis in children; and CASPR2 mediated autoimmune limbic encephalitis should be considered as a cause of irritability with intractable itching/neuropathic pain in a child, as its recognition is important because they respond well to immunosuppressive therapy.

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Subtle Clinical Presentation of Pulmonary Alveolar Microlithiasis

Pulmonary alveolar microlithiasis (PAM) is characterized by the formation of calcium microlith in the alveoli due to defective clearance of phosphates. The clinical traits of PAM are heterogeneous and lung deterioration progresses at different speeds even when microliths appear early. The hallmark of PAM is clinico-radiological dissociation with typical imaging findings that correlate with specific pathological findings [1]. This report highlights the clinico-radiological dissociation and the tests available for the diagnosis of PAM.

Three children were diagnosed as PAM between 2015 and 2019. The first case was a 5-year-old girl (14.6 kg) who presented with complaints of fever, cough and poor appetite for seven months. Physical examination was normal. Imaging showed lung infiltrates suggestive of interstitial lung disease (***Fig.* 1** and **2**) but the open lung wedge biopsy showed calcific nature of the lesions confirming the diagnosis (***Web Fig.* 1**). The second one was a 4½-year-old girl (16.3 kg) who presented with recurrent fever, poor appetite and cough. The radiological imaging showed micronodular mottling on both lung fields. Broncho-alveolar lavage (BAL) demonstrated pus cells with moderate Streptococci. Video assisted thoracoscopic lung biopsy demonstrated air-spaces with innumerable tiny calcified bodies that are concentrically laminated with radial striations in the intra alveolar lumen consistent with pulmonary alveolar microlithiasis. The third case was a 12-year-old girl (17.3 kg) who presented with fever, weight loss and poor appetite for one year. She was treated for tuberculosis. Imaging showed calcified micronodule lesions on the midzones of both the lungs and ground glass opacities. Pulmonary function testing showed a mild restrictive pattern. BAL demonstrated *Moraxella spp*.

PAM is reported worldwide with more than half of the cases from five countries (Turkey, China, Japan, India, and Italy) [2]. It is often diagnosed incidentally during radiography of the chest [3]. All the three children in the present study were girls, contrary to a study from Turkey which reported six boys with PAM and a familial inheritance [4]. Studies have identified mutations causing decreased cellular uptake of phosphate leading to formation of intra-alveolar microliths [5].

The clinico-radiological dissociation with significant radiological findings in the absence of lower respiratory features like dyspnea and retractions have been reported earlier [6]. As the evolution of PAM is insidious, diffuse micronodular opacities may appear as miliary shadows in the chest radiograph leading to misdiagnosis of tuberculosis. CT chest demonstrated multiple calcified micronodules in both lungs, subpleural regions, and in the cardiac margins in all three affected children. PAM is usually diagnosed on the basis of a typical radiological pattern like a sand-like micronodulation of calcific density diffusely involving both lungs with basal predominance. Presence of this pattern may preclude the need for a lung biopsy. *SLC34A2* is implicated as the defective gene [5]. Paucity of symptoms and clinico-radiological dissociation may invite unnecessary investigations in the initial stages of the disease when PAM should be kept as a close differential.

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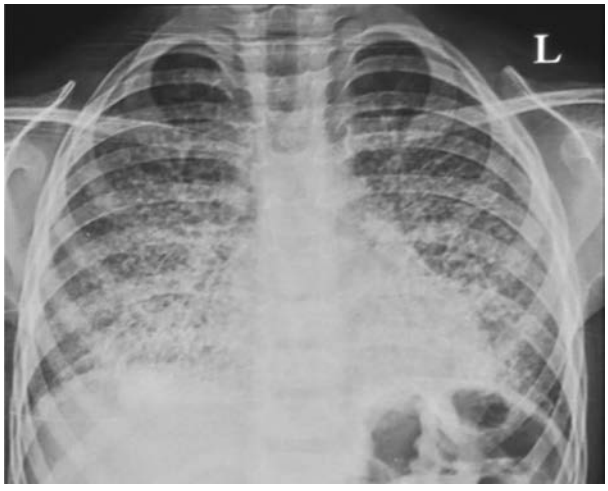

Fig. 1 *Chest radiograph showing diffuse reticulonodular opacities in both lung fields.*

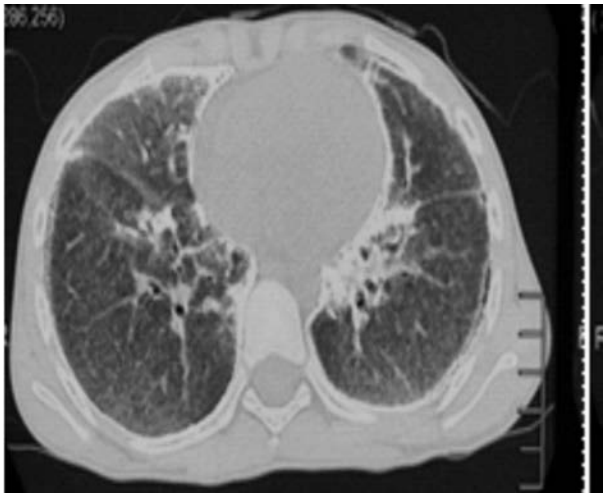

Fig. 2 *Computed tomography lung window showing calcified micronodule lesions on the midzones with ground glass opacities.*

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Plastic Bronchitis: A Manifestation of Dander Hypersensitivity

Plastic bronchitis, an entity with grave prognosis, is characterized by formation of large, branching bronchial casts obstructing the tracheobronchial tree. It has been previously reported in children with cyanotic congenital heart disease (CHD), asthma, allergic broncho-pulmonary aspergillosis (ABPA) and cystic fibrosis. A 9-month old boy presented with severe respiratory distress which required invasive ventilation for type-2 respiratory failure. He had received inhaled bronchodilators twice for recurrent cough in last 3 months. His grandfather had asthma and required inhaled medications. The child had exposure to animal dander (buffalo and cow) and wheat dust from nearby farm since birth. At admission, child had end-expiratory wheeze in left axillary area. High requirement of lung inflation pressures and loss of alpha-angle on end-tidal carbon dioxide graphic suggested obstructive airway. Salbutamol and ipratropium bromide nebulization along with systemic glucocorticoids partially reduced ventilatory requirements. Negative sepsis screen (CRP, procalcitonin, blood and tracheal culture) ruled out possibility of infection. Chest imaging (*X*-ray and CECT) suggested complete left lung collapse. Flexible bedside-bronchoscopy revealed thick, tenacious mucus plug, completely occluding the left main bronchus which could not be aspirated by multiple lavage attempts. A tree shaped, branching bronchial cast was removed from the left main bronchus via rigid bronchoscopy (***Fig.* 1a**). Baby was weaned off from ventilator over next 24 hours. Nebulized 3% saline was used along with chest physiotherapy for pulmonary toileting. Charcot-Leyden crystals with eosinophils and polymorphs were demonstrated on cytopathological examination of broncho-alveolar lavage (BAL) (***Fig.* 1b**). Cast histopathology showed eosinophils with necrotic background (type1 variety). Total immunoglobulin E (IgE) level was raised (276 IU/ml) with positive skin prick test (SPT) for buffalo dander. SPT for milk, egg, house dust, house dust mite, cow dander and wheat grass were negative. SPT was done with commercially available allergen extracts (Alcure Pharma) for local flora and fauna with valid positive and negative controls. Sweat chloride test and echocardiography was normal. The eczematous lesions over his back responded well to topical therapy. With a suggestive history of atopy, skin and respiratory manifestations, presence of Charcot Leyden crystals with eosinophils in cast and positive SPT for buffalo dander, an IgE-mediated allergic phenomenon was the most appropriate possibility. He was discharged on inhaled corticosteroids and oral montelukast with instructions to avoid buffalo dander, by shifting to maternal grandparents' home, and an emergency action-plan. Inhaled steroids were tapered over next 9 months. The child remained asymptomatic at 1-year in follow-up on montelukast alone and allergen avoidance measures.

Fig.1 *(a) Branching bronchial cast removed from left main bronchus; (b) red-colored diamond shaped Charcot-Leyden crystal (arrow) seen in the background of eosinophils and polymorphs in cytopathological examination of BAL fluid.*

Plastic bronchitis is a rare disease, with unknown prevalence, characterized by formation of thick, cohesive casts leading to complete or partial occlusion of the airway. Type I casts have cellular infiltrates, fibrin and are primarily associated with pulmonary disease like asthma, cystic fibrosis and ABPA, while type II are acellular casts with, mucin and few mononuclear cells, mainly seen in cardiac conditions. It has been classified as per underlying etiology into mucinous (structural CHDs), chylous (lymphatic disorders), inflammatory (atopy-asthma), and fibrinous casts in sickle cell acute chest syndrome (SCACS) [1]. It has also been documented after Fontan procedure, probably due to maladaptation to cavo-pulmonary circulation [2]. Casts associated with atopy or asthma are described as inflammatory with eosinophils, Charcot Leyden crystals and occasional neutrophils in a fibrinous background [1]. While expectoration of thick, rubbery, branching casts is pathognomic, patients usually present with cough, dyspnea or sometimes respiratory failure with suspicion of foreign body aspiration [3]. Management involves cast removal, chest physiotherapy, Dornase-α or hypertonic-saline or N-Acetylcysteine nebulization along with treatment of underlying disease [4]. With adequate supportive management, allergen identification and targeted measures (including immunotherapy) play an important role. Only one case of a 10-month infant with milk allergy and mucinous cast has been reported earlier [5].

Plastic bronchitis is one of the extreme presentations of allergic airway disorders. Animal dander exposure is common in developing world where increasing number of allergies are being recognized. The index case highlights the unique presentation of possible buffalo dander hypersensitivity in an atopic infant.

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Diabetic Striatopathy in a Child: A Cause of Reversible Chorea

The neurological manifestations of diabetes mellitus (DM) include altered mentation, convulsions, dyskinesia, paresthesia and coma. Dyskinesia including chorea and ballism are rare manifestations often encountered in elderly females of Asian descent [1], concurrently with hyperglycemic hyperosmolar state or diabetic ketoacidosis. Incidence of diabetic striatopathy in children is rare and characterized by myriad of symptoms including movement abnormalities like chorea-ballism and abnormality in striatum on neuroimaging. Till date, only six pediatric cases have been documented worldwide with two from India [1,2].

A twelve-year-old girl was brought with complaints of easy fatiguability, fever and two episodes of generalized convulsions for last two days. She was a patient of chronic calcific pancreatitis and was on treatment since the age of four years. She had polyuria, polydipsia and significant weight loss (body mass index-10.3 kg/m^2). Ophthalmic examination was normal. Investigations showed normal blood counts, renal function tests, serum electrolytes, liver function tests, calcium level, blood gas analysis and thyroid functions. Random blood glucose was 348 mg/dL, glycated hemoglobin was 16.4% and urine was positive for glucose and negative for ketones. CSF analysis was normal. A diagnosis of type 2c DM was made. She was started on insulin (basal-bolus) regimen. Seizures were controlled with anticonvulsant therapy.

On day-9 of admission, she developed low amplitude irregular abnormal movements of fingers, high frequency jerks involving both upper limbs, progressing to involve lower limbs and face. MRI brain showed T1-hyperintensities involving bilateral striatal regions with abnormal T2/ FLAIR signals with blooming on gradient-echo images and minimal restriction on diffusion-weighted images, consistent with non-ketotic hyperglycemic hemichorea syndrome. Symptomatic treatment with haloperidol, tetrabenezine, clonazepam and valproate was given. A reduction in the dyskinetic symptoms was seen by day 30 with adequate glycemic control. She was discharged on day 45 with minimal symptoms.

Diabetic striatopathy is a known complication of chronic uncontrolled hyperglycemia in adults with type 2 DM, which may be the only manifestation of underlying diabetes in the elderly [3]. This is the first case of a child with diabetic striatopathy secondary to type 2c DM. Affected patients are non-ketonemic, probably attributable to severe wasting with uncontrolled diabetes. Striatum is sensitive to acute changes in blood glucose, which manifest as hyperintensities, usually seen in unilateral than bilateral areas on neuroimaging [4].

Pathophysiology is unknown, probable mechanisms include metabolic changes like deposition of proteins, myelin

degradation products, calcium and other minerals. These products decrease as blood glucose levels are controlled and hence the recovery is explainable. Hyperglycemia causes hyperviscosity which leads to local tissue hypoperfusion, and depletion of gamma-aminobutyric acid secondary to a non-ketotic state. Hyaline degeneration, vascular proliferation and arteriolar thickening can be seen in striatal biopsy [5]. Astrocyte ballooning and neuronal degeneration can explain the hyper-intensities on imaging [1]. Similar hyperintensities in the basal nuclei can be seen in hepatic encephalopathy, toxic exposure to manganese, Wilson disease, intracerebral hemorrhage, carbon monoxide poisoning and methanol toxicity.

More than 90% patients show complete resolution between 2 to 28 days with or without radiological improvement [1]. Recovery was seen at 21 days with adequate glycemic control in the index case.

In conclusion, diabetic striatopathy is a rare entity in children, more so with bilateral presentation. This case highlights the need to suspect diabetic striatopathy in any child with uncontrolled diabetes and acute onset of chorea-ballism, irrespective of its cause.

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Transient Elastography to Represent Hepatic Copper Accumulation in Wilson Disease

Wilson disease is an autosomal recessive disorder characterized by abnormal copper accumulation, diagnosed based on clinical and laboratory features and treated with copper chelation [1,2]. Recent studies show that transient elastography (TE) could be used to predict liver fibrosis and monitor the disease progression [3]; however, many other conditions may lead to overestimation of the liver stiffness. Herein, we report a large reduction of liver stiffness after chelation therapy, that might be suggestive of effect of D-penicillamine and zinc on reducing copper load.

A 13-year-old girl presenting with jaundice, poor scholastic performance and coagulopathy for 6 months, was referred to our hospital for liver transplantation. Physical examination revealed Kayser-Fliescher (KF) rings with naked eye examination, splenomegaly, and pedal edema. However, her neurological system was otherwise normal. Laboratory investigations for Wilson disease and its complications were performed including complete blood count (hemoglobin 11 g/dL, white blood cell count 3,790/µL and platelet count 68,000/µL); liver function tests (total bilirubin 3.8 mg/dL, direct bilirubin, 2.1 mg/dL, albumin 2.0 g/dL, globulin 3.6 g/dL, AST 85 units/L, ALT 56 units/L and ALP 419 units/L); serum copper 0.42 pm; zinc 0.296 pm; PT 31.5 sec, INR 2.68; ceruloplasmin 9 mg/dL; and 24-hour urine copper 115.2 µg. Liver biopsy was omitted due to uncorrectable coagulopathy. TE (FibroScan; Echosens, Paris, France) was measured in preprandial state by a trained operator which showed a value of 50.6 kilopascals (kPa).

The patient was diagnosed with Wilson disease with 6 marks from the scoring system and the Wilson index score (WI) of 7, which implies good outcome without liver transplantation. Hence, D-penicillamine and zinc were initiated, and child closely followed-up for deterioration, and worsening coagulopathy. Six weeks later, while her clinical features and laboratory parameters did not improve, the TE value dramatically decreased (36.8 kPa). After one year, she rejoined school and performed well academically; her attention and memory were improved as per feedback from parents and teachers. However, KF rings were still present, even though tests of liver function were normal. The TE value was 34.3, 22.8 and 15.7 kPa at 6, 12 and 18 months, respectively, after the chelation therapy.

TE was used to measure liver stiffness by using a share wave method which determines the fibrosis level. TE has been studied as a non-invasive parameter to assess change in hepatic fibrosis during treatment in patients with Wilson disease; the cut-off values of mild and significant hepatic fibrosis were 6.6 and 8.4 kPa, respectively [3,4]. The decreasing value after copper chelation was ascribed to reduction in hepatic fibrosis [4]. However, liver elasticity may be influenced not only by fibrosis but also by other factors such as liver inflammation, the accumulation of various materials in liver tissue [4,5] and

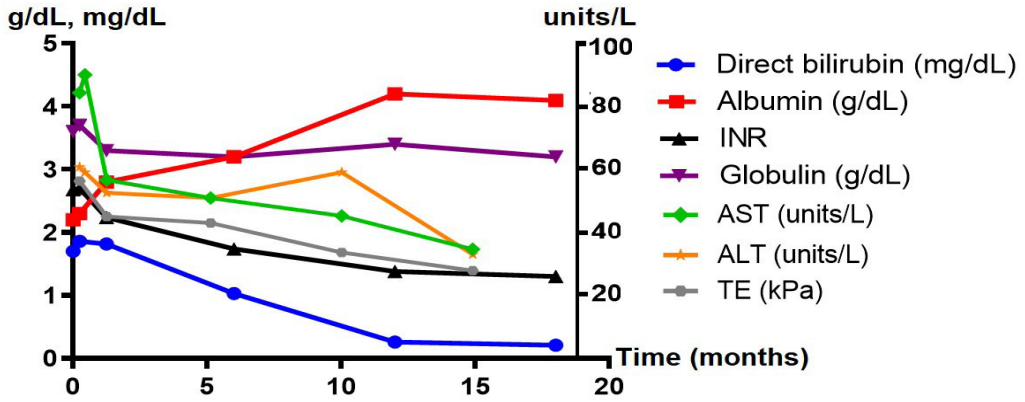

Fig. 1 *Trend of laboratory parameters and transient elastography (TE) in the index patient with Wilson disease.*

liver congestion [6]. Mikund, *et al*. [6] found a rapid decrease of liver stiffness from 73 kPa to 31 kPa in patients diagnosed with Budd Chiari syndrome after endovascular procedure that suggested the usefulness of TE in assessing hepatic congestion. Stefanescu, *et al*. [4] also reported reduction of liver stiffness after one year of treatment in children diagnosed Wilson disease. This study implied that intrahepatic copper deposit might be involved in the high liver stiffness before chelation therapy was initiated [4]. The present case demonstrated the reduction of liver stiffness after chelation therapy, with values comparable with the previous studies [4-6].

Consequently, the very high value of TE in the present case might reflect not only fibrosis but also the copper accumulation and inflammation in liver. Unfortunately, we could not measure the liver copper content as the patient had uncorrectable coagulopathy at the time of presentation. However, after chelation therapy, the TE value steadily decreased, which was associated with an improving clinical status. This report suggests the possibility of using TE to represent hepatic copper accumulation and to monitor treatment of Wilson disease.

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Steroid Resistant Nephrotic Syndrome with Clumsy Gait Associated With *INF2* Mutation

There has been significant improvement in our understanding of steroid resistant nephrotic syndrome (SRNS) with identification of multiple newer genes that are involved in regulating podocyte protein and maintaining podocyte architecture. Mutations in these genes have been linked to various forms of SRNS [1]. We hereby describe a child with SRNS whose associated neurological problem gave us a clue to the underlying etiology and genetic analysis provided the most likely link between his SRNS and neurological manifestations.

A 3-year-old boy born out of a non-consanguineous marriage presented with generalized swelling of body and decreased urine output. Blood and urine investigations confirmed the diagnosis of nephrotic syndrome. Remission was not achieved despite six weeks of full dose (2 mg/kg bodyweight) prednisolone and child was classified as SRNS. Renal biopsy revealed focal segmental glomerulosclerosis (FSGS) histopathology and tacrolimus therapy was added. Proteinuria failed to respond to tacrolimus as well as to subsequent addition of rituximab. During this period the child's gait was noticed to be clumsy. Neurological examination was unremarkable apart from mild bilaterally diminished ankle jerks. Nerve conduction velocity study was consistent with bilateral motor axonal neuropathy in lower limbs suggestive of Charcot Marie Tooth disease (CMTD). Next Generation Sequencing (NGS) and analysis of the exome data for any copy number variation revealed a heterozygous deletion (chr14:105181575-105196577) involving the *INF2* gene, which was extending to the proximal part of an adjoining gene *ADSSL1*. *ADSSL1* gene is implicated for distal myopathy-5 but as this is inherited in autosomal recessive fashion; the heterozygous deletion in our child was deemed insignificant. On the other hand, *INF2* mutation, which is inherited in an autosomal dominant mode, has been linked with FSGS coexisting with CMTD. Financial constraint precluded further confirming the CNV by cytogenetic microarray or to look for inheritance.

Large deletions involving the region described in our patient has been reported as pathogenic in genetic databases but phenotype similar to ours were not reported. Considering the above facts, the CNV found in our patient was classified as VUS (Variation of unknown significance). NGS can sometimes fail to detect deletions or duplication beyond few nucleotides and even in our case initial report was negative. In view of high suspicion, re-analysis of data was undertaken wherein with the aid of newer bio-informatic tools, the large deletion was identified. Currently the child is on regular albumin / frusemide infusion through a portacath along with anti-proteinuric agents like angiotensin converting enzyme inhibitor (ACEi). Although the association of CMTD with FSGS has been known for quite some time, the molecular pathogenesis linking the two has only been recently described with reports of *INF2* mutations in up to 75% of cohorts with this combination [2,3]. The INF2 protein is a member of the formin family of actin-regulatory proteins with an N-terminal Diaphenous Inhibitory Domain (DID) formin homology 1 and 2 domains and a C-terminal WASP Homology 2 domain, which has the hallmarks of the diaphanous autoregulatory domain (DAD) similar to other formins [4]. The CNV although currently classified as VUS in our child is particularly important as unlike all other previously reported mutations which were in the DID region, our case had it in the DAD region. Although interaction between the DID and DAD has been reported to be crucial in regulating INF2 depolymerization [5], clinical cases with mutations in DAD region have not been described earlier. *INF2* is strongly expressed in Schwann cell cytoplasm and interacts with myelin and lymphocyte protein (MAL2) and with GTP binding protein CDC42. These are essential for myelination and maintaining myelin structural integrity explaining the pathogenesis of CMTD. Proteinuria probably results from disruption in cytoskeletal dynamics due to defect in actin polymerization depolymerization balance secondary to INF2 mutation [2]. *INF2* mutation has also been reported to result in isolated autosomal dominant FSGS without CMTD and it is postulated that the relative positions of the deletions results in different clinical manifestation [2,3].

Our case not only highlights the interesting association of CMTD with FSGS, it also underscores the importance of NGS and the application of newer bio-informatic tools in the current genetic era. Among children with SRNS, recent guidelines strongly advocate the use of NGS for exome sequencing [1]. As in our child, additional clinical clues such as involvement of other systems are important and failure of a child with SRNS to respond to multiple immunosuppressant like calcineurin inhibitors (tacrolimus) and rituximab further augments the need to extensively search for an underlying genetic etiology.

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Hyperuricemia and Early-onset Chronic Kidney Disease in a 7-year-old Child

Familial juvenile hyperuricemic nephropathy (FJHN) is a rare and difficult to manage disease. Early diagnosis, early treatment with allopurinol and regular follow up can ameliorate the long-term progression to end-stage renal disease. We report a 7-year-old, asymptomatic patient with anemia, reduced renal function and hyperuricemia. Genetic screening played a crucial role in establishing the diagnosis.

A 7-year-old girl was referred to our clinic due to abnormal renal function tests noted in laboratory work-up for assessment of chronic anemia. Her past medical history was otherwise unremarkable. She was the fourth child of her family and there was no family history of renal pathology, except for diabetic nephropathy in the grandfather. Upon admission, her blood pressure was 123/93 mmHg (99th centile), weight 24 kg (25th centile) and height 127.5 cm (75th centile). Systemic examination was normal. Laboratory examination revealed increased blood urea nitrogen (BUN) (61 mg/dL), serum creatinine (1.23 mg/dL) and serum uric acid (8.52 mg/dL). The hematocrit was 32.3%, hemoglobin 10.8 g/dL, MCV 79.7 fL, MCH 26.7 pg and MCHC 33.5. Rest of the laboratory results were within normal limits. The glomerular filtration rate (GFR) was calculated as 65.5 mL/min/1.73 m^2 (stage 2 chronic renal disease). Fractional excretion of uric acid was 6 % (normal range 18±5%). Urine analysis results showed no abnormality. On sonographic examination, right kidney length was 7 cm and left kidney length 6.9 cm (25th centile), with increased cortical echogenicity of both kidneys, suggestive of parenchymal renal disease. Further imaging with mercaptoacetyltriglycine (MAG3) diuretic renogram indicated a moderate loss in renal function of both kidneys.

The laboratory and imaging findings were consistent with renal cortical necrosis. However, there was no previous medical history indicating a causative factor such as, chronic lead nephropathy or exposure to toxins and drugs for above findings. Moreover, the increased level of blood uric acid combined with its reduced fractional renal excretion could not be attributed only to the degree of renal failure. Thus, the possible diagnosis seemed to be genetic, most likely FJHN. In order to confirm the diagnosis, genetic testing was carried out. Mutation analysis of *Uromodulin*, the gene known to encode Tamm-Horsfall protein, revealed an in-frame deletion between nucleotides 668-767 and a replacement of conservative Glu 188 by an irrelevant valine, leading to a defective protein with 2 of 24 consecutive cysteine residues being removed. Cysteine residues are thought to be crucial in the cross-linking of the protein. Mutations involving them alter its tertiary structure and are commonly found in autosomal dominant tubulointerstitial kidney diseases (ADTKD) such as FJHN [1]. Our patient progressed to end stage renal failure at the age of 12 years. Her anemia, which was normocytic, normochromic was attributed to the chronic renal disease and was treated with erythropoetin. With the exception of *renin (REN)* gene-related ADTKD and nephronophthisis, anemia is not considered a typical finding of the condition [2]. The patient received a cadaver kidney transplant after 1 year of peritoneal dialysis, since the disease does not recur in the graft.

Familial juvenile hyperuricemic nephropathy is a rare genetic disease which falls into the category of ADTKD [2]. It is characterized by reduced renal excretion of urate, early onset hyperuricemia and slow progression to chronic end-stage renal disease. The exact pathophysiologic mechanism of the disease is not yet fully understood. It is presumed that mutations in the gene encoding Tamm-Horsfall protein, the most abundant protein in the urine, induce tubular dysfunction and subsequent uric acid retention [3,4]. FJHN is an autosomal-dominant disorder but this particular case did not have the expected findings of chronic renal disease, early hyperuricemia and episodes of gout [2]. Even in this case, ADTKD should still be considered in the differential diagnosis, as these may be *de novo* cases or with wrong diagnoses in other relatives [5,6]. Attempts can be made to select patients at risk of chronic kidney disease as a part of appropriate genetic counseling, which aims to detect them and improve their prognosis.

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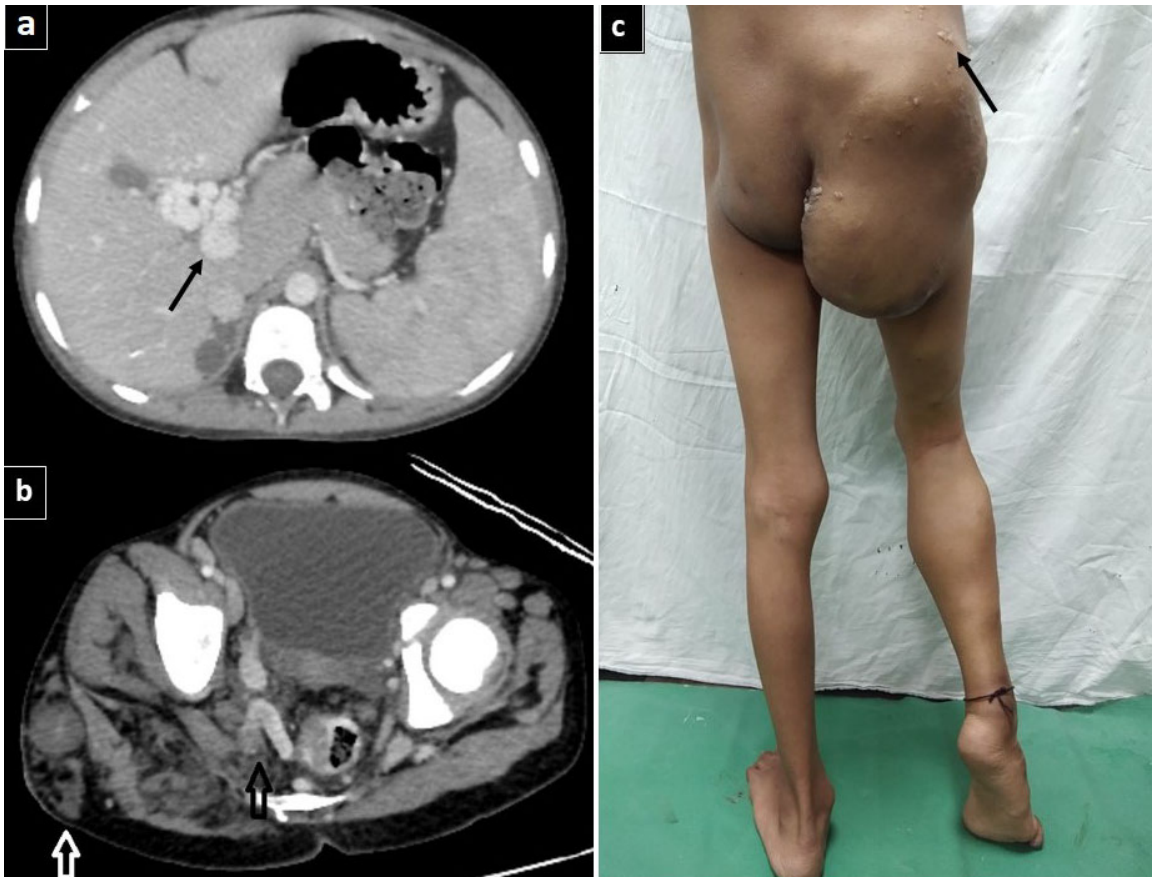
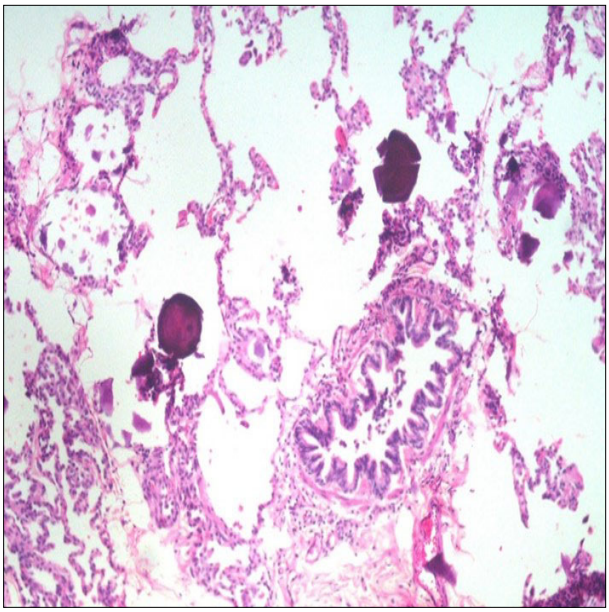


Web Fig. 1 *(a) Axial CECT abdomen showing dilated portal vein with periportal and peripancreatic collaterals (black arrow); (b) Axial CECT of the pelvis showing abnormal soft tissue and channels (white arrow) within the subcutaneous and intramuscular plane of right gluteal region. Right internal iliac veins are grossly enlarged with presence of abnormal draining vein (black arrow) arising from soft tissue; (c) Asymmetric limb hypertrophy with deformity and lymphanghioma circumscriptum (black arrow).*



Web Fig. 1 *HPE showing alveolar spaces contain basophilic calcified concretions with normal architecture suggestive of pulmonary alveolar microlithiasis.*

Encountering COVID in a Cancer Ward: Lessons in Infection Prevention

The coronavirus disease (COVID-19) pandemic has brought in unique issues for healthcare workers (HCW) managing health services of non-COVID patients [1]. This infection poses a double-edged sword while managing immuno-compromised patients [2]. As these individuals are at an increased risk of mortality from infections, most centers treating cancer have reduced hospital visits, modified chemotherapy protocols and rely heavily on tele-consultations [3]. The other major concern that has emerged while managing these children is the safety of healthcare workers (HCW) and sustainability of the non-COVID treatment centers.

We recently managed a child with acute lymphoblastic leukemia (ALL) who was subsequently diagnosed as being COVID positive. On examination in our daycare, she was afebrile, mildly tachypneic (respiratory rate of 25 breaths/min) with saturation of 88-89% in room air, and bilateral consolidation in lower lobes on *X*-ray chest. Although acute leukemia presenting with pneumonia is not unusual, the presence of hypoxia in room air was more suggestive of viral/ pneumocystis etiology. She was shifted to the COVID isolation ward without admission in the cancer ward. On evaluation, she was detected to be RT-PCR positive for severe acute respiratory syndrome coronavirus 2 (SARS-COV-2) and was managed as per standard guidelines. She recovered and was discharged after few weeks. Bone marrow evaluation confirmed ALL and she was started on treatment which is presently ongoing.

All HCWs in our team had been on hydroxychloroquine prophylaxis as per Indian Council of Medical Research (ICMR) advisory, since 2-3 weeks prior to this exposure [4]. On analysis of the exposure of healthcare staff to this patient, only six were high-risk and rest were low-risk [5]. The three primary nurses who initially interacted with her were quarantined from the day of the exposure. All HCWs who managed her, except the initial four, used appropriate personal protective equipment (PPE) [5]. The patient and her attendant wore triple-layer mask on hospital entry and all doctors and nurses in the team used N95 masks. All contacts were tested between 5-14 days of exposure and were negative. The services of the department were temporarily discontinued for a period of 14 days from last exposure to the contact. The patients admitted in the same ward and their attendants were observed for a period of 14 days from exposure and were asymptomatic. The daycare area was sanitized as per protocol on the same day after shifting the patient.

We wish to highlight certain issues related to containing COVID-19 infection in a non-COVID facility:

Non COVID facilities- low risk or high risk? In hospitals catering to both COVID-19 and non-COVID patients, albeit in separate buildings, COVID areas are considered high-risk, and non-COVID areas aree considered low risk. As per the MOHFW initial and revised guidelines on rational use of PPE, wards and ICUs in non-COVID areas continue to be marked low-risk [6.7]. As majority of COVID-19 positive in our country have been asymptomatic, it is only logical that many patients/attendants we encounter in non-COVID areas may be carriers of the virus, thus placing all the HCW in these areas at risk of infection. Thus the demarcation between low- and high-risk areas is no longer absolute. All HCW encountering patients (COVID or non-COVID) should take utmost precautions for personal protection to reduce exposure to the virus.

PPE rationalization: In resource-limited settings, rationalizing PPE use to ensure adequate supply to those HCW working in COVID hospitals and to prevent misuse, seems judicious [8]. However, without adequate PPE, accidental encounter with patients places HCW to high-risk category of exposure with mandatory quarantine of entire units, thus resulting in closure of treatment centers. Additionally, there is a bigger risk of exposure of other patients in the same facility, which could be more dangerous. Hence, when non-COVID areas are made functional in hospitals, adequate supply of PPE needs to be ensured for all HCWs.

Holding area in non-COVID hospitals: The recommendation is to develop a common holding area (after screening for COVID suspects) for non-COVID pa tients coming for routine treatment came later [8]. These patients could either be treated and sent home from this area itself or be tested for COVID and admitted to their respective wards after negative report is confirmed [8].

Universal testing of non-COVID patients: Although universal testing of patients who come for non-COVID treatment may be a safe strategy for healthcare workers [9], it does raise some concerns. Unlike in government facilities, most private laboratories charge between Rs 3500-4500 per RTPCR test. The turnaround time is usually 24 hours, which makes it difficult to get this test before providing acute treatment. For patients who need non-COVID treatment regularly such as those on dialysis, chemotherapy, blood transfusions *etc*, it will not be feasible to do tests before every hospital admission. The revised ICMR guidelines also do not recommend testing of asymptomatic individuals [10]. Although, real time PCR is the gold standard for diagnosis of this infection, false negative reports are known to occur, especially following early testing, improper collection and sample transport and primer related concerns [11]. In suspected COVID patients with corroborative symptoms, a repeat test is recommended. However, in the

current argument for mandatory testing in non-COVID patients, a proportion of patients may thus be missed despite testing.

As India transitions into a 'living with COVID' strategy, we will be encountering more of these patients in non-COVID settings. As patients and HCW are equally at risk of life-threatening complications of COVID infections, all efforts must still be made to protect all from getting infected. Also, HCW need to adapt themselves and work with each patient and attendant as if they are encountering a potential COVID carrier with universal precautions, appropriate PPE and standard steps for infection prevention. Another helpful strategy would be to develop teams of HCWs with 1-2 weeks of work followed by two weeks of quarantine, as is followed for COVID areas, even for non-COVID areas. In the event of an accidental exposure, this will prevent shutting down of services due to quarantining of staff.

Above all, a positive frame of mind is of utmost importance to tide over this difficult phase of for us and patients alike.

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Seasonal Influenza Vaccination and the Heightened Risk of Coronavirus and Other Pandemic Virus Infections: Fact or Fiction?

During this ongoing severe acute respiratory illness coronavirus 2 (SARS-CoV-2) pandemic, few speculative reports on significant association of influenza vaccines with an increased risk of coronavirus infection appeared both in media and academic circles. The speculation of vaccines paradoxically increasing the risk of infections possibly originated first following 2009 influenza A (H1N1pdm09) pandemic when four Canadian studies suggested that receipt of seasonal influenza vaccine increased the risk of laboratory-confirmed 2009 pandemic influenza A (H1N1pdm09) virus infection [1]. This led to five additional studies, each of which substantiated these initial findings. One proposed mechanism behind this phenomenon is 'original antigenic sin' which was first used to describe how first exposure to influenza virus shapes the outcome of subsequent exposures to antigenically related strains. When an individual is

infected by an 'evolved' strain with a new dominant antigen, slightly different from the 'original' strain against which the person has been vaccinated, the immune system produces antibodies against the 'original' strain through preformed high-affinity memory B cells that inhibit activation of naïve B cells resulting in a weak immune response against the new 'dominant' strain. Hence, the risk of infection paradoxically increased in vaccinated individuals as compared to unvaccinated individuals [2].

Besides, viruses are known to interfere with the circulation of other viruses. For example, there is evidence that the circulation of rhinovirus in the community interferes and decreases the spread of seasonal and pandemic influenza viruses [3,4]. Viral interference is also well-known to interfere with "take" of oral polio vaccine. However, more recently a new phenomenon, 'vaccine-associated virus interference' has been suggested whereby a vaccine can paradoxically increase the circulation of other viruses. That is, vaccinated individuals may be at increased risk for other respiratory viruses because they do not receive the non-specific immunity associated with natural infection [5,6]. Rikin, *et al*. [5] found an increased incidence of acute respiratory infection in children by non-influenza respiratory viruses among 999 participants (out of which 68.8% were children) following influenza vaccination compared to unvaccinated children during the same period. In a study of 115 children [6], a significantly increased risk of virologically confirmed non-influenza respiratory virus infections was found to be associated with receipt of inactivated influenza vaccine. Coronavirus was one of the non-influenza respiratory viruses [6]. Wolff, *et al*. [7] recently performed a large study among defence personnel to investigate respiratory virus interference during the 2017-2018 influenza season by comparing respiratory virus status with their influenza vaccination status. They concluded that overall, receipt of influenza vaccination was not associated with virus interference among the study population. However, vaccine-derived virus interference by specific respiratory viruses was significantly associated with coronavirus and human metapneumo-virus [7]. However, studies that have looked into the interference of influenza vaccine with specific non-influenza viral infections are scarce.

It is hypothesized that a respiratory virus infection confers immunity against the same and other respiratory viruses for a short time, perhaps a few weeks. This immune protection is associated with activation of the innate immune response to viral infection mediated by the release of type I interferons and other cytokines that have broad protective effects against a range of viruses [8]. This immunologic mechanism, known as heterosubtypic 'temporary non-specific immunity', has been proposed as the biological mechanism behind the paradoxical findings. Natural influenza infection that could have provided the host with some temporary immunity against other respiratory viruses is prevented by influenza vaccination. Hence, the risk of infection by non-influenza viruses (including the coronaviruses) is paradoxically increased [6].

The contentious issue of higher risk of non-influenza respiratory viruses to influenza vaccinated individuals has gained traction during the ongoing SARS-CoV-2 pandemic, which is also a coronavirus infection. Currently, we do not have sufficient data to establish or refute the association between influenza vaccination and higher susceptibility to coronavirus infection. We need to perform systematic studies urgently to find an answer to this question with regard to SARS-CoV-2. This is of vital importance since it is going to have far-reaching implications.

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Active Telephonic Follow-up During COVID-19 Lockdown: Initial Experience

The severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) pandemic forced a lockdown in India in late March, 2020. Prior to this, our department was working normally with 30-35 pediatric surgeries per week. As the lockdown was announced, a lot of patients who were discharged recently were lost to follow-up in the immediate post-operative period. We herein present our initial experience with telephonic follow-up and management of these children.

We prospectively collected the details of telephonic follow-of pediatric surgical patients discharged in the preceding two weeks period (10-24 March, 2020) before the nationwide lockdown. Data of children with day-care procedures was not collected. Parents of children eligible for study were called on the mobile numbers provided at the time of admission. In case of failure to contact, it was decided to stop calling after three days of twice daily calls. The problems identified, advice given and the outcome were recorded.

A total of 32 children were discharged during this period, out of which 26 families (81.2%) could be contacted. Of these, 10 (38.5%) had already paid a visit in the postoperative period and did not require any intervention, whereas the remaining had not visited since discharge and were not under follow-up. Seven children (26.9%) fared well in the post-operative period and were advised stitch removal from local health center. Nine (34.6% of contactable patients) parents had complaints *viz,* ostomy diarrhea with dehydration in three neonates (responded well to intravenous fluid administration with the help of local medical practitioner); two children with urethral stent *in situ* (stent was removed by telephonic coordination with local practitioner following which the patients did well), and one neonate with Peri-gastrostomy tube leakage and dehydration (managed successfully with telephonic advice for gastrostomy tube removal at nearby Primary Health Centre). However, three patients were advised to come to the hospital. One patient having *in situ* DJ stent for seven weeks with symptoms of pyelonephritis was managed by DJ stent removal, and a patient of adhesive obstruction was successfully managed conservatively and discharged after 6 days. The third child with slipped feeding jejunostomy was re-operated. Exploratory laparotomy with T-tube closure of perforation was performed but the child died on day 12 due to septicemia.

Postoperative follow-up of pediatric general surgical patient is important for ensuring and maintaining optimal patient outcomes [1], as complications in the early postoperative period have high morbidity and mortality [2]. Although there was no movement restriction for patients during the lockdown, still due to non-availability of public transport, travel was difficult for most patients [3].

Communication on phone can be an effective option for consultation during follow-up [4]. In the present study, we received good response both from the parents and the locally available doctors. Even in this era of telecommunication, with a lot of people having a mobile phone, surprisingly none of the patients with problems contacted us. This may be because patients do not feel comfortable in contacting the treating doctor on phone, and probably need to be proactively informed about the availability of this mode for problem resolution.

Most parents informed that initially the local general practitioners, when contacted, were not willing to handle these patients due to the notion of complication in a post-operative child and apparent lack of skills to treat them. With telephonic contact with the local general practitioners, we were able to manage 6 out of 9 complications (66.6%) with their help. Though there are chances of miscommunication on phone and difficulty in interpretation of all the instruction, one can avoid it if standard operating procedures are developed and followed diligently [5].

We propose from our experience that in wake of any event affecting the movement of patients, active efforts and call from the treating doctors can make a lot of difference with minimal effort and energy. Recent guidelines on telemedicine will go a long way in strengthening this model of patient care [6].

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Postnatally-Acquired COVID-19 in Central India

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infections have achieved pandemic proportions; although, pediatric manifestations still remain poorly delineated [1]. We describe two infants with postnatally acquired SARS-CoV-19 infection and discuss pertinent issues.

A 21-day-old girl was admitted with history of tachypnea for 1 day. She was born at 39 weeks of gestation with a birth weight of 3000 g to a primigravida mother who had an uneventful antenatal history. On admission, the temperature was 36.5°C, with pulse 140 beats per minute and respiratory rate 60 breaths per minute (oxygen saturation 90% in room air). Chest radiograph showed bilateral diffuse infiltrates. Laboratory investigations revealed hemoglobin of 11.1 g/dL, total leukocyte count 15,700/cumm (polymorphs 26%, lymphocytes 55%), platelets 6.69 $\times 10^9$/L, alanine transaminase 28 IU/L, serum ferritin 178 ng/mL, lactate dehydrogenase (LDH) 320 U/L and C-reactive protein (CRP) 0.5 mg/dL. Her nasal swab Reverse transcriptase polymerase chain reaction (RT-PCR) was positive for SARS-CoV-2. On contact tracing, the father and paternal grandfather were reported to be positive for SARS-CoV-2.

A 2-and-a-half-month-old boy was admitted with history of fever for two days. Neonatal period was uneventful and he was exclusively breastfed. At admission, his pulse rate was 112/min, respiratory rate of 52/min and oxygen saturation in room air of 88%. His chest radiograph showed right sided infiltrates. Laboratory investigations showed hemoglobin 9.8 g/dL, total leucocyte count 6710/cu mm (polymorphs 24%, lymphocytes 66%), platelets 4.5 $\times 10^9$/L, alanine aminotransferase 23 IU/dL, and CRP 0.5 mg/dL. His nasal swab RT-PCR for SARS-CoV-2 tested positive on the day of admission. His grandfather had recently tested positive for SARS-CoV-2. However, his parents and other family members were negative for the infection.

Both the infants received oxygen, broad spectrum antibiotics and syrup azithromycin (10 mg/kg/day) and syrup oseltamivir (3 mg/kg/day in two divided doses). None of the patients required ventilation. Both the infants were nursed by their mothers during hospital stay and were exclusively breastfed. Mothers were taught about the careful disposal of diapers and hand hygiene before and after handling the infants. There was no transmission of infection and nasal swabs for RT-PCR for SARS-COV-2 were negative for each mother twice. The babies were discharged home without supplemental oxygen after two repeat samples were negative.

The cases are presented to highlight the importance of mutual transmission of disease between mother, infant and other caregivers in the family. Infection was suspected in both infants based on clinical presentation and family history of infection in one or more family members. Recent research has shown that apart from droplets, infection can be transmitted through saliva of the infant [2] during breastfeeding, and stool of the infant [3-5], as viral shedding continues for several weeks in neonates. However, breastfeeding needs to be continued as per current recommendations [6], with regular hand washing with soap and water and proper diaper disposal. The presented cases signify the importance of proper hygiene in preventing transmission of infection from infected infants to nursing mothers, caregivers and *vice versa*.

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COVID-19 and Congenital Heart Disease: Perspectives From a Resource-limited Setting

Due to the emerging nature of the coronavirus disease (COVID-19), its effect on children/adults with congenital heart disease (CHD) are yet unknown. In developed countries, the majority of patients undergo effective surgical and/or catheter interventions in childhood. Thus, only a small proportion of patients have residual defects, and may be more prone to COVID-19 complications [1]. However, in a country like India, where large numbers of patients either remain unoperated or are just palliated, there is a possibility that severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection may be detrimental to such patients. Not only the risk of SARS-CoV-2 infection may be higher as compared to age-matched controls, the additive burden of COVID-19 can further compromise the facilities in already scarce cardiac care programs [2]. The categories of pediatric patients with cardiac disease, likely to be at a higher risk of severe COVID-19 disease are: cyanotic congenital heart disease with pulmonary artery hypertension (PAH) or severe cyanosis (SpO2 <80%), acyanotic congenital heart disease with PAH, acyanotic congenital heart disease with severe stenotic lesion, primary or secondary pulmonary hypertension, Eisenmenger syndrome, cardiomyopathy with severe ventricular dysfunction, and post-cardiac transplant patients [3,4].

Using recommended clinical criteria for hospital admission [5,6] in children with congenital heart disease might lead to many of these being hospitalized, who could otherwise have been managed at home. Children with acyanotic CHD with increased pulmonary blood flow have higher than normal resting respiratory rates even in healthy state, and some signs of respiratory distress are present due to heart failure. Children with cyanotic CHD, have low baseline saturation (<92%) and cyanosis due to their cardiac pathology. Due to these reasons, differentiating COVID-19 pneumonia from congenital heart disease can be very difficult. Therefore, in children with CHDs, the admission criteria [5,6] should not be used in isolation for hospitalization. Rather a wholesome clinical evaluation will help in triaging such patients.

Apart from the hemodynamic burden, some of these children might have reduced immunity, due to Down syndrome, DiGeorge syndrome and asplenia and therefore, may be at even higher risk for poor outcomes with COVID-19 infection [7]. Tele-consultations are being promoted for patients to maintain social distancing to avoid disease spread. In pediatric cardiology programs, teleservices do not suffice because many children suffering from congenital heart disease require surgical intervention or percutaneous intervention or diagnostic catheterization.

As children are less susceptible to COVID-19, the threat is indirect *i.e*. the delay in surgical/per-cutaneous interventions. Depending on local circumstances, many pertinent factors have to be weighed on case-to-case basis. Factors which need to be focused upon include resource utilization, such as anticipated ventilator duration, and ICU stay; clinical status of the patient and risk of delaying intervention, and; risk of exposure for the patient, family, and healthcare staff [8]. During the current situation, where healthcare personnel are themselves contracting the disease, optimal timings for congenital heart surgery [9] may not be practical.

We do not know much about the COVID-19 disease in children *per se* and specially in those with heart disease [4,5]. According to the local scenarios, such decisions have to be taken in individual capacity, keeping the interest of the patient as well as health care facility in mind. Providing teleservices and social distancing, triaging the patients into subgroups and focusing on the ones who need immediate intervention is a win-win situation for all the stakeholders *viz.* patient, family, health care personals, and the community at large.

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Medical Education During the COVID-19 Pandemics – Challenges Ahead

We read with interest the study on medical education during the coronavirus disease (COVID-19) Pandemic by Singh, *et al.* [1]. The authors have succinctly described the effect of social distancing on medical education in the wake of the pandemic.

Medical education is based essentially on pillars of knowledge, skills, behavior and communication skills, which need to be developed in medical students. While knowledge and deep learning actively require teacher-learner interaction in a conducive environment; skills, behavior and communication are difficult to cultivate without real time student-patient interaction.

Although didactic class room teaching, presentations, demonstrations and bed side teaching learning have largely been replaced by self-directed learning (SDL) and online teaching learning platforms, but the benefits of direct teacher student contact and real time two-way feedback are difficult to replicate at online forums [2]. Well-structured small group online teaching during this pandemic can improve teacher student interaction and initiate a deeper learning experience too [2]. Although adopting this format of teaching learning may not be easy for students with low motivation and from lower socioeconomic strata due to lack of equipment and connectivity, adapting to these new changes in medical education is the only way forward [2].

At our institute, we are using WebX platform for imparting online teaching to four hundred and twenty undergraduate students from four undergraduate medical batches. It is a real time large group teaching for all the subjects of the respective batch; each class lasting forty five minutes as per the approved curriculum. The presented lectures are posted online and are accessible to all for future reference. Small group teaching (4 to 5 learners in each group) for active participation and in-depth learning is also conducted through this platform. Some guidelines are available to conduct student assessments, but the modalities of conducting annual assessments is open to discussion [3,4].

In the era of social and physical distancing, actual student-patient interaction may not be possible but practical skills still can be facilitated by online videos on patient examination followed by students practicing on simulation and virtual reality platforms, while attributes like attitude and communication skills can be practiced on inpatients, maintaining social and physical distance. As development of clinical skills, attitude and communication in a medical student are integral part of medical education, we have to discuss and deliberate how these attributes can be addressed on online platforms.

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MMR Vaccine and Covid-19: A Myth or a Low Risk-High Reward Preventive Measure?

Published data highlights that immunity conferred by vaccination against Measles-Rubella (MR) vaccine persists for at least 20 years, and is generally thought to be lifelong for most individuals [1]. Emerging data speculates the role of live attenuated vaccines and the concept of 'trained innate immunity' offered by Bacillus Calmette Guerin (BCG) and MMR vaccine as an immune-prophylaxis against SARS-CoV 2 [1]. The molecular theory is based on overlapping of vaccine epitopes and amino acid residues between the Spike (S) glycoprotein of the SARS-CoV-2 virus with the Fusion (F1) glycoprotein of measles virus and the envelope (E1) glycoprotein of the rubella virus that possibly results in children presenting with a milder version of COVID-19 disease in contrast to adults [2]. As a result of these hypotheses, there has been an increase in adults opting to vaccinate themselves with the MMR vaccine.

We feel that this data may be more applicable to the Western population, particularly in the United States, and may not be applicable in India [3,4]. Firstly, the Serum Institute of India Ltd manufactures the MMR vaccine used in India. This contains the Edmonston-Zagreb measles virus strain, which provides long-term sero-persistence of antibodies against measles in a majority of vaccinees as compared to other brands found across Western countries [3]. Secondly, a study of 192 college students in India found that the sero-prevalence of antibodies (IgG) to the three components of MMR vaccine was 91%, 97%, and 88%, respectively, even though 96% of the study population did not recollect their vaccination history [5]. Both of these studies suggest that antibodies to measles might well persist into adulthood and the need for an MMR vaccine to boost immunity may not be essential.

Okada, *et al*. [6] compared the analysis of host responses related to immunosuppression following natural measles infection and vaccination with a live attenuated measles vaccine and concluded that in contrast to wild-type measles virus (natural infection), live measles vaccines did not provoke the host cytokine response that leads to apoptotic cytolysis of uninfected lymphocytes, lymphopenia and immuno-suppression, and thereby induced weaker immune responses to the virus. Published data from the United Kingdom (UK) revealed that macro domains of SARS-CoV-2 and rubella virus share 29% amino acid sequence identity, suggesting they have a similar protein fold. A cellular homolog of the methyl-transferase from SARS coronavirus can be found in *E.coli* with 65% sequence identity and viral homologs are detected in numerous coronaviruses, concluding that the observed homology is similar to an anamnestic reaction to SARS-CoV-2 by mounting IgG/IgM response of rubella antigen and that MMR will not prevent COVID-19 infection but could potentially reduce poor outcome, which may be more of a speculation without appropriate randomized control trials [7].

In summary, while there are many theoretical hypotheses to prove the concept of trained immunity offered by BCG and MMR vaccines, well-designed controlled studies are needed, before considering MMR vaccination as a low risk-high reward tool to prevent COVID-19.

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Lung Ultrasound in COVID-19

Chest *X*-ray is considered less sensitive in diagnosing coronavirus disease 2019 (COVID-19) pneumonia, and due to the milder disease and relatively subtle findings [1], the sensitivity may be further lowered in children. Though chest computerized tomography (CT) is the imaging modality of choice in COVID-19, difficulties in sparing a dedicated machine, transferring potentially infectious and sick patients to the CT room, disinfection of machine, and ionizing radiation exposure make it less appealing, especially in children. Therefore, a readily available, point-of-care tool that avoids radiation exposure is needed.

Lung ultrasound (LUS) is routinely used as a point-of-care imaging tool in emergency and intensive care units, and its role in COVID-19 is being explored. COVID-19 classically presents as diffuse bilateral pneumonia with asymmetric patchy lesions in the lung periphery that are amenable to ultrasound visualization [2]. Its easy availability, easy decontamination, freedom from radiation, and portability favor its use in COVID-19.

All 14 lung areas (three posterior, two lateral, and two anterior) should be scanned. B-lines are the most classical findings of COVID-19 pneumonia and some authors describe COVID-19 pneumonia as a "storm of clusters of B-lines", sometimes appearing as shining white lung [2]. In a meta-analysis of seven studies (122 patients), almost all patients had abnormal LUS [3]. The common abnormalities were interstitial involvement/B-pattern (97%), pleural line abnormalities (70%), pleural thickening (54%), consolidation (39%), and pleural effusion (14%) [3]. The number and appearance of B-lines also correlate with the disease severity. As disease progresses, the B-lines increase in number and become more confluent. In severe disease, extensive areas of subpleural consolidations and pleural effusion may be visualized. On serial monitoring, a decrease in the B-lines and appearance of A-lines indicate recovery [4].

Till now only three studies (23 patients) have evaluated the role of LUS in pediatric patients. Most common findings were pulmonary interstitial syndrome (82%) followed by consolidation. Only one study (5 patients) directly compared chest CT, *X*-ray, and LUS and found that ultrasound fares better than chest *X*-ray [5].

Few important limitations of LUS are the inability to detect deep and intrapulmonary lesions, difficult to scan posterobasal regions in sick patients, and relatively lower sensitivity than CT scan. Point of care lung ultrasound, where available, may be utilized in the management of children with COVID-19.

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Pubertal Menorrhagia – A Rare Presentation of Congenital Factor XIII Deficiency

Factor XIII deficiency, with an estimated incidence of 1 in 1-2 million, is an extremely rare congenital bleeding disorder with autosomal recessive inheritance and often with a history of consanguinity [1]. It can present with myriad bleeding manifestations in different age groups, with varying severity. This is partly due to multiple possible mutations of the two subunits of Factor XIII (subunit A mutations are associated with severe manifestations than those of subunit B) and partly because factor XIII is a multifunctional protein (required in wound healing, maintenance of pregnancy, angiogenesis and hemostasis). Umbilical cord bleeding, present in 73% of patients, is highly suggestive of this condition [1,2]. Pubertal menorrhagia was present in nearly 31% patients as per an Indian study [1].

A14-year-old girl presented with severe anemia and

menorrhagia, refractory to antifibrinolytic therapy, during the third menstrual cycle, with significant past history of having menorrhagia in every cycle after the onset of menarche. These episodes were being treated with blood transfusions, antifibrinolytics, hematinics and oral contraceptive pills. She was born as a third issue of third degree consanguineous couple with no relevant family history and bleeding diathesis. Ultrasound of pelvis, thyroid profile, platelet counts and morphology, coagulation profile (PT-12.1sec, PTT-28sec, INR-1) and clot retraction tests were normal. With this background, qualitative assay for factor XIII was considered and clot solubility test was positive. Quantitative assay, platelet function test, von Will ebrand factor assay and molecular genetic test were not performed because of affordability issues.

Although, coagulopathy is the second most common cause of pubertal menorrhagia [3] with congenital factor XIII deficiency being the commonest amongst the 'rare congenital factor deficiencies', it stays under diagnosed due to its rarity, heterogeneity and absence of diagnostic facilities [1]. Qualitative assay with clot solubility in 5M urea detects only severe form, but quantitative functional assay detects all forms and it is the recommended first line screening method as it has no false positivity [4]. The available treatment options include replacement and prophylactic therapy with cryoprecipitate, fresh frozen plasma and factor XIII concentrates [5].

Awareness about all possible features of factor XIII deficiency is essential among clinicians while managing cases for prompt diagnosis, appropriate treatment, improving quality of life, decreasing the burden of further medical catastrophes, follow up and genetic counseling to prevent morbidity in affected children.

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Arrhythmias Associated with Administration of Anti-fungal Agents

An 8-year-old boy who had received a bone marrow transplant due to relapse of acute lymphoblastic leukemia was admitted for veno-occlusive disease. He also developed acute kidney injury and was dialysis dependent. His condition was gradually stabilized but he subsequently developed invasive pulmonary aspergillosis with multiple aspergillomas involving both lungs. He was then given oral posaconazole 300 mg twice daily and intravenous liposomal amphotericin B (Ambisome) 90 mg (3 mg/kg) daily infused through a Hickman catheter over one hour. The tip of the central catheter was located at the junction between superior vena cava (SVC) and right atrium (RA). However, he developed feeding intolerance with severe abdominal pain requiring temporary suspension of enteral feeding. Posaconazole was switched to intravenous voriconazole 210 mg every 12 hours. Five days after the co-administration of voriconazole and amphotericin B infusion, he developed an attack of non-sustained wide complex tachycardia lasting for 24 seconds after infusion of voriconazole and amphotericin B. Thirty seconds later, there were three similar attacks lasting for 15, 2 and 4 seconds, respectively with an interval duration of 1 second in between. He was asymptomatic during the attacks. He was receiving continuous renal replacement therapy at that juncture. An electrocardiogram performed immediately after the event failed to capture the ventricular tachycardia. It showed a QT interval of 0.42 seconds. Few days later, the asymptomatic ventricular ectopics appeared again during amphotericin B infusion. There were no other pro-arrhythmic medications, and the tacrolimus level was 5.3 µg/L. Voriconazole was then switched back to oral posaconazole as his enteral feeding was re-established and the administration duration of amphotericin B was lengthened to two hours, with no recurrence of arrthymias.

Several antifungal agents of triazole class are arrhythmogenic but ventricular tachycardia has only been rarely reported [1,2]. The underlying mechanism probably involves both direct blockage of hERG potassium channel and inhibition of channel trafficking, as demonstrated with ketoconazole [3]. The development of ventricular arrhythmia and hyperkalemia after rapid infusion of amphotericin B has been previously reported in those with impaired renal function [4]. The infusion through a central catheter located at SVC-RA junction appeared to increase the risk of inducing arrhythmia [5].

Although, there is no drug interaction between voriconazole and amphotericin B, the arrhythmogenic properties of both agents increase the risk of developing cardiac arrhythmia, if co-administered. A rapid infusion rate, the presence of acute kidney injury with low glomerular filtration rate, electrolyte disturbances and administration through a central catheter near the SVC-RA junction – all appeared to have increased the risk of cardiac toxicity in this child. Our experience suggests that amphotericin B-associated ventricular arrhythmias may be managed with a slower infusion rate and avoidance of co-infusion with other anti-fungal agents.

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Emotional Intelligence: An Important Attribute for the Physician Leader

Over the last two decades, emotional intelligence has been heralded as an indispensable component of success. Ever since renowned psychologist Daniel Goleman published his book titled 'Emotional Intelligence' in 1995 [1], there is a growing body of evidence on how great leadership and emotional intelligence are interlinked. So what exactly is emotional intelligence? In Goleman's own words, "*emotional intelligence is the capacity for recognizing our own feelings and those of others, for motivating ourselves, and for managing emotions well in us and in our relationships* [1]." Emotional intelligence describes abilities distinct from, but complementary to, academic intelligence or purely cognitive capacities measured by the intelligence quotient [1].

Years of research in the business field has shown that although technical abilities and cognitive capacities are important in leadership, they merely serve as an entry requirement. What sets apart great leaders from others is their wealth of emotional intelligence. The five components of emotional intelligence as identified by Goleman are – self-awareness, self-regulation, internal motivation, empathy and social skills [1]. It was once thought that these qualities were merely 'icing on the cake', but not essential to have in leadership. Two decades of research and study in this area has resulted in a paradigm shift, not only are these skills essential, they are the hallmark of great leadership.

A systematic review in 2014 [2] showed that there are more than 80 articles highlighting the connection between physician leadership and emotional intelligence. Many authors have identified the need for emotional intelligence in physician leadership development, mentoring and advancement within academic medicine, and developing effective social networks within the healthcare field [2].

Emotional intelligence is something that can be improved through deliberate practice and training [3]. Two recent studies from India highlight the need to incorporate emotional intelligence training in medical education [4,5]. Such curriculum innovation is critical in the development tomorrow's medical leaders. Physician leaders who possess a high degree of emotional intelligence are able to consistently identify the needs of both patients and colleagues. They are highly effective at reading social cues and gauging responses to their words and actions; they then use this data to improve relationships and achieve positive outcomes.

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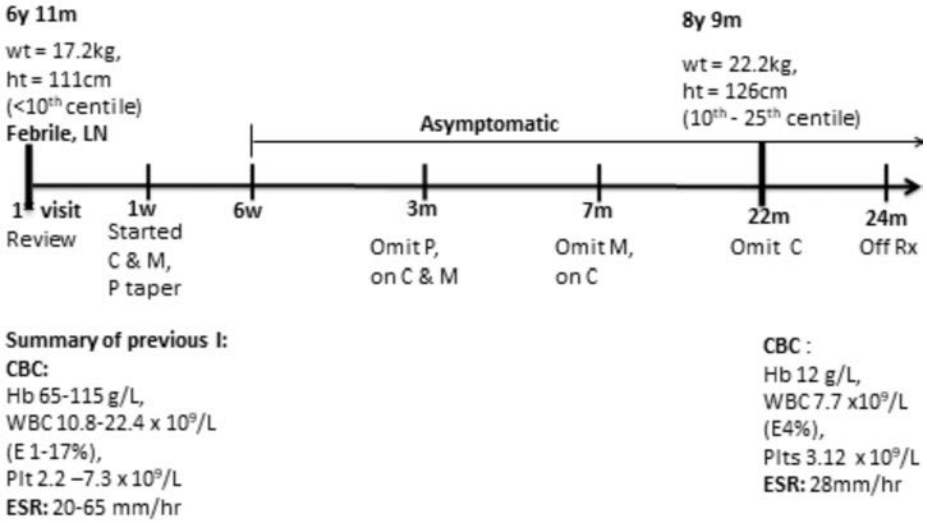
Steroid-dependent Kimura Disease in a Child Treated with Cetirizine and Montelukast

Kimura disease or eosinophilic lymphogranuloma, is a rare disease of unknown etiology, affecting middle-aged Asian men and occasionally children [1]. It is characterized by a triad of painless unilateral cervical adenopathy or subcutaneous masses predominantly in head or neck, blood and tissue eosinophilia, and elevated serum IgE levels [1]. We share our experience with using cetirizine and montelukast in a long-standing, steroid-dependent, severely growth retarded child with the disease, and propose this combination as an alternative to steroids, immunosuppressive drugs, radiation or surgery.

An 8-year-9-month-old male child, firstborn to third-degree consanguineous parents presented to his pediatrician with cervical, axillary, and inguinal lymphadenopathy and fever at the age of 3 years. Cervical lymph node biopsy had reported an ill-defined granulomatous lesion and with a positive Mantoux test, but no microbial confirmation, he had received anti-tuberculous therapy for 18 months. As the lymphadeno-pathy and fever persisted, his lymph node biopsy was repeated and he was diagnosed as Kimura disease at 5 years and 3 months of age. He was started on oral steroids to which he initially responded but the lymphadenopathy and fever would recur as the steroids were tapered to 10 mg (0.6 mg/kg/day). Methotrexate and azathioprine were tried sequentially as steroid-sparing agents, but were discontinued due to transaminitis. On steroids for about 20 months with significant growth retardation, he was referred to us for further management.

On examination, he was febrile with mild cushingoid facies and had non-tender left axillary and inguinal lymph nodes. The systemic examination was normal. His clinical features, anthropometry, investigations and course are summarized in ***Fig*. 1**. There were no features of renal involvement. His lymph node biopsy slides from both earlier biopsies were reviewed and



Abbreviations: C: Centirizine; M: Montelukast; P: Prednisolone; LN: Lymphadenopathy; wt: weight; ht: height; w: weeks; I: Investigations; CBC: Complete blood picture; ESR: Erythrocytesedimentation rate; Hb: Hemoglobin; WBC: white blood cell count; Plt: Platelets; E: eosinophils; Rx: treatment.

Fig. 1 *Disease course in the index patient with Kimura disease.*

were consistent with the diagnosis of Kimura disease.

After a extensive literature search [2-4] and detailed discussion with parents, we commenced a combination of oral cetirizine (0.29 mg/kg/day) and oral montelukast (0.58 mg/kg/day). We witnessed a rapid and sustained clinical response.

Kimura disease usually has an indolent course. The etiology remains unclear, although eosinophilia, increased IgE, tumor necrosis factor (TNF-α), interleukin (IL)-4, IL-5, IL-13 and mast cells are seen in the peripheral blood and tissue, leading to autoimmunity, allergy, neoplasm and parasite infestation being proposed as possible risk factors [5]. In localized disease, surgery is the mainstay of therapy. Regional or systemic corticosteroid therapy, immunosuppressive agents and radiation have been used [1]. Recurrences after surgery or on discontinuing steroid treatment are common [1]. Other agents tried with variable outcomes are oxpentifylline, cryotherapy, vinblastin, all-trans-retinoic acid and Imatinib [5].

Cetirizine, a selective histamine H1 receptor blocker is known for its antihistaminic and anti-inflammatory properties. It inhibits eosinophil chemotaxis, adhesion to endothelial cells, suppresses the generation of various proinflammatory cytokines and decreases intercellular adhesion molecule 1 expression [2]. Pranlukast and montelukast are leukotriene receptor antagonists known for their anti-inflammatory role [3]. Almost all the previous reports on these two drugs were in middle aged adult patients with Kimura disease, reflecting the disease demography, with little data on use of these safer drugs in children [3].

Due to financial constraints and poor access to health care resources, the patient was started on drug combination of montelukast and cetirizine, which were considered safe, affordable, easily available, orally administered and not needing any specific monitoring for side effects. After remission, we omitted montelukast first because we believed that cetirizine has a better safety profile for long term use.

Thus, while earlier reports suggest variable effect of cetirizine and montelukast in this disease, the dramatic response to these drugs suggests that inhibition of eosinophil recruitment and activity may be an important aspect in the treatment of Kimura disease.

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Beyond Hospital Boundary: A Novel Game-Changer Tool of Kayakalp for Community Participation in Sanitation, Hygiene, and Infection-control

Kayakalp initiative was launched in 2015 to promote cleanliness, hygiene, and infection control practices in public health facilities in India [1]. This innovative tool alongwith its annual incentives has the potential to make impactful behavior change amongst health caregivers eventually leading to desired transformation [2]. It has a standardized protocol and scoring pattern on given parameters under different sections for quality assessment, first by internal evaluation, then peer and final validation by an external assessment [3].

From 2018 onwards, a new section 'Beyond hospital boundary' has been added to this checklist. The name itself indicates the assessment of surroundings for sanitation and other parameters of Kayakalp. The ten sub-sections added are: promotion of *swachta* (cleanliness) in surroundings, coordination with local institutions, alternative in financing, leadership in governance, health facility approach, cleanliness of surroundings, public amenities in surrounding area, aesthetics of surrounding area, general waste management in surroundings and maintenance of surrounding area. All subsections have a maximum of ten marks, each based on five indicators. The aggregated maximum scores are 100 for district hospital Kayakalp checklist, and 60 each for bedded and non-bedded primary health centers, comprising 1/6th of the total kayakalp score.

By introducing this section, the government is emphatically promoting community participation. Earlier it had focused mainly on behavior change amongst health staff within the premises of an institute, and its impact was tremendous with incentivization of initiatives. Now the government has included

other stakeholders like community members, Panchayat Raj Institutions (PRI), Non-Government Organizations (NGO) and other public sector departments as a part of this campaign. Activities from within, like public rallies, marathons, *swachhata* walks, human chains, street plays/*nukkad nataks*/folk arts/folk-music, *etc.* will act as potent instruments of social advocacy and community participation. It ensures every stakeholder from outside health facility premises and communities are gradually involved for hygiene and infection control and thereby helping health promotion at the grass-root level. World Health Organization estimates that Swach Bharat Abhiyan in India would potentially have a spectacular impact on improving the sanitation of communities and thereby averting disease burden within five years of its launch [4]. The integration and extension of such activities will be another opportunity for healthcare providers to make an impact on health indicators and disease burden. Subsequently, as all stakeholders adopt these initiatives, there will be a visible and viable behavior change of the public at large.

To, summarize Beyond hospital boundary will act as a novel, innovative game-changer tool for community participation in sanitation, hygiene, and infection control.

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Spirometry in COVID-19 Times – An Emerging Dilemma

Spirometry is useful for the diagnosis, management and monitoring of chronic respiratory conditions in children, especially asthma. As severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) can be transmitted *via* aerosol generation, coughing or sneezing [1], spirometry can pose a risk for transmission of the virus as the procedure requires generation of high minute ventilation and flow, and for the patient to be in close contact with the technician and equipment. We have tried to extrapolate information from adult guidelines on spirometry during the COVID-19 pandemic.

As the pandemic evolves over time, prevalence can be classified to be in the pandemic phase, post-peak phase or post-pandemic phase, with high, low or controlled community prevalence, respectively. This can be determined by the local health authorities. Level 1 safety recommendations are suggested for those places in the pandemic phase, Level 2 in the post-peak phase, and Level 3 in the post-pandemic phase [2].

Indication for spirometry: During the pandemic phase and post-peak phase, clinicians should restrict referrals for spirometry to those patients who require it urgently or when it is essential for their diagnosis [3]. A pediatrician can teleconsult the patient and determine the need for spirometry, to reduce the number of visits of a child to the hospital. One should; however, not perform spirometry on patients with a clinical suspicion of COVID-19, influenza-like illness (ILI) or severe acute respiratory infections (SARI) [4]. In children who test positive for COVID-19 infection, all pulmonary function tests (PFTs) should be deferred for at least 30 days post-infection, as viral shedding can occur even after 10 days.

Guidelines for performing spirometry: The following are the Level 1 safety precautions one must follow while performing a spirometry in children during the pandemic phase. Similar precautions are advised for Level 2 in post-peak phase as it might be difficult to determine pre-test probability of infection in children.

- *Screening:* The clinician or technician performing the test, the child and the caregiver, should all be screened prior to entering the PFT room. A proposed triage questionnaire is available in the European Respiratory Society statement [2]. Patients who screen positive should not undergo spirometry.
- *Infrastructure:* Under ideal conditions, negative pressure rooms or HEPA filtration systems with UV germicidal lamps are recommended. However, this may not be available in most centres. Hence, at least a separate enclosed room with adequate ventilation should be designated for performing spirometry [2]. Waiting areas should be re-organized to ensure patients are not in contact with those who are febrile. Thorough cleaning and ventilation of both the room and equipment needs to be performed between each test [5]. The number of air exchanges between procedures need to be

determined by each facility to ensure removal of 99.0-99.9% of airborne microorganisms calculated as per CDC guidelines [6]. Only one caregiver, who must wear a face mask and follow hand hygiene procedures, should be allowed into the room [5].

- *Staff*: The person performing the spirometry in the pandemic and post-peak phases should wear full personal protective equipment (PPE) which includes a fit tested N95 mask, eye goggles or face shield, apron and disposable gloves [7]. Strict hand hygiene protocols must be followed by both the operator and the patient.
- *Equipment*: Equipment should be cleaned and disinfected by wiping down all surfaces that the patient comes in contact within a 2-metre radius, using a hospital grade antiviral disinfectant such as 70% isopropyl alcohol (IPA). Recalibration of the equipment after decontamination is suggested [2]. Single use bacterial and viral in-line filters of high specification are required to be used. The ideal filter is one with minimum proven efficiency for high expiratory flow of 600 to 700 L/min [8]. Replace all consumables to single use or disposable ones, wherever possible.

Appointments need to be staggered with a gap of 45-60 minutes, taking into consideration the time required for donning and doffing of PPE by the clinician/technician between each patient, post-test cleaning of the room and equipment, and recalibration of the spirometer [5].

All these safety recommendations for performing spirometry must be maintained till the local public health authorities can confirm that the community spread is controlled and the district is in the post-pandemic phase. More specific guidelines for performing lung function tests in children will need to be formulated by global organizations as the pandemic evolves.

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The Recovery Trial

The Randomized evaluation of COVID-19 therapy, the RECOVERY trial, has reiterated what many frontline workers had been experiencing. Steroids work! This was a 176 center, randomized control pragmatic trial comparing the effect of 6 mg dexamethasone for 10 days along with usual care to usual care alone in patients with COVID-19 infections.

There were 2104 patients who received dexamethasone and 4321 got only usual care. Primary outcome measured was mortality at 28 days and secondary outcomes were need for oxygen or ventilation. Overall, the dexamethasone group had a significantly lower mortality of 21.6% compared to the non-dexamethasone group (24.6%).

What was more striking was the reduction in mortality in those requiring oxygen or mechanical ventilation. Dexamethasone reduced deaths by one-third in patients receiving invasive mechanical ventilation (29.0% *vs.* 40.7%; $P<0.001$) and by one-fifth in patients receiving oxygen without invasive mechanical ventilation (21.5% *vs.* 25.0%; $P=0.002$). Patients who were not on oxygen did not benefit from dexamethasone, and, in fact there was a trend for increased mortality in those who received dexamethasone (not statistically significant). Patients who received dexamethasone after 7 days of onset of symptoms showed more benefit than those who received it early in the course of the disease.

Benefits accrued by patients on oxygen or mechanical ventilation and after 7 days of illness suggest that dexamethasone makes a difference at a stage when immune-mediated injury predominates over the initial viral replication. The low cost and wide availability of the drug make it an attractive option in serious COVID-19 infections.
(*MedRxiv 22 June 2020*)

Utility of Antibody Tests in COVID-19 Infections

Two recent meta-analyses have evaluated the utility of serological tests for SARS-CoV-2 infections. One included 40 studies and the other 54. Three types of antibody tests were used - Enzyme linked immunosorbent assays (ELISA), chemiluminescent immunoassays (CLIA) and lateral flow immunoassays (LFIA). The LFIA is typically used for point of care testing with either a dipstick format or cassette such as used in the common pregnancy test.

The sensitivities and specificities depended on the type of test done, the timing of the test after onset of illness, the type of antibody looked for (*e.g,* IgA, IgM or IgG) and the population characteristics. If the test was done between day 1-7, the sensitivity for a combination of IgG/IgM was 30.1%. It was 72.2% for day 8 to 14 and 91.4% for day 15 to 21. For day 21-35, the sensitivity was 96% but there was inadequate data for tests done beyond day 35.The sensitivity of the LFIA (which is the potential point of care test method) was lowest at 66%. Tests using ELISA had sensitivities of 84.3% and CLIA fared best at 97.8%. Specificities of all tests range from 92-98%.

The performance of the test also depends on the population being tested. For example, in healthcare workers with respiratory symptoms with an expected prevalence of 50%, in 1000 people tested, 43 would be missed and 7 would be falsely positive. In national surveys where one would expect a prevalence of 5%, of every 1000 people tested 4 would be missed and 12 would be falsely positive.

Overall it appears that antibody testing may be useful clinically after 15 days of onset of illness to complement other tests.
(Cochrane Database Syst Rev 2020;BMJ 1 July 2020)

Lessons from the Spanish Seroprevalence Study

A nationwide COVID-19 seroprevalence study of more than 60,000 people conducted in two stages in April and May, 2020 in Spain (ENE-COVID) has revealed valuable information. A history of symptoms was collated and point of care testing for antibodies using LFIA was done, with further testing using a chemiluminescent assay in those consenting.

The country-wide seroprevalence was 5% by point of care testing and 4.6% by chemiluminescent assay. Children had lower levels of seroprevalence with 1.1% in infants and 3.1% between 5-9 years. Seroprevalence in health care workers (10%) was higher than any other occupational group. About 32.7% of people with a positive serology were asymptomatic.

The key message from this well-conducted study was that though Spain incurred a huge burden of mortality in the pandemic, the overall seroprevalence was inadequate to provide herd immunity. This means further epidemic control by allowing natural infections will result in large number of deaths. Social distancing, and identifying and isolating new cases will continue to be important till an effective vaccine is available.
(*Lancet 6 July 2020*)

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Theme: Child Abuse and Neglect

The temporal impact of economic insecurity on child maltreatment: A systematic review *(Trauma Violence Abuse. 2020;21:157-78)*

Poverty and economic insecurity adversely affect child and adolescent health.18% of the global population and 22% of the Indian population fall below the poverty line. The ongoing COVID-19 pandemic has resulted in widespread socioeconomic deprivation. Economic hardships increase family stressors, marital conflicts and parental depression resulting in harsh parenting and child maltreatment.This systematic review of 26 longitudinal studies from Australia, USA, Japan and UK revealed that income losses, food insecurity, housing and bill paying hardships, and maternal depression predict child abuse and neglect. Parental employment buffered these effects. Recommendations are given for research, practice and policy emphasising on conducting robust scientific studies to strengthen correlation between various types of child maltreatment and economic insecurity, and for improving multisystem collaboration, child welfare services, cash incentives and parental employment opportunities to reduce prevalence of child maltreatment in underprivileged families.

Child maltreatment and depression: A meta-analysis of studies using the Childhood Trauma Questionnaire (*Child Abuse Negl. 2020;102:104361)*

Major depressive disorder (MDD) in childhood and adolescence increases morbidity and mortality in this age group and is known to track into adulthood. Early detection and timely management improves prognosis.This random effects meta-analysis is the largest ever study on defining the association of adult depression and childhood maltreatment using a single tool; the Childhood trauma questionnaire. 190 studies that included 68830 individuals were analyzed. Higher child maltreatment scores were associated with depression (g=1.07; 95% CI, 0.95-1.19) and with increased depression symptom score (z=0.35; 95% CI, 0.32-0.38). Although all forms of child abuse were associated with MDD, emotional abuse and emotional neglect were found to be more strongly associated compared to physical abuse, sexual abuse and physical neglect.Longitudinal studies are required to establish a causal relationship.

Clinicians should screen all cases of MDD for childhood maltreatment and strategize management modalities accordingly. Pediatricians should educate regarding positive parenting skills during well child visits.

Childhood maltreatment and its mental health consequences among Indian adolescents with a history of child work (*Aust N Z J Psychiatry. 2020;54:496-508)*

There are 11.72 million child workers in India as per Census, 2011. Socioeconomic deprivation, loss of educational opportunities, risky working environment and abuse results in poor health. This cross-sectional survey conducted on 132 working Indian adolescents aged 12 to 18 years estimated 83.3% prevalence of mental disorders in the study population. All reported at least one form of victimization and exposure to criminal activity. More than 80% reported one or more types of abuse or neglect (physical abuse 72.73%, emotional abuse 47.7%, general neglect 17.4%); 45.5% lived in unsafe homes. Emotional abuse was strongly associated with mental disorders. Juvenile victimization questionnaire was used to assess child abuse and the culturally adapted Hindi versions of the Youth's inventory–4R and the Strengths and difficulties questionnaire were used to diagnose mental disorders and emotional and behavioral problems.

Health professionals should screen for mental health issues in all working adolescents. Timely management of mental disorders would ensure emotional well-being over the entire life span.

Improving measurement of child abuse and neglect: A systematic review and analysis of national prevalence studies (*PLoS One. 2020;15:e0227884)*

United Nations sustainable development goal (SDG) 16.2 aims at ending abuse, violence, trafficking and exploitation of children. Hence, it is important for all nations to collect reliable data to monitor their progress towards reaching this SDG. This systematic review analyzed 30 national prevalence studies conducted in 22 countries. Though the studies provided useful prevalence data, the limitations noted included failure to assess all the five types of abuse, use of a validated and reliable instrument to collect data, inclusion of both children and adults and lack of longitudinal follow up regarding nature, severity and frequency of abuse. The authors have given recommendations and suggestions for planning and investing in robust scientific studies to precisely measure the prevalence of child abuse and neglect.

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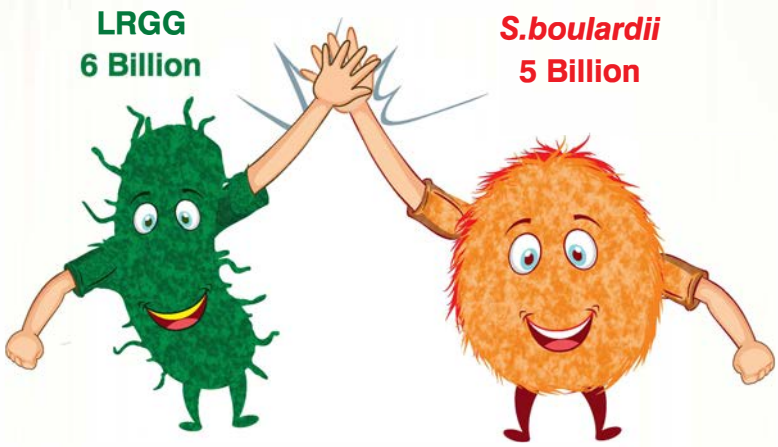

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