ISSN 2219-2808 (online)

World Journal of *Clinical Pediatrics*

World J Clin Pediatr 2022 May 9; 11(3): 215-320

Published by Baishideng Publishing Group Inc

WJCP

World Journal of Clinical Pediatrics

Contents

Bimonthly Volume 11 Number 3 May 9, 2022

World Journal of Clinical Pediatrics

Bimonthly Volume 11 Number 3 May 9, 2022

Contents

ABOUT COVER

Editorial Board Member of *World Journal of Clinical Pediatrics*, Judy Matta, Doctor, MD, Assistant Professor, Doctor, Department of Pediatrics, Saint George Hospital University Medical Center Beirut, Achrafieh 1100 2807, Lebanon. judymatta@gmail.com

AIMS AND SCOPE

The primary aim of the *World Journal of Clinical Pediatrics* (*WJCP*, *World J Clin Pediatr*) is to provide scholars and readers from various fields of pediatrics with a platform to publish high-quality clinical research articles and communicate their research findings online.

WJCP mainly publishes articles reporting research results and findings obtained in the field of pediatrics and covering a wide range of topics including anesthesiology, cardiology, endocrinology, gastroenterology, hematology, immunology, infections and infectious diseases, medical imaging, neonatology, nephrology, neurosurgery, nursing medicine, perinatology, pharmacology, respiratory medicine, and urology.

INDEXING/ABSTRACTING

The *WJCP* is now abstracted and indexed in PubMed, PubMed Central, Scopus, Reference Citation Analysis, China National Knowledge Infrastructure, China Science and Technology Journal Database, and Superstar Journals Database.

RESPONSIBLE EDITORS FOR THIS ISSUE

Production Editor: *Wen-Wen Qi*; **Production Department Director:** *Xu Guo*; **Editorial Office Director:** *Yu-Jie Ma.*

NAME OF JOURNAL
World Journal of Clinical Pediatrics

ISSN
ISSN 2219-2808 (online)

LAUNCH DATE
June 8, 2012

FREQUENCY
Bimonthly

EDITORS-IN-CHIEF
Toru Watanabe, Elena Daniela Serban, Surjit Singh, Consolato M Sergi

EDITORIAL BOARD MEMBERS
https://www.wjgnet.com/2219-2808/editorialboard.htm

PUBLICATION DATE
May 9, 2022

INSTRUCTIONS TO AUTHORS
https://www.wjgnet.com/bpg/gerinfo/204

GUIDELINES FOR ETHICS DOCUMENTS
https://www.wjgnet.com/bpg/GerInfo/287

GUIDELINES FOR NON-NATIVE SPEAKERS OF ENGLISH
https://www.wjgnet.com/bpg/gerinfo/240

PUBLICATION ETHICS
https://www.wjgnet.com/bpg/GerInfo/288

PUBLICATION MISCONDUCT
https://www.wjgnet.com/bpg/gerinfo/208

ARTICLE PROCESSING CHARGE
https://www.wjgnet.com/bpg/gerinfo/242

STEPS FOR SUBMITTING MANUSCRIPTS
https://www.wjgnet.com/bpg/GerInfo/239

ONLINE SUBMISSION
https://www.f6publishing.com

World Journal of Clinical Pediatrics

Submit a Manuscript: https://www.f6publishing.com

World J Clin Pediatr 2022 May 9; 11(3): 215-220

DOI: 10.5409/wjcp.v11.i3.215

ISSN 2219-2808 (online)

GUIDELINES

Preferred practice guidelines for retinopathy of prematurity screening during the COVID-19 pandemic

Anand Vinekar, Rajvardhan Azad, Mangat Ram Dogra, Subhadra Jalali, Pramod Bhende, Parijat Chandra, Narendran Venkatapathy, Sucheta Kulkarni

Specialty type: Pediatrics

Provenance and peer review: Invited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): 0
Grade B (Very good): 0
Grade C (Good): C
Grade D (Fair): 0
Grade E (Poor): 0

P-Reviewer: Lee SJ, South Korea

Received: March 1, 2021
Peer-review started: March 1, 2021
First decision: July 30, 2021
Revised: August 17, 2021
Accepted: March 16, 2022
Article in press: March 16, 2022
Published online: May 9, 2022

Anand Vinekar, Pediatric Retina, Narayana Nethralaya Eye Institute, Bangalore 560010, India

Rajvardhan Azad, Department of Ophthalmology, Regional Institute of Ophthalmology, IGIMS, Patna 800014, India

Mangat Ram Dogra, Department of Ophthalmology, Grewal Eye Institute, Chandigarh 160009, India

Subhadra Jalali, Department of Retina, LV Prasad Eye Institute, Hyderabad 500034, India

Pramod Bhende, Department of Retina, Sankara Nethralaya, Chennai 600006, India

Parijat Chandra, Department of Ophthalmology, All India Institute of Medical Sciences, New Delhi 110023, India

Narendran Venkatapathy, Department of Retina, Aravind Eye Hospital, Coimbatore 641014, India

Sucheta Kulkarni, Department of Ophthalmology, H.V.Desai Eye Hospital, Pune 411028, India

Corresponding author: Anand Vinekar, FRCS, MD, PhD, Attending Doctor, Director, Professor, Pediatric Retina, Narayana Nethralaya Eye Institute, 121/C, 1st R Block, Rajajinagar, Bangalore 560010, India. anandvinekar@yahoo.com

Abstract

Retinopathy of prematurity (ROP) is the leading cause of preventable infant blindness in the world and predominantly affects babies who are born low birth weight and premature. India has the largest number of surviving preterm births born annually. ROP blindness can be largely prevented if there is a robust screening program which detects treatment requiring disease in time. ROP treatment must be provided within 48 h of reaching this threshold of treatment making it a relative emergency. During the severe acute respiratory syndrome-coronavirus disease 2019 pandemic in 2020 ROP screening was disrupted throughout the world due to lockdowns and restriction of movement of these infants, their families, specialists and healthcare workers. The Indian ROP Society issued guidelines for ROP screening and treatment in March 2020, which was aimed at preserving the chain-of-care despite the potential limitations and hazards during the (ongoing) pandemic. This preferred practice guideline is

summarized in this manuscript.

Key Words: Retinopathy of prematurity; Screening; Preferred practice; COVID-19; Pandemic; Indian retinopathy of prematurity society

Core Tip: Retinopathy of prematurity (ROP) is a relative emergency in ophthalmology because if it's screening and treatment is delayed it can result in permanent vision impairment or even blindness in at risk infants. During the coronavirus disease 2019 pandemic, the Indian ROP society formulated these preferred practice guidelines with the aim of reducing this risk.

Citation: Vinekar A, Azad R, Dogra MR, Jalali S, Bhende P, Chandra P, Venkatapathy N, Kulkarni S. Preferred practice guidelines for retinopathy of prematurity screening during the COVID-19 pandemic. *World J Clin Pediatr* 2022; 11(3): 215-220
URL: https://www.wjgnet.com/2219-2808/full/v11/i3/215.htm
DOI: https://dx.doi.org/10.5409/wjcp.v11.i3.215

INTRODUCTION

The severe acute respiratory syndrome-coronavirus disease 2019 (COVID-19) pandemic that started in the last quarter of 2019 in China and spread thereafter reached epic proportions globally by early 2020 and is still ongoing in most regions of the world. This pandemic has become the greatest public health calamity in over a century or more. Lives and livelihood were lost globally and sadly the true quantum of loss and the impact on the future health and well-being of the human race is yet to be fully determined.

At the time of this submission, India had the second largest number of cases in the world. Like most parts of the world, the Government of India (GoI) mandated the first lockdown of all non-essential services between March 25, 2020 to April 14, 2020, which was followed by a series of continual lockdown periods with differing restrictions.

Like all other healthcare specialties, ophthalmology was impacted tremendously. While routine daycare surgeries were suspended initially only emergency services were offered. Since ophthalmology is a stand-alone specialty with very few life-threatening or relatively fewer eye emergencies, most ophthalmology set-ups were shut down. Aiming to strike a professional and ethical balance between controlling the spread of the virus and providing services for ophthalmic emergencies, the All India Ophthalmology Society (AIOS) developed.

A preferred practice pattern (PPP) based on consensus discussion between some of the leading ophthalmologists in India, major institutional representatives, and the AIOS leadership[1]. The PPP was initially for the specialty as a whole and was applicable to all practice settings including tertiary institutions, corporate and group practices, and individual eye clinics. Subsequently, sub-groups developed practice guidelines for eye-banking, glaucoma, vitreo-retina[2], and pediatric ophthalmology.

Retinopathy of prematurity (ROP), a bilateral vasoproliferative disease affecting the retinae of premature infants within a few weeks of birth is a relative Ophthalmic emergency. As this affects the vulnerable cohort of infants during their critical admission in the neonatal intensive care unit (NICU), ROP screening and often treatment requires to be made available in the NICU itself by the ROP team. Even in pre COVID times, ROP screening in India is not universal, with an acute shortage of trained specialists to carry out the screening[3,4]. The COVID lockdown restrictions of admissions into these NICUs, cessation of public transport, the shutdown of routine outpatient services added to the woes of ROP screening and treatment in an already fragile situation.

On March 24, 2020 a day prior to the national lockdown, the Indian ROP (IROP) society, a professional body of ROP specialists published the preferred guidelines for ROP screening on their website [5], and subsequently a summary was published in the vitreo-retinal diseases preferred practice guidelines[2]. These guidelines were circulated to all its members and subsequently were used in other countries with similar demographic profiles in the South and South-East Asian regions.

GUIDELINE METHOD

The Executive Founder members of the IROP contributed to the formation of the guidelines[3]. The

paper was then compiled and reviewed by the entire committee. In case there was any difference of opinion, a mutual consensus was reached by discussion amongst all the experts. The final version of the document was approved by all the authors.

Disclaimer by the authors

These guidelines are not sacrosanct and may be customized and modified depending on the regional situation in a particular district, state, or country. These guidelines are also not permanent and may be updated periodically depending on the prevailing condition, existing regulations and national and international scenario. These guidelines are not to be regarded as medico-legal advice.

The guidelines are summarized below and pertain to screening and treatment of ROP.

ROP screening criteria: (1) Who? This remains unchanged from the existing National ROP Operational Guidelines (2018)[6]. Eligible babies include: Those born ≤ 2000 g grams at birth/those born ≤ 34 wk of gestation; and outside the criteria if requested by the treating neonatologist; (2) When? We must strive to complete the first screening before the baby is 30 days old. If possible high-risk babies (< 1200 g and < 30 wk) may be screened earlier between 2-3 wk of life; (3) Where? In the NICU if admitted. In the NICU or Ophthalmic Office if discharged; and (4) How Often? With the aim of reducing the number of screening visits and restricting them to have the highest yield of detection of vision threatening ROP, the following modification to the screening schedule is suggested: Screening for ROP was initially restricted to the compliance of the below GoI guidelines. At the time of submission of this manuscript, these no longer are mandatory, but are included here for historical importance (Table 1).

CURRENT PREFERRED GUIDELINES-COMPLIANT WITH EXISTING RULES ON SOCIAL DISTANCING

Mother's with their infants waiting for screening must maintain social distance while undergoing dilatation, screening or counseling.

Mother must place the infant on a designated cot with a plastic/polythene sheet/large newspaper, uncovers the face of the infant and step away more than 6 feet. The screener walks to the baby and screens (using indirect ophthalmoscopy or a retinal camera).

Do not screen if the baby has conjunctivitis. ROP screening can be deferred until the infection is settled.

The assistant or nurse (wearing face mask) may handle the head only if needed during the screening.

After screening, screener must step back more than 6 feet. The mother then comes forward and picks up the baby and the ROP card with the findings documented.

The newspaper if used must be disposed in a yellow bin. The plastic/polythene sheet must be replaced or sanitized with an alcohol based sanitizer with a composition of, or similar to, a solution of liquid mixture of 1-Propanol and 2-Propanol (*e.g*. Sterillium or Bacillocid) before the next baby is screened.

Counseling the parents/other NICU staff must be done at a distance of 6 feet or more.

The designated cot must be sanitized using the above mentioned sanitizer. Other surfaces that may have been touched/handled by the physician/team/parent/must also be sanitized before the procedure is repeated for the next baby.

If an infant speculum is used during screening it should not be repeated unless sterilized before being re-used.

Eye drops used for dilating must be used carefully without touching the eye or eyelid and must be discarded at the end of the day or session.

The lens used for screening (20D or 28D) must be washed with water and soap and the rim should be cleaned with alcohol swabs. When a wide-field ROP camera is used, the lens tip should be cleaned with disposable alcohol swabs between each case.

Wherever possible, Personal Prophylaxis Equipment prescribed by the Indian Council of Medical Research must be used. The minimum protection that must be used by all members of the screening team are: Facial mask (preferably N95 grade), Head Cap, Eye protective glasses, Sterile gloves. However, these guidelines are constantly changing and the most updated recommendations must be followed.

Between each patient, hands must be washed and an alcohol based sanitizer as mentioned above must be used and allowed to dry before handling the patient.

The vehicle used for transporting the screening equipment are required to be sanitized every day before and after the screening sessions.

Tele-medicine must be encouraged. Tele-medicine platforms have been shown to be useful even in pre-COVID times[5,7].

To reduce the number of screening sessions, attempt must be made to pool infants of one district(s), region or center to maximize the efforts of the screening team.

Table 1 Mandatory questions that were required at the start of the pandemic in 2020

No.	Before screening, as the following 4 questions: (as *per* Govt guidelines in 2020)
1	International travel in last 4 wk
2	In quarantine period? (See stamp on hand or arm)
3	In isolation as some in family was COVID-19 positive or had contact with COVID positive patient
4	Fever, cough, cold

If yes to any of these 4, the parent/guardian must not enter the hospital and screening will not be performed. These are applicable to the physician, care giver, screening team and hospital staff as well fever is also checked at entry point with a non-contact thermometer (false negative if anti pyretic is taken).

Table 2 Suggested follow-up schedule for retinopathy of prematurity during the coronavirus disease 2019 pandemic

Finding in either eye with respect to zone	Next follow up	Comment
Immature retina in zone 3 and zone 2 anterior	3-4 wk or more	If the PMA is less than 34 wk/< 1500 grams/sick and admitted infant, consider a closer follow-up
Zone 3 and Zone 2 anterior disease	3-4 wk	Spontaneously regressing ROP can be watched
Zone 2 Posterior disease	2 wk	Unless associated with treatment requiring features (see below)
Zone 1 disease	1 wk or treat	Have a low threshold for treatment
Pre-plus	Consider early treatment or early follow-up if pupil does not dilate well and media is not clear	Individualize for each case based on the tempo of disease and PMA
Pre-plus	With good pupillary dilatation and clear media and other low risk features	Can delay the next screening by an additional 1 wk from the current guidelines

PMA: Post menstrual age; ROP: Retinopathy of prematurity.

Outreach specialists must be implored to take on a larger role to perform screening in centers that are in their proximity. Image based documentation and additional opinion from senior specialists must be encouraged.

Follow-up during ROP screening

Follow up visits are an integral part of ROP screening. On the average each infant requires 3-5 screening visits before the retinae are mature or the baby requires treatment. During the pandemic, the attempt was to reduce the number of follow-up visits without jeopardizing the ocular condition. The aim was to ensure that the most critical disease would not be missed and would be picked up at the appropriate time to avoid delayed treatment and is summarized in Table 2.

TREATMENT FOR ROP

The gold standard for ROP treatment is laser photoablation. Anti-Vascular endothelial growth factors injected intravitreally are also used in certain cases. The impact of delayed ROP screening and treatment has been reported from a tertiary care center more recently[8]. The aim of these guidelines was and are to prevent such a situation by optimizing the timing and modality of treatment and is summarized in Table 3.

Post treatment follow-up suggestions

Follow-up after treatment can be done by outreach specialists wherever feasible or by the treating physician's team if the former is not possible. The frequency of subsequent visits can be reduced and must be decided on case-to-case basis. Post intravitreal injection cases can be reviewed SOS/less frequently as normally followed in the initial phase. Recurrences can be addressed during the follow-up after the lock-down phase where possible.

Table 3 Suggested treatment guidelines for retinopathy of prematurity during the coronavirus disease 2019 pandemic

Disease	Comment
Type 1 ROP (ETROP)[9]	Treat as soon as you possible, preferably on the day that screening was done. Laser recommended
AROP[10]	Treat as soon as possible. Laser if disease is amenable. Intravitreal injections can be used, but caution to be exercised since follow-up may be a critical issue with travel restrictions for the family
Less than Type 1 ROP. Stage 2 with pre plus, stage 3 with no or early plus, high risk for APROP (but not yet full fledged), borderline Zone 1 disease/poor pupil dilatation, unclear media with pre-plus	Given the difficulty to closely follow-up consider treatment a 'little earlier' than classical Type 1 ROP
Stage 4A and 4B ROP[10]	Surgery must be performed as soon as treating ROP specialist feels it is required with adequate precautions taken while providing anesthesia
Stage 5 ROP[10]	Surgery is not urgent. Case-to-case based decision must be considered

ROP: Retinopathy of prematurity; AROP: Aggressive retinopathy of prematurity.

CONCLUSION

ROP is considered a relative emergency in Ophthalmology and as ROP specialists we understand our duty and responsibility towards mitigating the risk of blindness in infants who are at risk of this disease.

However, these are not normal times. In this unprecedented time, it is imperative that we also do everything possible to minimize the risk of COVID-19 (Corona Virus) transmission to our patients and our staff while simultaneously engaging in treating and preventing vision loss in our babies.

These guidelines are not designed to be ideal. In a restrictive time that the country is facing due to the *force de majeur* condition that we have encountered, it is important to understand that 'in good faith' and 'to the best of our ability' should be the driving dictum of the ROP care. Our aim should be to reduce and mitigate blindness without risking the lives of our patients and our health care givers.

FOOTNOTES

Author contributions: All authors read and approved the final manuscript.

Conflict-of-interest statement: No conflict-of-interest for any author.

Country/Territory of origin: India

ORCID number: Anand Vinekar 0000-0002-1989-9226; Rajvardhan Azad 0000-0001-6208-9280; Mangat Ram Dogra 0000-0001-9362-6595; Subhadra Jalali 0000-0003-4157-0539; Pramod Bhende 0000-0002-4303-7490; Parijat Chandra 0000-0002-7516-1410; Narendran Venkatapathy 0000-0001-7436-6783; Sucheta Kulkarni 0000-0002-3217-8108.

S-Editor: Fan JR
L-Editor: A
P-Editor: Fan JR

REFERENCES

1 **Sengupta S**, Honavar SG, Sachdev MS, Sharma N, Kumar A, Ram J, Shetty R, Rao GS, Ramasamy K, Khanna R, Jain E, Bhattacharjee K, Agarwal A, Natarajan S, Lahane TP; Writing Committee on behalf of the All India Ophthalmological Society - Indian Journal of Ophthalmology Expert Group for COVID-19 Practice Guidelines; Composition of the All India Ophthalmological Society - Indian Journal of Ophthalmology Expert Group for COVID-19 Practice Guidelines includes the Writing Committee (as listed) and the following members (in alphabetical order by the first name):. All India Ophthalmological Society - Indian Journal of Ophthalmology consensus statement on preferred practices during the

COVID-19 pandemic. *Indian J Ophthalmol* 2020; **68**: 711-724 [PMID: 32317433 DOI: 10.4103/ijo.IJO_871_20]

2 **Gupta V**, Rajendran A, Narayanan R, Chawla S, Kumar A, Palanivelu MS, Muralidhar NS, Jayadev C, Pappuru R, Khatri M, Agarwal M, Aurora A, Bhende P, Bhende M, Bawankule P, Rishi P, Vinekar A, Trehan HS, Biswas J, Agarwal R, Natarajan S, Verma L, Ramasamy K, Giridhar A, Rishi E, Talwar D, Pathangey A, Azad R, Honavar SG. Evolving consensus on managing vitreo-retina and uvea practice in post-COVID-19 pandemic era. *Indian J Ophthalmol* 2020; **68**: 962-973 [PMID: 32461407 DOI: 10.4103/ijo.IJO_1404_20]

3 **Vinekar A**, Azad R, Dogra MR, Narendran V, Jalali S, Bende P. The Indian retinopathy of prematurity society: A baby step towards tackling the retinopathy of prematurity epidemic in India. *Ann Eye Sci* 2017; **2**: 27

4 **Vinekar A**, Dogra M, Azad RV, Gilbert C, Gopal L, Trese M. The changing scenario of retinopathy of prematurity in middle and low income countries: Unique solutions for unique problems. *Indian J Ophthalmol* 2019; **67**: 717-719 [PMID: 31124475 DOI: 10.4103/ijo.IJO_496_19]

5 **Vinekar A**, Azad RV, Dogra MR. For the Indian Retinopathy of Prematurity Society. Retinopathy of Prematurity screening and treatment guidelines during the COVID-19 Lockdown 2020 available online. [cited 10 February 2021]. Available from: https://sites.google.com/view/iropsociety/newsroom?authuser=0

6 **Project operational guidelines**. Prevention of Blindness from Retinopathy of Prematurity in Neonatal Care Units. [cited 10 February 2021]. Available from: https://phfi.org/wp-content/uploads/2019/05/2018-ROP-operational-guidelines.pdf

7 **Vinekar A**, Jayadev C, Bauer N. Need for telemedicine in retinopathy of prematurity in middle-income countries: e-ROP *vs* KIDROP. *JAMA Ophthalmol* 2015; **133**: 360-361 [PMID: 25474398 DOI: 10.1001/jamaophthalmol.2014.4913]

8 **Katoch D**, Singh SR, Kumar P. Impact of the COVID-19 Pandemic on Retinopathy of Prematurity Practice: An Indian Perspective. *Indian Pediatr* 2020; **57**: 979-980 [PMID: 32893832 DOI: 10.1007/s13312-020-2017-1]

9 **Early Treatment For Retinopathy Of Prematurity Cooperative Group**. Revised indications for the treatment of retinopathy of prematurity: results of the early treatment for retinopathy of prematurity randomized trial. *Arch Ophthalmol* 2003; **121**: 1684-1694 [PMID: 14662586 DOI: 10.1001/archopht.121.12.1684]

10 **Chiang MF**, Quinn GE, Fielder AR, Ostmo SR, Paul Chan RV, Berrocal A, Binenbaum G, Blair M, Peter Campbell J, Capone A Jr, Chen Y, Dai S, Ells A, Fleck BW, Good WV, Elizabeth Hartnett M, Holmstrom G, Kusaka S, Kychenthal A, Lepore D, Lorenz B, Martinez-Castellanos MA, Özdek Ş, Ademola-Popoola D, Reynolds JD, Shah PK, Shapiro M, Stahl A, Toth C, Vinekar A, Visser L, Wallace DK, Wu WC, Zhao P, Zin A. International Classification of Retinopathy of Prematurity, Third Edition. *Ophthalmology* 2021; **128**: e51-e68 [PMID: 34247850 DOI: 10.1016/j.ophtha.2021.05.031]

World Journal of Clinical Pediatrics

Submit a Manuscript: https://www.f6publishing.com

World J Clin Pediatr 2022 May 9; 11(3): 221-238

DOI: 10.5409/wjcp.v11.i3.221

ISSN 2219-2808 (online)

REVIEW

Advances in pediatric non-alcoholic fatty liver disease: From genetics to lipidomics

Simona Riccio, Rosa Melone, Caterina Vitulano, Pierfrancesco Guida, Ivan Maddaluno, Stefano Guarino, Pierluigi Marzuillo, Emanuele Miraglia del Giudice, Anna Di Sessa

Specialty type: Pediatrics

Provenance and peer review: Invited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): 0
Grade B (Very good): 0
Grade C (Good): C, C
Grade D (Fair): 0
Grade E (Poor): 0

P-Reviewer: Zhou J, Singapore

Received: June 30, 2021
Peer-review started: June 30, 2021
First decision: July 30, 2021
Revised: August 5, 2021
Accepted: April 2, 2022
Article in press: April 2, 2022
Published online: May 9, 2022

Simona Riccio, Rosa Melone, Caterina Vitulano, Pierfrancesco Guida, Ivan Maddaluno, Stefano Guarino, Pierluigi Marzuillo, Emanuele Miraglia del Giudice, Anna Di Sessa, Department of Woman, Child, General and Specialized Surgery, University of Campania Luigi Vanvitelli, Naples 80138, Italy

Corresponding author: Anna Di Sessa, MD, PhD, Research Fellow, Department of Woman, Child, General and Specialized Surgery, University of Campania Luigi Vanvitelli, Via De Crecchio 2, Naples 80138, Italy. anna.disessa@libero.it

Abstract

As a result of the obesity epidemic, non-alcoholic fatty liver disease (NAFLD) represents a global medical concern in childhood with a closely related increased cardiometabolic risk. Knowledge on NAFLD pathophysiology has been largely expanded over the last decades. Besides the well-known key NAFLD genes (including the I148M variant of the *PNPLA3* gene, the E167K allele of the *TM6SF2*, the *GCKR* gene, the *MBOAT7-TMC4* rs641738 variant, and the rs72613567:TA variant in the *HSD17B13* gene), an intriguing pathogenic role has also been demonstrated for the gut microbiota. More interestingly, evidence has added new factors involved in the "multiple hits" theory. In particular, omics determinants have been highlighted as potential innovative markers for NAFLD diagnosis and treatment. In fact, different branches of omics including metabolomics, lipidomics (in particular sphingolipids and ceramides), transcriptomics (including micro RNAs), epigenomics (such as DNA methylation), proteomics, and glycomics represent the most attractive pathogenic elements in NAFLD development, by providing insightful perspectives in this field. In this perspective, we aimed to provide a comprehensive overview of NAFLD pathophysiology in children, from the oldest pathogenic elements (including genetics) to the newest intriguing perspectives (such as omics branches).

Key Words: Fatty; Liver; Genetics; Lipidomics; Pediatric

Core Tip: A large body of evidence supported a complex non-alcoholic fatty liver disease (NAFLD) physiopathology with several factors involved in this tangled puzzle. Considering the cardiometabolic burden of NAFLD even in childhood, a better knowledge of NAFLD physiopathology is fundamental for novel therapeutic strategies.

Citation: Riccio S, Melone R, Vitulano C, Guida P, Maddaluno I, Guarino S, Marzuillo P, Miraglia del Giudice E, Di Sessa A. Advances in pediatric non-alcoholic fatty liver disease: From genetics to lipidomics. *World J Clin Pediatr* 2022; 11(3): 221-238
URL: https://www.wjgnet.com/2219-2808/full/v11/i3/221.htm
DOI: https://dx.doi.org/10.5409/wjcp.v11.i3.221

INTRODUCTION

Due to the increasing rate in pediatric obesity worldwide, non-alcoholic fatty liver disease (NAFLD) has become the most common chronic liver disease in childhood[1,2]. Current pediatric estimates report a prevalence of 3%-10% in the general population, while a dramatic increase (up to 50%) has been observed in children and adolescents with obesity[2]. Owing to its strong relationship with the metabolic syndrome (MetS) and insulin resistance (IR), both metabolic and cardiovascular risks are increased in children with NAFLD[2-4].

Hepatic fat accumulation represents the hallmark of the disease, that includes a wide spectrum of progressive forms ranging from simple steatosis through non-alcoholic steatohepatitis (NASH) to fibrosis and cirrhosis[5]. Lipolysis of adipose tissue and *de novo* hepatic lipogenesis are the main biological pathogenic processes contributing to fatty liver and IR[3,6]. Taken together, they result in an increased flux of free fatty acids to the liver and skeletal muscle that might activate lipotoxic pathways responsible for more progressive forms of hepatocellular injury. Interestingly, recent studies have highlighted not only the role of lipotoxicity but also of fatty acid composition as central players in NAFLD[7-9].

Pathophysiological hypotheses of NAFLD have been resumed in the "multiple hits" theory, by assuming the role of genetics, microbial, metabolic, and environmental factors through a complex interplay[1,2,10-12].

Key genetic factors for NAFLD are represented by the I148M variant of the *PNPLA3* gene[13], the E167K allele of the *TM6SF2*[14,15], the *MBOAT7-TMC4* rs641738 variant[16], and the rs72613567:TA variant in the *HSD17B13* gene[17] (Table 1).

Recently, advances in the understanding of NAFLD pathogenesis have reported the role of specific lipid classes (in particular sphingolipids and ceramides) and their correlation also with IR, by underscoring the strength of the tangled link between NAFLD and IR[9,18-21].

For this reason, we aimed to provide a comprehensive overview from the oldest to the newest pathophysiological evidence on pediatric NAFLD.

NAFLD PATHOGENESIS: THE "MULTIPLE HITS" THEORY

One of the most recurrent questions regarding NAFLD concerns the potential progression to more severe forms in certain subjects. This seems to be relevant as hepatic inflammation or fibrosis determine the long-term prognosis of the disease, while simple steatosis does not seem to worsen the outcome[22,23], although some studies would seem to weaken this assumption[24,25].

In an attempt to explain NAFLD pathogenesis, Day *et al*[26] first proposed the ''two hit'' model theory, suggesting that after a first hit (*i.e.*, hepatic steatosis), another hit (*e.g.*, gut-derived endotoxin) contributed to NASH development. Later, a more complex model called the "multiple parallel hits model"[23] in which multiple factors (including genetics, obesity, insulin resistance, metabolic and environmental determinants) act together to induce NAFLD development and progression in genetically predisposed or high-risk individuals was proposed. In particular, increased lipid storage, lipogenesis, and adipokine synthesis in adipose and liver tissue, may act as stress signals for the endoplasmic reticulum (ER) with subsequent hepatocellular damage[27]. In addition, certain genes (such as *PNPLA3, TM6SF2, GCKR, MBOAT7*, and *HSD17B13*) have been strongly related to NAFLD susceptibility.

Genetics

PNPLA3: The *PNPLA3* gene, discovered by Hobbs and colleagues in 2008, has been largely accepted as the most important genetic determinant in NAFLD development. *PNPLA3* is located on chromosome 22

Table 1 Main genes and changes in methylation found in human epigenomics studies in non-alcoholic fatty liver disease

Gene	Changes	Methods	Ref.
FGFR2	Hypomethylation	Bisulfite pyrosequencing and liver biopsy	Zhang *et al*[112]
MAT1A	Hypomethylation	Bisulfite pyrosequencing and liver biopsy	Zhang *et al*[112]
CASP1	Hypomethylation	Bisulfite pyrosequencing and liver biopsy	Zhang *et al*[108]
MTND6	Hypomethylation	Methylation-specific PCR and liver biopsy	Pirola *et al*[109]
PARVB	Hypomethylation	Targeted-bisulfite sequencing and liver biopsy	Kitamoto *et al*[111]
PNPLA3	Hypomethylation	Targeted-bisulfite sequencing and liver biopsy	Kitamoto *et al*[111]
PPARα	Hypomethylation	Bisulfite pyrosequencing and liver biopsy	Zeybel *et al*[112]
TGFβ1	Hypomethylation	Bisulfite pyrosequencing and liver biopsy	Zeybel *et al*[112]
Collagen 1A1	Hypomethylation	Bisulfite pyrosequencing and liver biopsy	Zeybel *et al*[112]
PDGFα	Hypomethylation	Bisulfite pyrosequencing and liver biopsy	Zeybel *et al*[112]
PPARGC1A	Hypomethylation	Methylation-specific PCR and liver biopsy	Sookoian *et al*[113]
cg08309687 (LINC00649)	Hypomethylation	Illumina BeadChip for array analyses	Ma *et al*[114]
NPC1L1	Methylation	Illumina human methylation 450 Beadchip and liver biopsy	Mwinyi *et al*[116]
STARD	Methylation	Illumina human methylation 450 Beadchip and liver biopsy	Mwinyi *et al*[116]
GRHL	Methylation	Illumina human methylation 450 Beadchip and liver biopsy	Mwinyi *et al*[116]

PCR: Polymerase chain reaction.

and belongs to the patatin-like phospholipase family. Its expression seems to be influenced by several factors, including diet, obesity, insulin and glucose levels, and gene mutation[28]. *PNPLA3* encodes for a protein called adiponutrin, an enzyme found in liver and adipose tissue that appears to confer susceptibility to increased liver fat levels and liver inflammation[29]. The discovery of *PNPLA3* has brought new insights into the understanding of fatty liver, specifically lipid remodeling in intracellular droplets has been identified as a common mechanism underlying disease progression independent of environmental triggers. In particular, *PNPLA3* is involved in the remodeling of triglycerides, phospholipids, and retinyl ester release, acting as a lipase on lipid droplets[30]. Adiponutrin is an enzyme with retinyl-palmitate lipase function that, in response to insulin, has been shown to be responsible for the release of retinol from lipid droplets in hepatic stellate cells *in vitro* and *ex vivo*[31]. It is induced by diet and IR[32] and exhibits lipolytic activity on triglycerides[33].

Several studies have investigated the major pathogenic role of the *PNPLA3* rs738409 (*PNPLA3* I148M) single nucleotide polymorphism (SNP) in NAFLD development. It is a non-synonymous variant in which there is a cytosine to guanosine change leading to an amino acid substitution of isoleucine to methionine at amino acid position 148 of the coding sequence, in the active site of the enzyme (I148M). This amino acid substitution affects the function of the enzyme (loss of-function), leading to intrahepatic triglyceride accumulation and consequent development of microvesicular steatosis. On the other hand, adiponutrin might exhibit a gain of lipogenic function, which could further lead to hepatic fatty acid accumulation[34]. The I148M variant, due to the altered enzymatic activity, determines an altered lipid remodeling, with accumulation of polyunsaturated fatty acids in diacylglycerol and triglycerides, and a parallel depletion in phospholipids[30]. Several studies have reported that the *PNPLA3* SNP resulted in decreased retinol metabolism and decreased hepatic protein levels of retinol dehydrogenase 16, which correlate with fibrosis severity[31].

There is strong evidence in the literature for an association between the *PNPLA3* 148M allele and NAFLD in both adults and children. In 2008, Romeo *et al*[29] first reported the association between the *PNPLA3* gene polymorphism (rs738409C/G) and NAFLD in a multiethnic cohort of Hispanic, African American, and European American adults.

Similarly, a large body of evidence supported the role of this gene in NAFLD development in children. Santoro *et al*[35], in a multiethnic group of 85 obese youths with magnetic resonance imaging (MRI)-detected steatosis, demonstrated that the prevalence of the G allele was higher in subjects with hepatic steatosis. Another study investigating 1048 obese Italian children, reported that children carrying the 148M allele showed higher aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels, in particular homozygous 148M carriers with a high level of abdominal fat (expressed as Waist/Height ratio greater than 0.62) had a higher odds ratio (OR) for developing pathological ALT. Thus, it was observed for the first time that the extent of *PNPLA3* association with liver enzymes was determined by the amount of abdominal fat[36].

Romeo *et al*[37], in a 2010 study of 475 obese/overweight children and adolescents with steatosis evaluated by liver ultrasound, reported that the I148M variant of the *PNPLA3* gene was associated with increased ALT/AST levels in obese children and adolescents, suggesting that it conferred a genetic susceptibility to liver damage at an early age.

In addition, it has been demonstrated that the frequency of the *PNPLA3* risk allele rs738409 was lower in African Americans, by suggesting some protection from hepatic steatosis in obese African American youths[38]. In a 2018 study, Hudert *et al*[39] in a cohort of Berlin adolescents aged 10-17 years with NAFLD observed that the *PNPLA3* rs 73844078G variant was significantly associated with the severity of steatosis, with an increased risk of progression to fibrosis.

The association between *PNPLA3* gene and the other major genetic variants of NAFLD was also evaluated. Viitasalo *et al*[40] demonstrated higher serum ALT levels in children carrying the risk alleles for the polymorphisms *PNPLA3, MBOAT7* and *TM6SF2*. Grandone *et al*[15] reported that homozygous subjects for the PNPLA3 148M allele carrying the rare variant of TM6SF2 showed an OR of 12.2 (confidence interval 3.8-39.6, $P = 0.000001$) to have hypertransaminasemia compared with the remaining patients. Of interest, an Italian pediatric study also confirmed the combined effect of the 3 major risk variants (*PNPLA3, TM6F2* and *MBOAT7*) on NAFLD risk[16].

Besides, the interaction of the *PNPLA3* 148M allele with environmental risk factors for NAFLD such as obesity, nutrients (including carbohydrate and polyunsaturated fatty acids), physical activity, and sedentary behaviors have been demonstrated in children with NAFLD[41-45]. Dai *et al*[28], in a meta-analysis, reported a strong influence of the *PNPLA3* rs738409 polymorphism not only on fatty liver but also on histological damage.

More recently, compelling evidence has also supported an intriguing role of this gene in reducing the estimated glomerular filtration rate independently of common renal and metabolic factors both in adults and children[46-49]. This gene seems to promote both fibrogenesis and glomerulosclerosis through the activation of renal pericytes in which the 148M allele is highly expressed[47,48].

Considering its detrimental effect on renal function in childhood[46-48], these findings demonstrated that the *PNPLA3* gene acts not only as one of the major genetic player in NAFLD development but also as a harmful factor beyond the liver[46-48].

***GCKR*:** Several studies reported that variations at the *GCKR* gene locus are associated with NAFLD and appear to influence hepatic fat accumulation. The GCKR protein has an inhibitory action on the activity of the enzyme glucokinase that regulates the hepatic storage and disposal of glucose. In particular, *GCKR* forms an inactive complex with the enzyme glucokinase and transports it from the cytoplasm to the nucleus, thus controlling both activity and intracellular localization of this key enzyme of glucose metabolism[49].

Fructose-6-phosphate (F6P) enhances GCKR-mediated inhibition. By controlling glucose influx into hepatocytes, *GCKR* regulates *de novo* lipogenesis. The mechanism responsible for liver injury is probably due to the lack of inhibition of glucokinase enzymatic activity by F6P and consequently uncontrolled lipogenesis[50].

GCKR gene polymorphisms (rs780094 and rs1260326) have been identified that appear to be important in the pathogenesis of NAFLD. In particular, Beer *et al*[51] and Valenti *et al*[52] reported that in the association with NAFLD and consequently in the accumulation of hepatic fat, the common missense loss-of-function *GCKR* mutation (rs1260326 C>T) encoding for the P446L protein variant plays an important pathogenic role. The P446L variant blocks the inhibitory activity of GCKR on the enzyme glucokinase, resulting in a steady increase in hepatic glucokinase and glucose uptake by the liver. Hepatic glycolysis associated with the minor allele P446L results in lower levels of both glucose and insulin, but leads to increased levels of malonyl-CoA which in turn blocks fatty acid oxidation through inhibition of carnitine-palmytoyltransferase-1 and acts as a substrate for lipogenesis, thus promoting hepatic fat accumulation[53]. The *GCKR* rs780094 C>T variant has been found to be associated with increased intrahepatic fat accumulation and progressive forms of NAFLD[54,55].

A pediatric study involving 70 obese adolescents demonstrated that the *GCKR* rs780094 C>T variant was associated with NAFLD and decreased levels of *GCKR* protein, while the *GCKR* rs780094C>T and rs1260326C>T variants were associated with fibrosis and decreased levels of GCKR protein[39]. Lin *et al*[56], in a study examining 797 obese Taiwanese children, reported that the *GCKR* rs780094T variant was associated with an increased risk of NAFLD, by further demonstrating that the *GCKR* and *PNPLA3* variants were common NAFLD risk genetic factors in obese individuals. In fact, several studies have also reported a combined effect of the *PNPLA3* and *GCKR* SNPs as NAFLD risk polymorphisms. In particular, Santoro *et al*[57] in a study of 455 obese children and adolescents reported that the *GCKR* rs1260326 variant was associated with hepatic fat accumulation along with large levels of very-low-density lipoprotein (VLDL) and triglycerides, further demonstrating that *GCKR* and *PNPLA3* synergistically act to convey susceptibility to fatty liver in obese youths.

More recent studies confirmed the strong association of the three major genetic variants such as *TM6SF2* rs58542926, *PNPLA3*rs738409, and *GCKR* rs1260326 with NAFLD in obese children and adolescents[58].

***TM6SF2*:** *TM6SF2* is responsible for the regulation of lipid metabolism in the liver[59]. In particular,

TM6SF2 gene contributes to the secretion of VLDL from the liver[60]. As suggested by recent evidence [61], *TM6SF2* is a polytopic membrane protein acting as a lipid transporter. It is predominantly expressed in the liver, small intestine, and kidney. *TM6SF2* encodes a 351 amino acid protein with 7-10 predicted transmembrane domains[60]. Sliz *et al*[62] reported an association of the *TM6SF2* rs58542926-T allele with lower-risk lipoprotein lipid profile and lower levels of glycerol and glycoprotein acetylation. Specifically, the authors reported that the *TM6SF2* variant was associated with lower concentrations of all lipoprotein particle subclasses [including VLDL and low-density lipoprotein (LDL)]. In addition, there was an inverse association between this variant and total serum triglycerides and triglycerides in all lipoprotein subclasses, including high-density lipoprotein (HDL) subclasses. Finally, the *TM6SF2* rs58542926-T allele did not appear to affect apolipoprotein A-I concentration, whereas it was associated with lower apolipoprotein B concentration. Furthermore, it was also found to impair the secretory pathway leading to hepatic lipid accumulation and reduced levels of circulating lipids and lipoproteins.

In the last few years, a single nucleotide rs58542926 C>T polymorphism giving rise to the E167K *TM6SF2* variant was noted in the complex puzzle of NAFLD pathophysiology[34]. It was associated with increased liver fat content, NASH, advanced liver fibrosis, and cirrhosis[63]. This variant is characterized by an adenine-guanine substitution in nucleotide 499 that replaces glutamate at residue 167 with lysine (c.499A > G; p.Glu167Lys) leading to a loss of function in hepatic secretion of VLDL[61].

Another study on two large histologically characterized adult cohorts (including steatosis, steatohepatitis, fibrosis and cirrhosis) reported an association of the *TM6SF2* gene with advanced liver fibrosis, regardless of the *PNPLA3* genotype presence[64]. This association was also independently validated in another large European cohort[65].

Thus, *TM6SF2* might be considered as a regulator of liver fat metabolism with the opposite effects on triglyceride-rich lipoprotein secretion and hepatic lipid droplet content[34].

Chen *et al*[59] in a recent meta-analysis, on associations of TM6SF2 polymorphisms with chronic liver disease, suggested that rs58542926 polymorphism may be significantly associated with chronic liver disease in both Asians and Caucasians. In addition, Holmen *et al*[66] showed in a longitudinal adult Norwegian study an association of the E167K TM6SF2 variant with lower total cholesterol levels resulting in a reduced risk of myocardial infarction. Accordingly, Dongiovanni *et al*[65] showed an effect of this polymorphism on reducing the risk of carotid atherosclerosis in adults.

The effect of this polymorphism on ALT and cholesterol levels has also been confirmed in children and adolescents. Grandone *et al*[15] demonstrated in a cohort of 1010 obese Caucasian children and adolescents that the TM6SF2 167K allele in carriers was associated with hepatic steatosis, higher ALT levels and lower total cholesterol, LDL-cholesterol, triglycerides and non-high density lipoproteins. In addition, subjects homozygous for the *PNPLA3* 148M allele carrying the rare variant of *TM6SF2* showed an OR of 12.2 for presenting hypertransaminasemia compared with the remaining patients. Thus, the effect of *PNPLA3* and *TM6SF2* alleles appeared to be additive in determining pediatric NAFLD. As previously demonstrated in adults, the authors found that the TMS6SF2 E167K variant predisposed to NAFLD in obese children, with a relevant beneficial effect on cardiovascular risk[15].

It is noteworthy that recent data also showed a protective effect of the *TM6SF2* gene on renal function both in adults and children through the reduction of lipotoxicity[47,67].

In conclusion, the discovery of the E167K variant adds another piece not only in the complex pathophysiology of NAFLD but also in the larger context of NAFLD-related cardiometabolic risk.

***MBOAT7*:** The pathogenic role of this gene in NAFLD susceptibility has been largely studied both in adults and children. Findings demonstrated its effect in increasing not only the risk (and the severity) of NAFLD but also of other chronic liver diseases (*e.g.* hepatitis B and C virus-related). *MBOAT7* encodes lysophosphatidylinositol acyltransferase, involved in the inflammation cascade through the regulation of arachidonic acid levels and leukotriene synthesis in neutrophils. A combined effect of this gene with the major NAFLD risk polymorphisms (such as *PNPLA3* and *TM6SF2*) has also been highlighted in adult and pediatric studies[16]. Similar to renal effects observed for *PNPLA3* and *TM6SF2*, a role for this gene in kidney dysfunction has also been demonstrated[47].

***HSD17B13*:** The 17β-hydroxysteroid dehydrogenases (HSD17Bs) encompasses a large family of 15 members involved in various metabolic processes such as the metabolism of steroid hormones, cholesterol, fatty acids, and bile acids[68]. In 2008, Horiguchi identified *HSD17B13* as a novel lipid droplet (LD) associated protein. The human *HSD17B13* gene is located on chromosome 4 (4q22.1) and its expression is highly restricted to the liver, particularly in hepatocytes[69]. The human *HSD17B13* gene encodes a 300 amino acid protein, hydroxyl-steroid 17-beta dehydrogenase 13, a liver-specific LD-associated protein which is localized to lipid droplets[70].

To date, the physiological function of *HSD17B13* remains largely unclear. *HSD17B13* appears to have a role in estradiol metabolism and enzymatic activity against bioactive lipid mediators, such as leukotriene B4, that are involved in lipid-mediated inflammation[71].

In a 2019 study, Ma *et al*[72] reported that HSD17B13 exerts retinol dehydrogenase activity *in vitro*, which is closely linked to lipid droplets. Indeed, it was observed that *HSD17B13* catalyzes the oxidation of retinol to retinaldehyde, the rate-limiting step in all-trans retinoic acid biosynthesis.

The fact that *HSD17B13* is highly abundant in the liver and selectively expressed on the lipid droplet surface suggests a potential critical effect in lipid droplet function, as supported by growing data demonstrating the key role of the *HSD17B13* gene in hepatic lipid homeostasis and NAFLD pathogenesis[73].

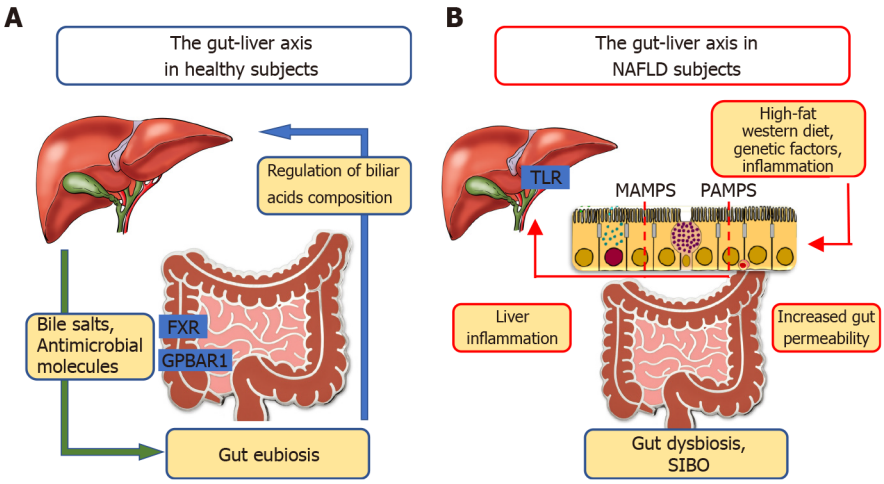
In contrast, inactivating variants in the *HSD17B13* gene have recently been linked with a reduced risk of chronic liver disease in several studies[63]. In 2018, Abul-Husn *et al*[71] reported that a loss-of-function variation in the *HSD17B13* (rs72613567:TA) gene resulting in a truncated protein confers protection against chronic liver damage and attenuates the progression of NAFLD and alcoholic liver disease (ALD) in European Americans through reduced enzymatic activity against several proinflammatory lipid species. Sookoian *et al*[74] in an exome-wide association study, confirmed that the *HSD17B13* rs72613567 variant had an influence on the susceptibility and histological severity of NAFLD. Furthermore, Pirola *et al*[75] observed a lower risk of progressive NASH in subjects carrying the rs72613567:TA variant compared to non-carriers. However, the exact role of *HSD17B13* in the NAFLD pathophysiology remains largely uncharacterized. Recently, interesting studies on the inactivation of *HSD17B13* in mice and the identification of an enzymatic active site that metabolizes retinol have been reported[76,77], but pathophysiological evidence on human models is still limited[74,78]. The rs72613567: TA *HSD17B13* variant seems to affect liver by modulating hepatic retinol metabolism and by reducing stellate cell activity[78]. Another study, examining a large adult population, reported a protective role of this variant against various liver diseases such as cirrhosis, and hepatocellular carcinoma (HCC). In particular, *HSD17B13* rs72613567 was associated with reduced inflammation and fibrosis, and milder disease severity of NAFLD. Thus, *HSD17B13* rs72613567 represents an important protective factor in distinct liver diseases (including ALD, cirrhosis, and HCC) and seems to be associated with milder histological progression of NAFLD[79,80]. In 2019, Yang *et al*[81] in a multicenter European study of a total of 3315 patients with or without HCC but with chronic liver disease, reported that the *HSD17B13* loss-of-function variant rs72613567 is protective of HCC development in patients with ALD. Taken together, these findings suggested the potential therapeutic role of the *HSD17B13* inhibition[79] in patients at high risk for liver diseases. The rs72613567 variant also appears to interact with PNPLA3 I148M through the additional *HSD17B13* TA alleles that reduce the effect of the additional *PNPLA3* I148M alleles on serum ALT levels. It also mitigated liver damage in individuals genetically predisposed to hepatic steatosis by *PNPLA3* I148M[71]. The protective effect of the rs72613567:TA *HSD17B13* variant in reducing liver damage has also been observed in children[17]. By analyzing a large cohort of Italian obese children, carriers of the *HSD17B13* variant showed lower NAFLD risk than noncarriers. It is noteworthy that this variant was found to protect against liver damage even among patients stratified on the basis of the number of the steatogenic alleles of the three major NAFLD risk polymorphisms (such as *PNPLA3, TM6SF2,* and *MBOAT7*genes). More interestingly, recent pediatric evidence[47,48,82] showed a similar protective effect of this gene also on renal function, by supposing its role in retinol metabolism through modulation of both inflammation and fibrogenesis. Another variant (rs143404524) in the *HSD17B13* gene, resulting in a truncated protein has also been associated with a reduced risk of chronic liver disease in the adult population[83]. Finally, it has also been demonstrated that the rs62305723 variant of the *HSD17B13* gene, a missense variant that confers loss of enzyme activity was associated with decreased steatohepatitis[72]. In conclusion, the *HSD17B13* gene represents a well-known genetic factor with a protective role against liver damage both in adults and children[68] that might be considered an important pharmacological target for NAFLD treatment [17,84].

NAFLD AND THE "GUT-LIVER AXIS"

Recently, compelling evidence has supported the close and interdependent relationship between the liver and gut axis in the pathogenesis of numerous chronic liver diseases such as chronic hepatitis B and C, ALD, NAFLD, NASH, development of liver cirrhosis, and HCC (Figure 1).

Bäckhed *et al*[85] for the first time described the role of gut microbiota in the context of NAFLD and obesity, taking part in the processes of absorption and storage of energy but also in the production of triglycerides, responsible for the infiltration of hepatocytes.

Crosstalk between the liver and gut occurs by means of the biliary tract, portal vein and systemic mediators[86]. The liver contributes to the maintenance of gut eubiosis through the transport of bile salts and antimicrobial molecules to the intestinal lumen. Conversely, the gut regulates bile acids (BAs) composition. In addition, BAs using farnesoid X receptor (FXR) in the enterocytes and G protein-coupled bile acid receptor 1 (also known as TGR5) are involved in the regulation of glucose and lipid metabolism, anti-inflammatory immune responses and host energy expenditure[87-91]. Furthermore, the gut through secretion of the incretin hormones glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic peptide influences the pancreas in regulating both insulin and glucagon secretion[92]. Moreover, GLP-1 interaction with its receptor (also located on the hepatocytes) results in reduced hepatic fat deposition and IR. Finally, it promotes energy expenditure and peripheral utilization of triglycerides for energy production[93].



DOI:10.5409/wjcp.v11i3.221

Figure 1 The role of the gut-liver axis in non-alcoholic fatty liver disease. A: In healthy patients, the liver through the transport of bile salts and antimicrobial molecules to the intestinal lumen contributes to the maintenance of gut eubiosis. Conversely, the gut regulates bile acids (BAs) composition. BAs using farnesoid X receptor in the enterocytes and G protein-coupled bile acid receptor 1 are involved in the regulation of glucose and lipid metabolism, anti-inflammatory immune responses and host energy expenditure; B: In subjects with non-alcoholic fatty liver disease, altered gut microbial composition (dysbiosis), small intestinal bacterial overgrowth, and increased intestinal permeability (resulting from different factors including high-fat Western diet, genetic, inflammation) promote the influx of microbial-associated molecular patterns or pathogen-associated molecular patterns into the portal system reaching the liver. These molecular patterns are able to induce inflammatory responses mediated by the activation of pattern recognition receptors, like toll-like receptor, in Kupffer cells and hepatic stellate cells, leading to liver inflammation and fibrosis.

BAs synthesis is regulated by two hepatic methods: the enterohepatic circulation (with a subsequent negative feedback loop on the expression of CYP7A1) and FGF19, (derived from the activation of FXR by BAs in the ileum and has an inhibitory effect on *CYP7A1* gene[94]).

Impaired FXR-FGF19 signaling and elevated circulating BA levels were described both in children and adults with NAFLD. However, experimental therapeutic interventions targeting BA signaling with FXR agonists (obeticholic acid) have produced contradictory results[95].

Some differences were reported in the composition of gut microbiota (*i.e.* dysbiosis) in healthy controls than in subjects with simple fatty liver disease (FLD) and NASH[96]. In fact, many pediatric studies have reported a decreased gut microbiota alpha diversity, measured with the Shannon index[45, 97-99].

In 2006, Turnbaugh *et al*[100] found that the ratio of *Firmicutes* to *Bacteroidetes* increased in obese mice, suggesting a putative role for *Firmicutes* as a group of obesity-related microbiomes.

Loomba *et al*[101] in an elegant study showed that NAFLD patients exhibited more *Gram-negative* and fewer *Gram-positive bacteria* compared to healthy subjects, with an increase in *Proteobacteria* and a decrease in *Firmicutes* in more progressive NAFLD forms.

Michail *et al*[102] noted that children with NAFLD had more abundant *Gammaproteobacteria* and *Prevotella* compared to obese children without NAFLD and healthy controls. In addition, no difference in *Firmicutes* and *Bacteroidetes* or their ratio was observed between the groups.

Del Chierico *et al*[97] in a complex study with an integrated meta-omics-based approach found a significant increment of *Actinobacteria* and a decrease of *Bacteroidetes* in NAFLD patients compared to healthy controls.

Stanislawski *et al*[102] examined 107 adolescents with MRI-detected hepatic steatosis and found that *Bilophila* was positively correlated with hepatic fat fraction (HFF), while *Oscillospira* and *Bacteroides* showed different patterns in relation to HFF.

Schwimmer *et al*[99] in a prospective, observational, cross-sectional study of 87 children with biopsy-proven NAFLD and 37 obese children without NAFLD noted that a high abundance of *Prevotella copri* was associated with more severe fibrosis.

In a metagenomic study of gut microbiota by Zhao *et al*[103] conducted in 58 children and adolescents with NAFLD diagnosed by magnetic resonance spectroscopy, the authors found no significant differences in terms of alpha diversity among the study groups (NAFLD children, obese children without NAFLD and healthy controls). However, *Proteobacteria* were found to be more represented in NAFLD children than in the control group, while *Bacteroidates* (*Alistipes*) were significantly reduced.

Finally, Kravetz *et al*[45] in a cross-sectional study including 73 obese children and adolescents with and without NAFLD, in which HFF was determined by MRI, the NAFLD group showed a higher *Firmicutes* to *Bacteroidetes* ratio and lower levels of *Bacteroidetes*, *Prevotella*, *Gemmiger* and *Oscillospira*.

Altered gut microbial composition and increased intestinal permeability are linked to several factors (*e.g.* high-fat Western diet, chronic alcohol consumption, and genetic factors) and promote the influx of microbial-associated molecular patterns or pathogen-associated molecular patterns into the portal

system reaching the liver. These molecular patterns are responsible for inflammatory responses mediated by the activation of pattern recognition receptors, like Toll-like receptor, in Kupffer cells and hepatic stellate cells, leading to liver injury and fibrosis[86,104-106].

Potential gut-microbiome-targeted therapies in hepatic diseases are represented by probiotics, prebiotics, antibiotics, fecal microbial transplantation and bacteriophages, but larger validation studies are needed[107].

ROLE OF "OMICS" IN NAFLD

Epigenomics

Several authors have studied the role of epigenetic modifications in the natural history of NAFLD. The main epigenomic modification studied in NAFLD is DNA methylation.

A recent systematic review[108] included twelve studies on DNA methylation and FLD of which two assessed global DNA methylation, five assessed DNA methylation for specific candidate genes and the remaining four used the EWAS approach. The review suggested no consistent associations with FLD in the studies of global DNA methylation evaluated in hepatic tissue samples by quantifying the methyl-cytosine (5-mC) present in the genome. One of the two studies assessing global DNA methylation found mitochondrial encoded NADH dehydrogenase 6 hypermethylation in the liver of NASH patients compared to those with simple steatosis, and this methylation was significantly associated with NAFLD activity score[109]. On the other hand, another study reported that global liver methylation based on genome-wide methylation arrays was not associated with NAFLD or NASH, but NASH was associated with long-interspersed nuclear element hypomethylation compared to simple steatosis or normal liver [110]. More, studies using a candidate gene approach found that NAFLD was associated with hypomethylation at *FGFR2*, *MAT1A*, *CASP1* and *PARVB* genes and hypermethylation at *PNPLA3*[111], *PPARα*, *TGFβ1*, *Collagen 1A1* and *PDGFα* genes[112]. Furthermore, PPARGC1A methylation status was significantly associated with NAFLD[113]. The epigenome-wide DNA methylation studies reported different associations of distinct methylation compounds with NAFLD[114,115]. Finally, a single study reported the role of methylation in NAFLD in the expression of three genes (*NPC1L1*, *STARD* and *GRHL*) involved in lipoprotein particle composition[116].

A recent and interesting prospective cohort study analyzed epigenome-wide DNA methylation data of 785 newborns and 344 10-year-old children in relation to liver fat fraction (measured by MRI) at 10 years. No differential DNA methylation at age 10 years in newborns or 10-year-old children were found [117].

Despite some causative evidence, little is still known about the relationship between these changes in hepatic epigenome and their repercussion in the bloodstream. As a result, the contribution of epigenomics in the non-invasive diagnosis of NAFLD is still very limited but promising.

Transcriptomics

A growing body of data is derived from micro RNAs (miRNAs), highly conserved noncoding small RNAs, involved in gene expression modulation at the post-transcriptional level (Table 2). MiRNAs are resistant to degradation as well as to several freeze–thaw cycles, suggesting their potential role as ideal biomarkers for use in clinical practice.

Several studies highlighted the association between miR-122 and the severity of steatosis[118]. A reduced hepatic expression of miR-122 was described[119,120], whereas miR-122 levels were upregulated in serum[120].

A systematic review reported 34 miRNAs associated with FLD. Among these, miR-122, miR-34a, miR-192, miR-21 and miR-99a were associated with FLD in two or more independent studies[108].

Specifically, circulating miR-122 and miR-192 not only reflected both histological and molecular processes occurring in the liver, but have also been considered to be able to differentiate simple steatosis from NASH[121].

A cross-sectional validation study disclosed that 15 specific circulating miRNAs were significantly deregulated in prepubertal obesity, including the decreased miR-221 and miR-28 -3p, and increased concentrations in plasma of miR-486-5p, miR-486-3p, miR-142-3p, miR-130b, and miR-423-5p[122].

Can *et al*[123] showed a significant association between circulating miR-370, miR-33, miR-378, miR-27, miR-335, miR-143 and miR-758 values, and childhood obesity. Low levels of miR-335, miR-143 and miR-758, and high levels of miR-27, miR-378, miR-33 and miR-370 may have been responsible for elevated triglycerides and LDL-C levels, and a low level of HDL-C in obese subjects.

An interesting work by Cui *et al*[124] highlighted the specific role of three miRNAs, miR-486, miR-146b and miR-15b, by demonstrating their increased circulating expression in obese children and adult patients with type 2 diabetes mellitus (T2DM). In particular, miR-486 was implicated in accelerating preadipocyte proliferation and myotube glucose intolerance, miR-146b and miR-15b were engaged in the suppression of high concentration glucose-induced pancreatic insulin secretion, and they all contributed to the pathological processes of obesity and T2DM.

Table 2 Main findings of human transcriptomics studies and microRNAs in non-alcoholic fatty liver disease

Ref.	Study design	Population (*n*)	Main findings
Yamada *et al*[118]	Cross-sectional study	403 male subjects (median age 68.2 ± 10.3 yr); 48 NAFLD subjects (median age 66.2 ± 9.1 yr); 221 female patients (median age 65.5 ± 9.6 yr); 44 women with NAFLD (median age 65.0 ± 8.93 yr). Hepatic steatosis was assessed by ultrasound	Increased serum levels of miR-21, miR-34a, miR-122, and miR-451 were found in NAFLD patients
Cheung *et al*[119]	Cross-sectional study	50 patients with NASH (median age 52.5 yr) and 25 normal controls (median age 40.3 yr). NAFLD was suspected if abnormal liver enzymes or radiological evidence of a fatty liver and negative study for other common causes of liver disease and absence of clinically significant alcohol consumption	miR-34a and miR-146b were overexpressed in the liver of NASH patients, while miR-122 was underexpressed; miR-451 was not significantly different among the two groups
Pirola *et al*[120]	Case-control study	48 control patients (median age 47.8 ± 6.81 yr); 16 patients with simple steatosis (median age 51.5 ± 6.81 yr); 16 patients with NASH (median age 49.1 ± 8.6 yr). NAFLD was proven by biopsy	Increased levels of miR-122, miR-19a, miR-192, miR-19b, miR-125b, and miR-375 in serum either in SS or NASH patients were found. Reduced miR-122 levels in the liver of NASH patients were detected
Prats-Puig *et al*[122]	Cross-sectional study	10 lean children (median age 9.9 ± 1 yr), 5 obese children (median age 8.8 ± 1.8 yr)	Increased miR-486-5p, miR-486-3p, miR-142-3p, miR-130b, miR-423-5p, miR-532-5p, miR140-5p, miR-16-1, miR-222, miR-363, and miR-122; decreased miR-221, miR-28–3p, miR-125b, and miR-328 in obese children
Can *et al*[123]	Case-control study	86 non obese children (median age 14.44 ± 1.62 yr); 45 obese children (median age 14.71 ± 1.76 yr)	Reduced miR-335, miR-143, miR-758 and increased miR-27, miR-378, and miR-370 in the serum of obese children were detected
Cui *et al*[124]	Cross-sectional study	535 obese patients (median age 61.0 ± 10.4 yr); 106 OW patients (median age 59.6 ± 11.0 yr); 101 patients with T2D (median age 57.5 ± 12.2 yr); 82 with NGT (median age 49.3 ± 7.73 yr); 146 normal controls (median age 60.4 ± 11.1 yr)	miR-486, miR-146b and miR-15b were increased in the serum of obese children and T2D patients
Iacomino *et al*[125]	Cross-sectional study	189 children (median age 12.0 ± 1.6 yr) and 94 OW/Ob children (median age 12.3 ± 1.8 yr)	Increased miR-551a and miR-501-5p and reduced miR-10b-5p, miR-191-3p, miR-215-5p, and miR-874-3p levels in the serum of OW/Ob children were found

NASH: Non-alcoholic steatohepatitis; miR: MicroRNA; NAFLD: Non-alcoholic fatty liver disease; SS: Simple steatosis; T2D: Type 2 diabetes; NGT: Normal glucose tolerance controls; OW/Ob: overweight/obese.

Iacomino *et al*[125] in a pilot study (FAMILY Study) conducted in 149 overweight/obese and 159 normal weight children and adolescents demonstrated a panel of miRNAs differentially expressed in these two groups (miR-551a and miR-501-5p were upregulated; miR-10b-5p, miR-191-3p, miR-215-5p, and miR-874-3p were downregulated).

In a transcriptomic study by Sheldon *et al*[126] a new candidate marker for distinguishing steatosis from NASH was proposed, the soluble factor FCER2, produced from NOCTH2 activation in B cells, whose expression was increased in NASH patients.

Finally, in a recent study interleukin-32 was found as the most significantly upregulated transcript in advanced NAFLD and NASH, being linked to lipid accumulation and disease severity[127].

Although many studies have been investigating the role of miRNAs in the pathogenesis of NAFLD in view of their potential use as non-invasive biomarkers, results are still controversial and scarce. However, the innovative role of transcriptomics in the non-invasive diagnosis of NAFLD contributes to the new "omics" path of NAFLD.

Proteomics

To date, few studies on proteomic analysis in NAFLD have been performed, probably due to technical limitations in the correct detection and identification of proteins and to the changing quantification of blood proteins[128].

Among these proteins, the caspase-generated cytokeratin-18 (CK-18) fragments have been proposed as a noninvasive alternative biomarker of NASH. CK-18 showed a relatively good specificity for NAFLD, NASH and fibrosis but limited overall sensitivity[129].

Another protein being studied is the soluble intercellular adhesion molecule-1, with promising results also in NASH detection[130].

The mitochondrial enzyme carbamoyl-phosphate synthase 1 and the heat shock protein family A member 5 have been indicated as potential tools to stratify the different phenotypes associated with liver disease severity[131-133].

In a recent study by Malecki *et al*[134], a proteome analysis in a group of 30 children (16 with a previous NAFLD diagnosis by ultrasound) identified a total of 297 proteins. Thirty-seven distinct proteins (responsible for inflammation, stress response, and regulation of these processes) were identified. Up-regulated proteins included afamin, retinol-binding protein-4, complement components,

and hemopexin, while serum protease inhibitors, clusterin, immunoglobulin chains, and vitamin D binding protein were found in the down-regulated group[134].

Bălănescu *et al*[135] confirmed the role of the heat shock protein-90 (Hsp90) isoforms as biomarkers for NAFLD in obese and overweight children. While the Hsp90β isoform was higher, the Hsp90α isoform was lower in overweight and obese NAFLD patients.

Hence, proteomics represents one of the most challenging fields that might contribute to the development of new noninvasive targeted tools for NAFLD diagnosis and treatment. See Table 3.

Glycomics

Most of the glycomics studies in NAFLD have tried to identify glycans or glycoproteins that can serve as blood biomarkers for differentiating between NAFLD and NASH or for detection of the presence of liver fibrosis and its stage.

Changes in glycosylation represent a potential good marker of liver damage due to the hepatic production of several serum glycoproteins[136].

The findings of these studies demonstrated that higher concentrations of fucosylated, sialylated and agalactosylated glycans were observed in NAFLD and its progressive forms. Circulating sialic acid levels were also positively associated with metabolic syndrome and with NAFLD[128].

Furthermore, changes in fucosylation were observed in other inflammatory conditions, such as in chronic pancreatitis, Crohn's disease, rheumatoid arthritis and sickle cell disease[137].

Finally, hypogalactosylation (especially of IgG) was also associated with some autoimmune diseases and inflammatory pathways[138].

The first glycomic analysis in a pediatric NAFLD population was conducted by Blomme *et al*[136]. In agreement with adult findings, B cells were found to play a dominant role in the N-glycan alterations of pediatric NASH patients. Serum protein N-glycosylation patterns of 51 pediatric NAFLD patients were assessed with deoxyribonucleic acid sequencer-assisted fluorophore-assisted capillary electrophoresis and compared with histology. Analysis of the N-glycans on IgG confirmed the under-galactosylation status typical of chronic inflammatory conditions.

Metabolomics and lipidomics

To date, both metabolomics and lipidomics represent the most investigated omics branches in NAFLD with promising results for the development of new targeted strategies (Figure 2). Of interest, robust and extensive changes were observed both in the hepatic as well as in the circulating lipidome, which have led to the development of numerous diagnostic models for NAFLD and the identification of novel therapeutic targets. Many studies have reported several diagnostic models based on metabolomics, lipidomics alone or combined with other biochemical and clinical parameters for the diagnosis and staging of NAFLD.

Lipidomic studies have described specific changes in hepatic lipidome in patients with NAFLD. The hepatic concentrations of triacylglycerols, saturated fatty acids (SFAs and specifically of palmitic acid, C16:0 and stearate acid, C18:0), free cholesterol, sphingolipids, glycerophospholipids and eicosanoids increase, whereas ω-3 polyunsaturated fatty acids (PUFAs) and specialized proresolving mediators of PUFAs decrease. Monounsaturated fatty acids, lysophosphatidylcholine (LPC) and ceramide are also increased[21].

SFAs accumulation is associated with liver disease severity. They work in two different ways: on the hepatocytes stimulating proinflammatory cytokine secretion, enhancing oxidative stress, inducing apoptosis and on nonparenchymal liver cells stimulating secretion of proinflammatory and profibrotic cytokines (Kupffer cells) and induce proinflammatory M1 polarization of macrophages. Finally, SFAs stimulate the secretion of chemokines from hepatic stellate cells that recruit more macrophages in the liver[128].

LPC also stimulates ER stress, causes mitochondrial dysfunction and increases apoptosis[139]. Increased activity of the enzyme phospholipase A2 that catalyzes the formation of LPC from PC, leads to the rapid depletion of PC which affects hepatocyte membrane integrity and results in hepatocyte apoptosis, high release of lipotoxic lipids and increased inflammation. Additionally, PC deficiency reduces VLDL secretion resulting in higher intrahepatic lipid degradation and the formation of toxic intermediates[140].

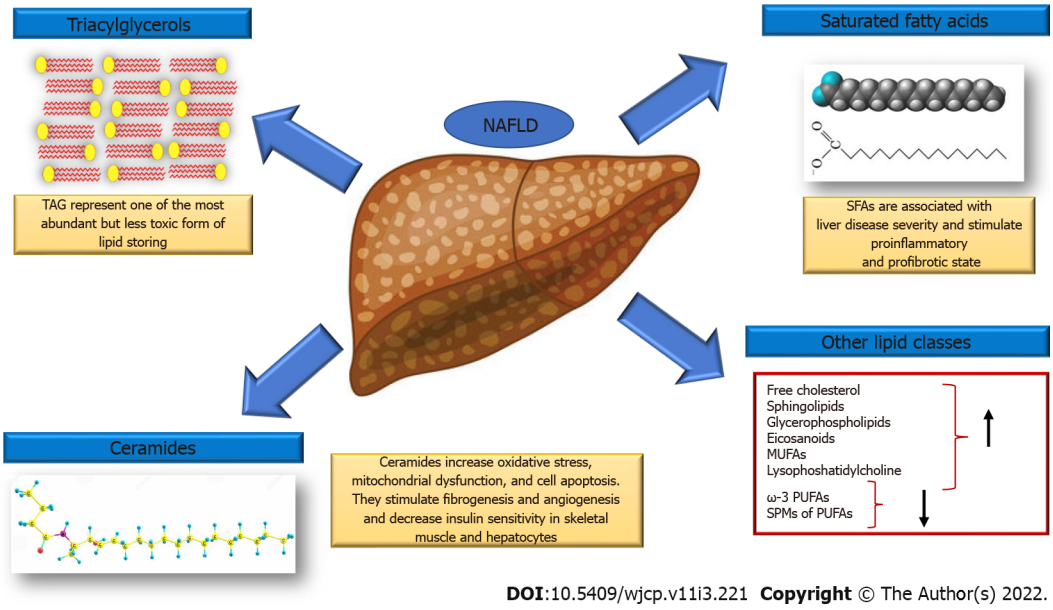
Ceramides correlate positively with hepatic disease severity[141]. These lipids have been found to decrease insulin sensitivity in skeletal muscle and hepatocytes[142] and are involved in increased oxidative stress, mitochondrial dysfunction, and cell apoptosis[142,143]. Finally, ceramide stimulates fibrogenesis and angiogenesis by increasing extracellular matrix deposition and the secretion of pro-angiogenic factors by hepatic stellate cells[144].

The attractive omics field might greatly contribute to improving not only knowledge on NAFLD pathophysiology but also its management.

Table 3 Main results of human proteomics studies in non-alcoholic fatty liver disease

Ref.	Study design	Population (*n*)	Main findings
Cusi *et al*[129]	Case-control study	300 subjects with NAFLD (median age 52 ± 1 yr) and 124 without NAFLD (median age 51 ± 1 yr). NAFLD was proven by MRS, biopsy, or US	Increased plasma CK-18 in steatosis, inflammation, and fibrosis
Sookoian *et al* [130]	Cross-sectional study	101 subjects with simple steatosis (median age 52.3 yr) and 60 NASH patients (median age 54.6 yr). NAFLD was proven by biopsy	sICAM-1 is able to differentiate between patients with simple steatosis and NASH
Rodriguez-Suarez *et al* [131]	Cross-sectional study	18 controls, 6 obese patients with NAFLD, 6 obese patients with early stage of NASH. Liver disease diagnosis was by biopsy	CPS1 could stratify different phenotypes associated with liver disease severity
Małecki *et al* [134]	Cross-sectional study	30 children (mean age 10.62 yr), 16 children with NAFLD (mean age 11.06 yr). NAFLD was proven by US	Afamin, retinol-binding protein-4, complement components, and hemopexin were upregulated; serum protease inhibitors, clusterin, immunoglobulin chains, vitamin D binding protein were down-regulated
Bălănescu *et al* [135]	Cross-sectional study	68 overweight and obese children (mean age 10 yr) and 10 healthy controls. NAFLD was proven by US or elevated alanine transaminase levels	HSP-90 isoforms could be used as NAFLD biomarkers in obese and overweight patients

NASH: Non-alcoholic steatohepatitis; miR: MicroRNA; NAFLD: Non-alcoholic fatty liver disease; MRS: Magnetic resonance spectroscopy; US: Ultrasound; CK-18: Cytokeratin-18; sICAM-1: Soluble intercellular adhesion molecule-1; CPS1: Carbamoyl-phosphate synthase 1; HSP-90: Heat shock protein-90.



DOI:10.5409/wjcp.v11i3.221

Figure 2 Main changes in hepatic lipid composition in non-alcoholic fatty liver disease. In non-alcoholic fatty liver disease subjects, hepatic concentrations of triacylglycerols, saturated fatty acids, free cholesterol, sphingolipids, glycerophospholipids and eicosanoids are increased, whereas ω-3 polyunsaturated fatty acids (PUFAs) and specialized proresolving mediators of PUFAs are decreased. Monounsaturated fatty acids, lysophosphatidylcholine and ceramide are also increased in the liver of these subjects.

CONCLUSION

Given the global relentless spread of childhood obesity, NAFLD and its cardiometabolic burden (including MetS, IR, cardiovascular disease, prediabetes, and type 2 diabetes) in childhood represent a major health challenge for clinicians[145]. Moreover, the close relationship of NAFLD with the metabolic milieu has recently been highlighted in the new definition of NAFLD as metabolic associated fatty liver disease[146,147].

To date, diet and lifestyle interventions remain the cornerstone of NAFLD treatment. Over the last few years, promising approaches have been proposed, but larger validation studies are required. In particular, omics represents the most intriguing strategy in this field, due to its potential effectiveness in preventing NAFLD as a noninvasive diagnostic and therapeutic tool.

Further novel therapeutic insights for this insidious disease might be provided only by advances in the knowledge of NAFLD pathophysiology.

FOOTNOTES

Author contributions: Riccio S and Di Sessa A wrote the manuscript; Miraglia del Giudice E, Di Sessa A, and Marzuillo P conceived the manuscript; Guarino S, Miraglia del Giudice E, Di Sessa A, and Marzuillo P supervised the manuscript drafting; Riccio S, Melone R, Vitulano C, Guida P, and Maddaluno I reviewed the literature data; Riccio S prepared the tables. Each author contributed important intellectual content during manuscript drafting or revision.

Conflict-of-interest statement: There is no conflict of interest.

Country/Territory of origin: Italy

ORCID number: Simona Riccio 0000-0003-3644-9259; Rosa Melone 0000-0001-6897-5742; Caterina Vitulano 0000-0002-5609-2843; Pierfrancesco Guida 0000-0002-8029-7814; Ivan Maddaluno 0000-0002-0238-1594; Stefano Guarino 0000-0002-0551-5236; Pierluigi Marzuillo 0000-0003-4682-0170; Emanuele Miraglia del Giudice 0000-0002-1492-076X; Anna Di Sessa 0000-0002-5877-3757.

S-Editor: Zhang H
L-Editor: Webster JR
P-Editor: Zhang H

REFERENCES

1 **Goldner D**, Lavine JE. Nonalcoholic Fatty Liver Disease in Children: Unique Considerations and Challenges. *Gastroenterology* 2020; **158**: 1967-1983.e1 [PMID: 32201176 DOI: 10.1053/j.gastro.2020.01.048]
2 **Shaunak M**, Byrne CD, Davis N, Afolabi P, Faust SN, Davies JH. Non-alcoholic fatty liver disease and childhood obesity. *Arch Dis Child* 2021; **106**: 3-8 [PMID: 32409495 DOI: 10.1136/archdischild-2019-318063]
3 **Morandi A**, Di Sessa A, Zusi C, Umano GR, El Mazloum D, Fornari E, Miraglia Del Giudice E, Targher G, Maffeis C. Nonalcoholic Fatty Liver Disease and Estimated Insulin Resistance in Obese Youth: A Mendelian Randomization Analysis. *J Clin Endocrinol Metab* 2020; **105** [PMID: 32841326 DOI: 10.1210/clinem/dgaa583]
4 **Di Bonito P**, Valerio G, Licenziati MR, Miraglia Del Giudice E, Baroni MG, Morandi A, Maffeis C, Campana G, Spreghini MR, Di Sessa A, Morino G, Crinò A, Chiesa C, Pacifico L, Manco M. High uric acid, reduced glomerular filtration rate and non-alcoholic fatty liver in young people with obesity. *J Endocrinol Invest* 2020; **43**: 461-468 [PMID: 31637675 DOI: 10.1007/s40618-019-01130-6]
5 **Barshop NJ**, Francis CS, Schwimmer JB, Lavine JE. Nonalcoholic fatty liver disease as a comorbidity of childhood obesity. *Ped Health* 2009; **3**: 271-281 [PMID: 20556232 DOI: 10.2217/phe.09.21]
6 **Flisiak-Jackiewicz M**, Lebensztejn DM. Update on pathogenesis, diagnostics and therapy of nonalcoholic fatty liver disease in children. *Clin Exp Hepatol* 2019; **5**: 11-21 [PMID: 30915402 DOI: 10.5114/ceh.2019.83152]
7 **Kim HY**. Recent advances in nonalcoholic fatty liver disease metabolomics. *Clin Mol Hepatol* 2021; **27**: 553-559 [PMID: 34098712 DOI: 10.3350/cmh.2021.0127]
8 **Kartsoli S**, Kostara CE, Tsimihodimos V, Bairaktari ET, Christodoulou DK. Lipidomics in non-alcoholic fatty liver disease. *World J Hepatol* 2020; **12**: 436-450 [PMID: 32952872 DOI: 10.4254/wjh.v12.i8.436]
9 **Pei K**, Gui T, Kan D, Feng H, Jin Y, Yang Y, Zhang Q, Du Z, Gai Z, Wu J, Li Y. An Overview of Lipid Metabolism and Nonalcoholic Fatty Liver Disease. *Biomed Res Int* 2020; **2020**: 4020249 [PMID: 32733940 DOI: 10.1155/2020/4020249]
10 **Bonsembiante L**, Targher G, Maffeis C. Non-alcoholic fatty liver disease in obese children and adolescents: a role for nutrition? *Eur J Clin Nutr* 2021 [PMID: 34006994 DOI: 10.1038/s41430-021-00928-z]
11 **Peng L**, Wu S, Zhou N, Zhu S, Liu Q, Li X. Clinical characteristics and risk factors of nonalcoholic fatty liver disease in children with obesity. *BMC Pediatr* 2021; **21**: 122 [PMID: 33711964 DOI: 10.1186/s12887-021-02595-2]
12 **Goyal NP**, Schwimmer JB. The Genetics of Pediatric Nonalcoholic Fatty Liver Disease. *Clin Liver Dis* 2018; **22**: 59-71 [PMID: 29128061 DOI: 10.1016/j.cld.2017.08.002]
13 **Tang S**, Zhang J, Mei TT, Guo HQ, Wei XH, Zhang WY, Liu YL, Liang S, Fan ZP, Ma LX, Lin W, Liu YR, Qiu LX, Yu HB. Association of PNPLA3 rs738409 G/C gene polymorphism with nonalcoholic fatty liver disease in children: a meta-analysis. *BMC Med Genet* 2020; **21**: 163 [PMID: 32811452 DOI: 10.1186/s12881-020-01098-8]
14 **Goffredo M**, Caprio S, Feldstein AE, D'Adamo E, Shaw MM, Pierpont B, Savoye M, Zhao H, Bale AE, Santoro N. Role of TM6SF2 rs58542926 in the pathogenesis of nonalcoholic pediatric fatty liver disease: A multiethnic study. *Hepatology* 2016; **63**: 117-125 [PMID: 26457389 DOI: 10.1002/hep.28283]

15 **Grandone A**, Cozzolino D, Marzuillo P, Cirillo G, Di Sessa A, Ruggiero L, Di Palma MR, Perrone L, Miraglia Del Giudice E. TM6SF2 Glu167Lys polymorphism is associated with low levels of LDL-cholesterol and increased liver injury in obese children. *Pediatr Obes* 2016; **11**: 115-119 [PMID: 25893821 DOI: 10.1111/ijpo.12032]

16 **Di Sessa A**, Umano GR, Cirillo G, Del Prete A, Iacomino R, Marzuillo P, Del Giudice EM. The Membrane-bound O-Acyltransferase7 rs641738 Variant in Pediatric Nonalcoholic Fatty Liver Disease. *J Pediatr Gastroenterol Nutr* 2018; **67**: 69-74 [PMID: 29601441 DOI: 10.1097/MPG.0000000000001979]

17 **Di Sessa A**, Umano GR, Cirillo G, Marzuillo P, Arienzo MR, Pedullà M, Miraglia Del Giudice E. The rs72613567: TA Variant in the Hydroxysteroid 17-beta Dehydrogenase 13 Gene Reduces Liver Damage in Obese Children. *J Pediatr Gastroenterol Nutr* 2020; **70**: 371-374 [PMID: 31789772 DOI: 10.1097/MPG.0000000000002573]

18 **Yki-Järvinen H**. Ceramides: A Cause of Insulin Resistance in Nonalcoholic Fatty Liver Disease in Both Murine Models and Humans. *Hepatology* 2020; **71**: 1499-1501 [PMID: 31899812 DOI: 10.1002/hep.31095]

19 **Samuel VT**, Shulman GI. Nonalcoholic Fatty Liver Disease, Insulin Resistance, and Ceramides. *N Engl J Med* 2019; **381**: 1866-1869 [PMID: 31693811 DOI: 10.1056/NEJMcibr1910023]

20 **Apostolopoulou M**, Gordillo R, Gancheva S, Strassburger K, Herder C, Esposito I, Schlensak M, Scherer PE, Roden M. Role of ceramide-to-dihydroceramide ratios for insulin resistance and non-alcoholic fatty liver disease in humans. *BMJ Open Diabetes Res Care* 2020; **8** [PMID: 33219119 DOI: 10.1136/bmjdrc-2020-001860]

21 **Di Sessa A**, Riccio S, Pirozzi E, Verde M, Passaro AP, Umano GR, Guarino S, Miraglia Del Giudice E, Marzuillo P. Advances in paediatric nonalcoholic fatty liver disease: Role of lipidomics. *World J Gastroenterol* 2021; **27**: 3815-3824 [PMID: 34321846 DOI: 10.3748/wjg.v27.i25.3815]

22 **Tiniakos DG**, Vos MB, Brunt EM. Nonalcoholic fatty liver disease: pathology and pathogenesis. *Annu Rev Pathol* 2010; **5**: 145-171 [PMID: 20078219 DOI: 10.1146/annurev-pathol-121808-102132]

23 **Tilg H**, Moschen AR. Evolution of inflammation in nonalcoholic fatty liver disease: the multiple parallel hits hypothesis. *Hepatology* 2010; **52**: 1836-1846 [PMID: 21038418 DOI: 10.1002/hep.24001]

24 **McPherson S**, Hardy T, Henderson E, Burt AD, Day CP, Anstee QM. Evidence of NAFLD progression from steatosis to fibrosing-steatohepatitis using paired biopsies: implications for prognosis and clinical management. *J Hepatol* 2015; **62**: 1148-1155 [PMID: 25477264 DOI: 10.1016/j.jhep.2014.11.034]

25 **Pais R**, Charlotte F, Fedchuk L, Bedossa P, Lebray P, Poynard T, Ratziu V; LIDO Study Group. A systematic review of follow-up biopsies reveals disease progression in patients with non-alcoholic fatty liver. *J Hepatol* 2013; **59**: 550-556 [PMID: 23665288 DOI: 10.1016/j.jhep.2013.04.027]

26 **Day CP**, James OF. Steatohepatitis: a tale of two "hits"? *Gastroenterology* 1998; **114**: 842-845 [PMID: 9547102 DOI: 10.1016/s0016-5085(98)70599-2]

27 **Friedman SL**, Neuschwander-Tetri BA, Rinella M, Sanyal AJ. Mechanisms of NAFLD development and therapeutic strategies. *Nat Med* 2018; **24**: 908-922 [PMID: 29967350 DOI: 10.1038/s41591-018-0104-9]

28 **Dai G**, Liu P, Li X, Zhou X, He S. Association between PNPLA3 rs738409 polymorphism and nonalcoholic fatty liver disease (NAFLD) susceptibility and severity: A meta-analysis. *Medicine (Baltimore)* 2019; **98**: e14324 [PMID: 30762732 DOI: 10.1097/MD.0000000000014324]

29 **Romeo S**, Kozlitina J, Xing C, Pertsemlidis A, Cox D, Pennacchio LA, Boerwinkle E, Cohen JC, Hobbs HH. Genetic variation in PNPLA3 confers susceptibility to nonalcoholic fatty liver disease. *Nat Genet* 2008; **40**: 1461-1465 [PMID: 18820647 DOI: 10.1038/ng.257]

30 **Trépo E**, Valenti L. Update on NAFLD genetics: From new variants to the clinic. *J Hepatol* 2020; **72**: 1196-1209 [PMID: 32145256 DOI: 10.1016/j.jhep.2020.02.020]

31 **Woodside M**. Research on children of alcoholics: past and future. *Br J Addict* 1988; **83**: 785-792 [PMID: 3061526 DOI: 10.1111/liv.14020]

32 **Marzuillo P**, Miraglia del Giudice E, Santoro N. Pediatric fatty liver disease: role of ethnicity and genetics. *World J Gastroenterol* 2014; **20**: 7347-7355 [PMID: 24966605 DOI: 10.3748/wjg.v20.i23.7347]

33 **Dongiovanni P**, Donati B, Fares R, Lombardi R, Mancina RM, Romeo S, Valenti L. PNPLA3 I148M polymorphism and progressive liver disease. *World J Gastroenterol* 2013; **19**: 6969-6978 [PMID: 24222941 DOI: 10.3748/wjg.v19.i41.6969]

34 **Marzuillo P**, Grandone A, Perrone L, Miraglia Del Giudice E. Understanding the pathophysiological mechanisms in the pediatric non-alcoholic fatty liver disease: The role of genetics. *World J Hepatol* 2015; **7**: 1439-1443 [PMID: 26085904 DOI: 10.4254/wjh.v7.i11.1439]

35 **Santoro N**, Kursawe R, D'Adamo E, Dykas DJ, Zhang CK, Bale AE, Calí AM, Narayan D, Shaw MM, Pierpont B, Savoye M, Lartaud D, Eldrich S, Cushman SW, Zhao H, Shulman GI, Caprio S. A common variant in the patatin-like phospholipase 3 gene (PNPLA3) is associated with fatty liver disease in obese children and adolescents. *Hepatology* 2010; **52**: 1281-1290 [PMID: 20803499 DOI: 10.1002/hep.23832]

36 **Giudice EM**, Grandone A, Cirillo G, Santoro N, Amato A, Brienza C, Savarese P, Marzuillo P, Perrone L. The association of PNPLA3 variants with liver enzymes in childhood obesity is driven by the interaction with abdominal fat. *PLoS One* 2011; **6**: e27933 [PMID: 22140488 DOI: 10.1371/journal.pone.0027933]

37 **Romeo S**, Sentinelli F, Cambuli VM, Incani M, Congiu T, Matta V, Pilia S, Huang-Doran I, Cossu E, Loche S, Baroni MG. The 148M allele of the PNPLA3 gene is associated with indices of liver damage early in life. *J Hepatol* 2010; **53**: 335-338 [PMID: 20546964 DOI: 10.1016/j.jhep.2010.02.034]

38 **Tricò D**, Caprio S, Rosaria Umano G, Pierpont B, Nouws J, Galderisi A, Kim G, Mata MM, Santoro N. Metabolic Features of Nonalcoholic Fatty Liver (NAFL) in Obese Adolescents: Findings From a Multiethnic Cohort. *Hepatology* 2018; **68**: 1376-1390 [PMID: 29665034 DOI: 10.1002/hep.30035]

39 **Hudert CA**, Selinski S, Rudolph B, Bläker H, Loddenkemper C, Thielhorn R, Berndt N, Golka K, Cadenas C, Reinders J, Henning S, Bufler P, Jansen PLM, Holzhütter HG, Meierhofer D, Hengstler JG, Wiegand S. Genetic determinants of steatosis and fibrosis progression in paediatric non-alcoholic fatty liver disease. *Liver Int* 2019; **39**: 540-556 [PMID: 30444569 DOI: 10.1111/liv.14006]

40 **Viitasalo A**, Eloranta AM, Atalay M, Romeo S, Pihlajamäki J, Lakka TA. Association of MBOAT7 gene variant with plasma ALT levels in children: the PANIC study. *Pediatr Res* 2016; **80**: 651-655 [PMID: 27411039 DOI: 10.1038/pr.2016.139]

41 **Marzuillo P**, Grandone A, Perrone L, del Giudice EM. Weight loss allows the dissection of the interaction between abdominal fat and PNPLA3 (adiponutrin) in the liver damage of obese children. *J Hepatol* 2013; **59**: 1143-1144 [PMID: 23845393 DOI: 10.1016/j.jhep.2013.06.027]

42 **Wang S**, Song J, Shang X, Chawla N, Yang Y, Meng X, Wang H, Ma J. Physical activity and sedentary behavior can modulate the effect of the PNPLA3 variant on childhood NAFLD: a case-control study in a Chinese population. *BMC Med Genet* 2016; **17**: 90 [PMID: 27905898 DOI: 10.1186/s12881-016-0352-9]

43 **Davis JN**, Lê KA, Walker RW, Vikman S, Spruijt-Metz D, Weigensberg MJ, Allayee H, Goran MI. Increased hepatic fat in overweight Hispanic youth influenced by interaction between genetic variation in PNPLA3 and high dietary carbohydrate and sugar consumption. *Am J Clin Nutr* 2010; **92**: 1522-1527 [PMID: 20962157 DOI: 10.3945/ajcn.2010.30185]

44 **Santoro N**, Savoye M, Kim G, Marotto K, Shaw MM, Pierpont B, Caprio S. Hepatic fat accumulation is modulated by the interaction between the rs738409 variant in the PNPLA3 gene and the dietary omega6/omega3 PUFA intake. *PLoS One* 2012; **7**: e37827 [PMID: 22629460 DOI: 10.1371/journal.pone.0037827]

45 **Monga Kravetz A**, Testerman T, Galuppo B, Graf J, Pierpont B, Siebel S, Feinn R, Santoro N. Effect of Gut Microbiota and PNPLA3 rs738409 Variant on Nonalcoholic Fatty Liver Disease (NAFLD) in Obese Youth. *J Clin Endocrinol Metab* 2020; **105** [PMID: 32561908 DOI: 10.1210/clinem/dgaa382]

46 **Mantovani A**, Zusi C, Sani E, Colecchia A, Lippi G, Zaza GL, Valenti L, Byrne CD, Maffeis C, Bonora E, Targher G. Association between PNPLA3rs738409 polymorphism decreased kidney function in postmenopausal type 2 diabetic women with or without non-alcoholic fatty liver disease. *Diabetes Metab* 2019; **45**: 480-487 [PMID: 30763699 DOI: 10.1016/j.diabet.2019.01.011]

47 **Di Sessa A**, Guarino S, Passaro AP, Liguori L, Umano GR, Cirillo G, Miraglia Del Giudice E, Marzuillo P. NAFLD and renal function in children: is there a genetic link? *Expert Rev Gastroenterol Hepatol* 2021; **15**: 975-984 [PMID: 33851883 DOI: 10.1080/17474124.2021.1906649]

48 **Marzuillo P**, Di Sessa A, Guarino S, Capalbo D, Umano GR, Pedullà M, La Manna A, Cirillo G, Miraglia Del Giudice E. Nonalcoholic fatty liver disease and eGFR levels could be linked by the PNPLA3 I148M polymorphism in children with obesity. *Pediatr Obes* 2019; **14**: e12539 [PMID: 31184438 DOI: 10.1111/ijpo.12539]

49 **Fang YL**, Chen H, Wang CL, Liang L. Pathogenesis of non-alcoholic fatty liver disease in children and adolescence: From "two hit theory" to "multiple hit model". *World J Gastroenterol* 2018; **24**: 2974-2983 [PMID: 30038464 DOI: 10.3748/wjg.v24.i27.2974]

50 **Nobili V**, Alisi A, Valenti L, Miele L, Feldstein AE, Alkhouri N. NAFLD in children: new genes, new diagnostic modalities and new drugs. *Nat Rev Gastroenterol Hepatol* 2019; **16**: 517-530 [PMID: 31278377 DOI: 10.1038/s41575-019-0169-z]

51 **Beer NL**, Tribble ND, McCulloch LJ, Roos C, Johnson PR, Orho-Melander M, Gloyn AL. The P446L variant in GCKR associated with fasting plasma glucose and triglyceride levels exerts its effect through increased glucokinase activity in liver. *Hum Mol Genet* 2009; **18**: 4081-4088 [PMID: 19643913 DOI: 10.1093/hmg/ddp357]

52 **Valenti L**, Alisi A, Nobili V. Unraveling the genetics of fatty liver in obese children: additive effect of P446L GCKR and I148M PNPLA3 polymorphisms. *Hepatology* 2012; **55**: 661-663 [PMID: 22281838 DOI: 10.1002/hep.25617]

53 **Eslam M**, Valenti L, Romeo S. Genetics and epigenetics of NAFLD and NASH: Clinical impact. *J Hepatol* 2018; **68**: 268-279 [PMID: 29122391 DOI: 10.1016/j.jhep.2017.09.003]

54 **Speliotes EK**, Butler JL, Palmer CD, Voight BF; GIANT Consortium; MIGen Consortium; NASH CRN, Hirschhorn JN. PNPLA3 variants specifically confer increased risk for histologic nonalcoholic fatty liver disease but not metabolic disease. *Hepatology* 2010; **52**: 904-912 [PMID: 20648472 DOI: 10.1002/hep.23768]

55 **Petta S**, Miele L, Bugianesi E, Cammà C, Rosso C, Boccia S, Cabibi D, Di Marco V, Grimaudo S, Grieco A, Pipitone RM, Marchesini G, Craxì A. Glucokinase regulatory protein gene polymorphism affects liver fibrosis in non-alcoholic fatty liver disease. *PLoS One* 2014; **9**: e87523 [PMID: 24498332 DOI: 10.1371/journal.pone.0087523]

56 **Lin YC**, Chang PF, Chang MH, Ni YH. Genetic variants in GCKR and PNPLA3 confer susceptibility to nonalcoholic fatty liver disease in obese individuals. *Am J Clin Nutr* 2014; **99**: 869-874 [PMID: 24477042 DOI: 10.3945/ajcn.113.079749]

57 **Santoro N**, Zhang CK, Zhao H, Pakstis AJ, Kim G, Kursawe R, Dykas DJ, Bale AE, Giannini C, Pierpont B, Shaw MM, Groop L, Caprio S. Variant in the glucokinase regulatory protein (GCKR) gene is associated with fatty liver in obese children and adolescents. *Hepatology* 2012; **55**: 781-789 [PMID: 22105854 DOI: 10.1002/hep.24806]

58 **Zusi C**, Mantovani A, Olivieri F, Morandi A, Corradi M, Miraglia Del Giudice E, Dauriz M, Valenti L, Byrne CD, Targher G, Maffeis C. Contribution of a genetic risk score to clinical prediction of hepatic steatosis in obese children and adolescents. *Dig Liver Dis* 2019; **51**: 1586-1592 [PMID: 31255630 DOI: 10.1016/j.dld.2019.05.029]

59 **Chen X**, Zhou P, De L, Li B, Su S. The roles of transmembrane 6 superfamily member 2 rs58542926 polymorphism in chronic liver disease: A meta-analysis of 24,147 subjects. *Mol Genet Genomic Med* 2019; **7**: e824 [PMID: 31309745 DOI: 10.1002/mgg3.824]

60 **Mahdessian H**, Taxiarchis A, Popov S, Silveira A, Franco-Cereceda A, Hamsten A, Eriksson P, van't Hooft F. TM6SF2 is a regulator of liver fat metabolism influencing triglyceride secretion and hepatic lipid droplet content. *Proc Natl Acad Sci U S A* 2014; **111**: 8913-8918 [PMID: 24927523 DOI: 10.1073/pnas.1323785111]

61 **Kozlitina J**, Smagris E, Stender S, Nordestgaard BG, Zhou HH, Tybjærg-Hansen A, Vogt TF, Hobbs HH, Cohen JC. Exome-wide association study identifies a TM6SF2 variant that confers susceptibility to nonalcoholic fatty liver disease. *Nat Genet* 2014; **46**: 352-356 [PMID: 24531328 DOI: 10.1038/ng.2901]

62 **Sliz E**, Sebert S, Würtz P, Kangas AJ, Soininen P, Lehtimäki T, Kähönen M, Viikari J, Männikkö M, Ala-Korpela M, Raitakari OT, Kettunen J. NAFLD risk alleles in PNPLA3, TM6SF2, GCKR and LYPLAL1 show divergent metabolic effects. *Hum Mol Genet* 2018; **27**: 2214-2223 [PMID: 29648650 DOI: 10.1093/hmg/ddy124]

63 **Carlsson B**, Lindén D, Brolén G, Liljeblad M, Bjursell M, Romeo S, Loomba R. Review article: the emerging role of genetics in precision medicine for patients with non-alcoholic steatohepatitis. *Aliment Pharmacol Ther* 2020; **51**: 1305-1320 [PMID: 32383295 DOI: 10.1111/apt.15738]

64 **Liu YL**, Reeves HL, Burt AD, Tiniakos D, McPherson S, Leathart JB, Allison ME, Alexander GJ, Piguet AC, Anty R, Donaldson P, Aithal GP, Francque S, Van Gaal L, Clement K, Ratziu V, Dufour JF, Day CP, Daly AK, Anstee QM. TM6SF2 rs58542926 influences hepatic fibrosis progression in patients with non-alcoholic fatty liver disease. *Nat Commun* 2014; **5**: 4309 [PMID: 24978903 DOI: 10.1038/ncomms5309]

65 **Dongiovanni P**, Petta S, Maglio C, Fracanzani AL, Pipitone R, Mozzi E, Motta BM, Kaminska D, Rametta R, Grimaudo S, Pelusi S, Montalcini T, Alisi A, Maggioni M, Kärjä V, Borén J, Käkelä P, Di Marco V, Xing C, Nobili V, Dallapiccola B, Craxi A, Pihlajamäki J, Fargion S, Sjöström L, Carlsson LM, Romeo S, Valenti L. Transmembrane 6 superfamily member 2 gene variant disentangles nonalcoholic steatohepatitis from cardiovascular disease. *Hepatology* 2015; **61**: 506-514 [PMID: 25251399 DOI: 10.1002/hep.27490]

66 **Holmen OL**, Zhang H, Fan Y, Hovelson DH, Schmidt EM, Zhou W, Guo Y, Zhang J, Langhammer A, Løchen ML, Ganesh SK, Vatten L, Skorpen F, Dalen H, Pennathur S, Chen J, Platou C, Mathiesen EB, Wilsgaard T, Njølstad I, Boehnke M, Chen YE, Abecasis GR, Hveem K, Willer CJ. Systematic evaluation of coding variation identifies a candidate causal variant in TM6SF2 influencing total cholesterol and myocardial infarction risk. *Nat Genet* 2014; **46**: 345-351 [PMID: 24633158 DOI: 10.1038/ng.2926]

67 **Marzuillo P**, Di Sessa A, Cirillo G, Umano GR, Pedullà M, La Manna A, Guarino S, Miraglia Del Giudice E. Transmembrane 6 superfamily member 2 167K allele improves renal function in children with obesity. *Pediatr Res* 2020; **88**: 300-304 [PMID: 31923913 DOI: 10.1038/s41390-020-0753-5]

68 **Su W**, Mao Z, Liu Y, Zhang X, Zhang W, Gustafsson JA, Guan Y. Role of HSD17B13 in the liver physiology and pathophysiology. *Mol Cell Endocrinol* 2019; **489**: 119-125 [PMID: 30365983 DOI: 10.1016/j.mce.2018.10.014]

69 **Dong XC**. A closer look at the mysterious HSD17B13. *J Lipid Res* 2020; **61**: 1361-1362 [PMID: 33008926 DOI: 10.1194/jlr.C120001160]

70 **Su W**, Wang Y, Jia X, Wu W, Li L, Tian X, Li S, Wang C, Xu H, Cao J, Han Q, Xu S, Chen Y, Zhong Y, Zhang X, Liu P, Gustafsson JÅ, Guan Y. Comparative proteomic study reveals 17β-HSD13 as a pathogenic protein in nonalcoholic fatty liver disease. *Proc Natl Acad Sci U S A* 2014; **111**: 11437-11442 [PMID: 25028495 DOI: 10.1073/pnas.1410741111]

71 **Abul-Husn NS**, Cheng X, Li AH, Xin Y, Schurmann C, Stevis P, Liu Y, Kozlitina J, Stender S, Wood GC, Stepanchick AN, Still MD, McCarthy S, O'Dushlaine C, Packer JS, Balasubramanian S, Gosalia N, Esopi D, Kim SY, Mukherjee S, Lopez AE, Fuller ED, Penn J, Chu X, Luo JZ, Mirshahi UL, Carey DJ, Still CD, Feldman MD, Small A, Damrauer SM, Rader DJ, Zambrowicz B, Olson W, Murphy AJ, Borecki IB, Shuldiner AR, Reid JG, Overton JD, Yancopoulos GD, Hobbs HH, Cohen JC, Gottesman O, Teslovich TM, Baras A, Mirshahi T, Gromada J, Dewey FE. A Protein-Truncating HSD17B13 Variant and Protection from Chronic Liver Disease. *N Engl J Med* 2018; **378**: 1096-1106 [PMID: 29562163 DOI: 10.1056/NEJMoa1712191]

72 **Ma Y**, Belyaeva OV, Brown PM, Fujita K, Valles K, Karki S, de Boer YS, Koh C, Chen Y, Du X, Handelman SK, Chen V, Speliotes EK, Nestlerode C, Thomas E, Kleiner DE, Zmuda JM, Sanyal AJ; (for the Nonalcoholic Steatohepatitis Clinical Research Network), Kedishvili NY, Liang TJ, Rotman Y. 17-Beta Hydroxysteroid Dehydrogenase 13 Is a Hepatic Retinol Dehydrogenase Associated With Histological Features of Nonalcoholic Fatty Liver Disease. *Hepatology* 2019; **69**: 1504-1519 [PMID: 30415504 DOI: 10.1002/hep.30350]

73 **Lin YC**, Wu CC, Ni YH. New Perspectives on Genetic Prediction for Pediatric Metabolic Associated Fatty Liver Disease. *Front Pediatr* 2020; **8**: 603654 [PMID: 33363067 DOI: 10.3389/fped.2020.603654]

74 **Sookoian S**, Arrese M, Pirola CJ. Genetics Meets Therapy? *Hepatology* 2019; **69**: 907-910 [PMID: 30102780 DOI: 10.1002/hep.30209]

75 **Pirola CJ**, Garaycoechea M, Flichman D, Arrese M, San Martino J, Gazzi C, Castaño GO, Sookoian S. Splice variant rs72613567 prevents worst histologic outcomes in patients with nonalcoholic fatty liver disease. *J Lipid Res* 2019; **60**: 176-185 [PMID: 30323112 DOI: 10.1194/jlr.P089953]

76 **Ma Y**, Karki S, Brown PM, Lin DD, Podszun MC, Zhou W, Belyaeva OV, Kedishvili NY, Rotman Y. Characterization of essential domains in HSD17B13 for cellular localization and enzymatic activity. *J Lipid Res* 2020; **61**: 1400-1409 [PMID: 32973038 DOI: 10.1194/jlr.RA120000907]

77 **Ma Y**, Brown PM, Lin DD, Ma J, Feng D, Belyaeva OV, Podszun MC, Roszik J, Allen JN, Umarova R, Kleiner DE, Kedishvili NY, Gavrilova O, Gao B, Rotman Y. 17-Beta Hydroxysteroid Dehydrogenase 13 Deficiency Does Not Protect Mice From Obesogenic Diet Injury. *Hepatology* 2021; **73**: 1701-1716 [PMID: 32779242 DOI: 10.1002/hep.31517]

78 **Gellert-Kristensen H**, Nordestgaard BG, Tybjaerg-Hansen A, Stender S. High Risk of Fatty Liver Disease Amplifies the Alanine Transaminase-Lowering Effect of a HSD17B13 Variant. *Hepatology* 2020; **71**: 56-66 [PMID: 31155741 DOI: 10.1002/hep.30799]

79 **Wang P**, Wu CX, Li Y, Shen N. HSD17B13 rs72613567 protects against liver diseases and histological progression of nonalcoholic fatty liver disease: a systematic review and meta-analysis. *Eur Rev Med Pharmacol Sci* 2020; **24**: 8997-9007 [PMID: 32964989 DOI: 10.26355/eurrev_202009_22842]

80 **Stickel F**, Lutz P, Buch S, Nischalke HD, Silva I, Rausch V, Fischer J, Weiss KH, Gotthardt D, Rosendahl J, Marot A, Elamly M, Krawczyk M, Casper M, Lammert F, Buckley TWM, McQuillin A, Spengler U, Eyer F, Vogel A, Marhenke S, von Felden J, Wege H, Sharma R, Atkinson S, Franke A, Nehring S, Moser V, Schafmayer C, Spahr L, Lackner C, Stauber RE, Canbay A, Link A, Valenti L, Grove JI, Aithal GP, Marquardt JU, Fateen W, Zopf S, Dufour JF, Trebicka J, Datz C, Deltenre P, Mueller S, Berg T, Hampe J, Morgan MY. Genetic Variation in HSD17B13 Reduces the Risk of Developing Cirrhosis and Hepatocellular Carcinoma in Alcohol Misusers. *Hepatology* 2020; **72**: 88-102 [PMID: 31630428 DOI: 10.1002/hep.30996]

81 **Yang J**, Trépo E, Nahon P, Cao Q, Moreno C, Letouzé E, Imbeaud S, Bayard Q, Gustot T, Deviere J, Bioulac-Sage P, Calderaro J, Ganne-Carrié N, Laurent A, Blanc JF, Guyot E, Sutton A, Ziol M, Zucman-Rossi J, Nault JC. A 17-Beta-Hydroxysteroid Dehydrogenase 13 Variant Protects From Hepatocellular Carcinoma Development in Alcoholic Liver Disease. *Hepatology* 2019; **70**: 231-240 [PMID: 30908678 DOI: 10.1002/hep.30623]

82 **Di Sessa A**, Umano GR, Cirillo G, Passaro AP, Verde V, Cozzolino D, Guarino S, Marzuillo P, Miraglia Del Giudice E. Pediatric non-alcoholic fatty liver disease and kidney function: Effect of *HSD17B13* variant. *World J Gastroenterol* 2020; **26**: 5474-5483 [PMID: 33024398 DOI: 10.3748/wjg.v26.i36.5474]

83 **Kozlitina J**, Stender S, Hobbs HH, Cohen JC. HSD17B13 and Chronic Liver Disease in Blacks and Hispanics. *N Engl J Med* 2018; **379**: 1876-1877 [PMID: 30403941 DOI: 10.1056/NEJMc1804027]

84 **Stender S**, Romeo S. HSD17B13 as a promising therapeutic target against chronic liver disease. *Liver Int* 2020; **40**: 756-757 [PMID: 32255570 DOI: 10.1111/liv.14411]

85 **Bäckhed F**, Ding H, Wang T, Hooper LV, Koh GY, Nagy A, Semenkovich CF, Gordon JI. The gut microbiota as an environmental factor that regulates fat storage. *Proc Natl Acad Sci U S A* 2004; **101**: 15718-15723 [PMID: 15505215 DOI: 10.1073/pnas.0407076101]

86 **Tripathi A**, Debelius J, Brenner DA, Karin M, Loomba R, Schnabl B, Knight R. The gut-liver axis and the intersection with the microbiome. *Nat Rev Gastroenterol Hepatol* 2018; **15**: 397-411 [PMID: 29748586 DOI: 10.1038/s41575-018-0011-z]

87 **Copple BL**, Li T. Pharmacology of bile acid receptors: Evolution of bile acids from simple detergents to complex signaling molecules. *Pharmacol Res* 2016; **104**: 9-21 [PMID: 26706784 DOI: 10.1016/j.phrs.2015.12.007]

88 **Sinal CJ**, Tohkin M, Miyata M, Ward JM, Lambert G, Gonzalez FJ. Targeted disruption of the nuclear receptor FXR/BAR impairs bile acid and lipid homeostasis. *Cell* 2000; **102**: 731-744 [PMID: 11030617 DOI: 10.1016/s0092-8674(00)00062-3]

89 **Pols TW**, Noriega LG, Nomura M, Auwerx J, Schoonjans K. The bile acid membrane receptor TGR5 as an emerging target in metabolism and inflammation. *J Hepatol* 2011; **54**: 1263-1272 [PMID: 21145931 DOI: 10.1016/j.jhep.2010.12.004]

90 **Broeders EP**, Nascimento EB, Havekes B, Brans B, Roumans KH, Tailleux A, Schaart G, Kouach M, Charton J, Deprez B, Bouvy ND, Mottaghy F, Staels B, van Marken Lichtenbelt WD, Schrauwen P. The Bile Acid Chenodeoxycholic Acid Increases Human Brown Adipose Tissue Activity. *Cell Metab* 2015; **22**: 418-426 [PMID: 26235421 DOI: 10.1016/j.cmet.2015.07.002]

91 **Slijepcevic D**, van de Graaf SF. Bile Acid Uptake Transporters as Targets for Therapy. *Dig Dis* 2017; **35**: 251-258 [PMID: 28249291 DOI: 10.1159/000450983]

92 **Svegliati-Baroni G**, Patrício B, Lioci G, Macedo MP, Gastaldelli A. Gut-Pancreas-Liver Axis as a Target for Treatment of NAFLD/NASH. *Int J Mol Sci* 2020; **21** [PMID: 32823659 DOI: 10.3390/ijms21165820]

93 **Jiang X**, Zheng J, Zhang S, Wang B, Wu C, Guo X. Advances in the Involvement of Gut Microbiota in Pathophysiology of NAFLD. *Front Med (Lausanne)* 2020; **7**: 361 [PMID: 32850884 DOI: 10.3389/fmed.2020.00361]

94 **Arab JP**, Karpen SJ, Dawson PA, Arrese M, Trauner M. Bile acids and nonalcoholic fatty liver disease: Molecular insights and therapeutic perspectives. *Hepatology* 2017; **65**: 350-362 [PMID: 27358174 DOI: 10.1002/hep.28709]

95 **Hernandez GV**, Smith VA, Melnyk M, Burd MA, Sprayberry KA, Edwards MS, Peterson DG, Bennet DC, Fanter RK, Columbus DA, Steibel JP, Glanz H, Immoos C, Rice MS, Santiago-Rodriguez TM, Blank J, VanderKelen JJ, Kitts CL, Piccolo BD, La Frano MR, Burrin DG, Maj M, Manjarin R. Dysregulated FXR-FGF19 signaling and choline metabolism are associated with gut dysbiosis and hyperplasia in a novel pig model of pediatric NASH. *Am J Physiol Gastrointest Liver Physiol* 2020; **318**: G582-G609 [PMID: 32003601 DOI: 10.1152/ajpgi.00344.2019]

96 **Mouzaki M**, Comelli EM, Arendt BM, Bonengel J, Fung SK, Fischer SE, McGilvray ID, Allard JP. Intestinal microbiota in patients with nonalcoholic fatty liver disease. *Hepatology* 2013; **58**: 120-127 [PMID: 23401313 DOI: 10.1002/hep.26319]

97 **Del Chierico F**, Nobili V, Vernocchi P, Russo A, De Stefanis C, Gnani D, Furlanello C, Zandonà A, Paci P, Capuani G, Dallapiccola B, Miccheli A, Alisi A, Putignani L. Gut microbiota profiling of pediatric nonalcoholic fatty liver disease and obese patients unveiled by an integrated meta-omics-based approach. *Hepatology* 2017; **65**: 451-464 [PMID: 27028797 DOI: 10.1002/hep.28572]

98 **Schwimmer JB**, Johnson JS, Angeles JE, Behling C, Belt PH, Borecki I, Bross C, Durelle J, Goyal NP, Hamilton G, Holtz ML, Lavine JE, Mitreva M, Newton KP, Pan A, Simpson PM, Sirlin CB, Sodergren E, Tyagi R, Yates KP, Weinstock GM, Salzman NH. Microbiome Signatures Associated With Steatohepatitis and Moderate to Severe Fibrosis in Children With Nonalcoholic Fatty Liver Disease. *Gastroenterology* 2019; **157**: 1109-1122 [PMID: 31255652 DOI: 10.1053/j.gastro.2019.06.028]

99 **Stanislawski MA**, Lozupone CA, Wagner BD, Eggesbø M, Sontag MK, Nusbacher NM, Martinez M, Dabelea D. Gut microbiota in adolescents and the association with fatty liver: the EPOCH study. *Pediatr Res* 2018; **84**: 219-227 [PMID: 29538359 DOI: 10.1038/pr.2018.32]

100 **Turnbaugh PJ**, Ley RE, Mahowald MA, Magrini V, Mardis ER, Gordon JI. An obesity-associated gut microbiome with increased capacity for energy harvest. *Nature* 2006; **444**: 1027-1031 [PMID: 17183312 DOI: 10.1038/nature05414]

101 **Loomba R**, Seguritan V, Li W, Long T, Klitgord N, Bhatt A, Dulai PS, Caussy C, Bettencourt R, Highlander SK, Jones MB, Sirlin CB, Schnabl B, Brinkac L, Schork N, Chen CH, Brenner DA, Biggs W, Yooseph S, Venter JC, Nelson KE. Gut Microbiome-Based Metagenomic Signature for Non-invasive Detection of Advanced Fibrosis in Human Nonalcoholic Fatty Liver Disease. *Cell Metab* 2019; **30**: 607 [PMID: 31484056 DOI: 10.1016/j.cmet.2019.08.002]

102 **Michail S**, Lin M, Frey MR, Fanter R, Paliy O, Hilbush B, Reo NV. Altered gut microbial energy and metabolism in children with non-alcoholic fatty liver disease. *FEMS Microbiol Ecol* 2015; **91**: 1-9 [PMID: 25764541 DOI: 10.1093/femsec/fiu002]

103 **Zhao Y**, Zhou J, Liu J, Wang Z, Chen M, Zhou S. Metagenome of Gut Microbiota of Children With Nonalcoholic Fatty Liver Disease. *Front Pediatr* 2019; **7**: 518 [PMID: 31921729 DOI: 10.3389/fped.2019.00518]

104 **Compare D**, Coccoli P, Rocco A, Nardone OM, De Maria S, Cartenì M, Nardone G. Gut--liver axis: the impact of gut microbiota on non alcoholic fatty liver disease. *Nutr Metab Cardiovasc Dis* 2012; **22**: 471-476 [PMID: 22546554 DOI: 10.1016/j.numecd.2012.02.007]

105 **He X**, Ji G, Jia W, Li H. Gut Microbiota and Nonalcoholic Fatty Liver Disease: Insights on Mechanism and Application of Metabolomics. *Int J Mol Sci* 2016; **17**: 300 [PMID: 26999104 DOI: 10.3390/ijms17030300]

106 **Seki E**, Schnabl B. Role of innate immunity and the microbiota in liver fibrosis: crosstalk between the liver and gut. *J Physiol* 2012; **590**: 447-458 [PMID: 22124143 DOI: 10.1113/jphysiol.2011.219691]

107 **Iruzubieta P**, Medina JM, Fernández-López R, Crespo J, de la Cruz F. A Role for Gut Microbiome Fermentative Pathways in Fatty Liver Disease Progression. *J Clin Med* 2020; **9** [PMID: 32392712 DOI: 10.3390/jcm9051369]

108 **Zhang X**, Asllanaj E, Amiri M, Portilla-Fernandez E, Bramer WM, Nano J, Voortman T, Pan Q, Ghanbari M. Deciphering the role of epigenetic modifications in fatty liver disease: A systematic review. *Eur J Clin Invest* 2021; **51**: e13479 [PMID: 33350463 DOI: 10.1111/eci.13479]

109 **Pirola CJ**, Gianotti TF, Burgueño AL, Rey-Funes M, Loidl CF, Mallardi P, Martino JS, Castaño GO, Sookoian S. Epigenetic modification of liver mitochondrial DNA is associated with histological severity of nonalcoholic fatty liver disease. *Gut* 2013; **62**: 1356-1363 [PMID: 22879518 DOI: 10.1136/gutjnl-2012-302962]

110 **de Mello VD**, Matte A, Perfilyev A, Männistö V, Rönn T, Nilsson E, Käkelä P, Ling C, Pihlajamäki J. Human liver epigenetic alterations in non-alcoholic steatohepatitis are related to insulin action. *Epigenetics* 2017; **12**: 287-295 [PMID: 28277977 DOI: 10.1080/15592294.2017.1294305]

111 **Kitamoto T**, Kitamoto A, Ogawa Y, Honda Y, Imajo K, Saito S, Yoneda M, Nakamura T, Nakajima A, Hotta K. Targeted-bisulfite sequence analysis of the methylation of CpG islands in genes encoding PNPLA3, SAMM50, and PARVB of patients with non-alcoholic fatty liver disease. *J Hepatol* 2015; **63**: 494-502 [PMID: 25776890 DOI: 10.1016/j.jhep.2015.02.049]

112 **Zeybel M**, Hardy T, Robinson SM, Fox C, Anstee QM, Ness T, Masson S, Mathers JC, French J, White S, Mann J. Differential DNA methylation of genes involved in fibrosis progression in non-alcoholic fatty liver disease and alcoholic liver disease. *Clin Epigenetics* 2015; **7**: 25 [PMID: 25859289 DOI: 10.1186/s13148-015-0056-6]

113 **Sookoian S**, Rosselli MS, Gemma C, Burgueño AL, Fernández Gianotti T, Castaño GO, Pirola CJ. Epigenetic regulation of insulin resistance in nonalcoholic fatty liver disease: impact of liver methylation of the peroxisome proliferator-activated receptor γ coactivator 1α promoter. *Hepatology* 2010; **52**: 1992-2000 [PMID: 20890895 DOI: 10.1002/hep.23927]

114 **Ma J**, Nano J, Ding J, Zheng Y, Hennein R, Liu C, Speliotes EK, Huan T, Song C, Mendelson MM, Joehanes R, Long MT, Liang L, Smith JA, Reynolds LM, Ghanbari M, Muka T, van Meurs JBJ, Alferink LJM, Franco OH, Dehghan A, Ratliff S, Zhao W, Bielak L, Kardia SLR, Peyser PA, Ning H, VanWagner LB, Lloyd-Jones DM, Carr JJ, Greenland P, Lichtenstein AH, Hu FB, Liu Y, Hou L, Darwish Murad S, Levy D. A Peripheral Blood DNA Methylation Signature of Hepatic Fat Reveals a Potential Causal Pathway for Nonalcoholic Fatty Liver Disease. *Diabetes* 2019; **68**: 1073-1083 [PMID: 30936141 DOI: 10.2337/DB18-1193]

115 **Nano J**, Ghanbari M, Wang W, de Vries PS, Dhana K, Muka T, Uitterlinden AG, van Meurs JBJ, Hofman A; BIOS consortium, Franco OH, Pan Q, Murad SD, Dehghan A. Epigenome-Wide Association Study Identifies Methylation Sites Associated With Liver Enzymes and Hepatic Steatosis. *Gastroenterology* 2017; **153**: 1096-1106.e2 [PMID: 28624579 DOI: 10.1053/j.gastro.2017.06.003]

116 **Mwinyi J**, Boström AE, Pisanu C, Murphy SK, Erhart W, Schafmayer C, Hampe J, Moylan C, Schiöth HB. NAFLD is associated with methylation shifts with relevance for the expression of genes involved in lipoprotein particle composition. *Biochim Biophys Acta Mol Cell Biol Lipids* 2017; **1862**: 314-323 [PMID: 27993651 DOI: 10.1016/j.bbalip.2016.12.005]

117 **Geurtsen ML**, Jaddoe VWV, Salas LA, Santos S, Felix JF. Newborn and childhood differential DNA methylation and liver fat in school-age children. *Clin Epigenetics* 2019; **12**: 3 [PMID: 31892367 DOI: 10.1186/s13148-019-0799-6]

118 **Yamada H**, Suzuki K, Ichino N, Ando Y, Sawada A, Osakabe K, Sugimoto K, Ohashi K, Teradaira R, Inoue T, Hamajima N, Hashimoto S. Associations between circulating microRNAs (miR-21, miR-34a, miR-122 and miR-451) and non-alcoholic fatty liver. *Clin Chim Acta* 2013; **424**: 99-103 [PMID: 23727030 DOI: 10.1016/j.cca.2013.05.021]

119 **Cheung O**, Puri P, Eicken C, Contos MJ, Mirshahi F, Maher JW, Kellum JM, Min H, Luketic VA, Sanyal AJ. Nonalcoholic steatohepatitis is associated with altered hepatic MicroRNA expression. *Hepatology* 2008; **48**: 1810-1820 [PMID: 19030170 DOI: 10.1002/hep.22569]

120 **Pirola CJ**, Fernández Gianotti T, Castaño GO, Mallardi P, San Martino J, Mora Gonzalez Lopez Ledesma M, Flichman D, Mirshahi F, Sanyal AJ, Sookoian S. Circulating microRNA signature in non-alcoholic fatty liver disease: from serum non-coding RNAs to liver histology and disease pathogenesis. *Gut* 2015; **64**: 800-812 [PMID: 24973316 DOI: 10.1136/gutjnl-2014-306996]

121 **Pirola CJ**, Sookoian S. Multiomics biomarkers for the prediction of nonalcoholic fatty liver disease severity. *World J Gastroenterol* 2018; **24**: 1601-1615 [PMID: 29686467 DOI: 10.3748/wjg.v24.i15.1601]

122 **Prats-Puig A**, Ortega FJ, Mercader JM, Moreno-Navarrete JM, Moreno M, Bonet N, Ricart W, López-Bermejo A, Fernández-Real JM. Changes in circulating microRNAs are associated with childhood obesity. *J Clin Endocrinol Metab* 2013; **98**: E1655-E1660 [PMID: 23928666 DOI: 10.1210/jc.2013-1496]

123 **Can U**, Buyukinan M, Yerlikaya FH. The investigation of circulating microRNAs associated with lipid metabolism in childhood obesity. *Pediatr Obes* 2016; **11**: 228-234 [PMID: 26223376 DOI: 10.1111/ijpo.12050]

124 **Cui X**, You L, Zhu L, Wang X, Zhou Y, Li Y, Wen J, Xia Y, Ji C, Guo X. Change in circulating microRNA profile of obese children indicates future risk of adult diabetes. *Metabolism* 2018; **78**: 95-105 [PMID: 28966078 DOI: 10.1016/j.metabol.2017.09.006]

125 **Iacomino G**, Russo P, Marena P, Lauria F, Venezia A, Ahrens W, De Henauw S, De Luca P, Foraita R, Günther K, Lissner L, Molnár D, Moreno LA, Tornaritis M, Veidebaum T, Siani A. Circulating microRNAs are associated with early childhood obesity: results of the I.Family Study. *Genes Nutr* 2019; **14**: 2 [PMID: 30651891 DOI: 10.1186/s12263-018-0622-6]

126 **Sheldon RD**, Kanosky KM, Wells KD, Miles L, Perfield JW 2nd, Xanthakos S, Inge TH, Rector RS. Transcriptomic differences in intra-abdominal adipose tissue in extremely obese adolescents with different stages of NAFLD. *Physiol Genomics* 2016; **48**: 897-911 [PMID: 27764764 DOI: 10.1152/physiolgenomics.00020.2016]

127 **Baselli GA**, Dongiovanni P, Rametta R, Meroni M, Pelusi S, Maggioni M, Badiali S, Pingitore P, Maurotti S, Montalcini T, Taliento AE, Prati D, Rossi G, Fracanzani AL, Mancina RM, Romeo S, Valenti L. Liver transcriptomics highlights interleukin-32 as novel NAFLD-related cytokine and candidate biomarker. *Gut* 2020; **69**: 1855-1866 [PMID: 32001554

DOI: 10.1136/gutjnl-2019-319226]

128 **Perakakis N**, Stefanakis K, Mantzoros CS. The role of omics in the pathophysiology, diagnosis and treatment of non-alcoholic fatty liver disease. *Metabolism* 2020; **111S**: 154320 [PMID: 32712221 DOI: 10.1016/j.metabol.2020.154320]

129 **Cusi K**, Chang Z, Harrison S, Lomonaco R, Bril F, Orsak B, Ortiz-Lopez C, Hecht J, Feldstein AE, Webb A, Louden C, Goros M, Tio F. Limited value of plasma cytokeratin-18 as a biomarker for NASH and fibrosis in patients with non-alcoholic fatty liver disease. *J Hepatol* 2014; **60**: 167-174 [PMID: 23973932 DOI: 10.1016/j.jhep.2013.07.042]

130 **Sookoian S**, Castaño G, Burgueño AL, Gianotti TF, Rosselli MS, Pirola CJ. A diagnostic model to differentiate simple steatosis from nonalcoholic steatohepatitis based on the likelihood ratio form of Bayes theorem. *Clin Biochem* 2009; **42**: 624-629 [PMID: 19071103 DOI: 10.1016/j.clinbiochem.2008.11.005]

131 **Rodriguez-Suarez E**, Mato JM, Elortza F. Proteomics analysis of human nonalcoholic fatty liver. *Methods Mol Biol* 2012; **909**: 241-258 [PMID: 22903720 DOI: 10.1007/978-1-61779-959-4_16]

132 **Younossi ZM**, Baranova A, Ziegler K, Del Giacco L, Schlauch K, Born TL, Elariny H, Gorreta F, VanMeter A, Younoszai A, Ong JP, Goodman Z, Chandhoke V. A genomic and proteomic study of the spectrum of nonalcoholic fatty liver disease. *Hepatology* 2005; **42**: 665-674 [PMID: 16116632 DOI: 10.1002/hep.20838]

133 **Ulukaya E**, Yilmaz Y, Moshkovskii S, Karpova M, Pyatnitskiy M, Atug O, Dolar E. Proteomic analysis of serum in patients with non-alcoholic steatohepatitis using matrix-assisted laser desorption ionization time-of-flight mass spectrometry. *Scand J Gastroenterol* 2009; **44**: 1471-1476 [PMID: 19883279 DOI: 10.3109/00365520903353379]

134 **Małecki P**, Tracz J, Łuczak M, Figlerowicz M, Mazur-Melewska K, Służewski W, Mania A. Serum proteome assessment in nonalcoholic fatty liver disease in children: a preliminary study. *Expert Rev Proteomics* 2020; **17**: 623-632 [PMID: 32921203 DOI: 10.1080/14789450.2020.1810020]

135 **Bălănescu A**, Stan I, Codreanu I, Comănici V, Bălănescu E, Bălănescu P. Circulating Hsp90 Isoform Levels in Overweight and Obese Children and the Relation to Nonalcoholic Fatty Liver Disease: Results from a Cross-Sectional Study. *Dis Markers* 2019; **2019**: 9560247 [PMID: 31885746 DOI: 10.1155/2019/9560247]

136 **Blomme B**, Fitzpatrick E, Quaglia A, De Bruyne R, Dhawan A, Van Vlierberghe H. Serum protein N-glycosylation in paediatric non-alcoholic fatty liver disease. *Pediatr Obes* 2012; **7**: 165-173 [PMID: 22434757 DOI: 10.1111/j.2047-6310.2011.00024.x]

137 **Li J**, Hsu HC, Mountz JD, Allen JG. Unmasking Fucosylation: from Cell Adhesion to Immune System Regulation and Diseases. *Cell Chem Biol* 2018; **25**: 499-512 [PMID: 29526711 DOI: 10.1016/j.chembiol.2018.02.005]

138 **Reily C**, Stewart TJ, Renfrow MB, Novak J. Glycosylation in health and disease. *Nat Rev Nephrol* 2019; **15**: 346-366 [PMID: 30858582 DOI: 10.1038/s41581-019-0129-4]

139 **Hollie NI**, Cash JG, Matlib MA, Wortman M, Basford JE, Abplanalp W, Hui DY. Micromolar changes in lysophosphatidylcholine concentration cause minor effects on mitochondrial permeability but major alterations in function. *Biochim Biophys Acta* 2014; **1841**: 888-895 [PMID: 24315825 DOI: 10.1016/j.bbalip.2013.11.013]

140 **Li Z**, Agellon LB, Allen TM, Umeda M, Jewell L, Mason A, Vance DE. The ratio of phosphatidylcholine to phosphatidylethanolamine influences membrane integrity and steatohepatitis. *Cell Metab* 2006; **3**: 321-331 [PMID: 16679290 DOI: 10.1016/j.cmet.2006.03.007]

141 **Zhou Y**, Orešič M, Leivonen M, Gopalacharyulu P, Hyysalo J, Arola J, Verrijken A, Francque S, Van Gaal L, Hyötyläinen T, Yki-Järvinen H. Noninvasive Detection of Nonalcoholic Steatohepatitis Using Clinical Markers and Circulating Levels of Lipids and Metabolites. *Clin Gastroenterol Hepatol* 2016; **14**: 1463-1472.e6 [PMID: 27317851 DOI: 10.1016/j.cgh.2016.05.046]

142 **Holland WL**, Summers SA. Sphingolipids, insulin resistance, and metabolic disease: new insights from in vivo manipulation of sphingolipid metabolism. *Endocr Rev* 2008; **29**: 381-402 [PMID: 18451260 DOI: 10.1210/er.2007-0025]

143 **Marí M**, Colell A, Morales A, Caballero F, Moles A, Fernández A, Terrones O, Basañez G, Antonsson B, García-Ruiz C, Fernández-Checa JC. Mechanism of mitochondrial glutathione-dependent hepatocellular susceptibility to TNF despite NF-kappaB activation. *Gastroenterology* 2008; **134**: 1507-1520 [PMID: 18343380 DOI: 10.1053/j.gastro.2008.01.073]

144 **Moles A**, Tarrats N, Morales A, Domínguez M, Bataller R, Caballería J, García-Ruiz C, Fernández-Checa JC, Marí M. Acidic sphingomyelinase controls hepatic stellate cell activation and in vivo liver fibrogenesis. *Am J Pathol* 2010; **177**: 1214-1224 [PMID: 20651240 DOI: 10.2353/ajpath.2010.091257]

145 **Marzuillo P**, Grandone A, Perrone L, Miraglia Del Giudice E. Controversy in the diagnosis of pediatric non-alcoholic fatty liver disease. *World J Gastroenterol* 2015; **21**: 6444-6450 [PMID: 26074683 DOI: 10.3748/wjg.v21.i21.6444]

146 **Kang SH**, Cho Y, Jeong SW, Kim SU, Lee JW; Korean NAFLD Study Group. From nonalcoholic fatty liver disease to metabolic-associated fatty liver disease: Big wave or ripple? *Clin Mol Hepatol* 2021; **27**: 257-269 [PMID: 33751877 DOI: 10.3350/cmh.2021.0067]

147 **Di Sessa A**, Guarino S, Umano GR, Arenella M, Alfiero S, Quaranta G, Miraglia Del Giudice E, Marzuillo P. MAFLD in Obese Children: A Challenging Definition. *Children (Basel)* 2021; **8** [PMID: 33806784 DOI: 10.3390/children8030247]

World Journal of Clinical Pediatrics

Submit a Manuscript: https://www.f6publishing.com

World J Clin Pediatr 2022 May 9; 11(3): 239-252

DOI: 10.5409/wjcp.v11.i3.239

ISSN 2219-2808 (online)

MINIREVIEWS

Management of sleep disorders among children and adolescents with neurodevelopmental disorders: A practical guide for clinicians

Michael O Ogundele, Chinnaiah Yemula

Specialty type: Pediatrics

Provenance and peer review: Invited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): 0
Grade B (Very good): B
Grade C (Good): 0
Grade D (Fair): 0
Grade E (Poor): 0

P-Reviewer: Xiao CF, China

Received: March 23, 2021
Peer-review started: March 23, 2021
First decision: July 27, 2021
Revised: August 9, 2021
Accepted: March 25, 2022
Article in press: March 25, 2022
Published online: May 9, 2022

Michael O Ogundele, Department of Community Paediatrics, Bridgewater Community Healthcare NHS Foundation Trust, Halton District, Runcorn WA7 1TW, United Kingdom

Chinnaiah Yemula, Department of Community Paediatrics, Bedfordshire Community Health Services, Cambridgeshire Community Services NHS Trust, St Ives PE27 4LG, United Kingdom

Corresponding author: Michael O Ogundele, MBBS, MRCPCH, MSc, Doctor, Department of Community Paediatrics, Bridgewater Community Healthcare NHS Foundation Trust, Halton District, Lister Road, Runcorn WA7 1TW, United Kingdom. m.ogundele@nhs.net

Abstract

There is a complex relationship between sleep disorders and childhood neurodevelopmental, emotional, behavioral and intellectual disorders (NDEBID). NDEBID include several conditions such as attention deficit/hyperactivity disorder, autism spectrum disorder, cerebral palsy, epilepsy and learning (intellectual) disorders. Up to 75% of children and young people (CYP) with NDEBID are known to experience different types of insomnia, compared to 3% to 36% in normally developing population. Sleep disorders affect 15% to 19% of adolescents with no disability, in comparison with 26% to 36% among CYP with moderate learning disability (LD) and 44% among those with severe LD. Chronic sleep deprivation is associated with significant risks of behavioural problems, impaired cognitive development and learning abilities, poor memory, mood disorders and school problems. It also increases the risk of other health outcomes, such as obesity and metabolic consequences, significantly impacting on the wellbeing of other family members. This narrative review of the extant literature provides a brief overview of sleep physiology, aetiology, classification and prevalence of sleep disorders among CYP with NDEBIDs. It outlines various strategies for the management, including parenting training/psychoeducation, use of cognitive-behavioral strategies and pharmacotherapy. Practical management including assessment, investigations, care plan formulation and follow-up are outlined in a flow chart.

Key Words: Sleep; Emotional; Behavioural difficulties; Neurodevelopmental disorders; Pharmacotherapy; Non-pharmacologic interventions; Cognitive therapy; Melatonin; Adolescents; Psychoeducation

Core Tip: Up to 75% of children and young people with neurodevelopmental, emotional, behavioural and intellectual disorders (NDEBID) are known to experience different types of insomnia, associated with significant behavioral, emotional, cognitive and academic impairments, as well as negative impact on the wellbeing of other family members. This paper provides a brief overview of sleep physiology, aetiology, classification and prevalence of sleep disorders among children and adolescents with NDEBIDs. It outlines different strategies for the management of sleep disorders, including parenting training/psychoeducation, the use of cognitive-behavioural strategies and pharmacotherapy. Practical management including clinical assessment, investigations, care plan formulation and follow-up are outlined.

Citation: Ogundele MO, Yemula C. Management of sleep disorders among children and adolescents with neurodevelopmental disorders: A practical guide for clinicians. *World J Clin Pediatr* 2022; 11(3): 239-252
URL: https://www.wjgnet.com/2219-2808/full/v11/i3/239.htm
DOI: https://dx.doi.org/10.5409/wjcp.v11.i3.239

INTRODUCTION

Sleep problems are common in children from preschool age to adolescence, especially among those who have recognizable neurodevelopmental (and related neurodisability), emotional, behavioural and intellectual disorders (NDEBID). The prevalence of sleep problems among typically developing children and adolescents ranges from 3% to 36%, while affecting up to three-quarters of children with NDEBIDs, depending on the diagnostic criteria used[1,2].

There is a complex relationship between sleep disorders and childhood NDEBID. Sleep deprivation is known to cause clinically elevated externalizing and internalizing behaviour disorders, including inattention, mood variability, disruptive and rule-breaking behaviours, and school problems[3]. It can also affect children's cognitive development and learning abilities, by exacerbating memory and concentration problems, and mood disorders[4,5]. There is clear evidence that various sleep disturbances among children and adolescents increase the risk of mental health disorders such as depression, suicidal and self-harm behaviours, as well as other psychiatric and health outcomes including obesity and metabolic disorders[6]. It can negatively impact the cardiovascular, immune and metabolic systems, including growth disorders[7].

Sleep disorders in children also significantly affect the wellbeing of other family members. Among a cohort of 156 care-givers of children aged 1.5 to 10 years with insomnia, 47% of primary caregivers had clinically significant parenting stress associated with bedtime resistance, daytime sleepiness, parent history of sleep problems, parent history of psychiatric conditions, and child externalizing behaviour[8].

Management of sleep problems is important for long-term mental health and optimization of functioning, prevention of deficits in daily functioning and for halting the progression of psychiatric pathology of affected children and young people (CYP) into adulthood[4,9]. However, healthcare professionals have insufficient training on sleep disorders[10].

This narrative literature review presents important themes identified from search of electronic databases including PubMed, PubMed Medical Central, OVID, EMBASE, PsycINFO and Cochrane databases up to October 2020, using combinations of keywords including 'melatonin', 'ASD', 'developmental disorder', 'ADHD', 'sleep disorder' and 'children'.

It provides a brief overview of the research evidence on the diagnosis and management of sleep disorders among CYP with NDEBID conditions such as attention deficit/hyperactivity disorder (ADHD), autism spectrum disorder (ASD), cerebral palsy (CP), epilepsy and learning (intellectual) disorders.

SLEEP PHYSIOLOGY

Definitions and classifications of sleep disorders

Various definitions of sleep disorders have been used in sleep studies in terms of age, frequency, severity, and duration of symptoms and sample populations[6]. Some studies define insomnia vaguely as parental report of difficulty falling and/or staying asleep[3]. Furthermore, there is considerable variability in children's sleep duration.

Both the 5th edition of Diagnostic and Statistical Manual of Mental Disorders (DSM-5)[11] and the 3rd edition of the International Classification of Sleep disorders (ICSD-3)[12] are the key reference standards for the diagnosis of sleep disorders. Paediatric insomnia has been defined as "repeated difficulty with sleep initiation, duration, consolidation, or quality that occurs despite age-appropriate time and opportunity for sleep and results in daytime functional impairment for the child and/or family"[6]. The ICSD-3 classification includes 6 categories (Table 1).

Why is sleep important?

Sleep is essential to refresh and rejuvenate the body and mind. An average person spends a third of their life sleeping (122 d every year). It is a good practice to emphasise the benefits of sleep to provide a positive message to children, parents, and carers. Table 2 illustrates positive effects of adequate sleep and negative consequences of insomnia.

How much sleep is ideal for children and adolescents?

There is a wide variation about sleep requirement dependent on the child's age. It is important for health professionals to discuss and provide parents/carers and children written information about sleep duration as in Table 3.

Aetiology and pathogenesis of sleep disorders

The aetiology of sleep disturbances in CYP with NDEBID is heterogeneous and often disease specific. The diagnosis and management of sleep disorders in this population are complex, and little high-quality data exist to guide a consistent approach to therapy[13]. Three main causes of insomnia are biologic, behavioural (including environmental) and psycho-medical[14]. Table 1 shows common causes and examples of sleep disorders.

Chronic sleep deprivation, insomnia, and delayed sleep phase disorder are the commonest sleep disorders in childhood[9]. Other common sleep problems in children with NDEBIDs include difficulty falling asleep, difficulty maintaining sleep, and early morning awakenings[15].

SLEEP DISORDERS AND NDEBID

What are NDEBID?

Childhood NDEBID such as ADHD, tic disorder/Tourettes syndrome, developmental delay, development coordination disorder are commonly managed by Community Child Health Paediatricians, working within integrated teams involving the education, social care and voluntary sectors[16]. Neurodisability describes a group of congenital or acquired long-term conditions that are attributed to disturbance of the brain and or neuromuscular system and create functional limitations in sensory, motor, speech, language, cognition or behaviour. The estimated prevalence of NDEBID reported in developed countries varies widely, ranging up to 15%, depending on the diverse methodologies and definitions used[17,18].

Prevalence of sleep disorders in NDEBID

Sleep disorders in ASD: ASD is an heterogenous group of neurodevelopmental disorders (NDD) caused by a combination of genetic variation with complex interactions with environmental factors.

Some studies have found that sleep disturbance is the second most common physical co-morbidity in children with ASD, with prevalence estimated to be between 33% and 81%[15,19,20]. Sleep disturbances in CYP with ASD are significantly associated with severity of autism symptoms and deterioration in daytime challenging behaviour including physical aggression, irritability, inattention, and hyperactivity [20-22].

The causes of poor sleep in CYP with ASD are multifactorial and include disturbances in neurotransmitters that promote sleep, including serotonin and melatonin, abnormal sensitization to environmental stimuli, behavioural insomnia and delayed sleep phase syndromes (DSPSs), rapid eye movement sleep behaviour disorders, decreased time in bed, increased proportion of stage 1 sleep, as well as coexisting psychiatric symptoms, such as anxiety, depression, and epilepsy[23-25]. The core behavioural deficits associated with ASD could impair the establishment of sound bedtime behaviours and routines. The parents may also struggle with arranging the sleep environment to promote sleep and conveying sleep expectations effectively, while trying to deal with multiple other priorities and stressors[15].

Recent meta-analysis of 38 published studies on various non-pharmacological strategies for management of sleep disorders among CYP with ASD has shown conclusively that no single intervention is reliably effective in managing all the wide range of sleep problems seen in this group of individuals[26]. A recent clinical guideline from the American Academy of Neurology (AAN) concluded that behavioural strategies should be offered as first-line treatment approach for sleep disturbance in CYP with autism, either alone or in combination with pharmacologic treatment with melatonin [with or without cognitive behavioural therapy (CBT)][27].

Table 1 Showing majority of sleep disorders can be grouped into 6 main categories

Category	Description		Conditions and causes, some examples
Insomnias	Inability to fall asleep or stay asleep		Environmental: Poor sleep hygiene, bedroom noise, bright light. Behavioural insomnia of childhood (sleep onset/limit setting/combined). Psychiatric, trauma and substance misuse: Anxiety, depression, OCD, PTSD, abuse or neglect, bullying, drug and substance misuse. Medical: Pain (headaches, joint pains), lung problems (asthma, cystic fibrosis), skin (eczema, allergies), neuromuscular, obesity, medication side effects
Sleep related breathing disorders	Breathing difficulties during sleep		Obstructive sleep apnoea. Central sleep apnoea
Central disorders of hypersomnolence	Excessively sleepy		Narcolepsy
Circadian rhythm sleep-wake disorders	Sleep times are out of alignment		Delayed sleep phase syndrome. Jet lag
Parasomnias	Unwanted events or experiences that occur at the time of falling asleep, sleeping or waking up	During NREM sleep	Confusional arousals. Sleep terrors. Sleep-walking
		During REM sleep	Nightmares
		Others	Enuresis
Sleep related movement disorders	Unusual body movements during sleep		Bruxism. Restless legs syndrome. Periodic limb movement disorder. Rhythmic movement disorder (head banging, body rocking)

OCD: Obsessive-compulsive disorder; PTSD: Post traumatic stress disorder; NREM: Non-rapid eye movement; REM: Rapid-eye-movement.

Table 2 Showing positive effects and negative consequences

Positive effects of adequate and good quality sleep	Negative consequences of lack of adequate and good quality sleep
Promotes growth. Strengthens immunity. Helps cell growth and body repair. Consolidates memory (https://www.sleepscotland.org/support/gateway-to-good-sleep/why-is-sleep-important/). Promotes learning and cognitive development[69]. Maintains physical health and emotional wellbeing	Increased association with excess weight gain and obesity [69]. Impairs immune function. Affects physical coordination. Affects ability to learn new information and problem solve. Affects mood and emotional regulation and increases risk of mental health problems *e.g.*, mood or anxiety disorder, suicidal ideation

Table 3 Showing National Sleep Foundation's sleep duration recommendations (https://www.sleepfoundation.org/press-release/national-sleep-foundation-recommends-new-sleep-times)

Age of the child	Recommended	May be appropriate	Not recommended
Pre-schoolers (3-5 yr)	10-13 h	8-14 h	Less than 8 h or more than 14 h
School-aged children (6-13 yr)	9-11 h	7-12 h	Less than 7 h or more than 12 h
Teenagers (14-17 yr)	8-10 h	7-11 h	Less than 7 h or more than 11 h

Sleep disorders in ADHD: ADHD affects approximately 5% of CYP worldwide[28]. The brain regions, such as dorsolateral and ventrolateral prefrontal and dorsal anterior cingulate cortices, implicated in ADHD pathophysiology, are known to be sensitive to sleep deprivation. Genetics studies have also pointed to the involvement of the catecholaminergic system in both ADHD and sleep regulation[29].

Common sleep problems affecting up to 70% of paediatric ADHD patients include behaviourally based insomnia (limit-setting disorder), bedtime resistance, latency of sleep onset, dim light melatonin onset delay, decreased duration of sleep, increased number of overnight awakenings, daytime somnolence, sleep-disordered breathing, and restless legs syndrome (RLS)/periodic limb movement disorder (PLMD)[30,31]. They may also have sleep disturbances due to co-morbid psychiatric disorders or ADHD medications such as delayed sleep onset and shortened sleep duration[32,33]. In a study of 195 children with ADHD aged 5 to 13 years, sleep problem was observed to be variable over a 12-mo period in 60% of the children and transient in most cases but it was more persistent in a sub-group (10%) of the children[34].

Table 4 Below illustrates some useful resources

Users	Resources	Free access	Website links
Parents and carers	CEREBRA-Sleep Advice service	Free access	https://cerebra.org.uk/get-advice-support/sleep-advice-service/
	Sleep for better day ahead-leaflet	Free access	https://www.qvh.nhs.uk/wp-content/uploads/2020/08/Sleep-for-a-better-day-ahead-0127.pdf
	Sleep hygiene in children and young people: Information for families-leaflet	Free access	https://media.gosh.nhs.uk/documents/Sleep_hygiene_F1851_FINAL_Jun20.pdf
	Encouraging good sleep habits in children with learning disabilities-leaflet	Free access	https://www.oxfordhealth.nhs.uk/wp-content/uploads/2014/05/Good-sleep-habits-for-children-with-Learning-Difficulties.pdf
	Sleep problems and sleep disorders in school aged children	Free access	https://www.sleephealthfoundation.org.au/sleep-problemsand-sleep-disorders-in-school-aged-children.html
	Further useful facts sheets and resources-website	Free access	https://www.sleephealthfoundation.org.au/fact-sheets.html
	Other websites	Free access	https://www.nhs.uk/live-well/sleep-and-tiredness/healthy-sleep-tips-for-children/; https://www.sleepscotland.org/
Adolescents	How to sleep well and stay healthy-A guide for teenagers. This is an interactive guide with animations, sounds and external links to useful educational video clips	Free access	https://books.apple.com/gb/book/how-to-sleep-well-and-stay-healthy-a-guide-for-teenagers/id1397176909
	Sleep tips for teenagers	Free access	https://www.nhs.uk/live-well/sleep-and-tiredness/sleep-tips-for-teenagers/
Children	Sleep poster: Interactive pdf for children and parents/carers	Free access	https://www.cambscommunityservices.nhs.uk/docs/default-source/Luton---NDD-Webpages/Sleep/sleep-poster76ddec06f4f66239b188ff0000d24525.pdf?sfvrsn=2
	I see the animals sleeping: A bedtime story-an app	Free on Google play, App store and Kindle store	http://school.sleepeducation.com/childrensapps.aspx
	The animal sleep: A bedtime book for biomes-an app	Free on Google play, App store and Kindle store	http://school.sleepeducation.com/childrensapps.aspx; https://www.youtube.com/watch?v=zLQ3bkn8Gu8

ADHD is most commonly treated using psychostimulants, with potential side-effects including sleep disorders. Use of psychostimulants may however improve some aspects of sleep in ADHD children[2].

Moderate to severe sleep problems have been associated with increasing ADHD severity and poorer child quality of life (QoL), daily functioning and caregiver mental health, increased likelihood of missed/being late for school, and the caregivers being late for work[30]. Disorders of sleeping pattern is associated with inattention, problematic behaviour, progressive psychopathology, and attenuated emotional regulation, all of which can mimic the symptoms of ADHD. It is therefore necessary for the clinician to assess for sleep problems before confirming a diagnosis of ADHD[33].

The management of sleep disorders in ADHD children include recommendation of good sleep hygiene and other behavioural interventions as the first-line treatment option[33]. There is ample evidence for the effectiveness of behavioural interventions from several studies. Sixty-seven percent of parents of children with ADHD reported complete resolution, with improved child QoL, daily functioning and parental anxiety, five months after randomization into two groups of either brief (1 session, n = 13) or extended (2-3 sessions, n = 14) behavioural sleep programme[30]. Similar findings as well as improvement of ADHD symptoms have been reported[35].

Other strategies include modifying the dose regimens, formulation, or use of alternative to stimulants such non-stimulant atomoxetine and alpha agonists guanfacine or clonidine, and melatonin[32]. Combined strategy of behaviour modification techniques with use of stimulant medication have been reported to yield sustained improvement in ADHD symptoms, sleep duration, and QoL in a randomized controlled trial (RCT) of 244 children with ADHD[36].

There is lack of robust and reliable evidence for prescribing drugs for behavioural insomnia in children with ADHD. A systematic review of 12 studies, mostly of low quality, was recently reported for the pharmacological treatment of insomnia in CYP with ADHD[37]. The strongest evidence from published literature supports the use of melatonin in reducing sleep-onset delay, but the evidence for

other medications is weaker, with reported significant advancement of sleep onset by 26.9 ± 47.8 min and advancement of dim light melatonin onset by 44.4 ± 67.9 min, when compared to placebo[33,38]. From a recent systematic review of 12 studies including RCTs and observational studies, clonidine, melatonin and L-Theanine demonstrated positive responses in sleep-onset latency and total sleep duration while zolpidem, eszopiclone and guanfacine failed to show significant efficacy when compared with placebo. Zolpidem was associated with neuropsychiatric adverse effects[37].

Sleep disorder in epilepsy and other chronic disabilities: Insomnia, especially maintenance insomnia, is widely prevalent in epilepsy and other chronic conditions. Some expert opinions and a few small studies have presented inconclusive findings suggesting that melatonin either lowers or increases seizure thresholds[39].

MANAGEMENT OF SLEEP DISORDER IN CYP WITH NDEBID

Published clinical guidelines

The American Academy of Pediatrics published a consensus document on pharmacologic management of insomnia in 2006, which focused mainly on future research recommendations[40]. The Sleep Committee of the Autism Treatment Network later developed an expert consensus practice pathway in 2012, which documented best practices for screening, identification, and treatment for sleep problems in people with autism[41].

Other recommendations and clinical guidance have been published more recently for the management of chronic insomnia in children associated with NDD in children including Autism, CP, and genetic syndromes like Rett syndrome, Angelman syndrome, Williams syndrome, and Smith-Magenis syndrome, mostly based on consensus opinions[13]. A consensus statement has been produced by multidisciplinary professional associations in Spain[7]. A clinical practice guideline has recently been published by the AAN for management of insomnia and disrupted sleep behaviour in CYP with autism [27]. An evidence-based sleep management clinical guidance and flow chart designed by the authors is included as Supplementary Material.

Clinical assessment and triage

The diagnosis of sleep disorders in CYP is essentially clinical, based on the information provided by the parents/caregivers and the child and from detailed clinical examination[7]. In view of the high prevalence of sleep problems among CYP, it has been suggested that clinicians need to ensure that questions about sleep are incorporated into their routine health assessment of children, and try to distinguish sleep disturbances from normal age-related changes[42,43].

A clear and comprehensive history that includes all the relevant family, social, academic and lifestyle information is essential to provide an accurate differential diagnosis. History should include the sleep/wake schedule, sleeping environment and bedtime routines, abnormal movements or behaviour during sleep, daytime effects of sleep deprivation, and sleep onset latency (SOL) (which need to be differentiated from delayed circadian rhythm)[1,42]. Clinical assessment should also evaluate the primary and secondary contributing factors and maladaptive behaviours related to sleep[42]. Common parameters to be documented include: (1) Sleep-onset latency; (2) Number and duration of night wakings; and (3) Sleep efficiency (total time of sleep divided by the total time in bed). Box 2 outlines common items to be included in a detailed clinical assessment in Supplementary Material.

Previously rarely reported sleep disorders among children with NDEBID such as narcolepsy and nocturnal epilepsy should be explored, as they have been identified to be commoner than previously thought[44]. Use of validated sleep problems questionnaires including BEARS screening tool and Children's Sleep Habit Questionnaire is recommended to supplement the clinical assessments.

Detailed clinical assessment should lead to formulation of a sleep disorder diagnosis or consideration of potential differential diagnosis (see Table 1) and exclude other physical explanations for insomnia including obesity, tonsillar hypertrophy, facial dysmorphism, nasal septal deviation, craniofacial abnormalities, hypotonia, chronic rhinitis or other physical illness or discomfort (for example, reflux, ear or toothache, bedwetting, constipation or eczema).

This assessment should lead to the formulation of a sleep plan with the parents or carers. A sleep plan should include specific behavioural interventions which address the identified sleep problems and help restore a regular sleep pattern. This plan needs to be reviewed regularly until a regular sleep pattern is established.

Investigations

Clinical assessment should be supplemented by sleep diary over a 2-wk period. Diagnostic tools such as validated questionnaires, sleep diary and actigraphy are essential to properly detect sleep disorders at early stages[9].

Actigraphy monitors body motion, sleep and wake patterns in individuals. It can measure the total sleep time (TST), sleep efficiency, wake after sleep onset, and SOL, help to determine sleep patterns and document response to treatment in the patient's normal sleep environment[7].

Major indications for polysomnography include strong clinical suspicion of sleep-related breathing disorder, atypical parasomnia, PLMD, clinically unconfirmed RLS or nocturnal seizures when the clinical history and conventional encephalography are inconclusive.

Differential diagnosis

Detailed assessment should lead to formulation of a sleep disorder diagnosis or consideration of potential differential diagnosis including as follows.

RLS and PLMD: Common causes of childhood onset RLS include familial predisposition and systemic iron deficiency. Treatment options include iron supplementation and Gabapentin (researched mainly in adults). PLMD is a sleep disorder that is associated with periodic and repetitive movements of legs and less often arms during sleep. These include bending of toes, foot or ankle, kicking or jerking of legs. There is conflicting evidence on using iron therapy for RLS and PLMD in children[45]. Dopamine agonists and anticonvulsants have not been trialled in children.

Parasomnias: Arousal parasomnias such as confusional arousals are often triggered by sleep apnoea, RLS, or acid reflux. They often respond to specific treatment of these disorders. Parasomnias should be managed with reassurance and safety measures, using benzodiazepines sparingly for severe, potentially dangerous cases. Low dose clonazepam at bedtime may help resolve sleep walking and confusional arousals[46].

Obstructive sleep apnoea: Obstructive sleep apnoea (OSA) affects about 2 percent of children and any suspicion should trigger a referral to the ENT surgeons. Adeno-tonsillar hypertrophy, cranio-facial anomalies, and obesity are common predisposing factors. Mild symptoms of OSA often responds to management with a combination of nasal corticosteroids and a leukotriene antagonist. Moderate to severe OSA would require surgery (adeno-tonsillectomy), positive airway pressure breathing devices or weight reduction as required[47].

DSPS: DSPS is common and can be treated with chronotherapy, light therapy and potentially melatonin as long as the patient is motivated.

COMPREHENSIVE MANAGEMENT STRATEGIES

Most authors and professional guidelines have consistently emphasized the role of effective sleep hygiene strategies, parent and care-giver education and training and behavioral interventions as first line in the management of childhood sleep disorders, with pharmacotherapeutic treatment only considered if sleep hygiene strategies alone have failed[13,48]. The flow chart shows a recommended sleep management guidance based on the published evidence in Supplementary Material.

NON-PHARMACOLOGICAL/BEHAVIOURAL INTERVENTIONS

Non-pharmacological treatment options include sleep hygiene, behavioural interventions, parent education/training programmes, alternative therapies (such as massage therapy, aromatherapy, nutrients and multivitamin or iron supplementation) and CBT for older children and adolescents[9,26,42]. There is sufficient evidence to support the recommendation of these cognitive-behavioral strategies as the most effective approach in the management of paediatric insomnia[7,49].

The most common behavioural interventions are different types of extinction ranging from complete (total removal of reinforcement to reduce a behaviour) to various forms of graduated extinction, bedtime fading/positive routines (including positive bedtime routines, delaying the child's bedtime to match when he/she is currently falling asleep, and stimulus control techniques) and scheduled awakenings (deliberately waking and then soothing a child back to sleep 15-30 min before their typical spontaneous nocturnal awakening) (definitions and practical tips are listed in Box 4 in Supplementary Material).

Previous literature reviews have shown strong empirical evidence for the effectiveness of behavioural interventions based on learning principles when implemented in the short- or medium-term, but long-term evidence for their efficacy is limited. It is not yet possible to postulate any long-term conclusions about the effects of these treatments over time. A recent review confirmed a significant overall effect with small to medium effect size on different sleep outcomes among typically developing children of all ages, but limited evidence is available for CYP with NDEBIDs. For example, there were no clinically significant improvements for any of the studied sleep outcome measures for two trials involving children with autism or Down syndrome[6]. A meta-analysis of 16 controlled trials found small to large

Table 5 Showing drugs used to treat insomnia[17]

Pharmaceutical	Class	Mechanism of action	Half life (h)	Site of metabolism	Peak concentration	Interactions	Effect on sleep
Diphenhydramine	Antihistamine	H1 agonist. Crosses blood-brain barrier	4-6	Hepatic	Fast absorption. Fast onset of action. Peak at 2-4 h	CNS depressants	Reduces latency. May decrease quality
Hydroxyzine	Antihistamine	H1 agonist. Crosses blood-brain barrier	6-24	Hepatic	Fast absorption. Fast onset of action. Peak at 2-4 h	CNS depressants	Reduces latency. May decrease quality
Melatonin	Neuro-hormone	Hypnotic	90% excreted in 4	Hepatic	30-60 min	Unknown	Reduces latency. Maximum circadian effect
Clonazepam	Benzodiazepine	Central GABA receptors	30-40	CYP 450 3A oxidation	60-240 min	Fluoxetine	Suppresses slow-wave sleep. Reduces arousal
Flurazepam	Benzodiazepine	Central GABA receptors	2-100	CYP 450 3A oxidation	30 min to 13 h	Fluoxetine	Suppresses slow-wave sleep. Reduces arousal
Zolpidem	Z-drug	Benzodiazepine-like	2.5-3	CYP 450 3A oxidation	90 min		Reduces latency. Weak effect on sleep architecture
Clonidine	Alpha agonist	Inhibits noradrenaline release	6-24	50%-80% in urine	Fast absorption 100% bioavailability. Onset of action: 1 h. Peak effect: 2-4 h		Reduce REM. Reduces slow-wave sleep

REM: Rapid-eye-movement; CYP: Children and young people.

effect sizes for a number of sleep outcomes including SOL, number of night wakings, duration of night wakings, and sleep efficacy among typically developing children. Two studies conducted with special needs populations also showed no evidence of significant improvements[6].

A recent trial of sleep clinics offered by specialists' advice to parents over the phone and in one to one sessions, based on Behavioural non-medication social prescribing, led to CYP gaining an extra 2.4 h sleep per night, significant improvement in their mental state, time taken to get to sleep falling by more than half, and improved QoL and wellbeing of the parents and carers (NHS England, 2019). The RCT of melatonin in children with NDD and impaired sleep (MENDS) study showed that about 40% of the initial cohort of CYP with NDD did not need to proceed to randomization for melatonin treatment as they responded to one-month parent-led behavioural sleep hygiene strategies[50].

Parent-training and psychoeducation

Psychoeducation is considered a fundamental part of managing sleep problems/disorders in children and adolescents and can contribute towards better understanding of their condition, self-management strategies, partnership working, and improved compliance, resulting in positive outcomes. Table 4 below illustrates some useful resources for parents and adolescents.

Good sleep hygiene

Sleep hygiene involves proven practical strategies that parents and adolescents can implement to attain more optimal sleeping patterns. These include modifiable daytime, bedtime, and night-time practices such as diet, exercises and sleeping environment[42]. There is insufficient data to support sleep hygiene strategies as an evidence-based, stand-alone treatment[9]. Parents can also use reward charts, objects of reference such as applying parents pyjamas or perfume on teddy bear, pink or white noise (or music), night or daytime indicators such as Glo-clock or side lamps[10]. Box 1 shows tips for effective sleep hygiene in Supplementary Material.

Neurofeedback to improve sleep onset insomnia

Some authors have suggested that that Sensory-Motor Rhythm and Slow-Cortical Potential neurofeedback may have positive effect on the normalization of sleep onset insomnia, especially in children with ADHD[51].

Pharmacological treatments

Many hypnotics are widely prescribed for the management of paediatric insomnia, mostly as off-label prescriptions, with limited research evidence to determine the efficacy and safety of their use in the

medium and long term basis (Table 5)[37].

Antihistamines (alimemazine, promethazine, diphenhydramine, hydroxyzine): Antihistamine agents, including hydroxyzine or diphenhydramine, represent the most widely prescribed sedatives in the paediatric population, despite the lack of research evidence to back up their use. There is a risk of paradoxical reaction with some antihistamines. A single, small RCT of diphenhydramine reported small effect size efficacy in sleep outcomes (8-10 min improvement in sleep latency and duration) after a 1 wk trial[52].

Clonidine: Clonidine is a central alpha2-adrenergic receptor agonist, with a half-life of 6-24 h. The mechanism of its sedative effect is unclear but it has been a favorite agent employed in the treatment of sleep disorders among children with NDD despite little evidence in literature regarding its efficacy[10]. Clonidine, melatonin and L-theanine showed some improvements in SOL and TST for children with ADHD, while zolpidem, eszopiclone and guanfacine did not reveal any improvement when compared with placebo[37].

Limited evidence supports the use of alpha-agonists such as clonidine to improve SOL, especially in ADHD subjects. In a United States National survey, alpha agonists were the most commonly prescribed insomnia medication for children with ADHD (81%)[29].

Z-drugs: Only few studies have been carried out in CYP regarding use of zolpidem, zaleplon, and eszopiclone, with contrasting results[42]. In a recent study, children taking eszopiclone or zolpidem experienced more frequent undesirable effects compared with melatonin or placebo[52].

Benzodiazepines like clonazepam and flurazepam: Benzodiazepines are not recommended for routine management of sleep disorders in children but may have a place for treatment of transient insomnia, especially if associated with daytime anxiety[42]. Clonazepam may be used for severe parasomnia/night terrors with specialist advice from a tertiary sleep centre[10].

Tricyclic antidepressants: Tricyclic antidepressants are frequently used in adults with insomnia but not recommended in children because of their poor safety profile. Trazodone and mirtazapine have potential use in the paediatric population but their wider application require further studies[42]. Trazodone may be considered in children with Angelman syndrome with specialist advice from a tertiary sleep centre[10].

Selective serotonin reuptake inhibitors: Use of selective serotonin reuptake inhibitors such as sertraline may be considered for disabling bedtime anxiety. Benzodiazepines and tricyclic antidepressants are not recommended in children[10].

ALTERNATIVE THERAPIES

Many parents self-manage with a wide range of herbal and other counter formulations for relieving sleep disturbances, including use of Valerian, Lavender, Chamomile and Kava. In the absence of research-based evidence, their use remains largely based on empirical tradition[7].

BRIGHT LIGHT THERAPY

Sleep-onset insomnia associated with late melatonin onset is one of the common causes of chronic sleep disorders in childhood. Studies have shown that melatonin or bright light therapy (BLT) is effective in treating these sleep problems, both decreasing sleep latency and advancing dim-light melatonin onset (effects on sleep onset was stronger for melatonin[53].

Fargason *et al*[54] reported the efficacy of 2-wk trial of 30-min morning 10000-lux BLT commencing 3 h after mid-sleep period among a group of adult ADHD patients. BLT significantly advanced the phase of dim light melatonin onset by 31 min mean time SEM, and mid-sleep time by 57 min, associated with significantly decreased ADHD rating scale total scores (P = 0.027 and 0.044) and hyperactive-impulsivity sub-scores (P = 0.014 and 0.013) respectively. There was however no evidence of significant effects in TST, sleep efficiency, wake after sleep onset, or proportion of wakefulness during sleep.

MELATONIN

What is melatonin

Melatonin is an endogenous neurohormone produced by the pineal gland, with its secretion being regulated by the hypothalamic suprachiasmatic nucleus, which controls the circadian physiological

rhythms in response to the ambient 24 h light-dark cycle, for example, controlling sleep/wake blood pressure, body temperature and metabolism[39]. The circadian cycle of high levels of melatonin secretion at night and low levels during the day begins in infants at the age of 3 mo. Melatonin helps in maintaining and synchronizing the circadian rhythm through its daily pattern of secretion[39]. Its rate of secretion generally declines after the first 12 mo as the pineal gland remains static in size while the pituitary gland continues to grow with age[55]. Melatonin has a chronobiotic effect, and acts by its circadian phase-shifting effect, but a less established hypnotic and sleep-promoting effect. Melatonin is also reported to have some immunomodulating properties and is not recommended in children with immune and lymphoproliferative disorders, and in those taking immunosuppressants[56].

Circadin (slow-release melatonin) is currently only licensed for patients with primary insomnia aged 55 and over, and its widespread use for the treatment of sleep disorders especially in the paediatric population is practically "off-label"[57]. The European Medicines Agency has recently granted paediatric-use authorisations for a brand of melatonin (Slenyto), which is available in age-appropriate forms as small tablets[58]. Box 2 shows list of licensed melatonin products in Supplementary Material.

Side effects of melatonin treatment are known to be relatively uncommon and mild in nature[59]. While melatonin is generally considered to be safe in the short term, its long-term safety is yet to be extensively researched. There is limited evidence to suggest that exogenous melatonin suppresses the hypothalamic-pituitary-gonadal axis, due to the observation that endogenous levels of melatonin were elevated in 7 male patients with gonadotropin-releasing hormone deficiency. Sudden termination of melatonin treatment might potentially lead to sleep phase shift in the absence of effective behavioural sleep hygiene implementation. CYP with NDEBID managed with melatonin would require regular follow-up by clinicians to re-evaluate insomnia and determine if continuation of melatonin is still necessary[39].

Use of melatonin for paediatric insomnia and NDEBID

A number of studies and review articles have demonstrated the effectiveness of melatonin treatment in children with NDEBID. Studies have documented significantly shorter sleep onset latencies with melatonin treatment, especially in children with autism. Ayyash *et al*[60] reported a cohort of children with NDEBID (including intellectual disability; autism and ADHD) and sleep disturbances, with 69% of them responding to either low or moderate doses of melatonin (2.5-6 mg), with significantly increased total hours of sleep per night, decreased sleep onset delay and decreased number of awakenings (all: P = 0.001), identified with the use of sleep diaries. Only 9% of them benefited from any dose above 6 mg.

A recent systematic review and meta-analysis of thirteen randomized controlled trials showed that melatonin significantly improved TST compared with placebo [mean difference (MD) = 48.26 min]. In 11 studies (n = 581), SOL improved significantly with melatonin use (MD = -28.97). However, the overall quality of the evidence is limited due to study heterogeneity and inconsistency[61].

Limitations of melatonin effectiveness

There is limited availability of high quality published evidence on the management of sleep disorders among children with NDEBIDs[62]. Despite the widespread use of melatonin for the management of sleep disturbances in children with NDEBIDs, there is limited evidence on effective dosage and lack of documentation on type-specific efficacy on different categories of sleep problems. There is no evidence that extended-release melatonin confers advantage over immediate release. There is convincing evidence that melatonin decreases SOL and increases TST but does not decrease night awakenings. From a systematic review of 19 RCTs, melatonin was shown to significantly improve sleep latency (median 28 min; range: 11-51 min), sleep duration (median 33 min; range: 14-68 min), and wake time after sleep onset (range: 12-43 min), but did not significantly reduce the number of sleep interruptions per night (range: 0-2.7)[52].

Decreased CYP1A2 activity, either genetically determined or from use of certain concomitant medication, can slow down melatonin metabolism, with loss of day-night time variation and loss of effectiveness[63]. Limited studies have shown reduced activities of cytochrome P450 enzyme, CYP1A2 in the liver, with slow metabolization of exogenous melatonin is almost exclusively responsible for the loss of response to treatment. In patients with loss of response to melatonin, a period of melatonin clearance for up to 3 wk and a considerable dose reduction has been advised[64].

The initial MENDS trial was based on a cohort of children who failed to fall asleep within 1 h of lights out or who slept for less than 6 h of continuously[65]. The efficacy of melatonin is likely going to be less significant for children who are able to sleep more than 6 h at night. The overall effectiveness of melatonin compared to placebo was also modest, increasing TST by 22.43 min and reduced SOL by (-37.49 min) using sleep diary or (-45.34 min) by actigraphy. Using a definition of one hour as the minimum clinically worthwhile difference after the intervention, the upper limit of the confidence interval for increased TST did not reach the level of clinical significance. The children fell asleep slightly faster but they gained little additional sleep duration on melatonin. Overall behaviour rating and family functioning outcomes showed no significant improvement[50]. It is also worth considering some potential and reported side effects associated with melatonin use. There are some areas of uncertainties including long-term effects on puberty development and immune system[66].

Melatonin 1 mg/mL oral solution (Colonis Pharma Ltd) contains propylene glycol excipients which may be potentially problematic when used in children[3]. These are generally safe for children above the age of 5 to 6 years, unless they are requiring very high doses[58].

COMBINED TREATMENT MODALITIES

Only limited studies have assessed the efficacy combining behavioural and pharmacological therapies. The combination of controlled-release melatonin over 12 wk and four sessions of cognitive-behavioural therapy among a group of ASD children aged 4-10 years, revealed a better efficacy compared to other treatment modalities, fewer participant dropouts and higher rate of clinically significant response to treatment[67].

A similar small Canadian study among 27 ADHD children reported the effect size of the combined sleep hygiene and melatonin intervention was 1.7 after 90 d of treatment, compared to 0.6 on average for either sleep hygiene or melatonin alone. However, the decreased sleep latency and improved sleep had no demonstrable effect on ADHD symptoms[68].

CONCLUSION

Sleep difficulties and sleep disorders are more prevalent in children and adolescents with NDEBID. They can result in a significant impact on the child's cognitive development, behaviour, physical and mental health. This can also affect peer and family relationships.

It is important for clinicians to evaluate for sleep disorders when assessing children and adolescents with cognitive, behavioural, and emotional problems. Assessment can include screening tools such as BEARS questionnaire, Child Sleep Habit Questionnaire, a 2-wk sleep diary and relevant physical examination in order to identify sleep schedule and duration and any underlying potential sleep disorders. Parents/carers should be provided with sleep/psychoeducation. Sleep hygiene measures and also specific behavioural interventions where appropriate should be offered as first line management for sleep disorders such as behavioural insomnia and certain parasomnias. Management of DSPS involves a combination of strategies including, chronotherapy, light therapy and melatonin. In children and adolescents with NDD and insomnia, use of melatonin should be carefully considered only following an unsuccessful trial of sleep hygiene and behavioural measures and emphasis should remain on continuing the appropriate sleep hygiene measures. Referrals should be made to appropriate specialist/sleep centre for further evaluation and management of sleep disorders, including OSA, PLMD and narcolepsy.

FOOTNOTES

Author contributions: Ogundele MO conceived the idea, performed the literature review and prepared part of the manuscript; Yemula C reviewed the literature and prepared rest of the manuscript.

Conflict-of-interest statement: The authors declare no conflict of interest for this article.

Country/Territory of origin: United Kingdom

ORCID number: Michael O Ogundele 0000-0003-0744-1434; Chinnaiah Yemula 0000-0002-8249-0714.

Corresponding Author's Membership in Professional Societies: Bridgewater Community Healthcare NHS Foundation Trust, Halton District, WA7 1TW, UK; Cambridgeshire Community Services NHS Trust, Meadow Lane, St Ives, Cambridgeshire, PE27 4LG.

S-Editor: Wang JJ
L-Editor: A
P-Editor: Wang JJ

REFERENCES

1 **Meltzer LJ**, Valerie McLaughlin Crabtree. Pediatric Sleep Problems: A Clinician's Guide to Behavioral Interventions. *J Clin Sleep Med* 2016; **12**: 633-634 [DOI: 10.5664/jcsm.5710]

2 **Vélez-Galarraga R**, Guillén-Grima F, Crespo-Eguílaz N, Sánchez-Carpintero R. Prevalence of sleep disorders and their relationship with core symptoms of inattention and hyperactivity in children with attention-deficit/hyperactivity disorder. *Eur J Paediatr Neurol* 2016; **20**: 925-937 [PMID: 27461837 DOI: 10.1016/j.ejpn.2016.07.004]

3 **Calhoun SL**, Fernandez-Mendoza J, Vgontzas AN, Mayes SD, Liao D, Bixler EO. Behavioral Profiles Associated with Objective Sleep Duration in Young Children with Insomnia Symptoms. *J Abnorm Child Psychol* 2017; **45**: 337-344 [PMID: 27245765 DOI: 10.1007/s10802-016-0166-4]

4 **Licis A**. Sleep Disorders: Assessment and Treatment in Preschool-Aged Children. *Child Adolesc Psychiatr Clin N Am* 2017; **26**: 587-595 [PMID: 28577611 DOI: 10.1016/j.chc.2017.02.009]

5 **Scammell TE**. Overview of sleep: the neurologic processes of the sleep-wake cycle. *J Clin Psychiatry* 2015; **76**: e13 [PMID: 26035194 DOI: 10.4088/JCP.14046tx1c]

6 **Meltzer LJ**, Mindell JA. Systematic review and meta-analysis of behavioral interventions for pediatric insomnia. *J Pediatr Psychol* 2014; **39**: 932-948 [PMID: 24947271 DOI: 10.1093/jpepsy/jsu041]

7 **Pin Arboledas G**, Soto Insuga V, Jurado Luque MJ, Fernandez Gomariz C, Hidalgo Vicario I, Lluch Rosello A, Rodríguez Hernández PJ, Madrid JA. [Insomnia in children and adolescents. A consensus document]. *An Pediatr (Barc)* 2017; **86**: 165.e1-165.e11 [PMID: 27476002 DOI: 10.1016/j.anpedi.2016.06.005]

8 **Byars KC**, Yeomans-Maldonado G, Noll JG. Parental functioning and pediatric sleep disturbance: an examination of factors associated with parenting stress in children clinically referred for evaluation of insomnia. *Sleep Med* 2011; **12**: 898-905 [PMID: 21940206 DOI: 10.1016/j.sleep.2011.05.002]

9 **Shatkin JP**, Pando M. Diagnosis and Treatment of Common Sleep Disorders in Adolescence. *Adolesc Psychiatry* 2015; **5**: 146-163 [DOI: 10.2174/2210676605666150521232247]

10 **McDonald A**, Joseph D. Paediatric neurodisability and sleep disorders: clinical pathways and management strategies. *BMJ Paediatr Open* 2019; **3**: e000290 [PMID: 30957021 DOI: 10.1136/bmjpo-2018-000290]

11 **Meghan A Marty**, Daniel L Segal. DSM-5: Diagnostic and Statistical Manual of Mental Disorders. Fifth Edition. In: R. Cautin, S. Lilienfeld. Encyclopedia of Clinical Psychology. Wiley-Blackwell, 2015: 965-970

12 International Classification of Sleep Disorders – Third Edition (ICSD-3) (Online). [cited 7 February 2021]. Available from: https://learn.aasm.org/Public/Catalog/Details.aspx?id=%2fgqQVDMQIT%2fEDy86PWgqgQ%3d%3d&returnurl=%2fUsers%2fUserOnlineCourse.aspx%3fLearningActivityID%3d%252fgqQVDMQIT%252fEDy86PWgqgQ%253d%253d

13 **Blackmer AB**, Feinstein JA. Management of Sleep Disorders in Children With Neurodevelopmental Disorders: A Review. *Pharmacotherapy* 2016; **36**: 84-98 [PMID: 26799351 DOI: 10.1002/phar.1686]

14 **Esposito D**, Belli A, Ferri R, Bruni O. Sleeping without Prescription: Management of Sleep Disorders in Children with Autism with Non-Pharmacological Interventions and Over-the-Counter Treatments. *Brain Sci* 2020; **10** [PMID: 32664572 DOI: 10.3390/brainsci10070441]

15 **Reed HE**, McGrew SG, Artibee K, Surdkya K, Goldman SE, Frank K, Wang L, Malow BA. Parent-based sleep education workshops in autism. *J Child Neurol* 2009; **24**: 936-945 [PMID: 19491110 DOI: 10.1177/0883073808331348]

16 **Ogundele MO**. A Profile of Common Neurodevelopmental Disorders Presenting in a Scottish Community Child Health Service –a One Year Audit (2016/2017). *Health Res Policy Sy* 2018; **2**: 1 [DOI: 10.31058/j.hr.2018.21001]

17 **Morris C**, Janssens A, Tomlinson R, Williams J, Logan S. Towards a definition of neurodisability: a Delphi survey. *Dev Med Child Neurol* 2013; **55**: 1103-1108 [PMID: 23909744 DOI: 10.1111/dmcn.12218]

18 **World health Organization**. Children and Neurodevelopmental Behavioural Intellectual Disorders (NDBID). [cited 10 February 2021]. Available from: https://apps.who.int/iris/handle/10665/336959

19 **Matson JL**, Goldin RL. Comorbidity and autism: Trends, topics and future directions. *Res Autism Spectr Disord* 2013; **7**: 1228-1233 [DOI: 10.1016/j.rasd.2013.07.003]

20 **Mannion A**, Leader G. Sleep Problems in Autism Spectrum Disorder: A Literature Review. *Rev J Autism Dev Disord* 2014; **1**: 101-109 [DOI: 10.1007/s40489-013-0009-y]

21 **Hollway JA**, Aman MG. Sleep correlates of pervasive developmental disorders: a review of the literature. *Res Dev Disabil* 2011; **32**: 1399-1421 [PMID: 21570809 DOI: 10.1016/j.ridd.2011.04.001]

22 **Mazurek MO**, Sohl K. Sleep and Behavioral Problems in Children with Autism Spectrum Disorder. *J Autism Dev Disord* 2016; **46**: 1906-1915 [PMID: 26823076 DOI: 10.1007/s10803-016-2723-7]

23 **Malow BA**, McGrew SG. Sleep Disturbances and Autism. *Sleep Med Clin* 2008; **3**: 479-488 [DOI: 10.1016/j.jsmc.2008.04.004]

24 **Kotagal S**, Broomall E. Sleep in children with autism spectrum disorder. *Pediatr Neurol* 2012; **47**: 242-251 [PMID: 22964437 DOI: 10.1016/j.pediatrneurol.2012.05.007]

25 **Devnani PA**, Hegde AU. Autism and sleep disorders. *J Pediatr Neurosci* 2015; **10**: 304-307 [PMID: 26962332 DOI: 10.4103/1817-1745.174438]

26 **Cuomo BM**, Vaz S, Lee EAL, Thompson C, Rogerson JM, Falkmer T. Effectiveness of Sleep-Based Interventions for Children with Autism Spectrum Disorder: A Meta-Synthesis. *Pharmacotherapy* 2017; **37**: 555-578 [PMID: 28258648 DOI: 10.1002/phar.1920]

27 **Williams Buckley A**, Hirtz D, Oskoui M, Armstrong MJ, Batra A, Bridgemohan C, Coury D, Dawson G, Donley D, Findling RL, Gaughan T, Gloss D, Gronseth G, Kessler R, Merillat S, Michelson D, Owens J, Pringsheim T, Sikich L, Stahmer A, Thurm A, Tuchman R, Warren Z, Wetherby A, Wiznitzer M, Ashwal S. Practice guideline: Treatment for insomnia and disrupted sleep behavior in children and adolescents with autism spectrum disorder: Report of the Guideline Development, Dissemination, and Implementation Subcommittee of the American Academy of Neurology. *Neurology* 2020; **94**: 392-404 [PMID: 32051244 DOI: 10.1212/WNL.0000000000009033]

28 **Polanczyk G**, Rohde LA. Epidemiology of attention-deficit/hyperactivity disorder across the lifespan. *Curr Opin*

Psychiatry 2007; **20**: 386-392 [PMID: 17551354 DOI: 10.1097/YCO.0b013e3281568d7a]

29 **Owens J**, Gruber R, Brown T, Corkum P, Cortese S, O'Brien L, Stein M, Weiss M. Future research directions in sleep and ADHD: report of a consensus working group. *J Atten Disord* 2013; **17**: 550-564 [PMID: 22982880 DOI: 10.1177/1087054712457992]

30 **Sciberras E**, Fulton M, Efron D, Oberklaid F, Hiscock H. Managing sleep problems in school aged children with ADHD: a pilot randomised controlled trial. *Sleep Med* 2011; **12**: 932-935 [PMID: 22005602 DOI: 10.1016/j.sleep.2011.02.006]

31 **Ogundele MO**. Management of sleep difficulties among a cohort of children with adhd in a scottish local authority. *Arch Dis Child* 2018; **103** Suppl 1: A190.3-A191 [DOI: 10.1136/archdischild-2018-rcpch.455]

32 **Tsai MH**, Hsu JF, Huang YS. Sleep Problems in Children with Attention Deficit/Hyperactivity Disorder: Current Status of Knowledge and Appropriate Management. *Curr Psychiatry Rep* 2016; **18**: 76 [PMID: 27357497 DOI: 10.1007/s11920-016-0711-4]

33 **Cortese S**, Brown TE, Corkum P, Gruber R, O'Brien LM, Stein M, Weiss M, Owens J. Assessment and management of sleep problems in youths with attention-deficit/hyperactivity disorder. *J Am Acad Child Adolesc Psychiatry* 2013; **52**: 784-796 [PMID: 23880489 DOI: 10.1016/j.jaac.2013.06.001]

34 **Lycett K**, Mensah FK, Hiscock H, Sciberras E. A prospective study of sleep problems in children with ADHD. *Sleep Med* 2014; **15**: 1354-1361 [PMID: 25194583 DOI: 10.1016/j.sleep.2014.06.004]

35 **Peppers KH**, Eisbach S, Atkins S, Poole JM, Derouin A. An Intervention to Promote Sleep and Reduce ADHD Symptoms. *J Pediatr Health Care* 2016; **30**: e43-e48 [PMID: 27614815 DOI: 10.1016/j.pedhc.2016.07.008]

36 **Hiscock H**, Sciberras E, Mensah F, Gerner B, Efron D, Khano S, Oberklaid F. Impact of a behavioural sleep intervention on symptoms and sleep in children with attention deficit hyperactivity disorder, and parental mental health: randomised controlled trial. *BMJ* 2015; **350**: h68 [PMID: 25646809 DOI: 10.1136/bmj.h68]

37 **Anand S**, Tong H, Besag FMC, Chan EW, Cortese S, Wong ICK. Safety, Tolerability and Efficacy of Drugs for Treating Behavioural Insomnia in Children with Attention-Deficit/Hyperactivity Disorder: A Systematic Review with Methodological Quality Assessment. *Paediatr Drugs* 2017; **19**: 235-250 [PMID: 28391425 DOI: 10.1007/s40272-017-0224-6]

38 **Van der Heijden KB**, Smits MG, Van Someren EJ, Ridderinkhof KR, Gunning WB. Effect of melatonin on sleep, behavior, and cognition in ADHD and chronic sleep-onset insomnia. *J Am Acad Child Adolesc Psychiatry* 2007; **46**: 233-241 [PMID: 17242627 DOI: 10.1097/01.chi.0000246055.76167.0d]

39 **Janjua I**, Goldman RD. Sleep-related melatonin use in healthy children. *Can Fam Physician* 2016; **62**: 315-317 [PMID: 27076541]

40 **Mindell JA**, Emslie G, Blumer J, Genel M, Glaze D, Ivanenko A, Johnson K, Rosen C, Steinberg F, Roth T, Banas B. Pharmacologic management of insomnia in children and adolescents: consensus statement. *Pediatrics* 2006; **117**: e1223-e1232 [PMID: 16740821 DOI: 10.1542/peds.2005-1693]

41 **Souders MC**, Zavodny S, Eriksen W, Sinko R, Connell J, Kerns C, Schaaf R, Pinto-Martin J. Sleep in Children with Autism Spectrum Disorder. *Curr Psychiatry Rep* 2017; **19**: 34 [PMID: 28502070 DOI: 10.1007/s11920-017-0782-x]

42 **Bruni O**, Angriman M, Melegari MG, Ferri R. Pharmacotherapeutic management of sleep disorders in children with neurodevelopmental disorders. *Expert Opin Pharmacother* 2019; **20**: 2257-2271 [PMID: 31638842 DOI: 10.1080/14656566.2019.1674283]

43 **Esposito S**, Laino D, D'Alonzo R, Mencarelli A, Di Genova L, Fattorusso A, Argentiero A, Mencaroni E. Pediatric sleep disturbances and treatment with melatonin. *J Transl Med* 2019; **17**: 77 [PMID: 30871585 DOI: 10.1186/s12967-019-1835-1]

44 **Miano S**, Esposito M, Foderaro G, Ramelli GP, Pezzoli V, Manconi M. Sleep-Related Disorders in Children with Attention-Deficit Hyperactivity Disorder: Preliminary Results of a Full Sleep Assessment Study. *CNS Neurosci Ther* 2016; **22**: 906-914 [PMID: 27255788 DOI: 10.1111/cns.12573]

45 **Rosen GM**, Morrissette S, Larson A, Stading P, Barnes TL. Does Improvement of Low Serum Ferritin Improve Symptoms of Restless Legs Syndrome in a Cohort of Pediatric Patients? *J Clin Sleep Med* 2019; **15**: 1149-1154 [PMID: 31482837 DOI: 10.5664/jcsm.7810]

46 **Simon SL**, Byars KC. Behavioral Treatments for Non-Rapid Eye Movement Parasomnias in Children. *Curr Sleep Med Rep* 2016; **2**: 152-157 [DOI: 10.1007/s40675-016-0049-9]

47 **Kotagal S**. Treatment of dyssomnias and parasomnias in childhood. *Curr Treat Options Neurol* 2012; **14**: 630-649 [PMID: 23011807 DOI: 10.1007/s11940-012-0199-0]

48 **Grau K**, Plener PL. [Pharmacotherapy for children and adolescents with sleep disorders: an overview]. *Z Kinder Jugendpsychiatr Psychother* 2018; **46**: 393-402 [PMID: 29239270 DOI: 10.1024/1422-4917/a000562]

49 **Blake MJ**, Sheeber LB, Youssef GJ, Raniti MB, Allen NB. Systematic Review and Meta-analysis of Adolescent Cognitive-Behavioral Sleep Interventions. *Clin Child Fam Psychol Rev* 2017; **20**: 227-249 [PMID: 28331991 DOI: 10.1007/s10567-017-0234-5]

50 **Gringras P**, Gamble C, Jones AP, Wiggs L, Williamson PR, Sutcliffe A, Montgomery P, Whitehouse WP, Choonara I, Allport T, Edmond A, Appleton R; MENDS Study Group. Melatonin for sleep problems in children with neurodevelopmental disorders: randomised double masked placebo controlled trial. *BMJ* 2012; **345**: e6664 [PMID: 23129488 DOI: 10.1136/bmj.e6664]

51 **Arns M**, Kenemans JL. Neurofeedback in ADHD and insomnia: vigilance stabilization through sleep spindles and circadian networks. *Neurosci Biobehav Rev* 2014; **44**: 183-194 [PMID: 23099283 DOI: 10.1016/j.neubiorev.2012.10.006]

52 **McDonagh MS**, Holmes R, Hsu F. Pharmacologic Treatments for Sleep Disorders in Children: A Systematic Review. *J Child Neurol* 2019; **34**: 237-247 [PMID: 30674203 DOI: 10.1177/0883073818821030]

53 **van Maanen A**, Meijer AM, Smits MG, van der Heijden KB, Oort FJ. Effects of Melatonin and Bright Light Treatment in Childhood Chronic Sleep Onset Insomnia With Late Melatonin Onset: A Randomized Controlled Study. *Sleep* 2017; **40** [PMID: 28364493 DOI: 10.1093/sleep/zsw038]

54 **Fargason RE**, Fobian AD, Hablitz LM, Paul JR, White BA, Cropsey KL, Gamble KL. Correcting delayed circadian phase with bright light therapy predicts improvement in ADHD symptoms: A pilot study. *J Psychiatr Res* 2017; **91**: 105-110

[PMID: 28327443 DOI: 10.1016/j.jpsychires.2017.03.004]

55 **Schmidt F**, Penka B, Trauner M, Reinsperger L, Ranner G, Ebner F, Waldhauser F. Lack of pineal growth during childhood. *J Clin Endocrinol Metab* 1995; **80**: 1221-1225 [PMID: 7536203 DOI: 10.1210/jcem.80.4.7536203]

56 **Pelayo R**, Yuen K. Pediatric sleep pharmacology. *Child Adolesc Psychiatr Clin N Am* 2012; **21**: 861-883 [PMID: 23040905 DOI: 10.1016/j.chc.2012.08.001]

57 **Chua HM**, Hauet Richer N, Swedrowska M, Ingham S, Tomlin S, Forbes B. Dissolution of Intact, Divided and Crushed Circadin Tablets: Prolonged vs. Immediate Release of Melatonin. *Pharmaceutics* 2016; **8** [PMID: 26751472 DOI: 10.3390/pharmaceutics8010002]

58 **European Medicines Agency**. Two new paediatric-use marketing authorisations recommended by CHMP. European Medicines Agency. [cited 20 February 2021]. Available from: https://www.ema.europa.eu/en/news/two-new-paediatric-use-marketing-authorisations-recommended-chmp

59 **Schwichtenberg AJ**, Malow BA. Melatonin Treatment in Children with Developmental Disabilities. *Sleep Med Clin* 2015; **10**: 181-187 [PMID: 26055866 DOI: 10.1016/j.jsmc.2015.02.008]

60 **Ayyash HF**, Preece P, Morton R, Cortese S. Melatonin for sleep disturbance in children with neurodevelopmental disorders: prospective observational naturalistic study. *Expert Rev Neurother* 2015; **15**: 711-717 [PMID: 25938708 DOI: 10.1586/14737175.2015.1041511]

61 **Abdelgadir IS**, Gordon MA, Akobeng AK. Melatonin for the management of sleep problems in children with neurodevelopmental disorders: a systematic review and meta-analysis. *Arch Dis Child* 2018; **103**: 1155-1162 [PMID: 29720494 DOI: 10.1136/archdischild-2017-314181]

62 **Parker A**, Beresford B, Dawson V, Elphick H, Fairhurst C, Hewitt C, Scantlebury A, Spiers G, Thomas M, Wright K, Mcdaid C. Oral melatonin for non-respiratory sleep disturbance in children with neurodisabilities: systematic review and meta-analyses. *Dev Med Child Neurol* 2019; **61**: 880-890 [PMID: 30710339 DOI: 10.1111/dmcn.14157]

63 **Bruni O**, Alonso-Alconada D, Besag F, Biran V, Braam W, Cortese S, Moavero R, Parisi P, Smits M, Van der Heijden K, Curatolo P. Current role of melatonin in pediatric neurology: clinical recommendations. *Eur J Paediatr Neurol* 2015; **19**: 122-133 [PMID: 25553845 DOI: 10.1016/j.ejpn.2014.12.007]

64 **Braam W**, Didden R, Maas AP, Korzilius H, Smits MG, Curfs LM. Melatonin decreases daytime challenging behaviour in persons with intellectual disability and chronic insomnia. *J Intellect Disabil Res* 2010; **54**: 52-59 [PMID: 19888921 DOI: 10.1111/j.1365-2788.2009.01223.x]

65 **Appleton RE**, Jones AP, Gamble C, Williamson PR, Wiggs L, Montgomery P, Sutcliffe A, Barker C, Gringras P. The use of MElatonin in children with neurodevelopmental disorders and impaired sleep: a randomised, double-blind, placebo-controlled, parallel study (MENDS). *Health Technol Assess* 2012; **16**: i-239 [PMID: 23098680 DOI: 10.3310/hta16400]

66 **Rossignol DA**, Frye RE. Melatonin in autism spectrum disorders. *Curr Clin Pharmacol* 2014; **9**: 326-334 [PMID: 24050742 DOI: 10.2174/1574884713086660072]

67 **Cortesi F**, Giannotti F, Sebastiani T, Panunzi S, Valente D. Controlled-release melatonin, singly and combined with cognitive behavioural therapy, for persistent insomnia in children with autism spectrum disorders: a randomized placebo-controlled trial. *J Sleep Res* 2012; **21**: 700-709 [PMID: 22616853 DOI: 10.1111/j.1365-2869.2012.01021.x]

68 **Weiss MD**, Wasdell MB, Bomben MM, Rea KJ, Freeman RD. Sleep hygiene and melatonin treatment for children and adolescents with ADHD and initial insomnia. *J Am Acad Child Adolesc Psychiatry* 2006; **45**: 512-519 [PMID: 16670647]

69 **Bruce ES**, Lunt L, McDonagh JE. Sleep in adolescents and young adults. *Clin Med (Lond)* 2017; **17**: 424-428 [PMID: 28974591 DOI: 10.7861/clinmedicine.17-5-424]

World Journal of Clinical Pediatrics

Submit a Manuscript: https://www.f6publishing.com

World J Clin Pediatr 2022 May 9; 11(3): 253-269

DOI: 10.5409/wjcp.v11.i3.253

ISSN 2219-2808 (online)

MINIREVIEWS

Food allergy in children—the current status and the way forward

Ahmed Elghoudi, Hassib Narchi

Specialty type: Allergy

Provenance and peer review: Invited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): 0
Grade B (Very good): 0
Grade C (Good): C
Grade D (Fair): 0
Grade E (Poor): 0

P-Reviewer: Gomez-Torrijos E, Spain

Received: April 6, 2021
Peer-review started: April 6, 2021
First decision: July 6, 2021
Revised: July 16, 2021
Accepted: March 16, 2022
Article in press: March 16, 2022
Published online: May 9, 2022

Ahmed Elghoudi, Paediatric Department, Sheikh Khalifa Medical City, Abu Dhabi NA, Abu Dhabi, United Arab Emirates

Ahmed Elghoudi, Hassib Narchi, College of Medicine and Health Sciences, United Arab Emirates University, Alain, Abu Dhabi, United Arab Emirates

Corresponding author: Ahmed Elghoudi, FRCP, MSc, Doctor, Paediatric Department, Sheikh Khalifa Medical City, Al Karama Street, Abu Dhabi, United Arab Emirates.
ahmed.elghoudi1@gmail.com

Abstract

Food allergy in children is a major health concern, and its prevalence is rising. It is often over-diagnosed by parents, resulting occasionally in unnecessary exclusion of some important food. It also causes stress, anxiety, and even depression in parents and affects the family's quality of life. Current diagnostic tests are useful when interpreted in the context of the clinical history, although cross-sensitivity and inability to predict the severity of the allergic reactions remain major limitations. Although the oral food challenge is the current gold standard for making the diagnosis, it is only available to a small number of patients because of its requirement in time and medical personnel. New diagnostic methods have recently emerged, such as the Component Resolved Diagnostics and the Basophil Activation Test, but their use is still limited, and the latter lacks standardisation. Currently, there is no definite treatment available to induce life-long natural tolerance and cure for food allergy. Presently available treatments only aim to decrease the occurrence of anaphylaxis by enabling the child to tolerate small amounts of the offending food, usually taken by accident. New evidence supports the early introduction of the allergenic food to infants to decrease the incidence of food allergy. If standardised and widely implemented, this may result in decreasing the prevalence of food allergy.

Key Words: Oral food challenge; Oral immunotherapy; Allergens; Anaphylaxis; Desensitisation; Immunoglobulin E; Eosinophilic gastrointestinal diseases; Histamine; Mast cells; Basophil activation test

Core Tip: Food allergy in children is a potentially serious condition with an increasing prevalence. Current diagnostic tests are useful when interpreted in the context of the clinical history. The oral food challenge is the current gold standard for making the diagnosis, but its use is limited. New diagnostic methods have recently emerged. Currently, there is no definite treatment to induce life-long natural tolerance and cure for this condition, and available treatments only aim to decrease the occurrence of anaphylaxis. New evidence supports the early introduction of the allergenic food to infants to decrease the incidence of food allergy.

Citation: Elghoudi A, Narchi H. Food allergy in children—the current status and the way forward. *World J Clin Pediatr* 2022; 11(3): 253-269
URL: https://www.wjgnet.com/2219-2808/full/v11/i3/253.htm
DOI: https://dx.doi.org/10.5409/wjcp.v11.i3.253

INTRODUCTION

Reactions to foods, including allergies, have been known for many centuries. "What is food to one man may be fierce poison to others", was quoted by Lucretius (99-55 BC). In the 1920s and 30s, food intolerance was blamed for many disorders and became a common concern amongst parents in the 1980s. Not surprisingly, it was associated with a steady increase in the diagnosis of food allergy[1]. This was caused by an increase in the prevalence of atopic conditions as a result of different environmental and genetic factors and also by an increase in public awareness. Immunoglobulin (IgE)-mediated food allergy is a global health concern that affects millions of individuals, disrupting many aspects of their lives[2,3].

The condition may cause stress, fear, anxiety in affected children and their parents alike. It also negatively impacts the nutritional status of children with either proven or merely suspected food allergy by resulting in food restriction, elimination, or complete avoidance of particular important food. Stigma, bullying at school, regulation from normal social life, such as attending parties and dining out, also have a significant impact on the child and the family. As for parents, holiday planning becomes a nightmare, similar to when the child goes to school for the first time or moves to a university campus and becomes independent.

The worldwide prevalence of food allergy is estimated to be around 4% of children and 1% of adults, with an increased prevalence in the past two decades[2,4,5]. Differences in reported prevalence are because food allergy is not fully understood, and some of the adverse reactions to food are not allergic. Although in the Western world it is believed that approximately 25% of adults suffer from a food allergy, when accurately diagnosed by testing and oral food challenge (OFC), its true prevalence is found to be much lower, closer to 8% in young children and less than 4% in adults.

This review will revisit the definition, prevalence, and clinical presentation and evaluate the current management of food allergies in children, focusing essentially on the most common IgE-mediated food allergy.

DEFINITION

Different terminologies in food allergy are a source of confusion, with terms such as allergy, hypersensitivity, pseudo-allergy, and intolerance often being incorrectly used interchangeably. At first, a food allergy may sound to parents, and even some professionals, like a single simple disease. However, in reality, it is far more complex.

Adverse reactions to food are defined as an abnormal response related to the ingestion of that food. They can be classified as food intolerance or food allergy based on the pathophysiological mechanism of the reaction[6]. The vast majority of food allergic reactions reported by parents and the general population are, in fact, food intolerances.

The most acceptable and widely used definition of food allergy states that it is an adverse immune response to food proteins that occurs in a susceptible host[2]. Its manifestations are not dose-dependent but are reproducible[6]. Furthermore, food allergy is not one single distinct condition but a spectrum of clinicopathological disorders[7]. In addition to the classic fast (IgE-mediated) food allergy, other Hypersensitivity diseases cover medical problems such as acute allergic hives, allergic gastrointestinal diseases, *e.g.*, eosinophilic esophagitis, acute flare-up of eczema, and oral food pollen syndrome.

As a result, the manifestations of food allergy are very broad and entirely depend on the underlying immune mechanism involved and the affected target organ(s), resulting in a wide spectrum of manifestations commonly involving, alone or in combination, the skin, the respiratory and cardiovascular systems. Thus, diagnostic tools for food allergy, such as the skin prick test and the specific IgE test, have

limitations caused by cross-reactivity and the inability to predict the severity of the allergic reaction; their results must, therefore, always be interpreted in the context of the clinical symptoms to make an accurate disease assessment and, hence, a diagnosis.

In contrast to food allergy, food intolerance is defined as a non-immune reaction to food[2]. It encompasses adverse responses to food, caused by the inherent properties of that particular food (*i.e.*, contamination with a toxin or pathogen, presence of a pharmacologically active component such as caffeine, alcohol, monosodium glutamate), or more commonly caused by an abnormal response of the host, for instance, enzyme deficiencies such as lactase (lactose intolerance), or metabolic disorders (galactosemia, congenital fructose intolerance), or food aversion (due to psychological issues). The clinical manifestations tend to be dose-dependent and are not consistently reproducible.

EPIDEMIOLOGY

Accurate ascertainment of the prevalence of food allergy facilitates the planning of allergy services. Unfortunately, accurate prevalence statistics are notoriously difficult to obtain. The reasons include the existence of various definitions and methods of reporting food allergy and the pleiomorphic manifestations of food allergy with various degrees of severity. In addition, some reports may have also included confounding factors by either investigating specific populations, focusing on specific foods, or using different methodologies. Furthermore, there are wide geographic variations, diet exposures, differences according to age, race, ethnicity, and many other factors. Additionally, the presence of multiple food allergies in children is not often accounted for in prevalence studies.

Methods of reporting food allergy

The methods of reporting food allergy, either self-reported by the individual child, parents, or even by adult patients, lead to different prevalence estimates, as self-reported food allergy rates are notoriously higher than those confirmed by medically supervised OFCs[8].

Offending food and geographical variations

Food allergies disproportionately affect persons in industrialised or Western countries and are more common in children than adults. There is a relatively short list of foods that account for the majority of the more serious manifestations of food allergy, namely peanut, tree nuts, fish, shellfish, egg, milk, wheat, soy, and seeds[4,9,10]. A survey by the World Allergy Organization of 89 member countries using widely different methodologies reported wide variations in prevalence data revealing that prevalence rates for those < 5 years of age were lowest in Thailand and Iceland and highest in Canada, Finland, and Australia[11]. A birth cohort study from 9 European countries where 12049 infants were followed until the age of two years, and using OFC to confirm the diagnosis of food allergy, found an adjusted mean incidence of egg allergy of 1.23% (95%CI: 0.98% - 1.51%), with the highest rate in the United Kingdom (2.18%) and the lowest in Greece (0.07%)[12]. However, the prevalence rates of milk allergy were lower (0.54%; 95%CI: 0.41% - 0.70%), with the highest rates in The Netherlands and United Kingdom (1%) and the lowest rates in Lithuania, Germany, and Greece (< 0.3%)[12,13].

In a meta-analysis of food allergy by history alone, the prevalence of fish allergy was 0%-7% and shellfish allergy 0%-10.3%, whereas, when food challenges were used instead for the definition, the prevalence of fish allergy (0% to 0.3%) was similar worldwide, with shellfish allergy prevalence (0% to 0.9%) being higher in the Southeast Asia region[14]. A systematic review and meta-analysis of 36 studies from Europe and the United States on the prevalence of tree nut allergy showed a prevalence rate < 2% for oral challenge confirmed allergy and 0.05% to 4.9% for possible allergy. Hazelnut was the most common tree nut allergy in Europe, and walnut and cashew were the most common in the United States [15]. Some of the highest rates of food allergy, diagnosed by an OFC, were reported from Australia, where > 10% of one-year-old infants had challenge-proven IgE-mediated food allergy to one of the common allergenic foods of infancy. The prevalence of any sensitisation to peanut was 8.9% (95%CI: 7.9-10.0); raw egg white, 16.5% (95%CI: 15.1-17.9); sesame, 2.5% (95%CI: 2.0-3.1); cow's milk, 5.6% (95%CI: 3.2-8.0); and shellfish, 0.9% (95%CI: 0.6-1.5). The prevalence of challenge-proven peanut allergy was 3.0% (95%CI: 2.4-3.8); raw egg allergy, 8.9% (95%CI: 7.8-10.0); and sesame allergy, 0.8% (95%CI: 0.5-1.1) [16,17].

Ethnicity

A systematic review aiming to address potential racial and ethnic disparities showed that black persons, mainly children, had increased food sensitisation or food allergy. However, these results were tempered by the heterogeneity of the different reports and also by some inherent limitations of some of the included studies[18]. The rate of increase in self-reported paediatric food allergy was greater in non-Hispanic black subjects (2.1% per decade) compared with non-Hispanic white subjects (1% per decade) [19]. In a high-risk inner-city cohort of children, 74% black and 18% Hispanic, a very high rate of food allergy (9.9%) was reported[20]. African American children have higher odds than white children of having an allergy to wheat, soy, corn, fish, and shellfish. However, they had similar rates of peanut,

milk, and egg allergy; and lower rates of tree nut allergy, but they also had higher rates of anaphylaxis and emergency department visits[21]. In the United Kingdom, between 1990 and 2004, there was an increase, from 26.8% to 50.3%, in the proportion of non-white patients with peanut allergy (but not egg allergy). However, the proportion of black subjects attending the clinic had not changed during that period[22]. In New York City schools, no difference was found in food allergy rates between black and white children[23].

Multiple food allergies

An electronic household survey in the USA estimated that 8% of children have a food allergy, with 2.4% having multiple food allergies and 3% having experienced severe reactions[5].

It is, therefore, clear that different reports of prevalence are influenced by many factors, alone or in combination, such as race, ethnicity, country, geographical location, and offending allergens, as well as many other factors such as parents' education, development of health care and the reporting systems. Therefore, disparities need to be better characterised. They might reflect differing awareness of food allergy and access to health care, racial/ethnic or socioeconomic influences on childhood feeding practices, or true differences in prevalence[24].

PATHOPHYSIOLOGY AND MEDIATORS OF FOOD ALLERGY

The immune system plays an integral role in maintaining tolerance to innocuous antigens.

The primary exposure

IgE-mediated food allergies occur as a result of dysregulation in the immune system, which maintains a state of tolerance by preventing benign food antigens from being incorrectly identified as pathogens. Oral tolerance to food is defined as the trans-mucosal crossing of food antigens, processing by non-activated dendritic cells and the control of the inhibitory cytokines mediators as interleukin (IL) 10, by the cells taking the first role in the primary allergen exposure where the unknown protein particles taken by antigen-presenting cells to the lymphoid tissue proximal to the site of exposure. Usually, the process gives the all-clear to the new food protein through T regulatory cells and inhibiting Th2 cells production. It will also result in increased IgA and IgG_4 production with a decrease in IgE production. In addition, there is also an immune suppression of eosinophils, basophils, and mast cells effector cells responsible for causing symptoms. In such a scenario, the innocent food protein will be given the "all clear" by default by the balanced and non-incorrectly triggered immune system.

Sensitisation is defined when food-specific IgE is detectable in the blood. This immunological response is fundamental in the development of type 1 hypersensitivity reaction, and it is primed by the transfer of food protein across the deranged digestive tract membrane and leads to an unnecessary release of inflammatory cytokines, which activate dendritic cells, which will, in turn, trigger naïve T cells into acquiring a Th2 phenotype. The latter will promote inflammatory signals which induce food antigen-specific B cells to produce food antigen-specific IgE. Sensitisation, therefore, is the mistaken identification of a benign food antigen as a pathogen. The specific IgE to that particular protein binds to the surface receptors of mast cells, basophils, macrophages, and other antigen-presenting cells, priming the immune system to an allergic reaction should a second exposure occur to that specific allergen.

A second exposure to the allergen results in the burst of mast cells, leading to the release of histamine, which is one of the most important preformed mediators responsible for the symptomatology of both mild allergic reactions and anaphylaxis. When histamine is secreted from mast cells and resemblance cells, its effect immediately manifests through fades within a few minutes. Histamine causes all the signs and symptoms seen in mild and severe allergic reactions, such as an increased capillary leak, hives, tachycardia and drop in blood pressure.

Other inflammatory mediators such as prostaglandin D2, platelet-activating factor, and leukotrienes also contribute to the allergic reaction.

In summary, the five components of the immune system, the epithelium, innate immune cells, T cells, B cells, and effector cells (mast cells, eosinophils, and basophils), can either promote tolerance to food antigens or sensitisation to them, which will then lead to allergic manifestations.

The role of Epithelial Barriers

The role of the intestinal epithelial cells in the central regulatory mechanism controlling the absorption of ingested antigens is important. It helps maintain tolerance by preventing the unnecessary entry of antigens and thus avoiding the secondary production of inflammatory cytokines.

Antigens cannot freely pass an intact epithelial barrier but are often transported through mechanisms of net movement of particles present beneath the cells through several very specialised cells[25].

In the absence of pro-inflammatory or danger signals, food antigens recognised by specialised antigen-presenting cells will further promote the maintenance of tolerance through the release of mediators such as IL 10 and transforming growth factor-beta (TGF-β), which will promote the development of regulatory T cells[26-28].

The role of T Cells

The relevant cytokine and other mediators released by a nonspecific line of defence cells such as natural killer cells, macrophages, neutrophils, mast cells, dendritic. Cells, mast cells, and basophils all help by playing a role in tolerance production and the generation of T regulatory cells[26-28]. Moreover, products from the dendritic cells permit a complicated exchange process in the gastrointestinal lining to produce an inhibitory effect by binding the effector particles to CTLA-4[29,30]. In addition, inflammatory mediators such as IL-10 released T regulatory cells can also have an inhibitory effect on effector cells[31].Th2 cells periodically move from the local lymphoid glands into the thin layer lining of the exposed surface of the gastrointestinal tract, where inflammatory mediators such as IL5 and IL13 stimulate the process of B cell activation, leading to the development of the body action towards certain foods. At the same time, naïve T cells transform into helper -and the release of IL-9, which eventually result in the development of allergic reaction and an increase in the histamine secreting cells[32].

TYPES OF FOOD ALLERGY

Oral tolerance refers to a systemic immune non-responsiveness to antigens first encountered by the oral route. A failure to develop this homeostatic process in persons who are genetically and probably environmentally predisposed to atopy can result in the development of food allergy[33]. Based on the immunological mechanism involved, food allergies may be classified into three types[1-3,7,34,35].

IgE-mediated food allergy

This is the best-known and well-characterised type of food allergy. It is the most common food allergy in the Western world, with the highest prevalence in children below three years of age (6%-8%)[6]. There has been a steady increase in this type of food allergy and food induced-anaphylaxis in the Western world[36,37].

These allergic reactions are immediate, reproducible, and caused by food-specific IgE, which can usually be detected by *in vivo* or vitro tests to confirm the food allergy diagnosis[6]. These food-specific IgEs bind to high-affinity receptors for the Fc region of IgE (Fc□RI) on basophils, mast cells, dendritic cells, and Langerhans cells in the gut, skin, or through the respiratory system. When exposed to the offending food, the specific food antigen is recognised by two or more specific IgE bound to the Fc□RI, resulting in a cross-link of the receptor and activation of mast-cells to release histamine and other mediators. These released chemical mediators will cause vasodilation, hives, angioedema, low blood pressure, smooth muscle constriction, consequent bronchospasm, diarrhoea, and vomiting[38].

Food allergy-associated anaphylaxis is an IgE-mediated reaction. In a previously sensitised person with food-specific IgE on mast cells and basophils, the food allergen is ingested and absorbed into the local tissue, then cross-links with IgE resulting in immediate release of preformed mediators[2,39,40]. This immune response is rapid; the onset of symptoms typically occurs within 5 to 60 min after exposure to the food. An anaphylactic reaction affects multiple organ systems and may rapidly develop severe symptoms (*e.g.*, hypotension or respiratory collapse) and even death[41].

Although cutaneous manifestations such as hives and pruritus are the most common, they are absent in 20% of anaphylaxis persons. Thus, a high index of suspicion is required when other signs and symptoms such as cough, wheezing, laryngeal oedema, vomiting, diarrhoea, and hypotension are present. IgE-mediated food allergy is rarely associated with fatal anaphylaxis in children and adolescents. Recent data have linked cow's milk protein to several severe anaphylactic reactions, including a deadly anaphylactic reaction to baked milk following an OFC test.

Several devastating incidences of food allergy, which unfortunately results in fatality either at take away restaurants, school or even following supervised food challenges, made the issue of food allergy a major concern for parents, the public, school, and health authorities.

Up to one-third of the population now believes that they have food allergies, a much higher estimate than the actual prevalence based on physician diagnosis (5% of adults and 8% of children)[42].

The most common foods incriminated in IgE mediated food allergy are milk, egg, peanuts, tree nuts, seafood, soy, wheat, and seeds. Sesame has recently been emerging as a typical new food allergen in the Middle East and Europe[43].

Food pollen syndrome or Oral allergy syndrome: A unique and interesting form of IgE-mediated food allergy is a pollen-associated type of food allergy due to the cross-reactivity of epitopes shared between allergen molecules in certain pollens and some vegetables and fruits[44]. Here, the primary sensitisation occurs to pollen allergen, with the initial symptoms being allergic rhinitis. However, upon further exposure to that particular fruit or vegetable which shares epitopes or components with pollens responsible for the primary sensitisation, other symptoms then develop in addition to allergic rhinoconjunctivitis such as itching, redness and oedema of the lips, numbness in lips and tongue, itching, and swelling in the throat[44,45]. Pollen food syndrome (PFS) usually does not lead to anaphylaxis. Its symptoms can usually be avoided by either peeling the skin of the fruit or vegetables or by boiling them. Rinsing the mouth with water usually eases the symptoms. PFS is commonly misdiagnosed as a

true food allergy, with children being prescribed unnecessarily an adrenaline autoinjector[46,47].

Non-IgE food allergy

These are immunologic reactions to food that occur without demonstrable food-specific IgE antibodies in the skin or the serum and can therefore have several pathogenic mechanisms[48]. The non-IgE-mediated disease consists of a wide range of gastrointestinal conditions, generally of slow onset and with signs and symptoms very similar to other common conditions, especially in the first year of life, such as colic, gastroesophageal reflux, diarrhoea, and eczema, making it difficult to recognise.

This type of food allergy has been increasing worldwide. It encompasses eosinophilic oesophagitis (EoE), Non-eosinophilic gastrointestinal disorders (Non-EoE-EGID), food protein-induced enterocolitis (FPIES), and food protein-induced allergic proctocolitis (FPIAP). While EoE, Non-EoE-EGID, FPIAP are chronic, FPIES is always an acute disease. Although T cells may play a central role in non-IgE mediated food allergy and EoE, the pathogenesis of FPIES and FPIAP remains less clear[49,50]. Non-IgE mediated food allergies are usually managed by joint care between the paediatric allergist and the gastroenterologist.

Mixed IgE-cell-mediated food allergy

This occurs when both IgE and immune cells are involved in the reaction. Mixed and non-IgE-mediated food allergies, such as EoE, eosinophilic gastroenteritis (EG), and atopic dermatitis (AD), have a more prolonged onset and manifest primarily in the gastrointestinal tract and skin[51]. Infants and young children with EoE may present with feeding dysfunction and failure to thrive, whereas older children and adults often manifest vomiting, abdominal pain, dysphagia, and food impaction. EoE is diagnosed by oesophageal biopsy, demonstrating the presence of > 15 eosinophils per high-powered field. It is not uncommon in patients with EoE to have other allergic diseases, such as allergic rhinitis and IgE-mediated food allergy. Food allergens and possibly aeroallergens seem to play causative roles in the immunopathology of EoE, and food-avoidance diets are often effective in inducing clinical and histologic improvement. When eosinophils are found distal to the oesophagus in the gastrointestinal tract, the diagnosis of EG is then made. Symptoms of EG vary depending on the portion of the gastrointestinal tract involved and may include abdominal pain, nausea, diarrhoea, malabsorption, and weight loss. Unlike EoE, food-avoidance diets offer little or no benefit in EG[52].

AD also has features of mixed IgE- and non-IgE-mediated food allergy. The presentation includes a chronic pruritic rash distributed on flexor surfaces such as the antecubital and popliteal fossa, wrists, ankles, and neck. In approximately 35% of children with AD (typically young children with severe AD), food allergens may exacerbate their rash, causing increasing erythema and pruritus over a few hours if only IgE-mediated or over days if non-IgE mediated. Milk, soy, egg, wheat, and peanut are the most common culprit foods. Elimination of suspect foods often improves AD symptoms within a few weeks, whereas repeated exposure exacerbates symptoms[52].

Sensitivity to food chemicals: This is thought not to be a true allergy and is not immune-mediated. However, it is commonly described in food allergy as it shares similarities. It represents an adverse chemical reaction to either existing food chemicals such as amines, salicylates, natural food colourings and glutamate, or artificially added food chemicals such as sulfites, benzoates, and artificial food colourings. A small amount of these chemicals are well tolerated most of the time. Salicylates naturally exist in fruits, vegetables, nuts, and cereals and are also used to manufacture chewing gum, toothpaste, and mouthwashes. Amines such as histamine can occur naturally in food or are secondary to microbial contamination or fermentation. Histamine-associated symptoms include urticaria, angioedema, itching, rhinitis, conjunctivitis, abdominal cramps, palpitation, flushing, and headache[53]. Glutamate is an essential amino acid occurring naturally in many foods such as cheese, tomato, mushrooms, soy, and yeast extract. Monosodium glutamate MSG (E621) is commonly used in the manufacture of soaps, food and sauces, widely consumed in Asian restaurants. The "Chinese Restaurant Syndrome" is a condition caused by glutamate, widely used in Chinese food, and which manifests as headache, muscle tightness, nausea, tingling, flushing, and chest tightness.

A food additive is any substance not naturally present in that food, such as E 220, E 221, E 222 cited on some food labels. Sulfite is a very common food additive that exists in shrimps, beer, wine, dried grapes, and pizza dough and which may induce symptoms ranging from mild reactions to anaphylaxis. Food colourings can be found in tea, berries, and cinnamon, and, although they cause concern to the public, they rarely produce reactions. The exact mechanism of producing these reactions is not fully understood. Colourings have also been linked to hyperactivity in children, especially when combined with other food additives[54].

MANIFESTATIONS OF FOOD ALLERGY

We will focus exclusively on IgE-mediated food allergy, a type I hypersensitivity reaction that occurs

when ingestion of specific food triggers a response by preformed circulating IgE antibodies developed earlier against that same food[7,55].

When food molecules which have been wrongly appreciated as a pathogen in the atopic child, comes in contact with the lamina propria of the intestinal tract, it rapidly binds to basophils and mast cells, resulting in degranulation of these cells and the release of the inflammatory mediators such as histamine and tryptase[56]. These mediators are responsible for the signs and symptoms are seen in mild and severe food allergic reactions such as the development of urticarial rash, cough, hoarseness of voice, bronchospasm, hypotension, and collapse[57,58].

Cutaneous manifestations

They are the most common IgE-mediated food-allergic symptoms. Usually presents as redness of the skin, itchy rash, which commonly takes the form of papules or hives. When the inflammation involves the deeper layers of the skin, the skin manifestation is called angioedema. Hives can last for hours if not treated. Angioedema develops when the swelling extends below the skin's surface and fatty tissue. It usually presents eyelids, face, and lips swelling, causing significant discomfort. Eczema may develop or worsen in non-IgE-mediated food allergy, although pre-existing eczema can also worsen with IgE-mediated food allergy. The magnitude of the skin reactions in type 1 hypersensitivity food allergy is reflected by the surface area of the skin affected[59].

Respiratory manifestations

The entire respiratory tract from the nose to the mungs can be affected. Symptoms vary from runny nose, congested nose, sneezing, itchy nose to cough, stridor, wheeze, or breathing difficulty. Some children experience severe tightness in the throat and a feeling of impending death[7,58,60]. It has been reported that some patients who presented to the emergency room with anaphylaxis due to food allergy have been mistakenly diagnosed and treated as life-threatening asthma.

Gastrointestinal manifestations

Abdominal pain, vomiting, and diarrhoea are the cardinal gastrointestinal feature of IgE-mediated food-allergic reactions. These symptoms are usually quick in onset and could appear immediately following the exposure to the offending food up to 2 to 4 h later. Other problems such as constipation and failure to thrive are more common in non-IgE -mediated food allergy[7].

Cardiovascular and neurological manifestations

They are usually described as the most severe complications of IgE mediated food allergy. Children become pale dizzy with tachycardia and may experience a marked drop in their blood pressure, resulting in collapse. Cardiovascular involvement commonly goes hand in hand with skin or respiratory manifestation[7,57]. Death rates are very low but usually very tragic.

Anaphylaxis

This is a serious form of an IgE-mediated hypersensitivity allergic reaction involving more than one organ system, including the respiratory tract, gastrointestinal tract, and skin. It is rapid in onset and potentially fatal[41]. Even though it is rare, anaphylaxis can also present with only cardiovascular or neurological symptoms such as dizziness, weakness, tachycardia, hypotension, cardiovascular collapse, or unconsciousness[57]. The World Allergy Organization classified anaphylaxis into five grades The classification is based on the number of organ systems involved, the severity of the morbidity induced by the allergic reaction, subjective measurements such as the forced expiratory volume in 1 second (FEV1) and the response to treatment given. Grade1 describes mild morbidity; meanwhile, death is the outcome of grade 5[61] (Table 1). For simplicity, acute allergic reactions that involve skin such as urticarial rash, lips swelling, eye swelling, or abdominal pain and vomiting only are usually classified as mild to moderate. However, if one or more of the above symptoms are associated with cough, hoarseness of voice, stridor, wheeze, difficulty in breathing, pallor, or collapse, the reaction will be described as anaphylaxis. Anaphylaxis is life-threatening, but in most cases, it does not produce a severe outcome and rarely causes death. Despite the existence of many local and international guidelines for making its diagnosis, the diagnosis of anaphylaxis remains subjective to a greater extent. Biochemical testing such as serial measurements of serum tryptase during the acute presentation and at 1 h later has been introduced to help make an accurate diagnosis of anaphylaxis when in doubt.

The term biphasic reaction refers to a reaction that describes a second surge of histamine from degranulated mast cells after the initial symptoms of anaphylaxis settled. It is reported to occur in about 10% of patients who suffer an anaphylactic reaction. Hypotension is linked to severe morbidity and mortality when it happens, and its incidence is estimated to be 3% in the cases of anaphylaxis in children[62].

Risk factors for severe anaphylaxis

Risk factors most strongly associated with fatal or near-fatal anaphylaxis include the type of allergenic food, adolescence or young adulthood, the presence of concomitant asthma, and the delayed use of, or

Table 1 World Allergy Organization systemic allergic reaction grading system

System	Grade 1	Grade 2	Grade 3	Grade 4	Grade 5
	One organ system involved (cutaneous, respiratory, ocular or others)	Two organ systems involved, or lower respiratory tract involvement, gastrointestinal involvement, or uterine cramping			
Cutaneous	Generalised pruritus, urticaria, flushinor a sensation of heat or warmth, or angioedema not involving laryngeal, tongue, or uvular tissues. Localised hives or angioedema alone are not considered anaphylaxis				
Respiratory	Upper respiratory tract symptoms: sneezing, rhinorrhea, nasal pruritus and nasal congestion, throat clearing, itchy throat, and coughing	Lower respiratory tract symptoms: wheezing, shortness of breath. And a drop of 40% in the forced expiratory volume in one second (FEV1) and which responds to bronchodilators	Symptoms of laryngeal, uvular, or tongue tissue oedema. With or without stridor. Or FEV1 drops by 40% with no response to bronchodilators	Respiratory failure	
Cardiovascular				Hypotension	
Gastrointestinal		Abdominal cramping, vomiting or diarrhoea			
Conjunctival	Conjunctival erythema, pruritus, or tearing				
Other	Nausea, metallic taste, or headache	Uterine cramping			Death

lack of access to, an epinephrine autoinjector[41,62]. Also, several factors, including exercise, viral infections, menses, emotional stress, and alcohol consumption, place some persons at increased risk by lowering the reaction threshold after exposure to an allergen.

ALLERGY HISTORY TAKING

A good history is crucial for making an accurate diagnosis of food allergy. The use of "allergy-focused clinical history" is universally recommended, as it considers all the events and history related to the allergic reaction. A thorough inquiry about the personal and family history of atopy is also required. At least 30 min should be allocated to the first allergy consultation. In the case of IgE-mediated food allergy, the record is usually good enough for reaching the diagnosis. The National Institute for Health and Care Excellence produced a document on the quality standards of food allergy services in the United Kingdom, highlighting the importance of history taking in diagnosing food allergy and formulating any further management of the allergic patient[63].

Similarly, the European Academy of Allergy and Clinical Immunology had an essential role in standardising the allergy-focused history to maximise its value as an important diagnostic tool. It also considers the patient's age to produce age-specific standardised account-taking formats for children and adults, to be used by paediatricians, physicians, family physicians, allergists, and dietitians[64]. In 2009 the Royal College of Paediatrics and Child Health published its allergy care pathway, a reference for taking an allergy-focused clinical history in paediatrics[65].

INVESTIGATIONS IN FOOD ALLERGY

A variety of *in vivo* and *in vitro diagnostics* have been developed to assist with diagnosing food allergy.

In vivo tests

Skin prick test: It is the most commonly used test when investigating food allergy in children and adults. Usually, it is performed during the first visit and can also be repeated later to compare the size of the wheal produced by the allergen in question. This would help predict any development of natural tolerance or decide to conduct an OFC, which would help advise on either the continuation of avoidance or instead, to allow food reintroduction.

The skin prick test (SPT) is conducted using standardised extracts from different foods and environmental allergens such as house dust mites, pollens and animal dander. A drop of the standardised allergen solution is usually put on the child's forearm or back, then scratched with a lancet or a pointed device, aiming to prick the skin through the placed drop of the solution. Two drops of saline (negative

control) and histamine (positive control) are used to validate the results; *i.e.*, a positive impact for normal saline or a negative effect on histamine would void the test results. While the former development could occur in individuals with dermographism, the latter could indicate that the child has received antihistamines within 24-72 h of the test. After pricking the skin, the solution left on the skin is wiped by tissue as it may irritate the skin and sometimes makes it difficult to measure the reaction produced after 15 min. SPT uses standardised solutions produced by several manufacturers. The assessment of the wheal or erythema is used to determine the positivity of the test, with a wheal of ≥ 3 mm indicating a possible clinical allergy[65]. Another criterion for interpretation is to compare the wheal produced by the allergen solution to the one developed by the positive control (histamine). The test is considered positive if the wheal diameter made by the antigen extract is equal to or larger than the positive control. Most importantly, the SPT result should always be interpreted in the context of the patient's clinical history. A positive skin test only identifies sensitisation to the particular allergen but does not necessarily indicate a clinical allergy.

SPT is very informative for the child and the parents. It serves as a visual aid to reinforce the need for compliance with the avoidance of particular food, and it is usually performed in the clinic. Cost is low, especially when compared to other tests such as Component resolved diagnostics (CRD), where the number of tests, and thus prices, increase significantly with the required multiple components. Urticarial rash and itching can cause discomfort. Conducting and interpreting the test can also be challenging in individuals with eczema. A systematic review and meta-analysis found that the sensitivity of SPT ranges from 68 to 100 %, with a specificity of 70 to 91 %[66].

Recognisable wheals have been observed in 3-month-old infants, and some clinicians perform the test in infants from any age. However, the wheel size increases with age from infancy to adulthood, reflecting the change in the immune response. SPT is generally a safe procedure and is easily interpreted by trained professional staff. Emergency equipment and drugs should be readily accessible in case of any systemic adverse reactions to the allergen solution[67].

In vitro tests

Allergen-specific IgE: The serum's measurement of specific IgE, commonly known as the RAST test, through enzyme-linked immunosorbent assay (ELISA) technique, is being used nowadays, instead of the old RAST. In the allergen-specific IgE (sIgE) test, the allergen extracts of the allergen of interest are chemically attached to plastic test tubes or put into multiple wells in sensitised test plates, where a tiny volume of the patient's serum is added. As a result, any amount of the sIgE to the allergen will stick to the tube. A radiolabeled anti-IgE is then added and, after incubation and washing, the radioactivity is measured. The result is expressed in numbers, with a standard reference. It is more expensive than SPT, and despite the length of time to obtain its results, sIgE to the widely used. It remains an excellent alternative to SPT in patients with severe eczema or if systemic antihistamines have been taken within 1-3 d before the SPT[68]. The Immuno solid-phase allergen chip test uses microchip technology to detect specific IgE antibodies in a tiny blood sample to 112 food and airborne allergens using the same ELISA technique. Results need to be carefully interpreted due to the possibility of cross-reactivity between food proteins.

The total serum IgE concentration has lost its importance as one of the diagnostic tools in food allergy. It is commonly raised in atopic children with eczema, parasitic infestations, and immunodeficiencies. Additionally, low levels cannot rule out the existence of IgE-mediated food allergy.

CRD: Food components testing emerged in the 1990s as a diagnostic test capable of measuring IgE antibodies to specific components contained in the allergen of interest. Contrary to the basic old concept that cow's milk and food plants each have a single protein, determining its allergenicity, it became evident that every food plant has a range of heterogeneous protein components that may differ in their heat and acid stability as in their allergenicity.

By having specific information about the allergenicity, and heat stability of a particular component to which a child is sensitised, a more specific individualised action plan can be drawn up, depending on whether or not the child can tolerate the cooked/baked form of that food (if sensitised only to a heat-labile component(s). It can also help decide if the child or parents need to carry an adrenaline autoinjector, as determined by the allergenicity of the sensitised element (s)[69]. Sensitisation to peanut lipid transfer proteins, such as peanut Ara h1 and Ara h2, both heat and proteolytic resistant, would produce severe systemic reactions. At the same time, sensitisation to the peanut Ara h8 component alone would only produce oral symptoms[69]. Some data showed that sensitisation to certain proteins is linked to prolonged allergy, as in the case of Gald1 and Gald2 epitopes of hen eggs[70].

Like other allergy diagnostics, CRD should not be solely used to diagnose food allergy and should only be requested by clinicians competent in interpreting its results. More than 4700 food components have been discovered, adding complexity to the test. CRD can also help diagnose PFS and distinguish it from true IgE-mediated food allergy; later, the primary sensitisation occurs to food proteins rather than pollens. The two conditions' management, severity, and outcome are very different.

Basophil activation test: SPT, sIgE, and CRD cannot accurately diagnose food allergy because they only test for sensitisation by detecting specific IgE to whole food protein or its components. Sensitisation

does not mean allergy and cross-reactivity are common, especially in children with atopic dermatitis. Also, some non-allergenic components, such as polysaccharides, can trigger the production of specific IgE of no clinical importance, as it cannot produce an allergic reaction. The role of basophils is similar to mast cells in IgE-mediated food allergy, with both sharing similar high-affinity IgE receptors where specific IgE antibodies attach to their surface. As with mast cells, the basophil degranulates when re-exposed to food allergens. Detecting and measuring the degranulation of basophils by flow cytometry allows testing for allergy rather than sensitisation, likening the basophil activation test (BAT) to a "food challenge in a test tube". BAT can compensate for SPT, sIgE, and CRD testing deficiency. BAT results were 92% in line with the double-blind placebo-controlled food challenge in one study. This method is becoming increasingly used, and several commercial forms are currently available. However, its use is still restricted to research laboratories and some centres. Large-high-quality studies to standardise BAT are still lacking[69]. If BAT can replace the OFC, it would be considered one of the major successes in allergology.

OFC: Blood tests or skin prick tests cannot accurately predict the severity of the allergic reaction. Double-blind placebo-controlled OFC is the standard gold test for diagnosing food allergies. It consists of the oral administration of incremental doses of the suspected allergen, *e.g.*, cow's milk or peanut. It requires a physician, a nurse, space, and rescue medications. OFC is commonly performed in-office or clinic settings, especially for low-risk challenges. High-risk challenges, such as previous anaphylaxis and FPIES, should always be conducted in a safe environment with full resuscitation facilities to treat anaphylaxis.

OFC is an ideal diagnostic test used to either confirm or rule out food allergy when the history of alleged allergic reaction to food is inconsistent with the SPT and blood tests or when the clinician or parents want to explore food alternatives in children with multiple food allergies. Most importantly, it is used before food reintroduction, when the latest SPT or sIgE show that they may grow out of their allergy, which might have caused anaphylaxis in the past. The natural history of some food allergies, such as milk, is that it always occurs in the first year of life, but most children grow out of it quickly. Home reintroducing food to parents who witnessed their child suffering from anaphylaxis is not a good option for them or the child. Thus, they may never reintroduce cow's milk even with their doctor's reassurance and even when the allergy markers suggest the development of natural tolerance to it.

Any regular antihistamines should be discontinued at least 48 h before the challenge. When performing the challenge, the dose should be gradually increased until a typical food serving appropriate for the child's age is consumed. The total weight or portion of the food can be divided into four or six portions. A negative challenge is valid if no symptoms are observed following exposure to the problematic food, in an amount equivalent to a standard serving. The medical team will observe the patient for symptoms for several hours after the challenge. The procedure should be delayed if the child is unwell with cold, flu symptoms, or suffering from an asthma exacerbation. The latter is especially important when a child with asthma had received a short-acting beta-agonist or beta-blockers earlier. It may increase the risk of allergic reactions and antagonises the effect of anaphylaxis rescue treatment. Ideally, the child and parents are located in a calm and relaxing area, preferably with a play specialist available. Usually, parents will be requested to bring the food for the challenge, but this depends on the hospital policy. The paediatric dietitian will liaise with the allergy nurse or the doctor to determine the weight and portion of the food needed for the challenge. Usually, the food is given to parents under the observation of the medical staff. The challenge should be called off if the child develops symptoms of allergy such as hives, vomiting, change in behaviour, cough, stridor, pallor, or any other suggestive manifestations. The clinician responsible for the challenge should be immediately informed and treatment provided instantly[70]. If the symptoms or signs are very subtle, not convincing, or thought to be just a skin irritation to the food rather than an allergic reaction, the same dose can be repeated. The child is regularly observed throughout the procedure, with sets of vital signs and examinations, and whenever a reaction is suspected. If the child passes the challenge, it is recommended to continue to consume the challenged food regularly to prevent re-sensitisation.

OFC is time and staff-intensive. A single food challenge may take up to 4 h, and occasionally the child may refuse to eat or drink the challenging food. It is acceptable to stop the procedure should the parents request it, even without a valid reason.

References are available for serum level of sIgE and the wheal size of SPT to typical food to help predict and increase the rate of successful OFC. Although these diagnostics cannot be wholly relied upon, they may encourage clinicians and reluctant parents to accept the OFC. Passing the OFC would enable parents to reintroduce certain essential foods such as milk, egg, and fish, which could have been avoided earlier. It also reduces the stress experienced by the family and helps improve their quality of life and the child's nutritional status. The success rate of passing OFC is estimated to be around sixty per cent, with the most commonly encountered reactions being mild to moderate and the occurrence of anaphylaxis being infrequent[71]. Such responses should not undermine the clinician from doing further OFC. Anecdotally, some allergists even state that if a clinician does not see reactions while doing OFC, they may not be challenging suitable patients.

TREATMENT OF FOOD ALLERGY

There is no approved medical treatment for food allergy that develops a permanent tolerance. Treatment remains primarily based on counselling the patients and their families to avoid offending food, such as carefully reading food labels, taking precautions when dining out in restaurants and parties or being mindful of mistakes caused by a language barrier when travelling abroad. Parents of children with a food allergy should have a written allergy action plan. This usually includes the name of the offending food(s), information on how to detect an allergic reaction, when and how to use the rescue medications such as an oral antihistamine or an adrenaline autoinjector device, and what to do next. A copy should also be given to school nurses.

Oral immunotherapy

There has been a significant increase in oral food immunotherapy trials, with the majority focusing on developing oral immunotherapy (OIT) for peanut, cow's milk, and hen egg allergy. These trials were based on the old concept that the continuous introduction of small amounts of allergenic food would induce tolerance over weeks or months. These protocols were designed to induce tolerance while ensuring children's safety[71]. In real life, food OIT is still not widely available for all children with food allergy, and its use is minimal due to the absence of formally OIT-approved protocols in most countries.

Palforzia is the first FDA-approved food allergy medication designed to treat peanut allergy. It is a peanut allergen extract that has shown success in double-blinded placebo-controlled trials, with 67.2% of Palforzia recipients tolerating 600 mg of peanut protein when challenged, compared to 4% of the placebo arm of the study. The treatment risks provoking anaphylaxis, especially during the initial dose-escalation phase. A significant limitation of this treatment is that it does not provide a definite cure, as treated patients will only be able to tolerate a limited portion of peanut by the end of the treatment. However, many clinicians, parents, and patients consider it a success and a life-saving treatment. More research is required to develop a treatment that can produce long-term natural tolerance without exposing the patients to the risks of severe side effects and anaphylaxis during treatment.

OIT duration usually extends over many months or years. Some suitable treatment protocols are currently under, hoping to enable the allergic child to consume a total age-proportional volume of milk by the end of the treatment. So far, only a few treatment protocols have the formal approval of accredited bodies. It has also been shown that in a standard allergy clinic setting, 79% of the children with peanut allergy tolerated the desensitisation protocol and maintained it afterwards by consuming a daily dose of peanut[72]. Other studies had shown that some participants, who were successfully desensitised earlier, developed later eosinophilic oesophagitis due to regularly consuming the allergenic food at a sub-allergenic dose subsequently improved when the treatment was later aborted[73]. Similar protocols have been created for cow's milk and hen egg-allergic children. Although most participants did not experience significant reactions, a small number developed anaphylaxis during the treatment. It remains unclear if OIT would eventually produce tolerance similar to when these children naturally grow out of their allergy or if it only induces transient desensitisation. Some treated patients lose tolerance once stopped taking the maintenance amount of the offending food. Thus, some consider this kind of treatment an additional risk rather than a therapy[74]. More work is required to provide safe and efficient protocols that can be applied to everyday practise by addressing the encountered short and long-term complications of the OIT. Moreover, different routes such as epicutaneous or sublingual are currently under study.

Adjuvants

Adjuvants are frequently added to vaccines to boost their immune response and reduce some undesirable reactions. Their role has been investigated in OIT to improve the duration of tolerance and reduce side effects. Aluminium salt is one of the most popular vaccine adjuvants used. However, its use in treating peanut allergy has been disappointing due to the side effects encountered, especially with subcutaneous peanut therapy[75].

Probiotics

Studies have confirmed the role of intestinal microbiota in supporting the early establishment of immune tolerance and reducing the risk of developing food allergies in early life. In the first few days of life, the newborn baby's gut becomes inoculated by bifidobacteria, lactobacilli, and other non-aerobic bacteria. Also, in breastfed infants, other bacteria such as streptococci, staphylococci, lactobacilli, and bifidobacteria colonise the gut. Interacting with the host gut, these bacteria play a beneficiary role in absorbing nutrients, interfering with pathogenic organisms' growth, are essential for developing immune tolerance to different antigens, including food antigens[76,77]. Most of the studies that looked at probiotics' function in food allergy have focused on their role in managing cow's milk allergy. A systematic review of nine trials involving 985 patients with cow's milk protein allergy has demonstrated moderately encouraging results with probiotics, showing that the use of Lactobacillus rhamnoses GG can help induce tolerance in infants with suspected cow's milk protein allergy[76,77].

Biologicals

Omalizumab is one of the most common biologicals investigated to enforce and speed up tolerance during oral food immunotherapy. In a double-blind placebo-controlled trial comparing it as a catalyst in the treatment of cow's protein milk allergy to a placebo in 57 participants aged between 7 to 32 years; no difference was found in the number of participants desensitised to the cow's milk protein in the two arms of the study. However, there was a marked reduction in the allergic reaction that needed resuscitation with adrenaline in the omalizumab arm[77].

Studies of omalizumab have been performed to improve the safety of OIT and find out if it could speed up a tolerance to cow's milk and peanut in food allergic individuals. In the cow's milk study, 92% of the participants could reach the maintenance dose, although almost half suffered moderate to severe reactions during the induction phase. In the peanut study, 88% of the participants tolerated the induction phase, with only 2% continuing to encounter allergic reactions, with some requiring adrenaline to treat anaphylaxis[78,79].

From the available evidence, omalizumab offers some benefits as a mono booster for desensitisation in food allergy, but its benefit remains limited. More work is required to support biologicals either as monotherapy or other adjuvants in desensitising food allergy.

The role of baked food

Strong hypotheses state that introducing baked food, such as biscuits or cake containing milk or egg, in children with cow's milk and egg allergy would expedite the development of natural tolerance to these foods of high nutritional value. The baked form of the food is usually less allergenic than the raw or lightly cooked form, as cooking at high temperature alters the conformational epitope of the food proteins, making them less allergenic to the sensitised child[80]. It has been estimated that almost two-thirds of children with cow's milk allergy can tolerate the baked milk in biscuits, and the egg in cakes, and in addition, the continued ingestion of the baked form of these foods, speeds up the natural tolerance. This observation has been noticed with both IgE-mediated and non-IgE-mediated allergies. Some researchers are unconvinced of the role of baked food in inducing natural tolerance. One argument is the short and naturally self-limiting duration of cow's milk and egg allergy, and the other is that those children who tolerate the baked form of milk and egg may have an allergy phenotype that enables them to tolerate the baked forms of the food, facilitating a rapid tolerance oduction[81]. The benefit of introducing baked milk or egg remains a success, not only because of the added nutritional value of these foods to the allergic child but also to help reduce parental stress and anxiety and develop food aversion to milk and egg in these children.

The

role of **the** ***early dietary introduction of potentially allergenic food in food allergy prevention***

Until now, infant and young children's feeding and nutrition international advisory boards, such as the World Health Organization and United Nations International Children's Emergency Fund, advise parents and professionals dealing with children to delay the introduction of the common allergenic foods such as peanut, egg and cow's milk to breastfed babies. These recommendations were introduced in the last few decades due to the remarkable rise in food allergy prevalence among children. Also, in an attempt to promote natural breastfeeding, the introduction of cow's milk was discouraged in breastfed babies in the first 6 to 12 mo of life.

Unfortunately, the opposite effect was witnessed, as the prevalence of food allergy in children continued to rise despite that conservative approach. This led the international food and allergy community to revise the current recommendations a prompted researchers to conduct multicentre clinical trials to compare the effect of the early introduction of allergenic food with the current recommendations. Learning Early About Peanut Allergy (LEAP), a study published in 2015, gave concrete evidence that early introduction of peanuts can significantly prevent the development of peanut allergy in many infants and very young children[82]. The LEAP study was a game-changer that overshadowed all the previous international guidelines and recommendations. More studies followed, investigating how the early introduction of egg and cow's milk resulted in similar outcomes. The introduction of the egg at the age of 4-6 mo helped reduce the development of egg allergy compared to those who had egg introduced at 10-12 mo[83]. Some countries, such as Canada, took the lead and started to change infants' feeding recommendations, with the early introduction of peanut butter for children with mild to moderate eczema, at 6 mo. For infants with severe eczema or severe egg allergy (< 1%), the introduction needs to be done under medical supervision along with a skin prick test done initially, followed by a home or hospital-graded introduction. The introduction should be avoided in case of high sensitisation, especially in those with a skin prick test of ≥ 8 mm wheal[84].

We hope to see significant changes in the current recommendation concerning the age of introducing weaning food and the introduction of certain foods -considered highly allergenic- below the age of 6 and 12 mo. The development of new weaning guidance can be a challenge, especially when it comes to the recommended age of introducing the different allergenic foods the amount and frequency of meals to be given. Alternatively, the new guidelines could be more pragmatic and leave it to parents and the baby's tolerance to dictate when and how to introduce these foods as it is in the proper form and consistency for the infant to swallow them.

Food allergy and parental anxiety

Food allergy in infants and young children causes significant stress and puts a heavy burden on parents. The psychological effects vary from anxiety and stress to depression. These manifestations were observed more often in mothers, peaking at certain stages in their children's lives, such as when they join nursery or school. While in other medical conditions, control is achievable by using medications and avoiding specific obvious triggers, with food allergy, the accidental exposure to a hidden ingredient could happen "anytime and anywhere", as described by some parents.

The allergenic food could be a component of a benign food fed to the child unknowingly, or eaten due to poor labelling or simply because another child shared his food with the atopic child. The stress is amplified if parents have witnessed their child suffering an anaphylactic reaction, which could affect the parents' entire life and undermine their sound ability to make the right decision in their daily lives. It may even interfere with providing the optimal treatment during anaphylaxis.

Physicians, allergy nurses and dietitians are encouraged to spend part of the allergy consultation practising good listening to the parents and inviting them to talk about what they feel and think about their child's allergy. Studies have addressed this issue by evaluating the stress produced, quality of life, and how normal activities (school, work, dining out, attending events, travel, and normal social life) have been affected. Some studies have recommended mandatory referral of these parents to the local psychological service for support and counselling. Psychological support and cognitive therapy would support these parents in finding a balance between keeping their child safe and enjoying a nearly everyday life[85].

Natural history and prognosis of food allergy

Most children with cow's milk and egg allergies grow out of their allergies even before school age[86]. However, tree nuts, peanut, fish, and shellfish allergies persist. Peanut is widely known for the aggressiveness of its allergic reaction. However, data in recent years have shown that severe morbidity and mortality have been linked to cow's milk and sesame. Figures of children with sesame allergy are growing worldwide for no apparent reason[43]. Tolerance induction is expected when the level of specific IgE antibody drops during every6 to 12-monthly monitoring, usually followed by home or hospital supervised reintroduction[6]. Interestingly, sometimes tolerance is diagnosed with the child accidentally ingesting the offending food, but to the parent's surprise, it does not cause any signs or symptoms.

CONCLUSION

There is a pressing need to develop new allergy tests that can accurately diagnose and predict the severity of any potential reaction. Future research is also needed to create simple and quick diagnostic tests to inform clinicians and parents when these children grow out of their food allergies.

No approved definite treatment can produce lifelong natural tolerance, and adrenaline autoinjector (AAI) remains the drug of choice in treating anaphylaxis. Studies showed some parents might underuse AAI due to a lack of empowerment knowledge of when and how to use the device. Data also showed worrying attitudes by teenagers towards the use of AAI related to their risk-taking behaviour. Further studies are still needed to elucidate other reasons for the underuse of AAI. Better training with audio-visual aids and psychological support for the patients and parents to find a balanced lifestyle is required. Adrenaline autoinjectors need to be made available in public places such as malls, bus stations, and schools, as the child's first-ever noticeable food allergy reaction could be a severe and life-threatening one.

More research into feeding recommendations with early introduction of allergenic food such as peanut, egg, and cow's milk is also needed.

FOOTNOTES

Author contributions: Elghoudi A and Narchi H wrote the paper, revised it, and approved the final submitted version.

Conflict-of-interest statement: The authors declare that the review article was written in the absence of any financial conflict of interest.

Country/Territory of origin: United Arab Emirates

ORCID number: Ahmed Elghoudi 0000-0002-9078-6152; Hassib Narchi 0000-0001-6258-2657.

S-Editor: Chang KL
L-Editor: A
P-Editor: Chang KL

REFERENCES

1 **Sampson HA**. Food allergy. Part 1: immunopathogenesis and clinical disorders. *J Allergy Clin Immunol* 1999; **103**: 717-728 [PMID: 10329801 DOI: 10.1016/s0091-6749(99)70411-2]
2 **NIAID-Sponsored Expert Panel**, Boyce JA, Assa'ad A, Burks AW, Jones SM, Sampson HA, Wood RA, Plaut M, Cooper SF, Fenton MJ, Arshad SH, Bahna SL, Beck LA, Byrd-Bredbenner C, Camargo CA Jr, Eichenfield L, Furuta GT, Hanifin JM, Jones C, Kraft M, Levy BD, Lieberman P, Luccioli S, McCall KM, Schneider LC, Simon RA, Simons FE, Teach SJ, Yawn BP, Schwaninger JM. Guidelines for the diagnosis and management of food allergy in the United States: report of the NIAID-sponsored expert panel. *J Allergy Clin Immunol* 2010; **126**: S1-58 [PMID: 21134576 DOI: 10.1016/j.jaci.2010.10.007]
3 Finding a Path to Safety in Food Allergy: Assessment of the Global Burden, Causes, Prevention, Management, and Public Policy. Washington (DC): National Academies Press (US); 2016-Nov-30 [PMID: 28609025]
4 **Branum AM**, Lukacs SL. Food allergy among children in the United States. *Pediatrics* 2009; **124**: 1549-1555 [PMID: 19917585 DOI: 10.1542/peds.2009-1210]
5 **Gupta RS**, Springston EE, Warrier MR, Smith B, Kumar R, Pongracic J, Holl JL. The prevalence, severity, and distribution of childhood food allergy in the United States. *Pediatrics* 2011; **128**: e9-17 [PMID: 21690110 DOI: 10.1542/peds.2011-0204]
6 **Cianferoni A**, Spergel JM. Food allergy: review, classification and diagnosis. *Allergol Int* 2009; **58**: 457-466 [PMID: 19847094 DOI: 10.2332/allergolint.09-RAI-0138]
7 **Sampson HA**. Food allergy. Part 2: diagnosis and management. *J Allergy Clin Immunol* 1999; **103**: 981-989 [PMID: 10359874 DOI: 10.1016/s0091-6749(99)70167-3]
8 **Nwaru BI**, Hickstein L, Panesar SS, Roberts G, Muraro A, Sheikh A; EAACI Food Allergy and Anaphylaxis Guidelines Group. Prevalence of common food allergies in Europe: a systematic review and meta-analysis. *Allergy* 2014; **69**: 992-1007 [PMID: 24816523 DOI: 10.1111/all.12423]
9 **Burks AW**, Jones SM, Boyce JA, Sicherer SH, Wood RA, Assa'ad A, Sampson HA. NIAID-sponsored 2010 guidelines for managing food allergy: applications in the pediatric population. *Pediatrics* 2011; **128**: 955-965 [PMID: 21987705 DOI: 10.1542/peds.2011-0539]
10 **Chafen JJ**, Newberry SJ, Riedl MA, Bravata DM, Maglione M, Suttorp MJ, Sundaram V, Paige NM, Towfigh A, Hulley BJ, Shekelle PG. Diagnosing and managing common food allergies: a systematic review. *JAMA* 2010; **303**: 1848-1856 [PMID: 20460624 DOI: 10.1001/jama.2010.582]
11 **Prescott SL**, Pawankar R, Allen KJ, Campbell DE, Sinn JKh, Fiocchi A, Ebisawa M, Sampson HA, Beyer K, Lee BW. A global survey of changing patterns of food allergy burden in children. *World Allergy Organ J* 2013; **6**: 21 [PMID: 24304599 DOI: 10.1186/1939-4551-6-21]
12 **Xepapadaki P**, Fiocchi A, Grabenhenrich L, Roberts G, Grimshaw KE, Fiandor A, Larco JI, Sigurdardottir S, Clausen M, Papadopoulos NG, Dahdah L, Mackie A, Sprikkelman AB, Schoemaker AA, Dubakiene R, Butiene I, Kowalski ML, Zeman K, Gavrili S, Keil T, Beyer K. Incidence and natural history of hen's egg allergy in the first 2 years of life-the EuroPrevall birth cohort study. *Allergy* 2016; **71**: 350-357 [PMID: 26514330 DOI: 10.1111/all.12801]
13 **Schoemaker AA**, Sprikkelman AB, Grimshaw KE, Roberts G, Grabenhenrich L, Rosenfeld L, Siegert S, Dubakiene R, Rudzeviciene O, Reche M, Fiandor A, Papadopoulos NG, Malamitsi-Puchner A, Fiocchi A, Dahdah L, Sigurdardottir ST, Clausen M, Stańczyk-Przyłuska A, Zeman K, Mills EN, McBride D, Keil T, Beyer K. Incidence and natural history of challenge-proven cow's milk allergy in European children--EuroPrevall birth cohort. *Allergy* 2015; **70**: 963-972 [PMID: 25864712 DOI: 10.1111/all.12630]
14 **Moonesinghe H**, Mackenzie H, Venter C, Kilburn S, Turner P, Weir K, Dean T. Prevalence of fish and shellfish allergy: A systematic review. *Ann Allergy Asthma Immunol* 2016; **117**: 264-272.e4 [PMID: 27613460 DOI: 10.1016/j.anai.2016.07.015]
15 **McWilliam V**, Koplin J, Lodge C, Tang M, Dharmage S, Allen K. The Prevalence of Tree Nut Allergy: A Systematic Review. *Curr Allergy Asthma Rep* 2015; **15**: 54 [PMID: 26233427 DOI: 10.1007/s11882-015-0555-8]
16 **Osborne NJ**, Koplin JJ, Martin PE, Gurrin LC, Lowe AJ, Matheson MC, Ponsonby AL, Wake M, Tang ML, Dharmage SC, Allen KJ; HealthNuts Investigators. Prevalence of challenge-proven IgE-mediated food allergy using population-based sampling and predetermined challenge criteria in infants. *J Allergy Clin Immunol* 2011; **127**: 668-76.e1 [PMID: 21377036 DOI: 10.1016/j.jaci.2011.01.039]
17 **Peters RL**, Koplin JJ, Gurrin LC, Dharmage SC, Wake M, Ponsonby AL, Tang MLK, Lowe AJ, Matheson M, Dwyer T, Allen KJ; HealthNuts Study. The prevalence of food allergy and other allergic diseases in early childhood in a population-based study: HealthNuts age 4-year follow-up. *J Allergy Clin Immunol* 2017; **140**: 145-153.e8 [PMID: 28514997 DOI: 10.1016/j.jaci.2017.02.019]
18 **Greenhawt M**, Weiss C, Conte ML, Doucet M, Engler A, Camargo CA Jr. Racial and ethnic disparity in food allergy in the United States: a systematic review. *J Allergy Clin Immunol Pract* 2013; **1**: 378-386 [PMID: 24565543 DOI: 10.1016/j.jaip.2013.04.009]

19 **Keet CA**, Savage JH, Seopaul S, Peng RD, Wood RA, Matsui EC. Temporal trends and racial/ethnic disparity in self-reported pediatric food allergy in the United States. *Ann Allergy Asthma Immunol* 2014; **112**: 222-229.e3 [PMID: 24428971 DOI: 10.1016/j.anai.2013.12.007]

20 **McGowan EC**, Bloomberg GR, Gergen PJ, Visness CM, Jaffee KF, Sandel M, O'Connor G, Kattan M, Gern J, Wood RA. Influence of early-life exposures on food sensitization and food allergy in an inner-city birth cohort. *J Allergy Clin Immunol* 2015; **135**: 171-178 [PMID: 25129677 DOI: 10.1016/j.jaci.2014.06.033]

21 **Mahdavinia M**, Fox SR, Smith BM, James C, Palmisano EL, Mohammed A, Zahid Z, Assa'ad AH, Tobin MC, Gupta RS. Racial Differences in Food Allergy Phenotype and Health Care Utilization among US Children. *J Allergy Clin Immunol Pract* 2017; **5**: 352-357.e1 [PMID: 27888035 DOI: 10.1016/j.jaip.2016.10.006]

22 **Fox AT**, Kaymakcalan H, Perkin M, du Toit G, Lack G. Changes in peanut allergy prevalence in different ethnic groups in 2 time periods. *J Allergy Clin Immunol* 2015; **135**: 580-582 [PMID: 25441289 DOI: 10.1016/j.jaci.2014.09.022]

23 **Taylor-Black SA**, Mehta H, Weiderpass E, Boffetta P, Sicherer SH, Wang J. Prevalence of food allergy in New York City school children. *Ann Allergy Asthma Immunol* 2014; **112**: 554-556.e1 [PMID: 24768412 DOI: 10.1016/j.anai.2014.03.020]

24 **Soller L**, Ben-Shoshan M, Harrington DW, Knoll M, Fragapane J, Joseph L, St Pierre Y, La Vieille S, Wilson K, Elliott SJ, Clarke AE. Prevalence and predictors of food allergy in Canada: a focus on vulnerable populations. *J Allergy Clin Immunol Pract* 2015; **3**: 42-49 [PMID: 25577617 DOI: 10.1016/j.jaip.2014.06.009]

25 **McDole JR**, Wheeler LW, McDonald KG, Wang B, Konjufca V, Knoop KA, Newberry RD, Miller MJ. Goblet cells deliver luminal antigen to CD103+ dendritic cells in the small intestine. *Nature* 2012; **483**: 345-349 [PMID: 22422267 DOI: 10.1038/nature10863]

26 **Mazzini E**, Massimiliano L, Penna G, Rescigno M. Oral tolerance can be established *via* gap junction transfer of fed antigens from CX3CR1$^+$ macrophages to CD103$^+$ dendritic cells. *Immunity* 2014; **40**: 248-261 [PMID: 24462723 DOI: 10.1016/j.immuni.2013.12.012]

27 **Coombes JL**, Siddiqui KR, Arancibia-Cárcamo CV, Hall J, Sun CM, Belkaid Y, Powrie F. A functionally specialized population of mucosal CD103+ DCs induces Foxp3+ regulatory T cells *via* a TGF-beta and retinoic acid-dependent mechanism. *J Exp Med* 2007; **204**: 1757-1764 [PMID: 17620361 DOI: 10.1084/jem.20070590]

28 **Hadis U**, Wahl B, Schulz O, Hardtke-Wolenski M, Schippers A, Wagner N, Müller W, Sparwasser T, Förster R, Pabst O. Intestinal tolerance requires gut homing and expansion of FoxP3+ regulatory T cells in the lamina propria. *Immunity* 2011; **34**: 237-246 [PMID: 21333554 DOI: 10.1016/j.immuni.2011.01.016]

29 **Bakdash G**, Vogelpoel LT, van Capel TM, Kapsenberg ML, de Jong EC. Retinoic acid primes human dendritic cells to induce gut-homing, IL-10-producing regulatory T cells. *Mucosal Immunol* 2015; **8**: 265-278 [PMID: 25027601 DOI: 10.1038/mi.2014.64]

30 **Evans TI**, Reeves RK. All-trans-retinoic acid imprints expression of the gut-homing marker α4β7 while suppressing lymph node homing of dendritic cells. *Clin Vaccine Immunol* 2013; **20**: 1642-1646 [PMID: 23966557 DOI: 10.1128/CVI.00419-13]

31 **Sakaguchi S**, Yamaguchi T, Nomura T, Ono M. Regulatory T cells and immune tolerance. *Cell* 2008; **133**: 775-787 [PMID: 18510923 DOI: 10.1016/j.cell.2008.05.009]

32 **Sehra S**, Yao W, Nguyen ET, Glosson-Byers NL, Akhtar N, Zhou B, Kaplan MH. TH9 cells are required for tissue mast cell accumulation during allergic inflammation. *J Allergy Clin Immunol* 2015; **136**: 433-40.e1 [PMID: 25746972 DOI: 10.1016/j.jaci.2015.01.021]

33 **Berin MC**, Shreffler WG. Mechanisms Underlying Induction of Tolerance to Foods. *Immunol Allergy Clin North Am* 2016; **36**: 87-102 [PMID: 26617229 DOI: 10.1016/j.iac.2015.08.002]

34 **Nowak-Wegrzyn A**, Sampson HA. Adverse reactions to foods. *Med Clin North Am* 2006; **90**: 97-127 [PMID: 16310526 DOI: 10.1016/j.mcna.2005.08.012]

35 **Lee LA**, Burks AW. Food allergies: prevalence, molecular characterization, and treatment/prevention strategies. *Annu Rev Nutr* 2006; **26**: 539-565 [PMID: 16602930 DOI: 10.1146/annurev.nutr.26.061505.111211]

36 **Sheikh A**, Hippisley-Cox J, Newton J, Fenty J. Trends in national incidence, lifetime prevalence and adrenaline prescribing for anaphylaxis in England. *J R Soc Med* 2008; **101**: 139-143 [PMID: 18344471 DOI: 10.1258/jrsm.2008.070306]

37 **Turner PJ**, Gowland MH, Sharma V, Ierodiakonou D, Harper N, Garcez T, Pumphrey R, Boyle RJ. Increase in anaphylaxis-related hospitalizations but no increase in fatalities: an analysis of United Kingdom national anaphylaxis data, 1992-2012. *J Allergy Clin Immunol* 2015; **135**: 956-963.e1 [PMID: 25468198 DOI: 10.1016/j.jaci.2014.10.021]

38 **Cianferoni A**, Muraro A. Food-induced anaphylaxis. *Immunol Allergy Clin North Am* 2012; **32**: 165-195 [PMID: 22244239 DOI: 10.1016/j.iac.2011.10.002]

39 **Berin MC**. Pathogenesis of IgE-mediated food allergy. *Clin Exp Allergy* 2015; **45**: 1483-1496 [PMID: 26215729 DOI: 10.1111/cea.12598]

40 **Iweala OI**, Burks AW. Food Allergy: Our Evolving Understanding of Its Pathogenesis, Prevention, and Treatment. *Curr Allergy Asthma Rep* 2016; **16**: 37 [PMID: 27041704 DOI: 10.1007/s11882-016-0616-7]

41 **Sampson HA**, Muñoz-Furlong A, Campbell RL, Adkinson NF Jr, Bock SA, Branum A, Brown SG, Camargo CA Jr, Cydulka R, Galli SJ, Gidudu J, Gruchalla RS, Harlor AD Jr, Hepner DL, Lewis LM, Lieberman PL, Metcalfe DD, O'Connor R, Muraro A, Rudman A, Schmitt C, Scherrer D, Simons FE, Thomas S, Wood JP, Decker WW. Second symposium on the definition and management of anaphylaxis: summary report--Second National Institute of Allergy and Infectious Disease/Food Allergy and Anaphylaxis Network symposium. *J Allergy Clin Immunol* 2006; **117**: 391-397 [PMID: 16461139 DOI: 10.1016/j.jaci.2005.12.1303]

42 **Sicherer SH**, Sampson HA. Food allergy: Epidemiology, pathogenesis, diagnosis, and treatment. *J Allergy Clin Immunol* 2014; **133**: 291-307; quiz 308 [PMID: 24388012 DOI: 10.1016/j.jaci.2013.11.020]

43 **Garkaby J**, Epov L, Musallam N, Almog M, Bamberger E, Mandelberg A, Dalal I, Kessel A. The Sesame-Peanut Conundrum in Israel: Reevaluation of Food Allergy Prevalence in Young Children. *J Allergy Clin Immunol Pract* 2021; **9**: 200-205 [PMID: 32822919 DOI: 10.1016/j.jaip.2020.08.010]

44 **Jeon YH**. Pollen-food allergy syndrome in children. *Clin Exp Pediatr* 2020; **63**: 463-468 [PMID: 32403897 DOI: 10.3345/cep.2019.00780]

45 **Guvenir H**, Dibek Misirlioglu E, Buyuktiryaki B, Zabun MM, Capanoglu M, Toyran M, Civelek E, Kocabas CN. Frequency and clinical features of pollen-food syndrome in children. *Allergol Immunopathol (Madr)* 2020; **48**: 78-83 [PMID: 31601505 DOI: 10.1016/j.aller.2019.07.010]

46 **Skypala IJ**. Can patients with oral allergy syndrome be at risk of anaphylaxis? *Curr Opin Allergy Clin Immunol* 2020; **20**: 459-464 [PMID: 32842037 DOI: 10.1097/ACI.0000000000000679]

47 **Ota M**, Nishida Y, Yagi H, Sato K, Yamada S, Arakawa H, Takizawa T. Regional differences in the prevalence of oral allergy syndrome among Japanese children: A questionnaire-based survey. *Asian Pac J Allergy Immunol* 2020 [PMID: 32563230 DOI: 10.12932/AP-130120-0739]

48 **Dellon ES**, Gonsalves N, Hirano I, Furuta GT, Liacouras CA, Katzka DA; American College of Gastroenterology. ACG clinical guideline: Evidenced based approach to the diagnosis and management of esophageal eosinophilia and eosinophilic esophagitis (EoE). *Am J Gastroenterol* 2013; **108**: 679-92; quiz 693 [PMID: 23567357 DOI: 10.1038/ajg.2013.71]

49 **Cianferoni A**, Spergel J. Eosinophilic Esophagitis: A Comprehensive Review. *Clin Rev Allergy Immunol* 2016; **50**: 159-174 [PMID: 26194940 DOI: 10.1007/s12016-015-8501-z]

50 **Goswami R**, Blazquez AB, Kosoy R, Rahman A, Nowak-Węgrzyn A, Berin MC. Systemic innate immune activation in food protein-induced enterocolitis syndrome. *J Allergy Clin Immunol* 2017; **139**: 1885-1896.e9 [PMID: 28192147 DOI: 10.1016/j.jaci.2016.12.971]

51 **Calvani M**, Anania C, Caffarelli C, Martelli A, Miraglia Del Giudice M, Cravidi C, Duse M, Manti S, Tosca MA, Cardinale F, Chiappini E, Olivero F, Marseglia GL. Food allergy: an updated review on pathogenesis, diagnosis, prevention and management. *Acta Biomed* 2020; **91**: e2020012 [PMID: 33004782 DOI: 10.23750/abm.v91i11-S.10316]

52 **Sharma HP**, Bansil S, Uygungil B. Signs and Symptoms of Food Allergy and Food-Induced Anaphylaxis. *Pediatr Clin North Am* 2015; **62**: 1377-1392 [PMID: 26456438 DOI: 10.1016/j.pcl.2015.07.008]

53 **Maintz L**, Novak N. Histamine and histamine intolerance. *Am J Clin Nutr* 2007; **85**: 1185-1196 [PMID: 17490952 DOI: 10.1093/ajcn/85.5.1185]

54 **Bateman B**, Warner JO, Hutchinson E, Dean T, Rowlandson P, Gant C, Grundy J, Fitzgerald C, Stevenson J. The effects of a double blind, placebo controlled, artificial food colourings and benzoate preservative challenge on hyperactivity in a general population sample of preschool children. *Arch Dis Child* 2004; **89**: 506-511 [PMID: 15155391 DOI: 10.1136/adc.2003.031435]

55 **Collins MH**, Martin LJ, Alexander ES, Boyd JT, Sheridan R, He H, Pentiuk S, Putnam PE, Abonia JP, Mukkada VA, Franciosi JP, Rothenberg ME. Newly developed and validated eosinophilic esophagitis histology scoring system and evidence that it outperforms peak eosinophil count for disease diagnosis and monitoring. *Dis Esophagus* 2017; **30**: 1-8 [PMID: 26857345 DOI: 10.1111/dote.12470]

56 **Noel RJ**, Putnam PE, Rothenberg ME. Eosinophilic esophagitis. *N Engl J Med* 2004; **351**: 940-941 [PMID: 15329438 DOI: 10.1056/NEJM200408263510924]

57 **Tringali A**, Thomson M, Dumonceau JM, Tavares M, Tabbers MM, Furlano R, Spaander M, Hassan C, Tzvinikos C, Ijsselstijn H, Viala J, Dall'Oglio L, Benninga M, Orel R, Vandenplas Y, Keil R, Romano C, Brownstone E, Hlava Š, Gerner P, Dolak W, Landi R, Huber WD, Everett S, Vecsei A, Aabakken L, Amil-Dias J, Zambelli A. Pediatric gastrointestinal endoscopy: European Society of Gastrointestinal Endoscopy (ESGE) and European Society for Paediatric Gastroenterology Hepatology and Nutrition (ESPGHAN) Guideline Executive summary. *Endoscopy* 2017; **49**: 83-91 [PMID: 27617420 DOI: 10.1055/s-0042-111002]

58 **Gonsalves N**, Policarpio-Nicolas M, Zhang Q, Rao MS, Hirano I. Histopathologic variability and endoscopic correlates in adults with eosinophilic esophagitis. *Gastrointest Endosc* 2006; **64**: 313-319 [PMID: 16923475 DOI: 10.1016/j.gie.2006.04.037]

59 **Schaefer ET**, Fitzgerald JF, Molleston JP, Croffie JM, Pfefferkorn MD, Corkins MR, Lim JD, Steiner SJ, Gupta SK. Comparison of oral prednisone and topical fluticasone in the treatment of eosinophilic esophagitis: a randomized trial in children. *Clin Gastroenterol Hepatol* 2008; **6**: 165-173 [PMID: 18237866 DOI: 10.1016/j.cgh.2007.11.008]

60 **Hwang JB**, Sohn SM, Kim AS. Prospective follow-up oral food challenge in food protein-induced enterocolitis syndrome. *Arch Dis Child* 2009; **94**: 425-428 [PMID: 18829623 DOI: 10.1136/adc.2008.143289]

61 **Cardona V**, Ansotegui IJ, Ebisawa M, El-Gamal Y, Fernandez Rivas M, Fineman S, Geller M, Gonzalez-Estrada A, Greenberger PA, Sanchez Borges M, Senna G, Sheikh A, Tanno LK, Thong BY, Turner PJ, Worm M. World allergy organization anaphylaxis guidance 2020. *World Allergy Organ J* 2020; **13**: 100472 [PMID: 33204386 DOI: 10.1016/j.waojou.2020.100472]

62 **Bock SA**, Muñoz-Furlong A, Sampson HA. Further fatalities caused by anaphylactic reactions to food, 2001-2006. *J Allergy Clin Immunol* 2007; **119**: 1016-1018 [PMID: 17306354 DOI: 10.1016/j.jaci.2006.12.622]

63 **Walsh J**. NICE food allergy and anaphylaxis quality standards: a review of the 2016 quality standards. *Br J Gen Pract* 2017; **67**: 138-139 [PMID: 28232362 DOI: 10.3399/bjgp17X689833]

64 **Skypala IJ**, Venter C, Meyer R, deJong NW, Fox AT, Groetch M, Oude Elberink JN, Sprikkelman A, Diamandi L, Vlieg-Boerstra BJ; Allergy-focussed Diet History Task Force of the European Academy of Allergy and Clinical Immunology. The development of a standardised diet history tool to support the diagnosis of food allergy. *Clin Transl Allergy* 2015; **5**: 7 [PMID: 25741437 DOI: 10.1186/s13601-015-0050-2]

65 **Fox AT**, Lloyd K, Arkwright PD, Bhattacharya D, Brown T, Chetcuti P, East M, Gaventa J, King R, Martinez A, Meyer R, Parikh A, Perkin M, Shah N, Tuthill D, Walsh J, Waddell L, Warner J; Science and Research Department, Royal College of Paediatrics and Child Health. The RCPCH care pathway for food allergy in children: an evidence and consensus based national approach. *Arch Dis Child* 2011; **96** Suppl 2: i25-i29 [PMID: 22053063 DOI: 10.1136/adc.2011.214502]

66 **Nevis IF**, Binkley K, Kabali C. Diagnostic accuracy of skin-prick testing for allergic rhinitis: a systematic review and meta-analysis. *Allergy Asthma Clin Immunol* 2016; **12**: 20 [PMID: 27127526 DOI: 10.1186/s13223-016-0126-0]

67 **Bousquet J**, Heinzerling L, Bachert C, Papadopoulos NG, Bousquet PJ, Burney PG, Canonica GW, Carlsen KH, Cox L, Haahtela T, Lodrup Carlsen KC, Price D, Samolinski B, Simons FE, Wickman M, Annesi-Maesano I, Baena-Cagnani CE, Bergmann KC, Bindslev-Jensen C, Casale TB, Chiriac A, Cruz AA, Dubakiene R, Durham SR, Fokkens WJ, Gerth-van-Wijk R, Kalayci O, Kowalski ML, Mari A, Mullol J, Nazamova-Baranova L, O'Hehir RE, Ohta K, Panzner P, Passalacqua

G, Ring J, Rogala B, Romano A, Ryan D, Schmid-Grendelmeier P, Todo-Bom A, Valenta R, Woehrl S, Yusuf OM, Zuberbier T, Demoly P; Global Allergy and Asthma European Network; Allergic Rhinitis and its Impact on Asthma. Practical guide to skin prick tests in allergy to aeroallergens. *Allergy* 2012; **67**: 18-24 [PMID: 22050279 DOI: 10.1111/j.1398-9995.2011.02728.x]

68 **Peebles RS**, Church MK, Durham SR. Principles of allergy diagnosis. *Allergy* 2012; 129-146 [DOI: 10.1016/B978-0-7234-3658-4.00010-X]

69 **Ansotegui IJ**, Melioli G, Canonica GW, Caraballo L, Villa E, Ebisawa M, Passalacqua G, Savi E, Ebo D, Gómez RM, Luengo Sánchez O, Oppenheimer JJ, Jensen-Jarolim E, Fischer DA, Haahtela T, Antila M, Bousquet JJ, Cardona V, Chiang WC, Demoly PM, DuBuske LM, Ferrer Puga M, Gerth van Wijk R, González Díaz SN, Gonzalez-Estrada A, Jares E, Kalpaklioğlu AF, Kase Tanno L, Kowalski ML, Ledford DK, Monge Ortega OP, Morais Almeida M, Pfaar O, Poulsen LK, Pawankar R, Renz HE, Romano AG, Rosário Filho NA, Rosenwasser L, Sánchez Borges MA, Scala E, Senna GE, Sisul JC, Tang MLK, Thong BY, Valenta R, Wood RA, Zuberbier T. IgE allergy diagnostics and other relevant tests in allergy, a World Allergy Organization position paper. *World Allergy Organ J* 2020; **13**: 100080 [PMID: 32128023 DOI: 10.1016/j.waojou.2019.100080]

70 **De Martinis M**, Sirufo MM, Suppa M, Ginaldi L. New Perspectives in Food Allergy. *Int J Mol Sci* 2020; **21** [PMID: 32098244 DOI: 10.3390/ijms21041474]

71 **Anagnostou K**, Stiefel G, Brough H, du Toit G, Lack G, Fox AT. Active management of food allergy: an emerging concept. *Arch Dis Child* 2015; **100**: 386-390 [PMID: 25378378 DOI: 10.1136/archdischild-2014-306278]

72 **Wasserman RL**, Hague AR, Pence DM, Sugerman RW, Silvers SK, Rolen JG, Herbert M. Real-World Experience with Peanut Oral Immunotherapy: Lessons Learned From 270 Patients. *J Allergy Clin Immunol Pract* 2019; **7**: 418-426.e4 [PMID: 29859333 DOI: 10.1016/j.jaip.2018.05.023]

73 **Burk CM**, Dellon ES, Steele PH, Virkud YV, Kulis M, Burks AW, Vickery BP. Eosinophilic esophagitis during peanut oral immunotherapy with omalizumab. *J Allergy Clin Immunol Pract* 2017; **5**: 498-501 [PMID: 28017628 DOI: 10.1016/j.jaip.2016.11.010]

74 **Nowak-Węgrzyn A**, Sampson HA. Future Therapies for Food Allergies. *Food Allergy* 2012; 235-250 [DOI: 10.1016/B978-1-4377-1992-5.00017-X]

75 **Kramer MF**, Heath MD. Aluminium in allergen-specific subcutaneous immunotherapy--a German perspective. *Vaccine* 2014; **32**: 4140-4148 [PMID: 24892252 DOI: 10.1016/j.vaccine.2014.05.063]

76 **Tan-Lim CSC**, Esteban-Ipac NAR. Probiotics as treatment for food allergies among pediatric patients: a meta-analysis. *World Allergy Organ J* 2018; **11**: 25 [PMID: 30425779 DOI: 10.1186/s40413-018-0204-5]

77 **Wood RA**, Kim JS, Lindblad R, Nadeau K, Henning AK, Dawson P, Plaut M, Sampson HA. A randomized, double-blind, placebo-controlled study of omalizumab combined with oral immunotherapy for the treatment of cow's milk allergy. *J Allergy Clin Immunol* 2016; **137**: 1103-1110.e11 [PMID: 26581915 DOI: 10.1016/j.jaci.2015.10.005]

78 **Schneider LC**, Rachid R, LeBovidge J, Blood E, Mittal M, Umetsu DT. A pilot study of omalizumab to facilitate rapid oral desensitization in high-risk peanut-allergic patients. *J Allergy Clin Immunol* 2013; **132**: 1368-1374 [PMID: 24176117 DOI: 10.1016/j.jaci.2013.09.046]

79 **Nadeau KC**, Schneider LC, Hoyte L, Borras I, Umetsu DT. Rapid oral desensitization in combination with omalizumab therapy in patients with cow's milk allergy. *J Allergy Clin Immunol* 2011; **127**: 1622-1624 [PMID: 21546071 DOI: 10.1016/j.jaci.2011.04.009]

80 **Tan JW**, Campbell DE, Turner PJ, Kakakios A, Wong M, Mehr S, Joshi P. Baked egg food challenges - clinical utility of skin test to baked egg and ovomucoid in children with egg allergy. *Clin Exp Allergy* 2013; **43**: 1189-1195 [PMID: 24074337 DOI: 10.1111/cea.12153]

81 **Leonard SA**. Debates in allergy medicine: baked milk and egg ingestion accelerates resolution of milk and egg allergy. *World Allergy Organ J* 2016; **9**: 1 [PMID: 26839628 DOI: 10.1186/s40413-015-0089-5]

82 **Du Toit G**, Roberts G, Sayre PH, Bahnson HT, Radulovic S, Santos AF, Brough HA, Phippard D, Basting M, Feeney M, Turcanu V, Sever ML, Gomez Lorenzo M, Plaut M, Lack G; LEAP Study Team. Randomized trial of peanut consumption in infants at risk for peanut allergy. *N Engl J Med* 2015; **372**: 803-813 [PMID: 25705822 DOI: 10.1056/NEJMoa1414850]

83 **Skjerven HO**, Rehbinder EM, Vettukattil R, LeBlanc M, Granum B, Haugen G, Hedlin G, Landrø L, Marsland BJ, Rudi K, Sjøborg KD, Söderhäll C, Staff AC, Carlsen KH, Asarnoj A, Bains KES, Carlsen OCL, Endre KMA, Granlund PA, Hermansen JU, Gudmundsdóttir HK, Hilde K, Håland G, Kreyberg I, Olsen IC, Mägi CO, Nordhagen LS, Saunders CM, Skrindo I, Tedner SG, Værnesbranden MR, Wiik J, Jonassen CM, Nordlund B, Carlsen KCL. Skin emollient and early complementary feeding to prevent infant atopic dermatitis (PreventADALL): a factorial, multicentre, cluster-randomised trial. *Lancet* 2020; **395**: 951-961 [PMID: 32087121 DOI: 10.1016/S0140-6736(19)32983-6]

84 **Chan ES**, Abrams EM, Hildebrand KJ, Watson W. Early introduction of foods to prevent food allergy. *Allergy Asthma Clin Immunol* 2018; **14**: 57 [PMID: 30275847 DOI: 10.1186/s13223-018-0286-1]

85 **Knibb RC**. Effectiveness of Cognitive Behaviour Therapy for Mothers of Children with Food Allergy: A Case Series. *Healthcare (Basel)* 2015; **3**: 1194-1211 [PMID: 27417820 DOI: 10.3390/healthcare3041194]

86 **Fleischer DM**, Conover-Walker MK, Christie L, Burks AW, Wood RA. The natural progression of peanut allergy: Resolution and the possibility of recurrence. *J Allergy Clin Immunol* 2003; **112**: 183-189 [PMID: 12847497 DOI: 10.1067/mai.2003.1517]

World Journal of Clinical Pediatrics

Submit a Manuscript: https://www.f6publishing.com

World J Clin Pediatr 2022 May 9; 11(3): 270-288

DOI: 10.5409/wjcp.v11.i3.270

ISSN 2219-2808 (online)

MINIREVIEWS

Bleeding per rectum in pediatric population: A pictorial review

Karamvir Chandel, Rishabh Jain, Anmol Bhatia, Akshay Kumar Saxena, Kushaljit Singh Sodhi

Specialty type: Pediatrics

Provenance and peer review: Invited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): A
Grade B (Very good): 0
Grade C (Good): 0
Grade D (Fair): 0
Grade E (Poor): 0

P-Reviewer: Govindarajan KK, India

Received: June 27, 2021
Peer-review started: June 27, 2021
First decision: July 30, 2021
Revised: August 9, 2021
Accepted: March 16, 2022
Article in press: March 16, 2022
Published online: May 9, 2022

Karamvir Chandel, Rishabh Jain, Anmol Bhatia, Akshay Kumar Saxena, Kushaljit Singh Sodhi, Department of Radio Diagnosis and Imaging, Post Graduate Institute of Medical Education and Research, Chandigarh 160012, India

Corresponding author: Anmol Bhatia, MBBS, MD, Associate Professor, Department of Radio Diagnosis and Imaging, Post Graduate Institute of Medical Education and Research, Sector 12, Chandigarh 160012, India. anmol_bhatia26@yahoo.co.in

Abstract

Bleeding per rectum in children can be seen in congenital as well as acquired conditions that may require medical or surgical management. The present review article is aimed to discuss the imaging findings of some common and uncommon causes of bleeding per rectum in children.

Key Words: Bleeding; Per rectum; Children; Imaging; Congenital

Core Tip: Bleeding per rectum in children can be seen in congenital as well as acquired conditions. The referring clinicians as well the radiologists must be aware of the various radiological findings of common and uncommon causes of bleeding per rectum in children discussed in the article.

Citation: Chandel K, Jain R, Bhatia A, Saxena AK, Sodhi KS. Bleeding per rectum in pediatric population: A pictorial review. *World J Clin Pediatr* 2022; 11(3): 270-288
URL: https://www.wjgnet.com/2219-2808/full/v11/i3/270.htm
DOI: https://dx.doi.org/10.5409/wjcp.v11.i3.270

INTRODUCTION

Bleeding per rectum in children may be in the form of passage of either bright red or dark red blood (hematochezia) or black tarry stools (melena). It may occur due to congenital as well as acquired causes in the pediatric population which may require medical or surgical management.

The causes of bleeding per rectum in children across different age groups are summarized in Table 1. In the present review, we aim to discuss the imaging findings

Table 1 Causes of bleeding per rectum in children across different age groups

Up to 2 yr of age	2-5 yr	6-15 yr
Milk allergy	Polyps	Polyps
Necrotizing enterocolitis	Anal fissure	Anal fissure
Duplication cyst	Intussusception	Infectious enterocolitis
Polyps	Meckel diverticulum	Inflammatory bowel disease
Anal fissure	Infectious enterocolitis	Henoch-Schönlein purpura
Intussusception	Bleeding diathesis	Hemolytic-uremic syndrome
Hirschsprung disease related enterocolitis	Henoch-Schönlein purpura	Bleeding diathesis
Meckel diverticulum	Hemolytic-uremic syndrome	Angiodysplasia
Infectious enterocolitis	Angiodysplasia	Lymphonodular hyperplasia
Bleeding diathesis	Lymphonodular hyperplasia	Extrahepatic portal venous obstruction
	Extrahepatic portal venous obstruction	

of some common and uncommon causes of bleeding per rectum in children.

NECROTIZING ENTEROCOLITIS

Necrotizing enterocolitis (NEC) refers to acute severe inflammation of the bowel in the newborn. Its incidence ranges from 1%-5% in neonatal intensive care units[1,2]. Greater risk of NEC is noted in extreme preterm (less than 28 wk) and extremely low birth weight (birth weight less than 1000 g) babies [3]. Approximately 10% of NEC cases may be seen in term neonates with associated congenital heart diseases being the major risk factor for these patients[1,4-6].

Clinical findings vary with the severity of involvement, with feed intolerance, vomiting, hematochezia, and abdominal tenderness noted in the early stage. In advanced stages, it can lead to peritonitis, sepsis, and eventually shock.

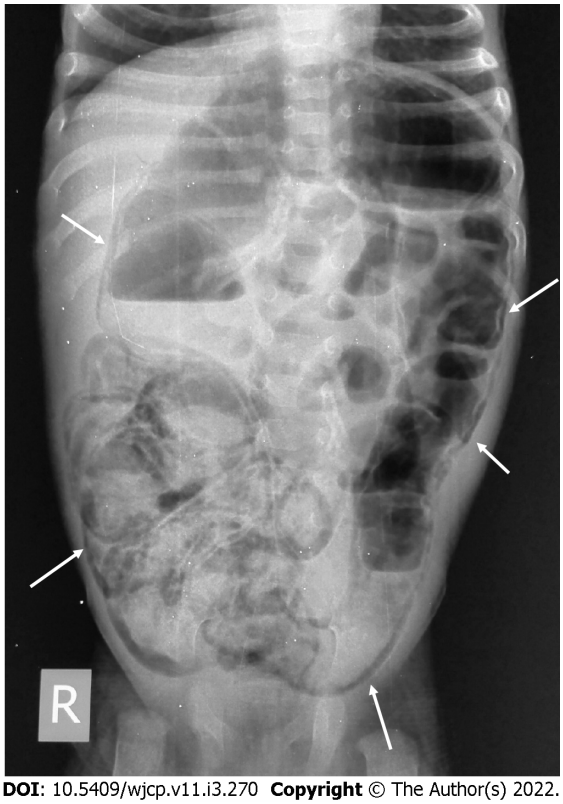
Plain abdominal radiographs are the initial radiological investigation and the mainstay of diagnosis of NEC in an appropriate clinical setting. Both supine views and cross-table lateral views are preferred. The earliest and most common sign of NEC even before clinical findings is the loss of normal "mosaic" pattern of bowel gas in neonates along with tubular or rounded dilatation of the loops. This is seen in 90% of the neonates. The degree of dilatation and clinical severity are correlated[7]. Furthermore, persistent dilated bowel loops are an ominous sign, with the "fixed bowel loop sign" reflecting transmural bowel necrosis and imminent perforation".

In an appropriate clinical setting, the presence of intramural gas is considered a pathognomonic sign of NEC and is seen in 19%-98% of the cases[8-10] (Figure 1). Two patterns can be seen - a bubbly pattern (representing air in the submucosa) or a linear pattern of intramural gas (suggesting subserosal gas).

Portal venous gas (seen in approximately 30% of cases on X-ray) is seen as linear, branching radiolucent lines radiating from the region of the hilum towards the periphery[11,12]. It must be differentiated from pneumobilia where the gas is seen more centrally as compared to portal venous gas where it extends more peripherally[12].

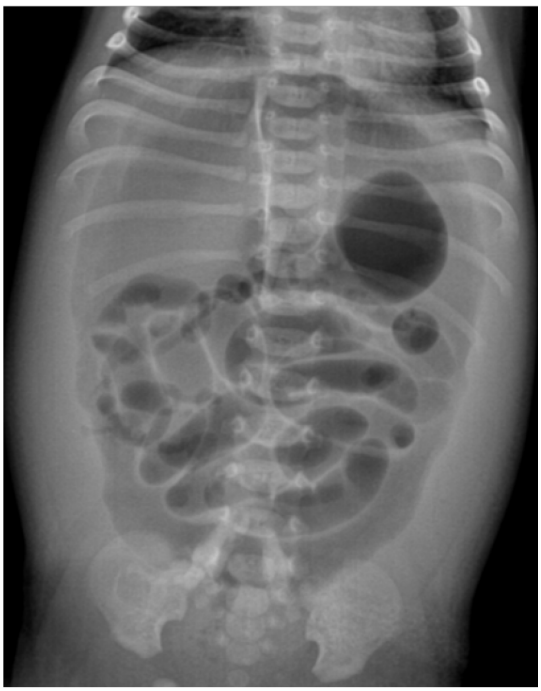
In later stages, bowel perforation may occur and is seen as pneumoperitoneum which becomes an indication for surgical intervention[13] (Figure 2). Various signs have described free intraperitoneal gas in the abdomen, namely, Rigler sign (air lining the bowel wall), football sign, Cupola sign (air under the central diaphragm), inverted V sign (air outlining the lateral umbilical ligaments), *etc.* Cross table lateral views are especially valuable in detecting small amounts of interbowel gas, which is seen as a triangular lucency between the bowel walls.

Ultrasonography (USG) provides valuable information in patients with NEC in the form of bowel wall thickness and echogenicity, free intraperitoneal fluid and its character, peristalsis, and bowel wall perfusion. Few studies have shown USG to be more sensitive in depicting intramural and portal venous gas[14,15]. However, the major limitation is that it is operator dependent. Early stages of NEC show bowel wall thickening with loss of normal gut signature (the hypoechoic rim of the muscularis propria) with an increase in the wall echogenicity (Figure 3). This is accompanied by an increase in the Doppler color flow in the bowel wall in early stages. Later stages show thinning of the wall with reduced and later absent flow[14]. Free intraperitoneal fluid may be seen. The presence of low-level internal echoes/septations suggests perforation[12,13].



DOI: 10.5409/wjcp.v11.i3.270

Figure 1 Abdominal X-ray in an infant with necrotizing enterocolitis showing diffuse intramural air (arrows).

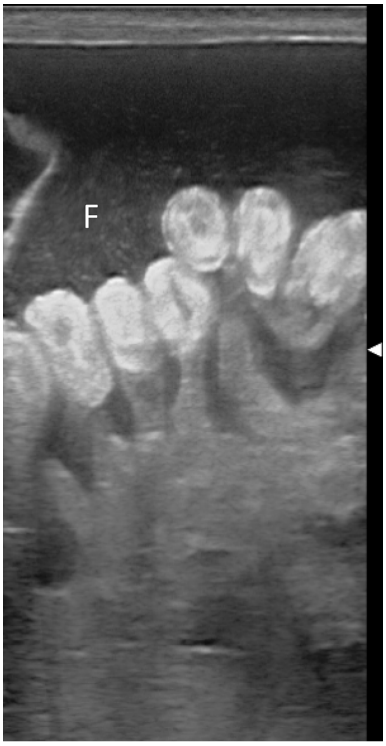


DOI: 10.5409/wjcp.v11.i3.270

Figure 2 Abdominal X-ray in an infant with necrotizing enterocolitis showing free air. The gas is seen outlining both sides of the bowel wall, *i.e.*, gas is seen within the bowel lumen as well as in the abdominal cavity. This sign is called Rigler sign and is suggestive of large amount of pneumoperitoneum.

MECKEL'S DIVERTICULUM

Meckel's diverticulum is a true diverticulum arising from the terminal ileum and is the result of persistence of the vitello-intestinal duct[16]. The lifetime risks of complications from Meckel's diverticulum are reported to be 6.4%[17], which include bleeding, diverticulitis, and intussusception.



DOI: 10.5409/wjcp.v11.i3.270

Figure 3 Ultrasound in an infant with complicated necrotizing enterocolitis showing diffusely echogenic bowel walls. In addition, free fluid (F) with internal echoes is also seen.

The risk of bleeding is more common in children in than adults[18] and is due to the presence of ectopic gastric mucosa causing ulceration.

High-resolution USG shows a fluid-filled anechoic blind ending tubular structure in the right lower quadrant with a typical "gut signature". The other non-blinding end connects to a peristaltic bowel loop.

On $Tc^{99}m$ pertechnate scintigraphy, the ectopic gastric mucosa within the diverticulum appears as a focal area of increased tracer uptake in the right iliac fossa (sensitivity, 85%; specificity, 95%). Other scintigraphy techniques like Tc-99m labeled sulfur colloid scan and RBC scan can also localize the site of LGIB, but neither is specific for Meckel's diverticulum[19,20].

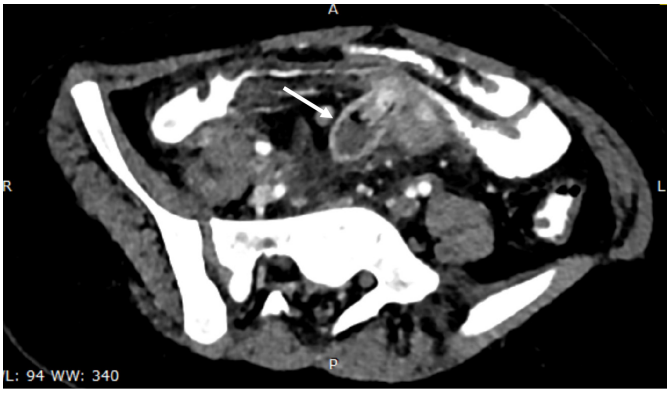
Complications of Meckel's diverticulum like diverticulitis, bowel obstruction, and in some cases intussusceptions are very well seen on computed tomography (CT)[21]. On CT, it appears as a fluid containing blind-ending pouch with variable mural thickness and adjacent fat stranding (Figure 4). Bowel obstruction can occur in Meckel's diverticulum secondary to intussusception, volvulus, the inclusion of diverticulum in the hernia, or foreign body impaction. Direct visualization of the diverticulum on computed tomography (CT) is difficult and features are similar to those caused by post-op adhesions, *i.e.,* dilated bowel loops with an abrupt change in caliber with the absence of soft tissue mass at the site of obstruction.

Surgical resection is the treatment of choice for symptomatic Meckel's diverticulum. However, management in asymptomatic incidentally detected diverticulum is controversial with some authors advocating conservative approaches owing to reduced lifetime risk of complications while others support early prophylactic diverticulectomy[17,18].

RECTAL POLYPS

Colorectal polyps are an important cause of lower gastrointestinal (GI) bleeding in children and adolescents with an estimated prevalence of 12% during pediatric colonoscopy for lower GI bleeding [22]. The majority of them are juvenile hamartomatous polyps[23] and an overwhelming majority of these are solitary and sporadic not associated with malignancy[24,25]. Most of these present as painless rectal bleeding[26]. Few of them may have lower abdominal pain. Most of these are located in the rectosigmoid and thus present as fresh red blood per rectum[27]. Multiple colonic polyps have been associated with polyposis syndromes.

On abdominal radiographs, they may appear as a rounded soft tissue mass in gas-filled bowel lumen. Barium enema may show polyps as a filling defect on the dependent wall[28]. Double contrast enema



DOI: 10.5409/wjcp.v11.i3.270

Figure 4 Axial computed tomography image showing blind ending tubular structure (arrow) in the midline in the mesentery coursing towards adjacent ileal loop suggestive of Meckel's diverticulum. Inflammation is also seen in the surrounding mesentery.

(DCE) better outlines the polyps which may be seen as ring shadow with barium coated white rim. "Bowler hat sign" on double contrast air enema refers to the appearance of sessile polyp formed by a ring of barium at the base of the polyp surrounding a domed layer of barium coating the surface of the polyp[29]. "Mexican hat sign" is the analogous appearance of pedunculation on DCE formed by pair of concentric rings with outer and inner rings representing head and stalk of polyp[28].

CT colonography with the help of advanced graphic software creates two dimensional and three dimensional images along with volumetric data of the colon[30]. Being non-invasive, it provides a virtual endoluminal image of the polyps which are seen as projections (Figure 5). Bright lumen and dark lumen techniques in magnetic resonance (MR) colonography are used to visualize the colon. In bright lumen technique, polyps are seen as hypointense filling defects in the bright lumen. In dark lumen techniques, polyps appear as enhancing soft- tissue masses against the background of dark intraluminal air/water. Dark lumen techniques have better sensitivity than bright lumen techniques[31,32].

CROHN'S COLITIS

Inflammatory bowel disease is a chronic disease of the gastrointestinal tract consisting of two separate but related entities, namely, ulcerative colitis and Crohn's disease (CD). The manifestations of CD vary depending upon the extent of the disease with isolated colonic involvement presenting similarly to UC, whereas small bowel CD presents as fever, weight loss, and fatigue more commonly than UC[33]. Symptoms of inflammatory bowel disease wax and wane, resulting in "flares" and "remission", respectively. Up to 30% of children with CD present with growth failure[34].

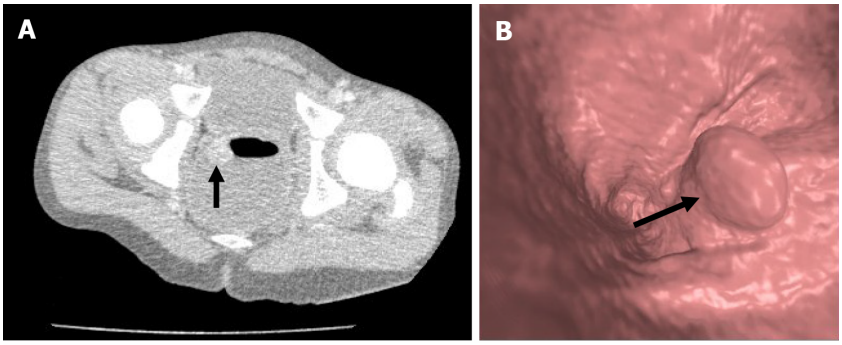
Imaging has been used to assess parts of the bowel not accessible by direct endoscopic visualization, namely, the small bowel. Fluoroscopic techniques like small bowel enteroclysis and barium meal follow-through have largely been replaced by non-invasive techniques like CT and MR enterography, which provide both mucosal and extraluminal information as well as extraintestinal manifestations.

Double contrast barium enema allows to obtain greater details of the colonic mucosa and shows irregular thickening and distortion of the valvulae conniventes, widely separated bowel loops due to fibro-fatty mesenteric proliferation and pseudo-sacculations at the ulcer site. Severe cases produce transverse and longitudinal ulcers giving rise to cobblestone appearance. Chronic cases result in multiple strictures, sinus tracts, and fistulae, which can be readily demonstrated on contrast studies.

USG has a limited role and shows predominantly bowel wall thickening with loss of mural stratification, bowel wall hyperemia, reactive mesenteric lymphadenopathy, and ascites[35]. Other complications like abscess formation and bowel obstruction can also be demonstrated on USG.

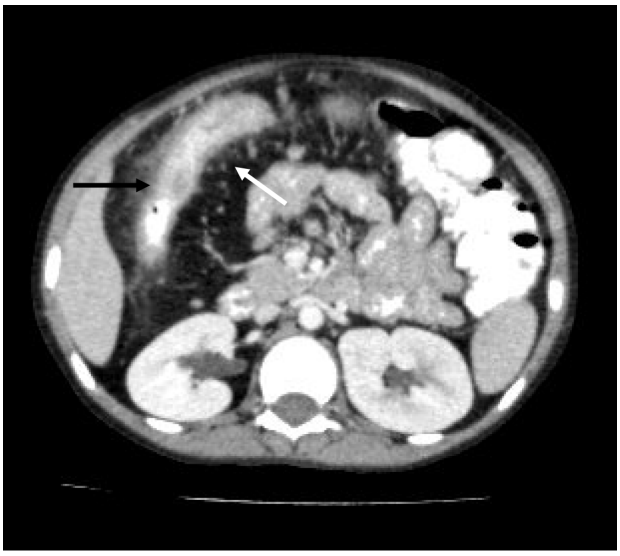
CT helps in the simultaneous assessment of extraluminal and extraintestinal complications and has emerged as one of the primary imaging modalities. Common signs of active CD include bowel wall thickening (> 3 mm) and mucosal hyperenhancment[36,37]. These are the most common and sensitive findings of CD[38,39]. Increased vascularity in form of "comb sign" (Figure 6) refers to increased vascularity of the distal mesenteric arterial arcades and the vasa recta of the affected ascending colon and small bowel and is a sign of active inflammation[40]. Perienteric fat stranding and engorged vasa recta are the most specific signs of active CD on CT enterography[41]. Chronic CD produces "creeping fat sign" due to fibrofatty proliferation. Complications like strictures, bowel obstruction, fistula formation, and an intra-abdominal abscess can also be readily demonstrated.

MR enterography offers the advantage of radiation free modality and is of utmost importance in the pediatric population. It is specifically the modality of choice for better evaluation of perianal disease and better distinction of acute disease from chronic disease. Findings on MR enterography such as bowel



DOI: 10.5409/wjcp.v11.i3.270

Figure 5 Axial contrast enhanced computed tomography colonoscopy image. A: in a child with bleeding per rectum showing an enhancing polypoidal soft tissue (arrow) suggestive of a rectal polyp, as well as endoluminal colonoscopy image B: in another child showing a rectal polyp (arrow).



DOI: 10.5409/wjcp.v11.i3.270

Figure 6 Axial contrast enhanced computed tomography image in a child with Crohn's disease showing mesenteric fat proliferation with increased vascularity (white arrow) with mural thickening and enhancement in the adjacent bowel loop (black arrow).

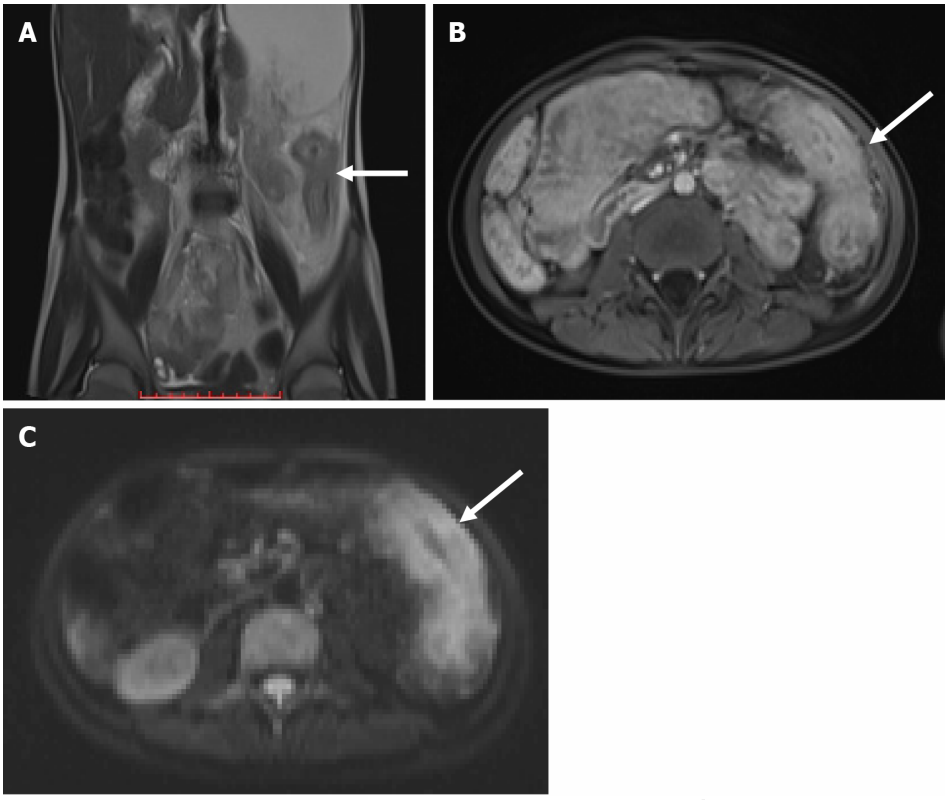
wall thickening, mucosal hyperenhancement, mural stratification, and perienteric fat stranding denote active disease (Figure 7A and B). Chronic fibrotic CD shows hypointense signal on T2 weighted images. Diffusion weighted imaging is unique and shows restricted diffusion with low apparent diffusion coefficient values in active disease[42](Figure 7C). Strictures are better evaluated on MR enterography due to its dynamic bowel examination in time and CINE imaging[39]. Perianal fistulas and other entero-cutaneous fistulas appear as enhancing tracts best visualized on post- contrast fat-saturated T1 weighted sequences[43,44] (Figure 8).

MIDGUT VOLVULUS

Midgut volvulus occurs due to intestinal malrotation. In malrotation, due to abnormal 270^{0}counter-clock rotation of the midgut around the superior mesenteric artery (SMA) axis, an abnormally long, narrow mesenteric pedicle is present from the ligament of Trietz to the ileocaecal valve and is more susceptible for midgut volvulus. It usually presents with bilious vomiting due to proximal small bowel obstruction, but occasionally bloody stools may be seen secondary to intestinal ischemia.

Plain abdominal radiographs show a paucity of bowel gases beyond the stomach and the duodenum and colonic gas if present is seen in the left hemi-abdomen.

Upper GI contrast studies are the preferred imaging tests for a suspected case. Typical findings on fluoroscopy include a corkscrew appearance of the duodenum with it not crossing the midline and duodeno-jejunal (DJ) flexure present on the right, below the level of the pylorus and to the right of the left pedicle of L1 vertebra[45](Figure 9).



DOI: 10.5409/wjcp.v11.i3.270

Figure 7 Diffusion weighted imaging. A: Coronal T2 weighted magnetic resonance image showing long segment mural thickening in the descending colon (arrow in A) which is showing post contrast enhancement in post contrast T1 image (arrow in B) and intense diffusion restriction in diffusion weighted image (arrow in C) suggestive of active disease.

On USG, the superior mesenteric vein (SMV) is present to the left of the SMA[46]; however, the absence of this finding does not rule out malrotation[47,48]. On color Doppler images, twisting or wrapping of the SMV and the mesentery around the SMA in a clockwise direction is suggestive of whirlpool sign (Figure 10). It has a sensitivity, specificity, and positive predictive value of 92%, 100%, and 100%, respectively[46].

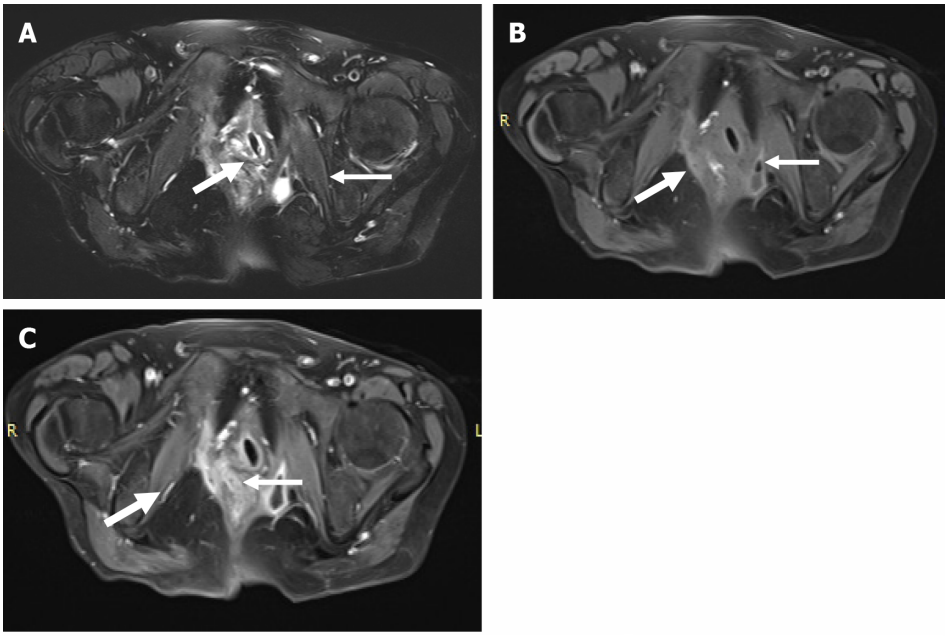
On CT and magnetic resonance imaging (MRI) at the site of volvulus, swirling of mesenteric vessels may be seen[49] (Figures 11 and 12). There is abnormally positioned duodenum, DJ flexure, cecum (in the left upper quadrant), and the large bowel (the majority of colonic loops in the right hemiabdomen) along with distention of the proximal duodenum and the stomach.

EXTRA-HEPATIC PORTAL VEIN OBSTRUCTION (EHPVO)

Extra-hepatic portal vein obstruction (EHPVO) is an important and common cause of non-cirrhotic portal hypertension (HTN). It is characterized by chronic thrombosis of the main portal vein, with or without intrahepatic portal vein, splenic vein, and SMV involvement with portal vein cavernoma formation. It is a disorder of children and young adults. In developing countries in the Asian region, it is the most common cause of portal HTN and upper GI bleeding in the pediatric population. Hypercoagulable state, infections, inflammation, portal venous anomalies, and perinatal umbilical vein catheterization are the most common etiologies; however, 70% of cases are idiopathic[50-52].

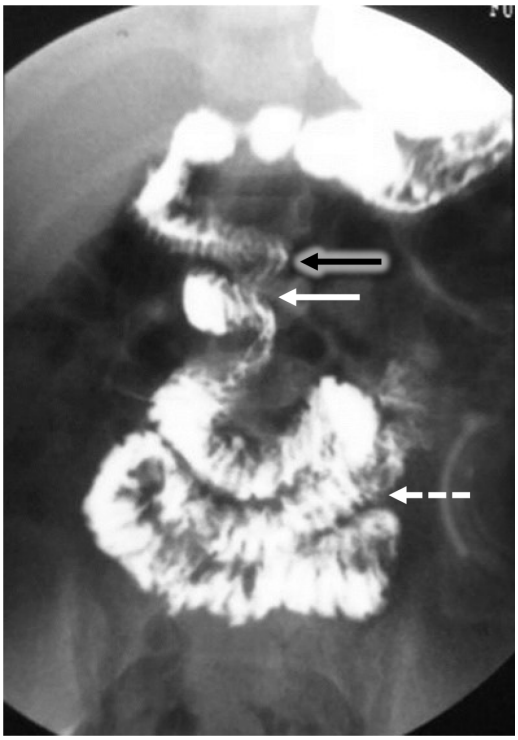
Clinically, these patients present with upper GI variceal bleeding with the associated feature of hypersplenism. Portal cavernous cholangiopathy (PCC) is seen in approximately 70%-100% of cases. Only 5%-28% of these are symptomatic due to biliary obstruction leading to intrahepatic biliary radical dilatation (IHBRD), choledocholithiasis, and hepatolithiasis[50,52]. Lower GI bleeding is rare with EHPVO (seen in 0.5%-10% of cases), but this is usually torrential and life-threatening. Anorectal varices (63%-95% of cases) and colopathy (approximately 54% of cases), secondary to increased portal venous pressure, are the two main causes for lower GI bleed in these patients[53]. Isolated inferior mesenteric vein portal hypertension secondary to EHPVO has been reported[54].

USG along with Doppler is usually the first investigation in a suspected case of EHPVO. The portal vein is not visualized and is replaced with multiple tortuous vascular channels suggestive of cavernoma formation. Depending on the extent of portal vein involvement, both intra- and extra-hepatic cavernoma



DOI: 10.5409/wjcp.v11.i3.270

Figure 8 Magnetic resonance image pelvis images in a child with Crohn's disease. A and B: T2 weighted fat suppressed and pre contrast T1 weighted images showing a fistulous tract (thick arrow in A and B) on the right side communicating with the rectum in the midline. In addition, a small collection (thin arrow in A and B) with air focus is seen in the left ischio-rectal fossa; C: Post contrast T1 weighted fat suppressed image showing enhancement of the tract (thick arrow) as well as peripheral enhancement of the collection (thin arrow).

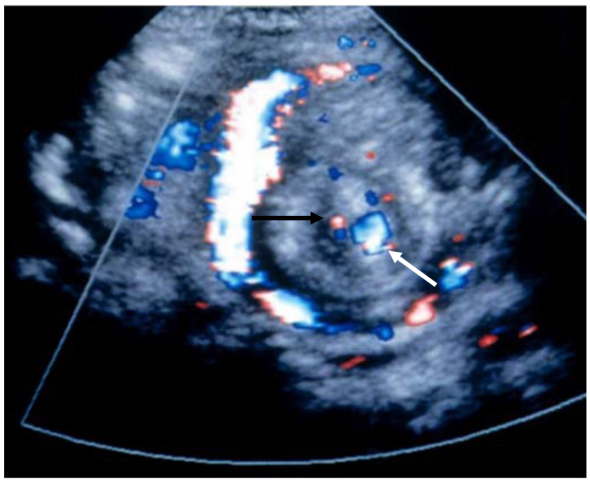


DOI: 10.5409/wjcp.v11.i3.270

Figure 9 Contrast study showing low lying duodenojejunal flexure (black arrow) with cork-screw appearance (solid white arrow) suggestive of malrotation with volvulus. The proximal jejunal loops (dashed white arrow) are seen in the midline and on the right side instead of the left side.

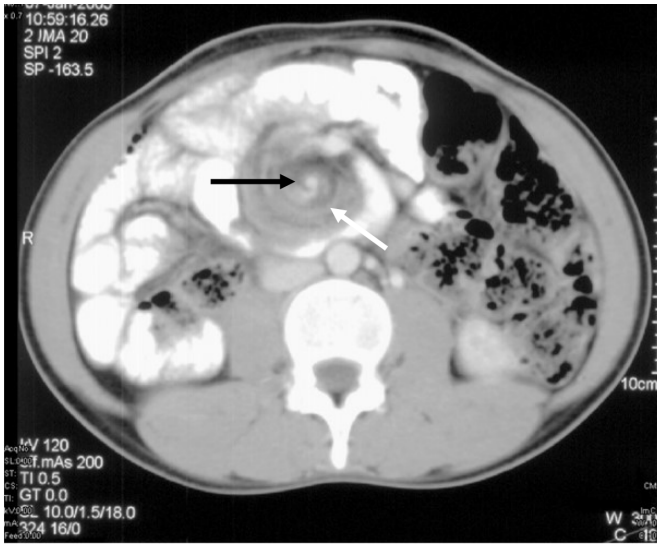
formation can be seen. Monophasic hepatopetal flow is noted in the collaterals. Pericholecystic collaterals are seen in approximately 30- 50% of cases. In patients with PCC, IHBRD, hepatolithiasis, and choledocholithiasis may be seen[50-52].

CT demonstrates vascular, biliary, and visceral changes. However, it is not routinely performed in children with EHPVO.



DOI: 10.5409/wjcp.v11.i3.270

Figure 10 Color Doppler image showing whirlpool sign due to rotation of the superior mesenteric vein (white arrow) along with bowel loops around the superior mesenteric artery (black arrow) suggestive of malrotation with midgut volvulus.



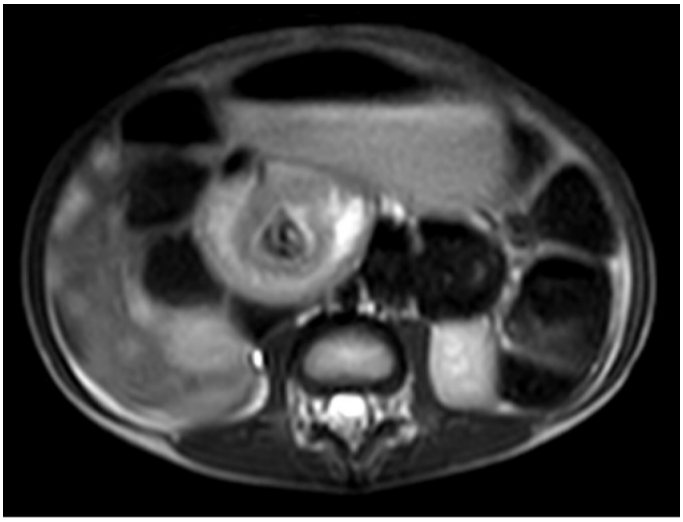
DOI: 10.5409/wjcp.v11.i3.270

Figure 11 Axial contrast enhanced computed tomography image showing whirlpool sign due to rotation of the superior mesenteric vein (white arrow) along with bowel loops around the superior mesenteric artery (black arrow) suggestive of malrotation with midgut volvulus.

Magnetic resonance cholangiopancreatography (MRCP) and MR portovenography provide valuable information regarding the biliary and splenoportal axis, respectively (Figure 13). MRI features suggestive of PCC include irregular wavy contour of bile ducts, biliary duct narrowing and strictures with or without dilatation, gall bladder and bile duct wall thickening, CBD angulation, hepatolithiasis and cholelithiasis, and choledocholithiasis. Paracholedochal and pericholecystic collaterals (Figure 14) are seen as enhancing tortuous collaterals causing smooth extrinsic impressions on bile duct[50,52]. MRI also demonstrates the presence of intra-splenic siderotic nodules (Gamna-Gandy bodies), which denotes long standing portal hypertension.

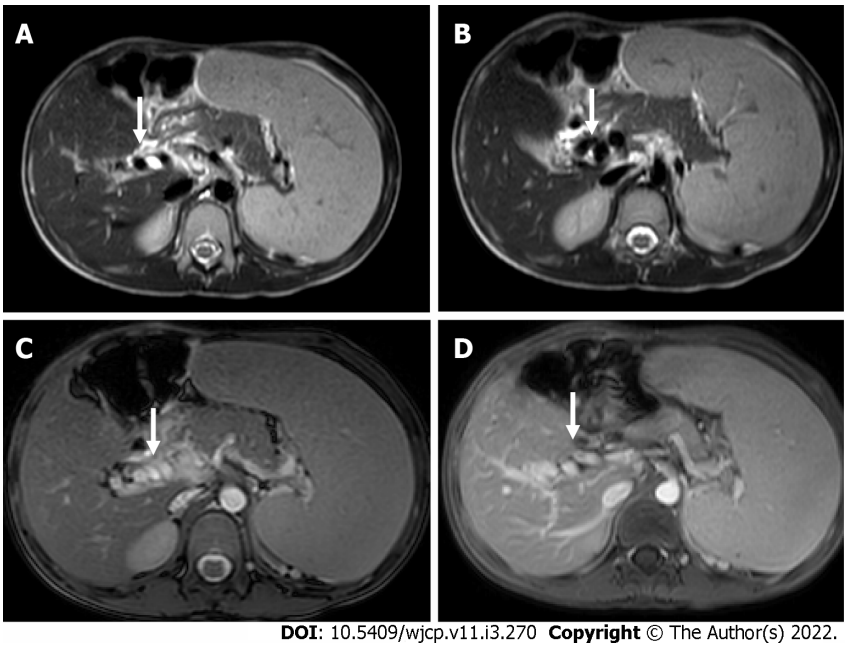
DUPLICATION CYST

Duplication cysts are a rare gastrointestinal tract developmental anomaly with an incidence of approximately 0.2% of all children and are most commonly seen in infancy. They may be contained within the gastrointestinal tract or lie outside to it and are usually seen on the mesenteric side. They are either cystic (80%) or tubular (20%). The ileum, esophagus, and colon are the common sites. Histologically, they show GI epithelial inner lining and smooth muscle outer layer. Presentation is variable and usually



DOI: 10.5409/wjcp.v11.i3.270

Figure 12 Axial T2 weighted magnetic resonance image showing whirlpool sign suggestive of malrotation with midgut volvulus.



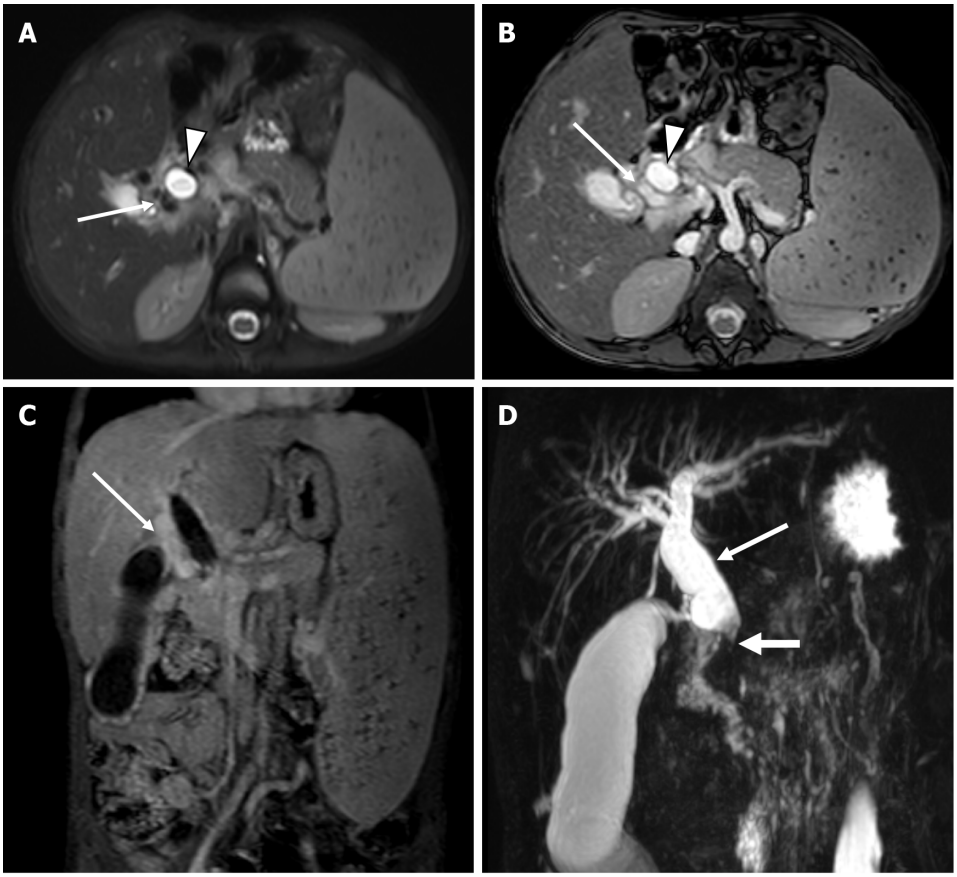
DOI: 10.5409/wjcp.v11.i3.270

Figure 13 A 7-year-old boy with extra-hepatic portal vein obstruction. A and B: Axial T2W images showing non visualized main portal vein with multiple collaterals (arrow) along the course of the portal vein; C and D: Axial BTFE and post-contrast T1 images show multiple collaterals (arrow) replacing the main portal vein at the porta. In addition, splenomegaly is also seen.

depends on location, size and mass effect, and complications. It may present as vomiting, abdominal distention, bleeding, abdominal mass, and increased urinary frequency and hesitancy. The cysts may be complicated by perforation and can act as a lead point for intussusception, volvulus, and bowel obstruction. GI bleeding occurs primarily because of ulceration of the gastric mucosa, intussusception, or pressure necrosis[55-58].

On USG, these are seen as well-defined anechoic lesions that demonstrate a classic gut signature (in about 50% of cases), *i.e.*, mucosal internal echogenic layer and muscular outer hypoechoic layer (Figure 15). This appearance is usually interrupted, due to non-uniform thickness[56]. USG appearance may vary in cases with hemorrhage. Barium contrast studies although not routinely used, may demonstrate a sub-mucosal filling defect with a mass effect on the gastrointestinal tract or rarely communicating with it[56].

On CT, a duplication cyst is seen as a well-defined non-enhancing mass with cystic attenuation adjacent to the GI tract (Figure 16). However, the central attenuation may vary, depending on hemorrhage or proteinaceous material, which usually show higher central attenuation[56] MRI will show a well-defined cystic lesion with heterogeneous signal density on TI weighted image and T2



DOI: 10.5409/wjcp.v11.i3.270

Figure 14 Portal cavernoma cholangiopathy. A and B: Axial T2 weighted and axial BTFE magnetic resonance images show dilated CBD [arrowhead] with multiple pericholedochal collaterals [white arrow]; C: Coronal post-contrast T1 image shows multiple enhancing pericholedochal collaterals [white arrow]; D: Massive splenomegaly. Thick slab coronal magnetic resonance cholangiopancreatography image shows dilated and tortuous CBD (thin arrow) with abrupt cut-off at the lower end (thick arrow).

homogenous high signal intensity[55].

RECTAL HEMANGIOMA

Gastrointestinal hemangiomas are benign vascular tumors. They are most commonly seen in pediatric and young adults where they present as GI bleeding in about 80% of cases and are a cause of life-threatening anemia. Most commonly are seen in the small bowel. They are also seen in the colon and rectum. They may be seen as part of Klippel-Trénaunay, Maffucci, blue rubber bleb nevus syndrome, and disseminated neonatal hemangiomatosis. Histologically, they can be of cavernous, capillary, and mixed type with the cavernous variety being the most common subtype[59-61].

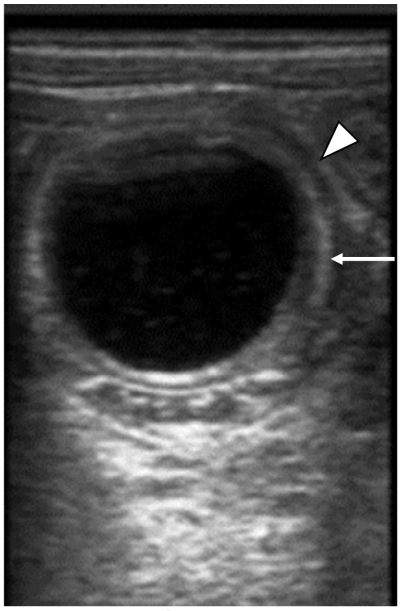
Plain radiograph being the first routine investigation may show phleboliths (evident in 50% of cases) along the course of the bowel. Extensive phleboliths are rare in young and this gives a clue for further investigations[59,60].

CT scan provides an intramural and extramural extension of the lesion. On CT, the involved bowel shows asymmetric bowel wall thickening with contrast enhancement. Phleboliths may or may not be seen[60] (Figure 17).

MRI shows rectal wall thickening, increased T2 signal intensity, and prominent perirectal serpiginous vascular channels. The perirectal vascular channels and atypical location help to differentiate rectal hemangiomas from hemorrhoids.

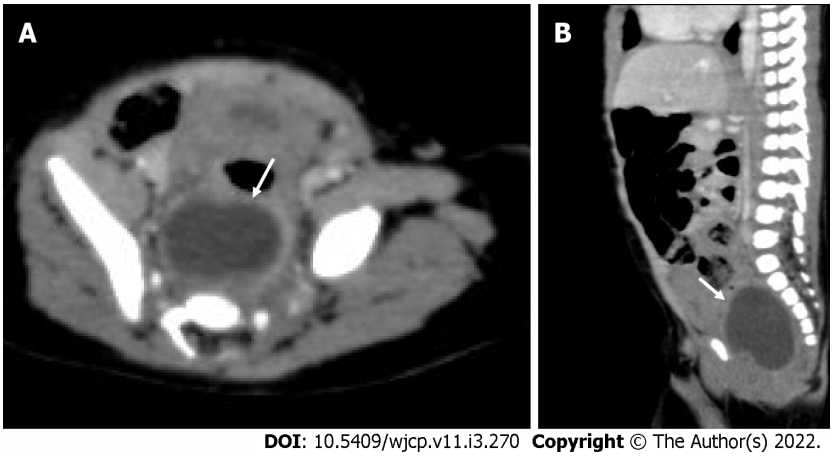
INTUSSUSCEPTION

Intussusception refers to telescoping of a bowel segment (intussusceptum) into the distal segment (intussuscipiens). It is among the most common abdominal emergencies in the pediatric age group with most cases (approximately 80%) occurring between 6 mo to 2 years of age[62-64]. It is also one of the



DOI: 10.5409/wjcp.v11.i3.270

Figure 15 Gray-scale ultrasonography image shows a well-defined anechoic lesion showing a classic gut signature with inner echogenic mucosa (arrow) and outer hypoechoic muscularis propria.



DOI: 10.5409/wjcp.v11.i3.270

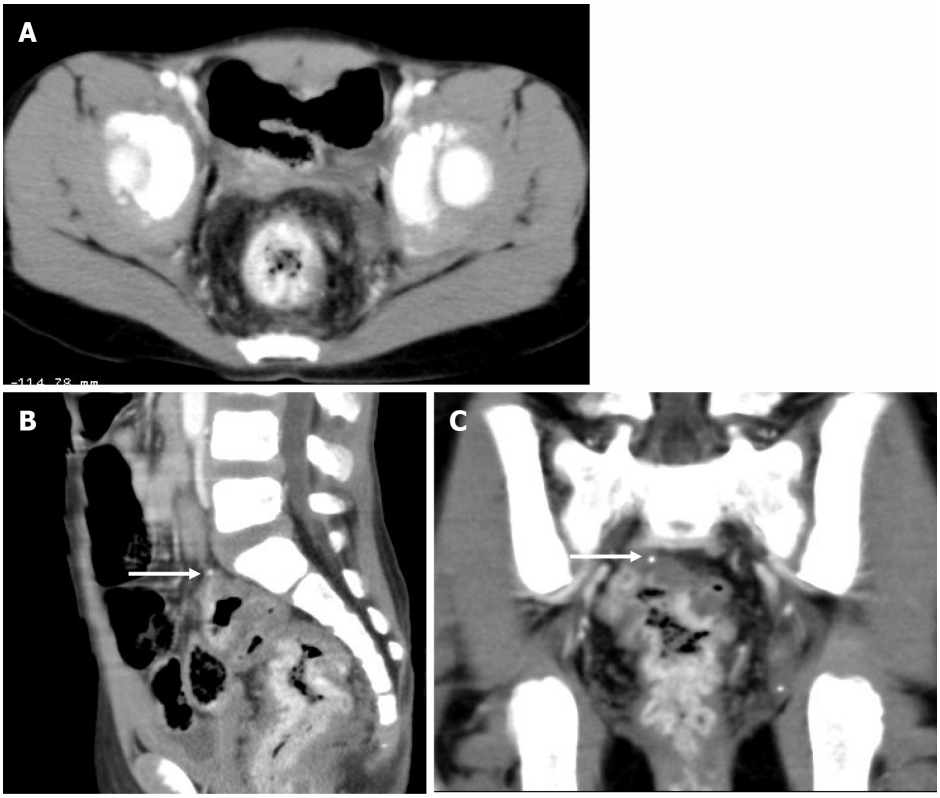
Figure 16 Rectal duplication cyst. A and B: Axial and sagittal contrast enhanced computed tomography images show a well-defined cystic structure in the presacral space pushing the rectum (arrow) anteriorly.

most common causes of small bowel obstruction in infants[63]. The clinical triad of acute abdominal pain, palpable abdominal mass, and currant jelly stools/hematochezia is noted only in 50% of patients [62]. Ileo-colic is the most common type, where an ileum segment invaginates across IC junction into the colon for variable length[64].

Many radiographic signs of intussusception have been described, which include soft tissue density mass in the right upper quadrant, gasless abdomen, small bowel obstruction, and meniscus sign (Figure 18). X-rays usually are the initial investigation and are primarily used to look for obstruction and perforation and to rule out any other causes of pain abdomen. On barium enema, the meniscus sign and the coiled spring sign are the classic signs explained in intussusception[62].

Ultrasound has a high sensitivity (98%-100%) and specificity (88%-100%) for diagnosis of intussusception. Multiple concentric ring sign and crescent in doughnut sign are seen on axial scans (Figure 19). On longitudinal scans, sandwich and hayfork signs are explained[65](Figure 20). The intussuscipiens contains the entering limb and the returning limb of the intussusceptum, along with the mesentery. This gives variable ultrasound features depending on the length of the involved segment.

Most cases of intussusception are idiopathic. However, duplication cyst, polyps, tumor, or Meckel's diverticulum can act as a lead point and are more common in neonates or older children. USG is very sensitive in picking up and characterization of lead points[62,65](Figure 21).



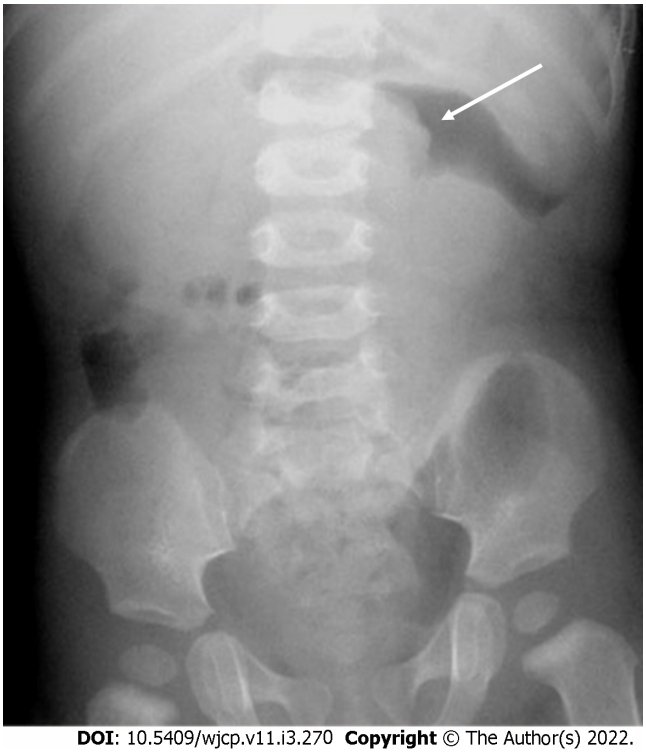
DOI: 10.5409/wjcp.v11.i3.270

Figure 17 An 8-year-old girl with bleeding per rectum. Axial (A), sagittal (B), and coronal (C) contrast enhanced computed tomography images showing diffuse rectal wall thickening with intense contrast enhancement. A tiny phlebolith is also seen (arrows in B and C). Findings are suggestive of rectal hemangioma.

Under real time fluoroscopy, uncomplicated ileocolonic and colocolonic intussusceptions can be reduced using barium enema, water-soluble contrast agents, and pneumatic reduction[62] (Figure 22).

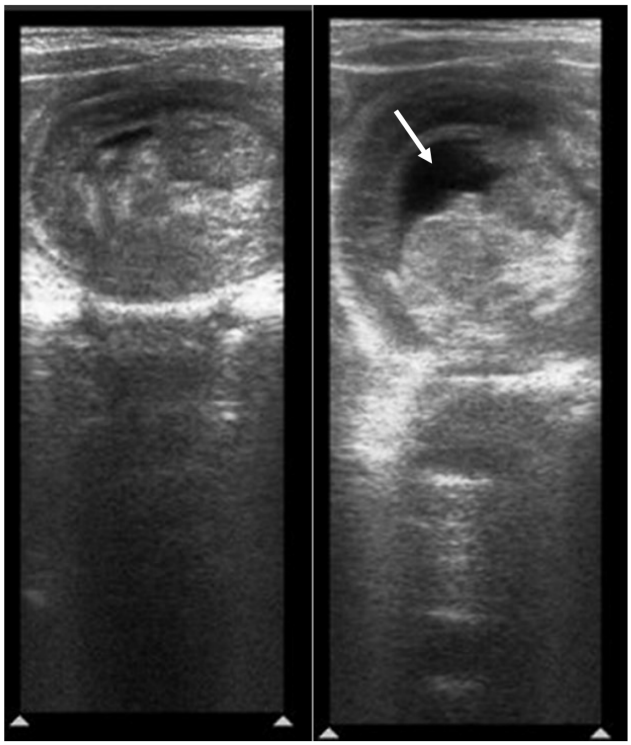
CONCLUSION

To conclude, bleeding per rectum in children can occur due to a variety of medical and surgical causes across the different age groups. The referring clinicians as well as the radiologists must be aware of the various radiological findings of common and uncommon causes of bleeding per rectum in children, which may require medical and surgical management at the time of presentation.



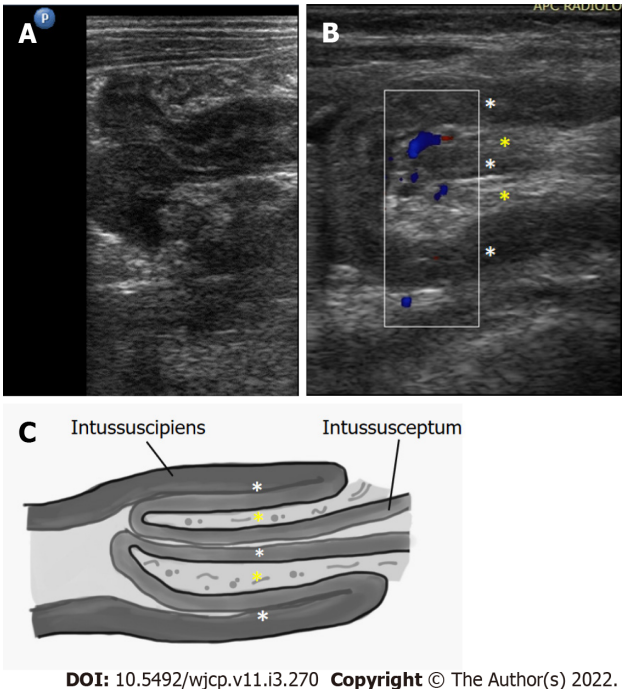
DOI: 10.5409/wjcp.v11.i3.270

Figure 18 Abdominal X-ray in a child with intussusception showing a gasless abdomen with meniscus of air outlining a soft tissue opacity (intussusceptum) in upper abdomen (arrow).



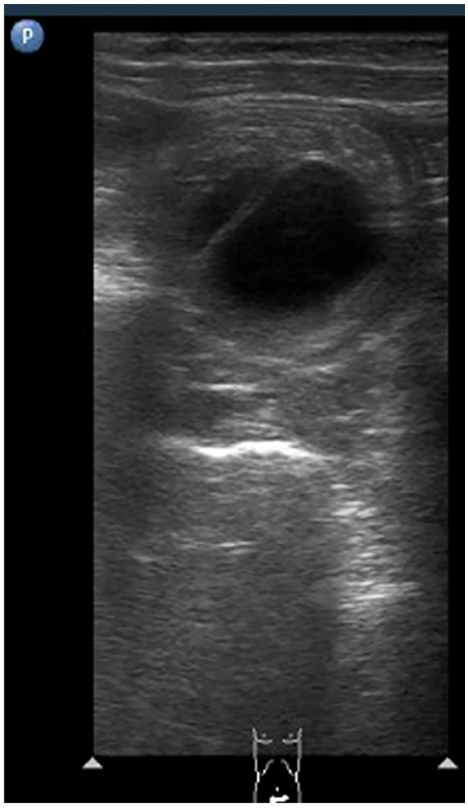
DOI: 10.5409/wjcp.v11.i3.270

Figure 19 Ultrasound image shows multiple concentric rings suggestive of intussusception. Minimal trapped fluid is also seen (arrow).



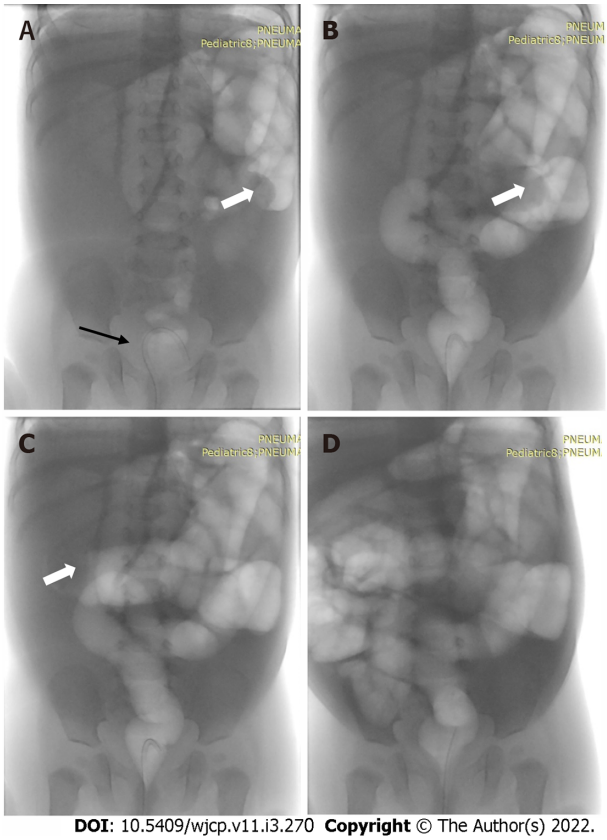
DOI: 10.5492/wjcp.v11.i3.270

Figure 20 Longitudinal ultrasound images of ileo-colic intussusception. A: Outer intussuscipiens and inner intussusceptum; B and C: Both show three hypoechoic lines (white asterixes) separating the two echogenic areas (yellow asterisks) giving sandwich sign/hay-fork sign. Longitudinal ultrasound images are important to delineate the length of the involved bowel segment.



DOI: 10.5409/wjcp.v11.i3.270

Figure 21 Ultrasound image shows cystic structure at the apex of the intussusceptum as the pathological lead point. Differentials include duplication cyst and Meckel's diverticulum.



DOI: 10.5409/wjcp.v11.i3.270

Figure 22 Pneumatic reduction of intussusception under fluoroscopy. A: The patient is positioned supine with a feeding tube within the rectum (black arrow); B and C: The intussusceptum is seen in the left hypochondrium (open arrow) given by the meniscus sign; subsequent spots after air inflation show that the intussusceptum has moved proximally, as well as reflux of air in the small bowel after successful reduction (D) of the intussusception.

ACKNOWLEDGEMENTS

We thank Dr. Bhujade H, Assistant Professor, Department of Radiodiagnosis and Imaging, Postgraduate Institute of Medical Education and Research, Sector-12, Chandigarh, 160012, India for designing the diagrammatic image for Figure 20.

FOOTNOTES

Author contributions: Bhatia A, Saxena AK, and Sodhi KS designed the research study; Chandel K, Jain R, and Bhatia A performed the research; Chandel K, Jain R, and Bhatia A wrote the manuscript; Bhatia A, Saxena AK, and Sodhi KS edited the manuscript; all authors have read and approved the final manuscript.

Conflict-of-interest statement: The authors have no conflicts of interest to declare.

Country/Territory of origin: India

ORCID number: Karamvir Chandel 0000-0002-7175-5203; Rishabh Jain 0000-0003-0281-3569; Anmol Bhatia 0000-0003-4630-5742; Akshay Kumar Saxena 0000-0002-9184-5687; Kushaljit Singh Sodhi 0000-0001-6419-2468.

S-Editor: Wang LL
L-Editor: Wang TQ
P-Editor: Qi WW

REFERENCES

1 **Stoll BJ**. Epidemiology of necrotizing enterocolitis. *Clin Perinatol* 1994; **21**: 205-218 [PMID: 8070222]
2 **Ballance WA**, Dahms BB, Shenker N, Kliegman RM. Pathology of neonatal necrotizing enterocolitis: a ten-year experience. *J Pediatr* 1990; **117**: S6-13 [PMID: 2362230 DOI: 10.1016/s0022-3476(05)81124-2]
3 **Rowe MI**, Reblock KK, Kurkchubasche AG, Healey PJ. Necrotizing enterocolitis in the extremely low birth weight infant. *J Pediatr Surg* 1994; **29**: 987-90; discussion 990 [PMID: 7965535 DOI: 10.1016/0022-3468(94)90264-x]
4 **Martinez-Tallo E**, Claure N, Bancalari E. Necrotizing enterocolitis in full-term or near-term infants: risk factors. *Biol Neonate* 1997; **71**: 292-298 [PMID: 9167850 DOI: 10.1159/000244428]
5 **Bolisetty S**, Lui K, Oei J, Wojtulewicz J. A regional study of underlying congenital diseases in term neonates with necrotizing enterocolitis. *Acta Paediatr* 2000; **89**: 1226-1230 [PMID: 11083380 DOI: 10.1080/080352500750027619]
6 **Lee JS**, Polin RA. Treatment and prevention of necrotizing enterocolitis. *Semin Neonatol* 2003; **8**: 449-459 [PMID: 15001117 DOI: 10.1016/S1084-2756(03)00123-4]
7 **Epelman M**, Daneman A, Navarro OM, Morag I, Moore AM, Kim JH, Faingold R, Taylor G, Gerstle JT. Necrotizing Enterocolitis: Review of State-of-the-Art Imaging Findings with Pathologic Correlation. *RadioGraphics March* 2007; **27**: 285-305 [DOI: 10.1148/rg.272055098]
8 **Leonidas JC**, Hall RT, Amoury RA. Critical evaluation of the roentgen signs of neonatal necrotizing enterocolitis. *Ann Radiol (Paris)* 1976; **19**: 123-132 [PMID: 988775]
9 **Pochaczevsky R**, Kassner EG. Necrotizing enterocolitis of infancy. *Am J Roentgenol Radium Ther Nucl Med* 1971; **113**: 283-296 [PMID: 4938604 DOI: 10.2214/ajr.113.2.283]
10 **Robinson AE**, Grossman H, Brumley GW. Pneumatosis intestinals in the neonate. *Am J Roentgenol Radium TherNucl Med* 1974; **120**: 333-341 [DOI: 10.2214/ajr.120.2.333]
11 **Bell RS**, Graham CB, Stevenson JK. Roentgenologic and clinical manifestations of neonatal necrotizing enterocolitis. Experience with 43 cases. *Am J Roentgenol Radium TherNucl Med* 1971; **112**: 123-134 [DOI: 10.2214/ajr.112.1.123]
12 **Kirks DR**, O'Byrne SA. The value of the lateral abdominal roentgenogram in the diagnosis of neonatal hepatic portal venous gas (HPVG). *Am J Roentgenol Radium TherNucl Med* 1974; **122**: 153-158 [DOI: 10.2214/ajr.122.1.153]
13 **Buonomo C**. The radiology of necrotizing enterocolitis. *Radiologic clinics of North America* 1999; **37**: 1187-1198 [DOI: 10.1016/s0033-8389(05)70256-6]
14 **Faingold R**, Daneman A, Tomlinson G, Babyn PS, Manson DE, Mohanta A, Moore AM, Hellmann J, Smith C, Gerstle T, Kim JH. Necrotizing enterocolitis: assessment of bowel viability with color doppler US. *Radiology* 2005; **235**: 587-594 [PMID: 15858098 DOI: 10.1148/radiol.2352031718]
15 **Kim WY**, Kim WS, Kim IO, Kwon TH, Chang W, Lee EK. Sonographic evaluation of neonates with early-stage necrotizing enterocolitis. *Pediatr Radiol* 2005; **35**: 1056-1061 [PMID: 16078076 DOI: 10.1007/s00247-005-1533-4]
16 **Levy AD**, Hobbs CM. From the archives of the AFIP. Meckel diverticulum: radiologic features with pathologic Correlation. *Radiographics* 2004; **24**: 565-587 [PMID: 15026601 DOI: 10.1148/rg.242035187]
17 **Cullen JJ**, Kelly KA, Moir CR, Hodge DO, Zinsmeister AR, Melton 3rd LJ. Surgical management of Meckel's diverticulum. An epidemiologic, population-based study. *Annals of surgery* 1994; **220**: 564 [DOI: 10.1097/00000658-199410000-00014]
18 **Sagar J**, Kumar V, Shah DK. Meckel's diverticulum: a systematic review. *J R Soc Med* 2006; **99**: 501-505 [PMID: 17021300 DOI: 10.1258/jrsm.99.10.501]
19 **Cooney DR**, Duszynski DO, Camboa E, Karp MP, Jewett TC Jr. The abdominal technetium scan (a decade of experience). *J Pediatr Surg* 1982; **17**: 611-619 [PMID: 6294268 DOI: 10.1016/s0022-3468(82)80121-8]
20 **Sfakianakis GN**, Conway JJ. Detection of ectopic gastric mucosa in Meckel's diverticulum and in other aberrations by scintigraphy: ii. indications and methods-a 10-year experience. *J Nucl Med* 1981; **22**: 732-738 [PMID: 6267233]
21 **Paulsen SR**, Huprich JE, Fletcher JG, Booya F, Young BM, Fidler JL, Johnson CD, Barlow JM, Earnest F 4th. CT enterography as a diagnostic tool in evaluating small bowel disorders: review of clinical experience with over 700 cases. *Radiographics* 2006; **26**: 641-57; discussion 657 [PMID: 16702444 DOI: 10.1148/rg.263055162]
22 **Thakkar K**, Fishman DS, Gilger MA. Colorectal polyps in childhood. *Curr Opin Pediatr* 2012; **24**: 632-637 [PMID: 22890064 DOI: 10.1097/MOP.0b013e328357419f]
23 **Thakkar K**, Alsarraj A, Fong E, Holub JL, Gilger MA, El Serag HB. Prevalence of colorectal polyps in pediatric colonoscopy. *Dig Dis Sci* 2012; **57**: 1050-1055 [PMID: 22147243 DOI: 10.1007/s10620-011-1972-8]
24 **Adolph VR**, Bernabe K. Polyps in children. *Clin Colon Rectal Surg* 2008; **21**: 280-285 [PMID: 20011439 DOI: 10.1055/s-0028-1089943]
25 **Nugent KP**, Talbot IC, Hodgson SV, Phillips RK. Solitary juvenile polyps: not a marker for subsequent malignancy. *Gastroenterology* 1993; **105**: 698-700 [PMID: 8395444 DOI: 10.1016/0016-5085(93)90885-g]
26 **Campbell AM**, Sugarman I. Does painless rectal bleeding equate to a colonic polyp? *Arch Dis Child* 2017; **102**: 1049-1051 [PMID: 28550146 DOI: 10.1136/archdischild-2016-311245]
27 **Lee BG**, Shin SH, Lee YA, Wi JH, Lee YJ, Park JH. Juvenile polyp and colonoscopic polypectomy in childhood. *Pediatr Gastroenterol Hepatol Nutr* 2012; **15**: 250-255 [PMID: 24010095 DOI: 10.5223/pghn.2012.15.4.250]
28 **Laufer I**, Levine MS. Principles of double contrast diagnosis. Double contrast gastrointestinal radiology, Philadelphia: Saunders, 1992: 9-54 [DOI: 10.1016/b978-1-4160-2332-6.50009-9]
29 **Miller WT**, Levine MS, Rubesin SE, Laufer I. Bowler-hat sign: a simple principle for differentiating polyps from diverticula. *Radiology* 1989; **173**: 615-617 [DOI: 10.1148/radiology.173.3.2813762]
30 **Vining DJ**, Gelfand DW. Noninvasive colonoscopy using helical CT scanning, 3D reconstruction, and virtual reality. Presented at the 23rd Annual Meeting and Postgraduate Course of the Society of Gastrointestinal Radiologists. *Maui HI* 1994; **127**: 310-316 [DOI: 10.1016/0363-8235(80)90045-9]
31 **Luboldt W**, Steiner P, Bauerfeind P, Pelkonen P, Debatin JF. Detection of mass lesions with MR colonography: preliminary report. *Radiology* 1998; **207**: 59-65 [DOI: 10.1148/radiology.207.1.9530299]

32 **Lauenstein TC**, Ajaj W, Kuehle CA, Goehde SC, Schlosser TW, Ruehm SG. Magnetic resonance colonography: comparison of contrast-enhanced three-dimensional vibe with two-dimensional FISP sequences: preliminary experience. *Invest Radiol* 2005; **40**: 89-96 [PMID: 15654253 DOI: 10.1097/01.rli.0000149489.56736.39]

33 **Mekhjian HS**, Switz DM, Melnyk CS, Rankin GB, Brooks RK. Clinical features and natural history of Crohn's disease. *Gastroenterology* 1979; **77**: 898-906 [DOI: 10.1016/0016-5085(79)90389-5]

34 **Motil KJ**, Grand RJ, Davis-Kraft L, Ferlic LL, Smith EO. Growth failure in children with inflammatory bowel disease: a prospective study. *Gastroenterology* 1993; **105**: 681-691 [PMID: 8359640 DOI: 10.1016/0016-5085(93)90883-e]

35 **Fraquelli M**, Colli A, Casazza G, Paggi S, Colucci A, Massironi S, Duca P, Conte D. Role of US in detection of Crohn disease: meta-analysis. *Radiology* 2005; **236**: 95-101 [PMID: 15987966 DOI: 10.1148/radiol.2361040799]

36 **Booya F**, Fletcher JG, Huprich JE, Barlow JM, Johnson CD, Fidler JL, Solem CA, Sandborn WJ, Loftus EV Jr, Harmsen WS. Active Crohn Disease: CT Findings and Interobserver Agreement for Enteric Phase CT Enterography. *Radiology* 2006; **241**: 787-795 [DOI: 10.1148/radiol.2413051444]

37 **Choi D**, Jin Lee S, Ah Cho Y, Lim HK, Hoon Kim S, Jae Lee W, Hoon Lim J, Park H, Rae Lee Y. Bowel wall thickening in patients with Crohn's disease: CT patterns and correlation with inflammatory activity. *Clin Radiol* 2003; **58**: 68-74 [PMID: 12565208 DOI: 10.1053/crad.2002.1068]

38 **Goldberg HI**, Gore RM, Margulis AR, Moss AA, Baker EL. Computed tomography in the evaluation of Crohn disease. *AJR Am J Roentgenol* 1983; **140**: 277-282 [PMID: 6600342 DOI: 10.2214/ajr.140.2.277]

39 **Wallihan DB**, Towbin AJ, Denson LA, Salisbury S, Podberesky DJ. Inflammatory bowel disease in children and adolescents: assessing the diagnostic performance and interreader agreement of magnetic resonance enterography compared to histopathology. *Acad Radiol* 2012; **19**: 819-826 [PMID: 22520509 DOI: 10.1016/j.acra.2012.02.023]

40 **Lee SS**, Ha HK, Yang SK, Kim AY, Kim TK, Kim PN, Lee MG, Myung SJ, Jung HY, Kim JH, Min YI. CT of prominent pericolic or perienteric vasculature in patients with Crohn's disease: correlation with clinical disease activity and findings on barium studies. *AJR Am J Roentgenol* 2002; **179**: 1029-1036 [PMID: 12239060 DOI: 10.2214/ajr.179.4.1791029]

41 **Bodily KD**, Fletcher JG, Solem CA, Johnson CD, Fidler JL, Barlow JM, Bruesewitz MR, McCollough CH, Sandborn WJ, Loftus EV Jr, Harmsen WS, Crownhart BS. Crohn Disease: mural attenuation and thickness at contrast-enhanced CT Enterography--correlation with endoscopic and histologic findings of inflammation. *Radiology* 2006; **238**: 505-516 [PMID: 16436815 DOI: 10.1148/radiol.2382041159]

42 **Oto A**, Zhu F, Kulkarni K, Karczmar GS, Turner JR, Rubin D. Evaluation of diffusion-weighted MR imaging for detection of bowel inflammation in patients with Crohn's disease. *Acad Radiol* 2009; **16**: 597-603 [PMID: 19282206 DOI: 10.1016/j.acra.2008.11.009]

43 **Koelbel G**, Schmiedl U, Majer MC, Weber P, Jenss H, Kueper K, Hess CF. Diagnosis of fistulae and sinus tracts in patients with Crohn disease: value of MR imaging. *AJR Am J Roentgenol* 1989; **152**: 999-1003 [PMID: 2705359 DOI: 10.2214/ajr.152.5.999]

44 **Essary B**, Kim J, Anupindi S, Katz JA, Nimkin K. Pelvic MRI in children with Crohn disease and suspected perianal involvement. *Pediatr Radiol* 2007; **37**: 201-208 [PMID: 17180366 DOI: 10.1007/s00247-006-0372-2]

45 **Dunn EA**, Olsen ØE, Huisman TA. The Pediatric Gastrointestinal Tract: What Every Radiologist Needs to Know. In Diseases of the Abdomen and Pelvis Springer, Cham, 2018: 157-166 [DOI: 10.1007/978-3-319-75019-4_15]

46 **Shimanuki Y**, Aihara T, Takano H, Moritani T, Oguma E, Kuroki H, Shibata A, Nozawa K, Ohkawara K, Hirata A, Imaizumi S. Clockwise whirlpool sign at color Doppler US: an objective and definite sign of midgut volvulus. *Radiology* 1996; **199**: 261-264 [DOI: 10.1148/radiology.199.1.8633156]

47 **Peterson CM**, Anderson JS, Hara AK, Carenza JW, Menias CO. Volvulus of the gastrointestinal tract: appearances at multimodality imaging. *Radiographics* 2009; **29**: 1281-1293 [PMID: 19755596 DOI: 10.1148/rg.295095011]

48 **Applegate KE**, Anderson JM, Klatte EC. Intestinal malrotation in children: a problem-solving approach to the upper gastrointestinal series. *Radiographics* 2006; **26**: 1485-1500 [PMID: 16973777 DOI: 10.1148/rg.265055167]

49 **Fisher JK**. Computed tomographic diagnosis of volvulus in intestinal malrotation. *Radiology* 1981; **140**: 145-146 [PMID: 7244217 DOI: 10.1148/radiology.140.1.7244217]

50 **Arora A**, Sarin SK. Multimodality imaging of primary extrahepatic portal vein obstruction (EHPVO): what every radiologist should know. *Br J Radiol* 2015; **88**: 20150008 [PMID: 26111208 DOI: 10.1259/bjr.20150008]

51 **Weiss B**, Shteyer E, Vivante A, Berkowitz D, Reif S, Weizman Z, Bujanover Y, Shapiro R. Etiology and long-term outcome of extrahepatic portal vein obstruction in children. World J Gastro 2010; 16: 4968 [DOI: 10.3748/wjg.v16.i39.4968]

52 **Pargewar SS**, Desai SN, Rajesh S, Singh VP, Arora A, Mukund A. Imaging and radiological interventions in extra-hepatic portal vein obstruction. *World J Radiol* 2016; **8**: 556-570 [PMID: 27358683 DOI: 10.4329/wjr.v8.i6.556]

53 **Khanna R**, Sarin SK. Non-cirrhotic portal hypertension-diagnosis and management. *J hepat* 2014; **60**: 421-441 [DOI: 10.1016/j.jhep.2013.08.013]

54 **Prasad GR**, Billa S, Bhandari P, Hussain A. Isolated inferior mesenteric portal hypertension with giant inferior mesenteric vein and anomalous inferior mesenteric vein insertion. *Journal of Indian Association of Pediatric Surgeons* 2013; **18**: 84 [DOI: 10.4103/0971-9261.109362]

55 **Liu R**, Adler DG. Duplication cysts: Diagnosis, management, and the role of endoscopic ultrasound. *Endosc Ultrasound* 2014; **3**: 152-160 [PMID: 25184121 DOI: 10.4103/2303-9027.138783]

56 **Tong SC**, Pitman M, Anupindi SA. Best cases from the AFIP. Ileocecal enteric duplication cyst: radiologic-pathologic correlation. *Radiographics* 2002; **22**: 1217-1222 [PMID: 12235349 DOI: 10.1148/radiographics.22.5.g02se221217]

57 **Sharma S**, Yadav AK, Mandal AK, Zaheer S, Yadav DK, Samie A. Enteric duplication cysts in children: A clinicopathological dilemma. *J Clin Diagnostic Res* 2015; **9**: EC08-EC11 [DOI: 10.7860/jcdr/2015/12929.6381]

58 **Flynn-O'Brien KT**, Rice-Townsend S, Ledbetter DJ. Structural anomalies of the gastrointestinal tract. In: Avery's Diseases of the Newborn Elsevier, Pjiladelphia, PA, 2018: 1039-1053 [DOI: 10.1016/b978-0-323-40139-5.00071-1]

59 **Dachman H**, Buck L, Shekitka KM, Olmsted W, Hinton CB. Colorectal Hemangloma: Radiologic Findings. *Radiology* 1988; **167**: 31-34 [DOI: 10.1148/radiology.167.1.3347741]

60 **Yoo S**. GI-Associated Hemangiomas and Vascular Malformations. *Clin Colon Rectal Surg* 2011; **24**: 193-200 [PMID: 22942801 DOI: 10.1055/s-0031-1286003]

61 **Han EC**, Kim SH, Kim HY, Jung SE, Park KW. Gastrointestinal hemangioma in childhood: a rare cause of gastrointestinal bleeding. *Korean J Pediatr* 2014; **57**: 245-249 [PMID: 25045368 DOI: 10.3345/kjp.2014.57.5.245]

62 **del-Pozo G**, Albillos JC, Tejedor D, Calero R, Rasero M, de-la-Calle U, López-Pacheco U. Intussusception in children: current concepts in diagnosis and enema reduction. *Radiographics* 1999; **19**: 299-319 [PMID: 10194781 DOI: 10.1148/radiographics.19.2.g99mr14299]

63 **Jiang J**, Jiang B, Parashar U, Nguyen T, Bines J, Patel MM. Childhood intussusception: a literature review. *PLoS One* 2013; **8**: e68482 [PMID: 23894308 DOI: 10.1371/journal.pone.0068482]

64 **Lioubashevsky N**, Hiller N, Rozovsky K, Segev L, Simanovsky N. Ileocolic *vs* small-bowel intussusception in children: can US enable reliable differentiation? *Radiology* 2013; **269**: 266-271 [PMID: 23801771 DOI: 10.1148/radiol.13122639]

65 **Verschelden P**, Filiatrault D, Garel L, Grignon A, Perreault G, Boisvert J, Dubois J. Intussusception in children: reliability of US in diagnosis--a prospective study. *Radiology* 1992; **184**: 741-744 [PMID: 1509059 DOI: 10.1148/radiology.184.3.1509059]

World Journal of Clinical Pediatrics

Submit a Manuscript: https://www.f6publishing.com

World J Clin Pediatr 2022 May 9; 11(3): 289-294

DOI: 10.5409/wjcp.v11.i3.289

ISSN 2219-2808 (online)

ORIGINAL ARTICLE

Observational Study

Turnaround times for molecular testing of pediatric viral cerebrospinal fluid samples in United Kingdom laboratories

Siba Prosad Paul, Varathagini Balakumar, Arangan Kirubakaran, Jothilingam Niharika, Paul Anthony Heaton, Paul Christopher Turner

Specialty type: Pediatrics

Provenance and peer review: Invited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): 0
Grade B (Very good): B, B
Grade C (Good): 0
Grade D (Fair): 0
Grade E (Poor): 0

P-Reviewer: Gravina AG, Italy; Matowicka-Karna J, Poland

Received: June 21, 2021
Peer-review started: June 21, 2021
First decision: July 30, 2021
Revised: August 1, 2021
Accepted: March 26, 2022
Article in press: March 26, 2022
Published online: May 9, 2022

Siba Prosad Paul, Paul Anthony Heaton, Department of Paediatrics, Yeovil District Hospital, Yeovil BA21 4AT, Somerset, United Kingdom

Varathagini Balakumar, Jothilingam Niharika, School of Medicine, Cardiff University, Cardiff CF14 4XN, United Kingdom

Arangan Kirubakaran, Department of Paediatrics, Hillingdon Hospital, Uxbridge UB8 3NN, United Kingdom

Paul Christopher Turner, Department of Medical Microbiology, Torbay Hospital, Torquay TQ2 7AA, United Kingdom

Corresponding author: Siba Prosad Paul, MBBS, DCH, MRCPCH, Consultant Paediatrician, Department of Paediatrics, Yeovil District Hospital, Higher Kingston, Yeovil BA21 4AT, Somerset, United Kingdom. siba.paul@nhs.net

Abstract

BACKGROUND

Rapid molecular testing has revolutionized the management of suspected viral meningitis and encephalitis by providing an etiological diagnosis in < 90 min with potential to improve outcomes and shorten inpatient stays. However, use of molecular assays can vary widely.

AIM

To evaluate current practice for molecular testing of pediatric cerebrospinal fluid (CSF) samples across the United Kingdom using a structured questionnaire.

METHODS

A structured telephone questionnaire survey was conducted between July and August 2020. Data was collected on the availability of viral CSF nucleic acid amplification testing (NAAT), criteria used for testing and turnaround times including the impact of the coronavirus disease 2019 pandemic.

RESULTS

Of 196/212 (92%) microbiology laboratories responded; 63/196 (32%) were excluded from final analysis as they had no on-site microbiology laboratory and outsourced their samples. Of 133 Laboratories included in the study, 47/133 (35%)

had onsite facilities for viral CSF NAAT. Hospitals currently undertaking onsite NAAT (n = 47) had much faster turnaround times with 39 centers (83%) providing results in ≤ 24 h as compared to those referring samples to neighboring laboratories (5/86; 6%).

CONCLUSION

Onsite/near-patient rapid NAAT (including polymerase chain reaction) is recommended wherever possible to optimize patient management in the acute setting.

Key Words: Cerebrospinal fluid; Nucleic acid amplification testing; Questionnaire survey; Turnaround times; Viral studies

Core Tip: Rapid diagnosis of viral meningitis in children through nucleic acid amplification testing (NAAT) of cerebrospinal fluid (CSF) can help in establishing a firm diagnosis, allowing early discontinuation of antibiotics and ensuring improved antibiotic stewardship. Turnaround times will be improved through availability of onsite NAAT facilities in the hospitals with inpatient pediatric units. All CSF samples in infants, irrespective of their white cell counts (actual/adjusted) should be offered NAAT, as viral meningitis due to enterovirus or human parechovirus can occur without pleocytosis.

Citation: Paul SP, Balakumar V, Kirubakaran A, Niharika J, Heaton PA, Turner PC. Turnaround times for molecular testing of pediatric viral cerebrospinal fluid samples in United Kingdom laboratories. *World J Clin Pediatr* 2022; 11(3): 289-294

URL: https://www.wjgnet.com/2219-2808/full/v11/i3/289.htm

DOI: https://dx.doi.org/10.5409/wjcp.v11.i3.289

INTRODUCTION

Timely diagnosis of meningitis is crucial to reduce mortality and long-term neurological disability[1,2]. The introduction of public health initiatives and immunization programs over the last 50 years have significantly decreased the incidence of bacterial meningitis in the United Kingdom[3]. In the United Kingdom, viral pathogens are the commonest cause of meningitis in both adult and pediatric populations[2]. The diagnosis of meningitis involves clinical assessment and a variety of laboratory investigations. Distinguishing between viral and bacterial causes can be challenging at initial presentation. For cases of suspected meningitis, in the absence of contraindications including coagulation disorders or raised intracranial pressure, a lumbar puncture should be performed[4,5]. Nucleic acid amplification testing (NAAT), predominantly through polymerase chain reaction (PCR) technology of the cerebrospinal fluid (CSF), is recognised as the gold standard for diagnosis in viral meningitis[6].

The aim of this study was to evaluate the use and availability of viral NAAT testing of CSF in microbiology laboratories across the United Kingdom.

MATERIALS AND METHODS

An electronic search of the National Health Service (NHS) database (http://www.nhs.uk/servicedirectories/pages/nhstrustlisting.aspx) was conducted to identify NHS trusts providing pediatric services across the United Kingdom (n = 212): England (n = 172), Scotland (n = 20), Wales (n = 12) and Northern Ireland (n = 8). Structured telephone surveys were conducted with either a Consultant Microbiologist (n = 3) or a Senior Biomedical Scientist (n = 193) in participating hospitals between July and August 2020. Twenty three of the 196 respondents submitted data *via* email citing data protection policy for their hospital. Sixteen laboratories did not respond, citing work pressures due to the coronavirus disease 2019 (COVID-19) pandemic, and were excluded from this study. The study was conducted and approved as an outcome audit. Ethical approval was considered not necessary as no confidential patient data was collected.

The survey consisted of a standardized questionnaire delivered by a single interviewer and the responses were collated electronically. The questionnaire asked the following details regarding NAAT of Paediatric CSF samples: (1) Whether the laboratory had onsite facilities to perform viral NAAT on CSF samples, type of assay used, criteria required to perform viral NAAT, the availability of point-of-

care (POC) testing, and the approximate turnaround time (TAT); (2) If the laboratory did not perform viral NAAT, they were asked where samples were sent, criteria required to perform testing, and the TAT for NAAT results; and (3) All the laboratories performing onsite testing were questioned about the impact of the COVID-19 pandemic on their ability to process CSF NAAT samples.

Statistical analysis was performed using standard Chi-squared analysis and a P value < 0.05 was considered to indicate significance.

RESULTS

In total, 196/212 hospitals (92%) responded to the questionnaire. Of those responding, 133 (68%) had an onsite microbiology laboratory within the same hospital site as the pediatric facility and were included in the study; 63 hospitals (32%) were covered by offsite microbiology services at a different hospital and were excluded from the study (Figure 1). More than one-third of onsite microbiology laboratories in the United Kingdom (n = 47) had facilities to perform viral CSF NAAT as well as cover neighboring onsite laboratories (n = 88) with no NAAT facilities. Other laboratories with no onsite microbiology laboratories (n = 63) outsourced samples elsewhere. The criteria used to perform viral NAAT amongst the 47 onsite laboratories were as follows: (1) Clinician request in 32% (n = 15); (2) Combination of CSF white blood cell count and clinician request in 28% (n = 13); (3) Performed on all samples if requested (referred to as "blanket testing") in 19% (n = 9); (4) Entirely dependent on CSF pleocytosis in 6% (n = 3); (5) Approval from a microbiologist in 4% (n = 2); and (6) Respondents unaware of the criteria for testing in 11% (n = 5).

The majority of microbiology laboratories (n = 86) that sent samples away did so on clinical request (n = 51; 59%). Other criteria included: CSF white cell counts (WCC) plus clinical request (n = 22; 26%), blanket testing (n = 8; 9%), and not known to respondent (n = 5; 6%). The TAT varied for CSF viral NAAT samples and is summarised in Table 1. The majority of laboratories (46 of 47) with onsite viral CSF NAAT facilities reported a sample processing time of ≤ 48 h, P < 0.00001. Four centers with onsite microbiology laboratories sent CSF samples to neighboring hospitals for more comprehensive NAAT targets as they offered limited facilities for viral PCR testing (only for enterovirus) performed through POC testing.

Onsite laboratories used a variety of assay kits to perform viral NAAT including BioFire® (n = 22), in-house kits (n = 8), various Multiplex PCR kits (n = 6), LightCycler® (n = 1), Altona diagnostics (n = 1), AusDiagnostics® (n = 3), EliTech® (n = 2), M2000 (n = 1) and kits not specified (n = 3). Most of the kits covered 4 common viruses: Enterovirus, Human parechovirus, Herpes simplex virus (HSV) 1 and 2. There were facilities for testing additional viruses such as Varicella zoster virus, Cytomegalovirus, Adenovirus, Human Herpes Virus-6, Epstein-Barr virus, which varied depending on the kit used.

The COVID-19 pandemic had minor effects on the turnaround time for viral CSF NAAT results for laboratories performing onsite tests (n = 4; 9%), primarily due to the sharing of PCR/NAAT machines for COVID-19 (severe acute respiratory syndrome coronavirus 2) analysis, as well as shortages of staff and/or manufacturer delays.

DISCUSSION

The diagnosis of pediatric meningitis can be fraught with difficulty, especially in neonates and infants. Although there are several suggestive clinical signs, there is no diagnostic isolated single finding or combination of features[7]. Clinical suspicion, with cytological and microscopic analysis of CSF samples are the mainstay of diagnosis; antibiotic treatment is often started empirically while these results are awaited.

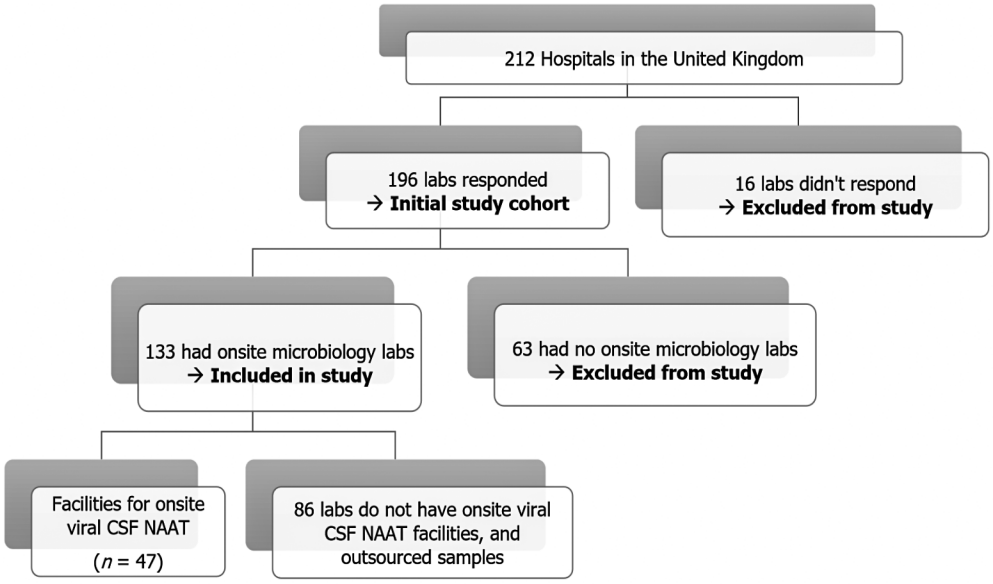
Enterovirus and Human Herpes Virus-6 (HHV-6) are the main pathogens causing viral meningitis in older neonates, infants and children. They usually have a favourable outcome, though neurological impairment has been observed, particularly following certain enterovirus strains such as D68 or human parechovirus[2]. HSV 1 and 2 infections typically cause severe encephalitis with serious sequelae if treatment with antivirals is delayed; evidence of these infections should be confirmed by NAAT as soon as possible.

The use of NAAT in the diagnosis of viral meningitis has been demonstrated to result in briefer parenteral antibiotic courses[5,8]. A positive CSF enterovirus result has also been associated with shorter lengths of hospital stay in infants with viral meningitis[5,9,10]. Most experts recommend that CSF PCR results for HHV-6 (due to potential for reactivation) should be interpreted with caution in the absence of readily attributable symptoms. A recent study reported that following detection of HHV-6 in 25 of 1005 children, five were subsequently diagnosed with either HHV-6 meningitis or meningoencephalitis based on HHV-6 detection in CSF, clinical presentation, and radiographic findings. These results led to early discontinuation of empirical acyclovir treatment in 12 children and appropriate initiation of ganciclovir therapy in 4 as a result of faster establishment of microbiological diagnosis[11]. NAAT remains an underutilised investigation: One observational study of 323 patients with a negative

Table 1 Turnaround times based on the presence of onsite laboratory nucleic acid amplification testing facilities

TAT (in hours)	Laboratories with onsite viral NAAT facilities (*n* = 47)	Laboratories without onsite viral NAAT facilities (*n* = 86)	*P* value
< 12	21	4	< 0.00001
12-24	18	1	< 0.00001
24-48	7	40	< 0.00001
48-72	0	23	NC
> 72	0	15	NC
Variable	1	3	NC

NC: Not calculated; NAAT: Nucleic acid amplification testing; TAT: Turnaround time.



DOI: 10.5495/wjcp .v11i3.289

Figure 1 Microbiology laboratories offering cerebrospinal fluid nucleic acid amplification testing. CSF: Cerebrospinal fluid; labs: Laboratories; NAAT: Nucleic acid amplification testing.

CSF gram stain reported that although PCR had the highest diagnostic yield it was only requested for 39.6% of patients[12].

The overwhelming majority of laboratories with onsite NAAT facilities (n = 47) reported a sample TAT of ≤ 24 h in 39/47 (89%), as compared to only 5/86 (6%) for samples sent elsewhere (P < 0.00001). The impact of transit times meant that only 52% (n = 45) of microbiology laboratories without NAAT facilities had a TAT of ≤ 48 h. Hopefully, as technology develops, turnaround times should improve, especially if POC tests become increasingly available. This has already been demonstrated as feasible for viral respiratory swab testing during the COVID-19 pandemic. A Canadian study using a model-based analysis of a retrospective cohort of all hospitalised children admitted with suspected enterovirus meningitis between November 2013 and 2017 demonstrated that same-day TAT of CSF enterovirus PCR was associated with a cost reduction of 342.83 Canadian dollar *per* patient in comparison to specimens sent to a reference laboratory. Further benefits such as decreased length of stay (LOS) and antibiotic therapy were also noted[13]. A retrospective study from the United States with 363 children who had HSV PCR tested on CSF samples demonstrated that the median duration of acyclovir therapy was significantly reduced in the group following implementation of a direct sample-to-answer assay technique (leading to faster TAT) as compared to laboratory-developed real-time PCR assay used in pre-implementation group [14.3 h *vs* 29.2 h (P < 0.01)] and marginal reduction in median LOS [4 d *vs* 5 d (P 0.23)][14].

There was also a wide variation in the acceptance criteria for performing NAAT analysis in the 133 centers with an onsite microbiology laboratory; the most popular approaches being based on clinician request (66/133, 50%) or a combination of CSF WCC with clinician request (35/133, 26%). Two recently published studies from the United Kingdom have suggested performing viral PCR testing of all CSF samples in infants, irrespective of their adjusted CSF WCC, has potential to reduce length of hospital

stay and antibiotic usage[5,9].

The COVID-19 pandemic had minimal effect on TAT with delay in sample analysis reported in 6% centers who had onsite testing facilities. Within the context of pediatrics, the cumulative effect of these delays can be lengthier hospital admissions, prolonged courses of parenteral antibiotics and diagnostic uncertainty.

CONCLUSION

Despite the widely documented benefits of using NAAT technology to aid the diagnosis and management of pediatric meningitis, onsite testing facilities for viral NAAT are limited in the United Kingdom. The lack of available NAAT facilities may have significant implications on patient outcomes, including increased LOS and duration of parenteral antibiotics. Early discontinuation of antibiotics in cases of viral meningitis should lead to improved antibiotic stewardship. Our study underlines the need for a national consensus on the role of PCR testing and emphasises the desirability of onsite PCR testing equipment for microbiology laboratories in the United Kingdom and elsewhere in the world.

ARTICLE HIGHLIGHTS

Research background

Viral pathogens are considered the major cause for meningitis worldwide. The use of nucleic acid amplification testing (NAAT), predominantly through polymerase chain reaction (PCR) in the diagnosis of meningitis has been demonstrated to result in faster turnaround times, shorter length of stay and briefer course of parenteral antibiotics.

Research motivation

NAAT remains an underutilized investigation and it is important to develop a national consensus on the role of PCR testing for diagnosing viral meningitis in children.

Research objectives

The aim of this study was to evaluate the use and availability of viral NAAT testing of cerebrospinal fluid (CSF) in microbiology laboratories across the United Kingdom.

Research methods

Structured telephone questionnaire survey was conducted to understand the availability of viral CSF NAAT in the United Kingdom with emphasis on the criteria used for testing and turnaround times including the impact of the coronavirus disease 2019 pandemic.

Research results

Onsite facilities for viral CSF NAAT was available in 35% centres with much faster turnaround times of ≤ 24 h as compared to those outsourcing to neighboring laboratories.

Research conclusions

Onsite/near-patient rapid NAAT [including polymerase chain reaction (PCR)] is recommended wherever possible to optimize patient management in the acute setting.

Research perspectives

Our study underlines the need for a national consensus on the role of NAAT and emphasizes the need for on-site PCR testing equipment for microbiology laboratories in the United Kingdom.

FOOTNOTES

Author contributions: Paul SP contributed to the Project concept, formulation of questionnaire survey, supervision, data analysis, manuscript revision, literature review and correspondence; Balakumar V, Kirubakaran A and Niharika J conducted interviews, data collection and analysis, prepared first draft; Heaton PA and Turner PC provided expert opinion, helped with formulation of questionnaire survey, edited manuscript.

Institutional review board statement: This is a national questionnaire survey and ethical approval wasn't considered necessary.

Conflict-of-interest statement: None for any of the authors.

Data sharing statement: All data has been included in the paper.

Country/Territory of origin: United Kingdom

ORCID number: Siba Prosad Paul 0000-0002-5263-1109; Varathagini Balakumar 0000-0001-9413-6838; Arangan Kirubakaran 0000-0001-8466-3642; Jothilingam Niharika 0000-0002-1010-0385; Paul Anthony Heaton 0000-0002-7948-1737; Paul Christopher Turner 0000-0002-4595-548X.

S-Editor: Fan JR
L-Editor: A
P-Editor: Fan JR

REFERENCES

1 **GBD 2016 Meningitis Collaborators**. Global, regional, and national burden of meningitis, 1990-2016: a systematic analysis for the Global Burden of Disease Study 2016. *Lancet Neurol* 2018; **17**: 1061-1082 [PMID: 30507391 DOI: 10.1016/S1474-4422(18)30387-9]
2 **Hudson JA**, Broad J, Martin NG, Sadarangani M, Galal U, Kelly DF, Pollard AJ, Kadambari S. Outcomes beyond hospital discharge in infants and children with viral meningitis: A systematic review. *Rev Med Virol* 2020; **30**: e2083 [PMID: 31524309 DOI: 10.1002/rmv.2083]
3 **Martin NG**, Sadarangani M, Pollard AJ, Goldacre MJ. Hospital admission rates for meningitis and septicaemia caused by Haemophilus influenzae, Neisseria meningitidis, and Streptococcus pneumoniae in children in England over five decades: a population-based observational study. *Lancet Infect Dis* 2014; **14**: 397-405 [PMID: 24631222 DOI: 10.1016/S1473-3099(14)70027-1]
4 **National Institute for Health and Care Excellence (NICE)**. Meningitis (bacterial) and meningococcal septicaemia in under 16s: recognition, diagnosis and management. [cited 15 May 2021]. Available from: https://www.nice.org.uk/guidance/cg102
5 **Turner PC**, Brayley J, Downing HC, Homfray GJ, Doolan G, Paul SP. Screening for enteroviral meningitis in infants and children-Is it useful in clinical practice? *J Med Virol* 2019; **91**: 1882-1886 [PMID: 31180138 DOI: 10.1002/jmv.25512]
6 **Sanaei Dashti A**, Alizadeh S, Karimi A, Khalifeh M, Shoja SA. Diagnostic value of lactate, procalcitonin, ferritin, serum-C-reactive protein, and other biomarkers in bacterial and viral meningitis: A cross-sectional study. *Medicine (Baltimore)* 2017; **96**: e7637 [PMID: 28858084 DOI: 10.1097/MD.0000000000007637]
7 **Curtis S**, Stobart K, Vandermeer B, Simel DL, Klassen T. Clinical features suggestive of meningitis in children: a systematic review of prospective data. *Pediatrics* 2010; **126**: 952-960 [PMID: 20974781 DOI: 10.1542/peds.2010-0277]
8 **Lyons TW**, McAdam AJ, Cohn KA, Monuteaux MC, Nigrovic LE. Impact of in-hospital enteroviral polymerase chain reaction testing on the clinical management of children with meningitis. *J Hosp Med* 2012; **7**: 517-520 [PMID: 22592976 DOI: 10.1002/jhm.1947]
9 **Chakrabarti P**, Warren C, Vincent L, Kumar Y. Outcome of routine cerebrospinal fluid screening for enterovirus and human parechovirus infection among infants with sepsis-like illness or meningitis in Cornwall, UK. *Eur J Pediatr* 2018; **177**: 1523-1529 [PMID: 30022279 DOI: 10.1007/s00431-018-3209-8]
10 **Dewan M**, Zorc JJ, Hodinka RL, Shah SS. Cerebrospinal fluid enterovirus testing in infants 56 days or younger. *Arch Pediatr Adolesc Med* 2010; **164**: 824-830 [PMID: 20819964 DOI: 10.1001/archpediatrics.2010.153]
11 **Pandey U**, Greninger AL, Levin GR, Jerome KR, Anand VC, Dien Bard J. Pathogen or Bystander: Clinical Significance of Detecting Human Herpesvirus 6 in Pediatric Cerebrospinal Fluid. *J Clin Microbiol* 2020; **58** [PMID: 32102858 DOI: 10.1128/JCM.00313-20]
12 **Nesher L**, Hadi CM, Salazar L, Wootton SH, Garey KW, Lasco T, Luce AM, Hasbun R. Epidemiology of meningitis with a negative CSF Gram stain: under-utilization of available diagnostic tests. *Epidemiol Infect* 2016; **144**: 189-197 [PMID: 25989841 DOI: 10.1017/S0950268815000850]
13 **Alghounaim M**, Caya C, Cho M, Beltempo M, Yansouni CP, Dendukuri N, Papenburg J. Impact of decreasing cerebrospinal fluid enterovirus PCR turnaround time on costs and management of children with suspected enterovirus meningitis. *Eur J Clin Microbiol Infect Dis* 2020; **39**: 945-954 [PMID: 31933018 DOI: 10.1007/s10096-019-03799-2]
14 **Van TT**, Mongkolrattanothai K, Arevalo M, Lustestica M, Dien Bard J. Impact of a Rapid Herpes Simplex Virus PCR Assay on Duration of Acyclovir Therapy. *J Clin Microbiol* 2017; **55**: 1557-1565 [PMID: 28275080 DOI: 10.1128/JCM.02559-16]

World Journal of Clinical Pediatrics

Submit a Manuscript: https://www.f6publishing.com

World J Clin Pediatr 2022 May 9; 11(3): 295-306

DOI: 10.5409/wjcp.v11.i3.295

ISSN 2219-2808 (online)

ORIGINAL ARTICLE

Observational Study

Serologic, endoscopic and pathologic findings in pediatric celiac disease: A single center experience in a low/middle income country

Hala H Mansour, Nabil A Mohsen, Mortada HF El-Shabrawi, Somia M Awad, Dalia Abd El-Kareem

Specialty type: Pediatrics

Provenance and peer review: Invited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): 0
Grade B (Very good): 0
Grade C (Good): C, C
Grade D (Fair): D
Grade E (Poor): 0

P-Reviewer: Han JM, China; Ma L, China

Received: October 11, 2021
Peer-review started: October 11, 2021
First decision: November 7, 2021
Revised: November 25, 2021
Accepted: April 2, 2022
Article in press: April 2, 2022
Published online: May 9, 2022

Hala H Mansour, Nabil A Mohsen, Mortada HF El-Shabrawi, Somia M Awad, Department of Pediatrics, Faculty of Medicine Kasr Al Ainy, Cairo University, Cairo 12411, Egypt

Dalia Abd El-Kareem, Department of Pathology, Cairo University, Cairo 11562, Egypt

Corresponding author: Mortada HF El-Shabrawi, MD, Professor, Department of Pediatrics, Faculty of Medicine Kasr Al Ainy, Cairo University, 3 Nablos Street, Off Shehab Street, Mohandesseen, Cairo 12411, Egypt. melshabrawi@medicine.cu.edu.eg

Abstract

BACKGROUND

Studies in Africa, Asia, and Latin America are needed to provide a comprehensive picture of the global incidence of celiac disease (CD).

AIM

To describe the serology, endoscopic and histological findings in typical and atypical presentations of pediatric CD at a tertiary referral hospital in an African low/middle income country (LMIC).

METHODS

This observational study was conducted on 199 patients with CD from 2010 to 2019. The patients were divided into typical and atypical groups according to the presenting symptoms including 120 and 79 patients respectively. Serology, upper gastrointestinal endoscopy with duodenal biopsy were performed for patients who had symptoms suggestive of CD. The severity of the intestinal damage was graded according to the histo-pathologic Marsh-Oberhuber classification.

RESULTS

Chronic diarrhea was the main intestinal presentation in the typical group. Anemia was the most common extraintestinal symptom in both the typical and atypical group. Marsh-Oberhuber type 3b and 3c was significantly higher in the seropositive patients with a P value of 0.007. A significant correlation was observed between the histological grade of the biopsied duodenal mucosa and the clinical presentation ($P < 0.001$). Age was significantly higher in the atypical group (P value < 0.001).

CONCLUSION

Although typical CD was observed in 120 patients in this study, the clinical

variability of the condition was frequently observed. Age only was a significant predictor for the appearance of atypical CD. Therefore, CD presentations in LMIC are not different from industrialized countries.

Key Words: Typical; Atypical celiac disease; Celiac serology; Marsh-Oberhuber histopathology

Core Tip: This study included 199 patients diagnosed with celiac disease (CD) over a 10-year period from 2010 to 2019 and was conducted at our tertiary hospital. Age, sex, clinical presentation, serological tests, and endoscopic findings were evaluated. We used the Marsh-Oberhuber classification to define the histopathological findings of the duodenal biopsies. The histopathological evaluation of intestinal biopsies revealed a statistically significant correlation between the histological grade of biopsied duodenal mucosa and the clinical presentation ($P < 0.001$). Those typical and atypical CD are not different from industrialized countries regarding age, clinical presentations, serology and pathology.

Citation: Mansour HH, Mohsen NA, El-Shabrawi MH, Awad SM, Abd El-Kareem D. Serologic, endoscopic and pathologic findings in pediatric celiac disease: A single center experience in a low/middle income country. *World J Clin Pediatr* 2022; 11(3): 295-306
URL: https://www.wjgnet.com/2219-2808/full/v11/i3/295.htm
DOI: https://dx.doi.org/10.5409/wjcp.v11.i3.295

INTRODUCTION

Celiac disease (CD) is an immune-mediated enteropathy caused by permanent sensitivity to gluten in genetically susceptible individuals. CD patients sustain an autoimmune reaction to the gliadin protein fraction of gluten[1]. The incidence of CD has been rising significantly in the second half of the 20th century and into the 21st century throughout the Western industrialized world. Population-based studies in Africa, Asia, and Latin America are needed to provide a comprehensive picture of the global incidence of CD[2].

CD is one of the most common causes of chronic malabsorption. This results from the injury to the small intestine with loss of absorptive surface area, reduction of digestive enzymes, and consequential impaired absorption of micronutrients, such as fat-soluble vitamins, iron, and potentially B_{12} and folic acid. Moreover, the inflammation exacerbates the symptoms of malabsorption by causing a net secretion of fluid that can result in diarrhea[3]. The clinical features of CD vary considerably. Typical or classic CD patients have predominant gastrointestinal manifestations, such as chronic diarrhea, abdominal distension, and failure to thrive. Typical CD is common in children diagnosed within the first 2 years of life. Many cases of CD present with predominant non-GI signs and symptoms, such as anemia, short stature, aphthous stomatitis, recurrent abdominal pain, pica, delayed puberty, osteopenia, and dental enamel hypoplasia and are called atypical or non-classic CD. The most common atypical presentations of CD are iron deficiency anemia unresponsive to iron therapy and short stature[4].

CD should be considered in the diagnosis of patients with an appropriate clinical history and patients from high-risk populations. Serological tests are used as initial tests for CD, and duodenal biopsies obtained during esophago-gastroduo-denoscopy (EGD) are considered the standard for diagnosis[5]. Endoscopy reveals grossly visible abnormalities in the proximal portion of the small intestine (scalloping of duodenal folds, mosaic mucosal pattern, and mucosal atrophy). The diagnosis is confirmed *via* histologic evaluation as per the Marsh-Oberhuber classification[6]. However, many recent studies have shown that the ingestion of uncontaminated oats is not only safe but can also improve the quality of the diet in most patients with CD or dermatitis herpetiformis. Other natural foods, such as vegetables, salads, pulses, fruits, nuts, meat, fish, poultry, cheese, eggs, and milk, can be consumed in a gluten free diet (GFD) without limitations[7].

This observational study aims to compare the serological, gastrointestinal endoscopic, and histopathologic findings in typical and atypical presentations of pediatric CD at a tertiary referral hospital in the capital city of Egypt; an African low/middle income country (LMIC). We also aimed to find whether these findings are different from presentations in industrialized countries.

MATERIALS AND METHODS

This hospital-based, cross-sectional observational study was conducted at Cairo University Children Hospital which is the largest pediatric tertiary hospital in the capital city of Egypt and one of the largest in the Middle East and North Africa (MENA) region. Data of the patients diagnosed with CD was collected in the period from January 2010 to December 2019. The study included 199 patients diagnosed with celiac disease; they were divided into two groups based upon the presenting symptoms: typical or classic CD (Group A) and atypical or non-classic CD patients (Group B). Patients with predominant GI features, such as chronic diarrhea, abdominal distension, and failure to thrive, were included in the typical CD group, whereas the patients with atypical intestinal or extraintestinal symptoms were included in the atypical CD group. The age of patients ranged from less than 1 year up to 18 years. Patients that were within the age group but on gluten-free diet, those within the age group without a history of gluten introduction before 6 mo, and those with other GIT pathology, such as inflammatory bowel disease (IBD) (*e.g.*, Crohn's disease) were excluded from the study. Detailed history was taken from each patient and/or care-takers with a special focus on the age of disease onset, history of diarrhea or constipation, abdominal distension, weight loss, anemia, bone pain, and neurological symptoms. Anthropometric measurements (height, weight and head circumference), and full systemic examination were performed.

Investigations of all patients included complete blood cell count, measurement and serum calcium and celiac serology (total immunoglobulin A, anti-tissue transglutaminase (anti-tTG) antibody IgA). EGD and duodenal biopsy were also performed. For the endoscopy to be accurate, the patients should have been on a gluten-containing diet. The patients were asked to fast (no food or drink) for 6-8 h before endoscopy. The amount and type of sedation are dependent on the patient's age, weight, and coexisting medical conditions. In the GI endoscopy laboratory of our tertiary hospital, UGI endoscopy was performed using pediatric-size flexible gastro-duodenoscopes with compatible biopsy forceps (standard gastroscope manufactured by KARL STORZ group (Tuttlingen, Germany). Four biopsies were obtained from the second and third part of the duodenum and one from the duodenal bulb. The endoscopic duodenal specimens were processed as formalin-fixed specimens embedded in paraffin blocks. The sections were cut with a thickness of 5 microns and stained with Hematoxylin and Eosin. The severity of the intestinal damage was graded by the pathologist as per the Marsh-Oberhuber classification[8]. After endoscopy was performed, the patient was transferred to a recovery room until any medication or sedation wears off. Most of the children were able to resume eating food within a few hours, after they fully recovered.

Diagnostic criteria for celiac disease patients in this study include (at least 4 of 5 or 3 of 4 if the HLA genotype is not performed)[9]

Typical symptoms of celiac disease: Chronic diarrhea, growth faltering and iron deficiency anemia.

Positivity of serum celiac disease IgA class autoantibodies at high titer: Both IgA class anti-tTG and endomyseal antibody (EMA) in IgA-sufficient subjects or IgG class anti-tTG and EMA in IgA deficient subjects. The finding of IgG class anti-deamidated gliadin peptide adds evidence to the diagnosis.

HLA-DQ2 or DQ8 genotypes: HLA-DQ2 positivity includes subjects with only half the heterodimer (HLA-DQB1*02 positive).

Celiac enteropathy at the small intestinal biopsy: Including Marsh-Oberhuber 3 Lesions, Marsh-Oberhuber 1-2 Lesions associated with positive celiac antibodies positive at low/high titer, or Marsh-Oberhuber 1-3 Lesions associated with IgA subepithelial deposits.

Response to the GFD: Data was statistically described in terms of mean ± SD, or frequencies (number of cases) and percentages when appropriate. Comparison of age between the study groups was done using Student *t* test for independent samples. For comparing categorical data, Chi-square (χ^2) test was performed. Exact test was used instead when the expected frequency is less than 5. Two-sided *P* values less than 0.05 was considered statistically significant. All statistical calculations were done using computer program IBM SPSS (Statistical Package for the Social Science; IBM Corp, Armonk, NY, United States) release 22 for Microsoft Windows.

RESULTS

This observational study enrolled 199 patients diagnosed as CD according to clinical presentations, serology and histopathology at our tertiary referral hospital. Data of the patients diagnosed with CD was collected in the period from January 2010 to December 2019. They were further divided into two groups: typical CD and atypical CD groups. The typical group (Group A) included 120 cases of CD patients who had typical intestinal symptoms, whereas the atypical group (Group B) included 79 cases of celiac disease patients who had atypical intestinal or extraintestinal symptoms. The mean age was

3.74 ± 2.93 years in Group A (typical) and 5.74 ± 4.0 years in Group B (atypical), being significantly higher in the atypical group (*P* value < 0.001).

Overall, 51.7% (*n* = 62) males and 48.3% (*n* = 58) females presented with typical symptoms (Group A), whereas 51.9% (*n* = 41) males and 48.1% (*n* = 38) females presented with atypical symptoms (Group B) without statistically significant difference (*P* value was 0.9).

Chronic diarrhea (increased frequency and/or fluidity of the stool for more than 4 wk) was the main intestinal presentation in the typical group (99.2%) (*n* = 119) than in the atypical group (1.3%) (*n* = 1), whereas constipation (infrequent passage of stool or passage of hard stool) was more common in the atypical group (16.5%) (*n* = 13) than in the typical group (5.0%) (*n* = 6). Overall, 69.2% (*n* = 83) of the patients in the typical group and 41.8% (*n* = 33) in the atypical group presented with abdominal distention. Such symptoms were statistically significant as shown in Table 1.

Anemia (low hemoglobin level for age and sex) was the most common extraintestinal symptom in both the typical and atypical group (99.5%) (*n* = 198). There were 99.2% (*n* = 119) of the typical group cases discovered during patient follow-up and 100% (*n* = 79) of the atypical group cases presented with anemia. The type of anemia was determined by mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH). The most common type of anemia was microcytic hypochromic anemia, detected in 93.3% (*n* = 111/119) of the patients in the typical group and 93.7% (*n* = 74/79) in the atypical group. Normocytic normochromic anemia was detected in 5.9% (*n* = 7) of the patients in the typical group and 5.1% (*n* = 4) in the atypical group. Four cases of normocytic normochromic anemia were diagnosed as glucose-6-phpsphate dehydrogenase (G6PD) deficiency, two of them in the typical group and two in the atypical group. Macrocytic anemia was detected in two patients, one was typical and the other was atypical (Table 2).

Hypocalcemia was reported in 70.8% (*n* = 85) of the typical group cases and 65.8% (*n* = 52) of the atypical group cases. Delayed puberty was detected in 75% (*n* = 9/12) of the typical group cases and detected in 73.1% (*n* = 19/26) of the atypical group cases. Dermatitis was detected in 12.5% (*n* = 15) of the typical group cases and 7.6% (*n* = 6) of the atypical group cases.

Failure to thrive was common in the typical group, whereas short stature was common in the atypical group. Failure to thrive (weight < 5 percentile) was found in 67.5% (*n* = 81) of the typical group cases and 62% (*n* = 49) of the atypical group cases. Short stature (height < 5 percentile) was found in 61.7% (*n* = 74) of the typical group cases and 65.8% (*n* = 52) of the atypical group cases as shown in Table 1.

The majority of our patients had normal to high total IgA level (84.9%) (*n* = 169/199): 81.7% (*n* = 98) of the typical group and 89.9% (*n* = 71) of the atypical group. Low level of total IgA was observed in 18.3% (*n* = 22) of the typical group cases and 10.1% (*n* = 8) of the atypical group cases. Anti-tTG IgA was positive in 48.3% (*n* = 58) of the typical group cases and 60.8% (*n* = 48) of the atypical group cases (Table 3).

Histopathological evaluation of the intestinal biopsies (Figure 1) revealed Marsh-Oberhuber type 0 in 8 patients (4.0%), Marsh-Oberhuber type 1 in 18 (9.0%), Marsh-Oberhuber type 3a in 53 (26.6%), Marsh-Oberhuber type 3b in 56 (28.1%), Marsh-Oberhuber type 3c in 57 (28.6%), and Marsh-Oberhuber type 3b-c in 7 (3.5%). Marsh-Oberhuber type 3a was more common in the atypical group, whereas Marsh-Oberhuber type 3c was more common in the typical group. Marsh-Oberhuber type 3a was diagnosed in 24 patients (20.0%) in the typical group and 29 (36.7%) in the atypical group. The intestinal biopsies revealed Marsh-Oberhuber type 3c in 47 patients (39.2%) of the typical group and only 10 (12.7%) of the atypical group. The intestinal biopsies revealed Marsh-Oberhuber type 3b in 31 patients (25.8%) of the typical group and 25 (31.6%) of the atypical group. Marsh-Oberhuber type 3b-c was found in four patients (3.3%) of the typical group and three (3.8%) of the atypical group. No cases were classified as Marsh-Oberhuber type 2. Six patients (5%) of the typical group and two (2.5%) of the atypical group were classified as Marsh-Oberhuber type 0, whereas eight patients (6.7%) of the typical group and ten (12.7%) of the atypical group were classified as Marsh-Oberhuber type 1 (Table 4).

Marsh-Oberhuber type 0 was found only in seropositive patients. Total and subtotal villous atrophy (VA) (Marsh-Oberhuber type 3b and 3c) were more common in seropositive patients. Overall, 54% of the seronegative patients (*n* = 34) had Marsh-Oberhuber type 3b and 3c, whereas 67% of the seropositive patients (*n* = 71) had Marsh-Oberhuber type 3b-c, being significantly higher in the seropositive patients (*P* value = 0.007) (Table 5).

Among the seronegative patients, no cases were classified as Marsh-Oberhuber type 0 or type 2 based on their histological findings. In the seronegative patients with typical symptoms, 4 patients (10%) had Marsh-Oberhuber type 1, whereas 13 patients (32.5%), 13 patients (32.5%), 9 patients (22.5%), and 1 patient (2.5%) had Marsh-Oberhuber type 3a, 3b, 3c, and 3b-c, respectively. In the seronegative patients with atypical symptoms, four patients (17.4%) had Marsh-Oberhuber type 1, whereas eight patients (34.8%), ten patients (43.5%), and one patient (4.3%) had Marsh-Oberhuber type 3a, 3b, and 3c, respectively (Table 6).

In seropositive patients with typical symptoms 27 patients (46.6%) had Marsh-Oberhuber type 3c, in seropositive patients with atypical symptoms 15 patients (31.3%) had Marsh-Oberhuber type 3b with *P* value = 0.022 (Table 7).

Logistic regression analysis for the predictors of atypical CD, including all statistically significant items including age, diarrhea, constipation, abdominal distention, Marsh-Oberhuber type 3a, and Marsh-Oberhuber type 3c) was conducted. Age only was significant with a *P* value = 0.013 (as age

Table 1 Clinical presentation of typical and atypical celiac disease

Clinical presentations	Typical, *n* = 120		Atypical, *n* = 79		*P* value
	n	%	*n*	%	
Male	62	51.7	41	51.9	0.974
Female	58	48.3	38	48.1	
Chronic diarrhea	119	99.2	1	1.3	< 0.001
Abdominal distention	83	69.2	33	41.8	< 0.001
Constipation	6	5	13	16.5	0.007
Weight loss	81	67.5	49	62	0.427
Anemia	119	99.2	79	100	0.931
Short stature	74	61.7	52	65.8	0.552
Hypocalcemia	85	70.8	52	65.8	0.455
Depression	0	0	2	2.5	0.08
Skin lesion	20	16.7	11	13.9	0.764

Table 2 Type of anemia in typical and atypical celiac disease

	Microcytic hypochromic		Normocytic normochromic		Macrocytic hypochromic		*P* value
	n	%	*n*	%	*n*	%	0.931
Typical	111	93.3	7	5.9	1	0.8	
Atypical	74	93.7	4	5.1	1	1.3	

Table 3 Serological finding in both the typical and atypical groups

	Typical, *n* = 120		Atypical, *n* = 79		*P* value
	n	%	*n*	%	
Total IgA deficient	22	18.3	8	10.1	0.074
Anti-tTG IgA-positive	58	48.3	48	60.8	0.086
Anti-tTG IgA-negative	40	33.3	23	29.1	0.315

Ig: Immunoglobulin; Anti-tTG: Anti-tissue transglutaminase.

progresses, the predictors of atypical CD will increase).

DISCUSSION

This study included 199 patients diagnosed with CD over a 10-year period from January 2010 to December 2019 and was conducted at our tertiary hospital. Age, sex, clinical presentation, serological tests, and endoscopic findings were evaluated. We used the Marsh-Oberhuber classification to define the histopathological findings of the duodenal biopsies.

The mean age was 3.74 ± 2.93 years in Group A (typical) and 5.74 ± 4.0 years in Group B (atypical), being significantly higher in the atypical CD group than in the typical CD group ($P < 0.001$). The study conducted by Semwal *et al*[4] reported a mean age of 4.83 ± 3.05 years in the typical group and 7.71 ± 3.46 years in the atypical group, being significantly higher in the atypical group ($P < 0.001$). Dinler *et al*[10] reported a mean age of 6.2 ± 4.4 years in the typical group and 11.5 ± 3.4 years in the atypical group, being significantly higher in the atypical group ($P < 0.001$). Moreover, Kuloğlu *et al*[11]. reported that the age of children with typical type (7.5 ± 4.3 years) was significantly lower than that of those with atypical type (10.8 ± 4.3 years) ($P = 0.001$).

Table 4 Histological findings in the typical and atypical celiac disease group

Marsh-Oberhuber classification	Typical, *n* = 120		Atypical, *n* = 79		*P* value
	n	%	*n*	%	
0	6	5	2	2.5	0.386
1	8	6.7	10	12.7	0.149
2	0	0	0	0	
3a	24	20	29	36.7	0.009
3b	31	25.8	25	31.6	0.372
3c	47	39.2	10	12.7	< 0.001
3b-c	4	3.3	3	3.8	0.862

Table 5 Histological findings in the seropositive (normal IgA level+ positive anti-tTG IgA) and seronegative (normal IgA level + negative anti-tTG IgA) celiac disease

Marsh-Oberhuber grade	CD seronegative, *n* = 63		CD seropositive, *n* = 106		*P* value
	n	%	*n*	%	0.007
0	0	0	7	6.6	
1	8	12.7	8	7.5	
2	0	0	0	0	
3a	21	33.3	20	18.9	
3b	23	36.5	29	27.4	
3c	10	15.9	36	34	
3b-c	1	1.6	6	5.7	

CD: Celiac disease.

Table 6 Histological findings in the seronegative typical and atypical celiac disease group

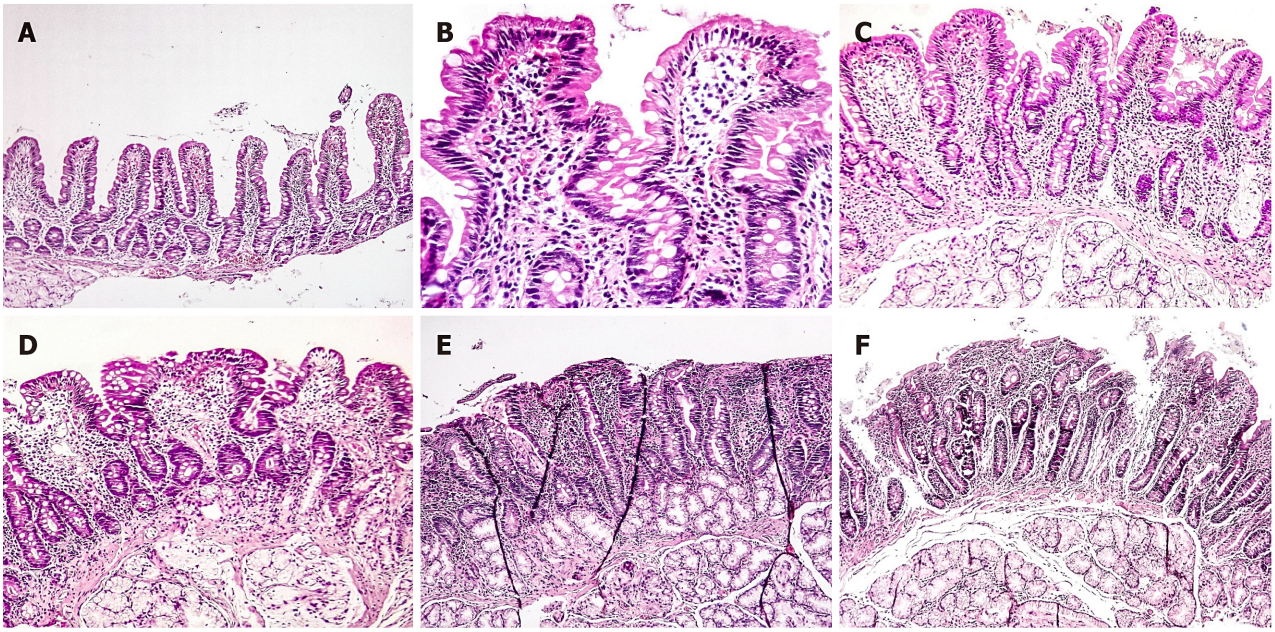
Marsh-Oberhuber classification	Typical, *n* = 40		Atypical, *n* = 23		*P* value
	n	%	N	%	0.315
0	0	0	0	0	
1	4	10	4	17.4	
2	0	0	0	0	
3a	13	32.5	8	34.8	
3b	13	32.5	10	43.5	
3c	9	22.5	1	4.3	
3b-c	1	2.5	0	0	

This difference is apparently due to the late diagnosis of the atypical cases because the typical presentations (diarrhea, abdominal distension) are usually noticed by the caretaker easily and therefore CD is diagnosed at an early age. Atypical presentations are diagnosed at a later age, due to the less awareness of the varied non-GI presentations of CD[4].

Of the studied cases, 51.7% (*n* = 103/199) were males and 48.2% (*n* = 96/199) were females, with a male/female ratio of 1.1:1. In the study conducted by Semwal *et al*[4], 48 males and 53 females had a male/female ratio of 1:1.1, whereas in the study of Dinler *et al*[10], 33 males and 54 females had a male/female ratio of 1:1.6. Kuloğlu *et al*[11] evaluated the features of 109 children with CD, in which the disease is known to be more frequent among females, with a male/female ratio of 1:1.5.

Table 7 Histological findings in the seropositive (106 patients) typical and atypical celiac disease group

Marsh-Oberhuber classification	Typical, *n* = 58		Atypical, *n* = 48		*P* value
	n	%	*n*	%	0.022
0	5	8.6	2	4.2	
1	3	5.2	5	10.4	
2	0	0	0	0	
3a	6	10.3	14	29.2	
3b	14	24.1	15	31.3	
3c	27	46.6	9	18.8	
3b-c	3	5.2	3	6.3	



DOI: 10.5492/wjcp.v11i3.295

Figure 1 Photomicrographs of duodenal mucosal biopsies. A: Preserved villi with increased intraepithelial lymphocytes (Marsh-Oberhuber type 1); B and C: Mild villous shortening and crypt hyperplasia with increased intraepithelial lymphocytes (Marsh-Oberhuber type 3a); D: Moderate villous atrophy and crypt hyperplasia with increased intraepithelial lymphocytes (Marsh-Oberhuber type 3b); E and F: Complete villous atrophy (Marsh-Oberhuber type 3c). Hematoxylin and eosin stained sections, original magnification ×40, ×200, ×100, ×100, ×100, ×100 respectively.

Anemia was the most common symptom (99.5%) (*n* = 198/199), followed by failure to thrive (65.3%) (*n* = 130/199), short stature (63.3%) (*n* = 126/199), chronic diarrhea (60.3%) (*n* = 120/199), and abdominal distention (58.3%) (*n* = 116/199).

In the study of Semwal *et al*[4], the most common symptom was anemia (54.5%) (*n* = 55/101) followed by chronic diarrhea (51.5%) (52/101) and short stature (50.5%) (51/101); in that of Dinler *et al*[10] chronic diarrhea (60.9%) (*n* = 53/87) followed by failure to thrive (49.4%) (*n* = 43/87), abdominal distention (41.3%) (*n* = 36/87), and short stature (33.3%) (*n* = 29/87); and in that of Kuloğlu *et al*[11], diarrhea (53.2%) (*n* = 58/109) followed by failure to thrive (45.9%) (*n* = 50/109), short stature (42.2%) (*n* = 46/109), and abdominal distention (26.6%) (*n* = 44/109).

Chronic diarrhea (*n* = 119/120) and abdominal distension (*n* = 83/120) were the most common presentations in the typical CD cases, whereas anemia (*n* = 79/79) and short stature (*n* = 52/79) were the most common presentations in the atypical CD cases. In the study of Semwal *et al*[4], chronic diarrhea (*n* = 52/55) and abdominal distention (*n* = 32/55) were the common presentations in the typical CD cases, whereas anemia (*n* = 36/46) and short stature (*n* = 29/46) were the most common presentations in the atypical CD cases. In the study of Dinler *et al*[10], chronic diarrhea (*n* = 53/55) and abdominal distention (*n* = 36/55) were the common presentations in the typical CD cases, whereas anemia (*n* = 10/32) and short stature (*n* = 20/32) were the most common presentations in the atypical CD cases. Moreover, in the study of Kuloğlu *et al*[11], chronic diarrhea (*n* = 58/66) and abdominal distention (*n* = 29/66) were the common presentations in the typical CD cases, whereas anemia (*n* = 23/41) and short stature (*n* =

22/41) were the most common presentations in the atypical CD cases.

In this study, the other common atypical presentations were constipation (9.5%) (*n* = 19/199), recurrent aphthous ulcers (5%) (*n* = 10/199), and bone changes in the form of rickets (10%) (*n* = 20/199). In the study conducted by Semwal *et al*[4], the most common atypical presentations were constipation (6.9%) (*n* = 7/101), recurrent aphthous ulcers (3.9%) (*n* = 4/101), and bone changes in the form of rickets (3.9%) (*n* = 4/101). Moreover, in the study conducted by Dinler *et al*[10], the most common atypical presentations were constipation (3.4%) (*n* = 3/87) and recurrent aphthous ulcers (2.2%) (*n* = 2/87), and in the study conducted by Kuloğlu *et al*[11], constipation (2.7%) (*n* = 3/109) and recurrent aphthous ulcers (1.8%) (*n* = 2/109).

Delayed puberty (14%) (*n* = 28/199), dermatitis (10.6%) (*n* = 21/199), and depression (1%) (*n* = 2/199). In the study of Semwal *et al*[4], delayed puberty (2.0%) (*n* = 2/101), chronic urticaria (1.0%) (*n* = 1/101), and depression (1.0%) (*n* = 1/101) were observed; in that of Dinler *et al*[10], chronic urticaria (1.1%) (*n* = 1/87) and delayed puberty (2.2%) (*n* = 2/87); and in that of Kuloğlu *et al*[11], delayed puberty (5.5%) (*n* = 6/109).

Anemia in CD is primarily caused by iron deficiency but also by the lack of other nutritional factors necessary for normal erythropoiesis, such as folic acid, vitamin B12, proteins, and copper[11]. Anemia was the most common presenting feature in the present study and was multifactorial. Based on the laboratory evaluation, anemia was prevalent in 198/199 patients (99.5%), with the microcytic hypochromic type being the most common in 185/198 patients (93.4%). In the study conducted by Berry *et al*[12], anemia was the most common presenting feature in their study and was multifactorial. Based on the laboratory evaluation, anemia was prevalent in 96/103 patients (93.2%), with iron deficiency anemia (IDA) being the most common in 84/103 patients (81.5%). In the study conducted by Dinler *et al*[10], iron deficiency with low iron stores was the most common presentation of anemia in both groups; IDA was found in 48/85 patients (56.5%). In the study conducted by Kuloğlu *et al*[11], iron deficiency anemia was a frequent finding in CD patients. It is seen in the majority of patients with one or more other findings and can also be the single finding of the disease. IDA was found in 80/98 patients (81.6%). Normocytic normochromic anemia was found in 5.6% of the patients (11 patients), one of them diagnosed with autoimmune hemolytic anemia and 4 cases (2%) diagnosed with G6PD deficiency. In the study conducted by Hosnut *et al*[13], the association between G6PD deficiency and CD was coincidental. The gene frequency of enzyme deficiency was 0.70 in the Mediterranean region. The prevalence of G6PD deficiency was high, reaching up to 10% in some ethnic populations in Turkey. Since G6PD deficiency is common in their country, the presence of CD and G6PD deficiency in their patients would be expected[13].

The prevalence of vitamin B12 deficiency is variable in different studies and ranges from 8 to 41% of the patients[14,15]. In this study, macrocytic hypochromic anemia was found in 2/199 patients (1%). One of them was diagnosed with megaloblastic anemia (vitamin B12 deficiency) by bone marrow biopsy (BMB). In the study conducted by Dinler *et al*[10] and Kuloğlu *et al*[11], vitamin B12 deficiency was observed in 3.4% (3/87) and 5.5% (6/109) of the child patients with CD, respectively. In the study conducted by Berry *et al*[12], Wierdsma *et al*[16], and McGowan *et al*[17], vitamin B12 deficiency was observed in 2.9% (3/103), 11% (5/50), and 19% (15/80) of the adult patients with CD, respectively. The mechanism of vitamin B12 deficiency in CD remains unclear. Various postulated mechanisms include ileal VA, pancreatic insufficiency in CD, autoimmune gastritis, small intestinal bacterial overgrowth, and decreased efficiency of mixing with transfer factors in the small intestine[12].

In this study, IgA deficiency was observed in 15% (*n* = 30/199) of the cases. In the study conducted by Kuloğlu *et al*[11], IgA deficiency was detected in 9.1% (*n* = 10/109) of the cases. The prevalence of CD in patients with selective IgA deficiency ranges from 10% to 30%[18]. Moreover, 53.3% (*n* = 106/199) of the cases had positive anti-tTG IgA. In the study conducted by Wolf *et al*[19], 76.4% (*n* = 404/529) of the patients had positive anti-tTG IgA.

In this study, we found a higher but non-significant proportion of patients with typical CD symptoms among the IgA anti-tTG-seronegative patients (63.5%) (*n* = 40/63) compared with the atypical CD seronegative cases (36.5%) (*n* = 23/63). In the study conducted on adults by Sugai *et al*[20], the proportion of patients with typical CD symptoms among the IgA anti-tTG-seronegative patients was 26.3% (*n* = 5/19) compared with the atypical CD seronegative cases (68.4%) (*n* = 13/19).

Furthermore, the histopathological evaluation of intestinal biopsies revealed total or subtotal VA (Marsh-Oberhuber type 3b and 3c) in 82/120 patients (68.3%) in the typical group and 38/79 (48%) in the atypical group. Partial VA (Marsh-Oberhuber type 3a) was observed in 24/120 patients (20%) in the typical group and 29/79 (36.7%) in the atypical group. The presence of total VA in the intestinal biopsies was significantly higher in the typical group than that in the atypical group ($P < 0.001$). In the study conducted by Dinler *et al*[10], the histopathological evaluation of intestinal biopsies revealed total or subtotal VA in 40/55 patients (72.7%) in the typical group and 12/32 (37.5%) in the atypical group. Partial VA was observed in 15/55 patients (27.3%) in the typical group and 20/32 (62.5%) in the atypical group. The presence of total or subtotal VA in the intestinal biopsies was significantly higher in the typical group than that in the atypical group ($P < 0.02$). Moreover, *Boskovica et al*[21] reported that the histopathological evaluation of intestinal biopsies revealed total or subtotal VA in 44/66 patients (66.6%) in the typical group and 24/37 (64.8%) in the atypical group. Partial VA was observed in 5/66 patients (7.5%) in the typical group and 1/37 (2.7%) in the atypical group.

Total and subtotal VA (Marsh-Oberhuber type 3b and 3c) were more common in the seropositive patients. Overall, 34/63 patients (54%) had Marsh-Oberhuber type 3b and 3c in the seronegative patients, whereas 71/106 patients (67%) had Marsh-Oberhuber type 3b and 3c in the seropositive patients, being significantly higher in the seropositive patients with a P value = 0.007.

In the study conducted on children by Hawamdeh *et al*[22], 40/51 seropositive patients (78.4%) had Marsh-Oberhuber type 3, whereas 9/30 seronegative patients (30%) had Marsh-Oberhuber type 3. A significant association between anti-tTG IgA titer and Marsh-Oberhuber grading was observed (P value 0.000). In the study of Donaldson *et al*[23], 3/26 seronegative patients (11.5%) had Marsh-Oberhuber type 3b and 3c, whereas 31/56 seropositive patients (55.3%) had Marsh-Oberhuber type 3b and 3c. IgA anti-tTG was significantly correlated with the Marsh-Oberhuber grades (P value 0.001). In the study conducted by Boskovica *et al*[21], the levels of tTG antibody were correlated significantly with Marsh-Oberhuber types in the entire population ($P < 0.0001$) and separately for typical ($P < 0.001$) and atypical ($P < 0.0001$) groups. In the study conducted on adults by Sugai *et al*[20], 7/19 seronegative patients (36.8%) had Marsh-Oberhuber type 3b and 3c, whereas 33/45 seropositive patients (73.3%) had Marsh-Oberhuber type 3b and 3c. In the study of *Dore et al*[24], severe duodenal mucosal damage (Marsh-Oberhuber type 2-3) was observed less frequently in patients with seronegative CD (n = 28/48) than in those with seropositive CD (n = 66/85) (58% *vs* 78%, P = 0.019).

In this study, 8/199 patients (4%) had Marsh-Oberhuber 0 and positive serology (potential CD): 6/120 patients had typical symptoms (5%) and 2/79 had atypical symptoms (2.5%). In the study conducted by Hawamdeh *et al*[22], 15/81 patients (18.5%) had Marsh-Oberhuber 0 and positive serology and were considered potential CD patients. In the study conducted by Boskovica *et al*[21], there were 12/37 patients with positive serology and Marsh-Oberhuber 0 (potential CD) (32.4%) had atypical CD symptoms and 11/66 (16.6%) had typical CD symptoms. In the present study, the histopathological evaluation of intestinal biopsies revealed Marsh-Oberhuber type 1 in 18/199 patients (9%), type 3a in 53/199 (26.6%), type 3b in 56/199 (28.1%), type 3c in 57/199 (28.6%), and type 3b-c in 7/199 (3.5%). Typical CD was more likely to have a significantly higher Marsh-Oberhuber grade based on the histological findings ($P < 0.001$). In the study conducted by Semwal *et al*[4], the histopathological evaluation of intestinal biopsies revealed Marsh-Oberhuber type 2 in 2/101 patients (2%), type 3a in 45/101 (44.6%), type 3b in 34/101 (33.7%), and type 3c in 20/101 (19.8%). Typical CD was more likely to have a significantly higher Marsh-Oberhuber grade based on the histological findings ($P < 0.001$).

In this study, the histopathological evaluation of intestinal biopsies revealed a statistically significant correlation between the histological grade of biopsied duodenal mucosa and the clinical presentation ($P < 0.001$). Dinler *et al*[10] and Semwal *et al*[4] also found that in children, total/subtotal VA was significantly higher in the typical group than in the atypical group. On the other hand, Brar *et al*[25] found that the degree of VA in the duodenal biopsies did not correlate with the mode of presentation, which might be due to the differences in the age of the study population since we studied children, while Brar *et al*[25] studied adult CD patients. Those typical and atypical CD in LMIC are not different from industrialized countries regarding age, clinical presentations, serology and pathology.

Limitations of our study: We didn't collect data about family history (as we focus on histopathology and clinical finding) and vomiting (as vomiting was not a common presentation in our patients and this was reported in Abu-Zekry[26]. We didn't collect data for laboratory test as anemia (hemoglobin, MCV, MCH) were taken through interpretation of result according to age and sex.

CONCLUSION

In conclusion, CD presentations in LMIC are not different from industrialized countries and late diagnosis is more common in atypical cases.

ARTICLE HIGHLIGHTS

Research background

Celiac disease (CD) is defined as an immune mediated systemic disorder elicited by gluten and related prolamines in genetically susceptible individuals. CD is one of the most common causes of chronic malabsorption. CD results from injury to the small intestine with loss of absorptive surface area, reduction of digestive enzymes, and consequential impaired absorption of micronutrients such as fat-soluble vitamins, iron, and potentially B12 and folic acid. Celiac disease presented with chronic diarrhea, failure to thrive and abdominal distention usually observed within the first 1-2 years of life. At the older age, atypical features such as anemia, short stature, bone disease and liver failure may occur. It should be considered in patients with an appropriate clinical history as well as in patients from high-risk populations. Serological tests are used as initial tests for CD and duodenal biopsies obtained during esophagogastroduodenoscopy (EGD) are considered the standard for diagnosis. The diagnosis of CD is based on the identification of histological lesions accompanied by clinical and serological consistent

data. On the basis of the presence of one or more of these elementary lesions the histopathology of CD is subdivided into different diagnostic categories according to Marsh classification.

Research motivation

Many cases of Celiac disease in our country with different clinical presentations motivate us to search for different histopathological examination in the disease sub-types.

Research objectives

To compare the serological, gastrointestinal endoscopic, and histopathologic findings in typical and atypical presentations of pediatric CD at a tertiary referral hospital in the capital city of Egypt; an African low/middle income country. We also aimed to find whether these findings are different from presentations industrialized countries.

Research methods

A hospital-based, cross-sectional observational study was conducted at Cairo University Children Hospital which is the largest pediatric tertiary hospital in the capital city of Egypt and one of the largest in the Middle East and North Africa (MENA) region. Data of the patients diagnosed with CD was collected in the period from 2010 to 2019. The study included 199 patients diagnosed with celiac disease; they were divided into two groups based upon the presenting symptoms: typical or classic CD (Group A) and atypical or non-classic CD patients (Group B).

Research results

Typical CD is more common than atypical, chronic diarrhea was common in typical group with *P* value < 0.001. sero-positive cases were 106 (typical 58, atypical 48cases). The most common histological finding in typical seropositive cases were March types 3c (27/58, 46.6%), The most common histological finding in atypical seropositive cases were March types 3b (15/48, 31.3%) (*P* value 0.022).

Research conclusions

CD clinical presentations in low/middle income country are not different from industrialized countries and late diagnosis is more common in atypical cases.

Research perspectives

Follow up of the CD cases and their prognosis, if there is changes in histological picture in future.

ACKNOWLEDGEMENTS

We would like to thank the parents and children for their cooperation in this study.

FOOTNOTES

Author contributions: El-Shabrawi MH, Mohsen NA, Abd El-Kareem D and Mansour HH contributed to the conception of the study, designed and executed the study, and wrote the final manuscript; El-Shabrawi MH contributed to the writing of the manuscript draft; Awad SM collected data and contributed to the writing of the manuscript; all authors read and approved the final manuscript.

Institutional review board statement: The study was reviewed and approved by the research ethics committee of Cairo university Institutional Review Board [(Approval No. 12-10-2019]).

Informed consent statement: All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2008 (5). Informed consent was obtained from all patients for being included in the study.

Conflict-of-interest statement: There are no conflicts of interest to report.

Data sharing statement: No additional data are available.

STROBE statement: The authors have read the STROBE Statement—checklist of items, and the manuscript was prepared and revised according to the STROBE Statement—checklist of items.

Country/Territory of origin: Egypt

ORCID number: Hala H Mansour 0000-0002-6394-7694; Nabil A Mohsen 0000-0002-4371-0956; Mortada HF El-Shabrawi 0000-0002-1995-4213; Somia M Awad 0000-0002-5350-1197; Dalia Abd El-Kareem 0000-0001-7838-7194.

S-Editor: Wang LL
L-Editor: A
P-Editor: Wang LL

REFERENCES

1 **Ammoury RF**, Croffie JM. Malabsorptive disorders of childhood. *Pediatr Rev* 2010; **31**: 407-15; quiz 415 [PMID: 20889735 DOI: 10.1542/pir.31-10-407]
2 **King JA**, Jeong J, Underwood FE, Quan J, Panaccione N, Windsor JW, Coward S, deBruyn J, Ronksley PE, Shaheen AA, Quan H, Godley J, Veldhuyzen van Zanten S, Lebwohl B, Ng SC, Ludvigsson JF, Kaplan GG. Incidence of Celiac Disease Is Increasing Over Time: A Systematic Review and Meta-analysis. *Am J Gastroenterol* 2020; **115**: 507-525 [PMID: 32022718 DOI: 10.14309/ajg.0000000000000523]
3 **Garg K**, Gupta RK. What a practitioner needs to know about celiac disease? *Indian J Pediatr* 2015; **82**: 145-151 [PMID: 25172576 DOI: 10.1007/s12098-014-1544-y]
4 **Semwal P**, Gupta RK, Sharma R, Garg K. Comparison of Endoscopic and Histological Findings between Typical and Atypical Celiac Disease in Children. *Pediatr Gastroenterol Hepatol Nutr* 2018; **21**: 86-92 [PMID: 29713605 DOI: 10.5223/pghn.2018.21.2.86]
5 **Kasirer Y**, Turner D, Lerman L, Schechter A, Waxman J, Dayan B, Bergwerk A, Rachman Y, Freier Z, Silbermintz A. Scalloping is a reliable endoscopic marker for celiac disease. *Dig Endosc* 2014; **26**: 232-235 [PMID: 23746050 DOI: 10.1111/den.12130]
6 **Schuppan D**, Zimmer KP. The diagnosis and treatment of celiac disease. *Dtsch Arztebl Int* 2013; **110**: 835-846 [PMID: 24355936 DOI: 10.3238/arztebl.2013.0835]
7 **Lionetti E**, Catassi C. New clues in celiac disease epidemiology, pathogenesis, clinical manifestations, and treatment. *Int Rev Immunol* 2011; **30**: 219-231 [PMID: 21787227 DOI: 10.3109/08830185.2011.602443]
8 **Oberhuber G**, Granditsch G, Vogelsang H. The histopathology of coeliac disease: time for a standardized report scheme for pathologists. *Eur J Gastroenterol Hepatol* 1999; **11**: 1185-1194 [PMID: 10524652 DOI: 10.1097/00042737-199910000-00019]
9 **Catassi C**, Fasano A. Celiac disease diagnosis: simple rules are better than complicated algorithms. *Am J Med* 2010; **123**: 691-693 [PMID: 20670718 DOI: 10.1016/j.amjmed.2010.02.019]
10 **Dinler G**, Atalay E and Kalayci AG. Celiac disease in 87 children with typical and atypical symptoms in Black Sea region of Turkey. *World J Pediatr* 2009; **5**: 282-286 [PMID: 19911143 DOI: 10.1007/s12519-009-0053-y]
11 **Kuloğlu Z**, Kirsaçlioğlu CT, Kansu A, Ensari A, Girgin N. Celiac disease: presentation of 109 children. *Yonsei Med J* 2009; **50**: 617-623 [PMID: 19881963 DOI: 10.3349/ymj.2009.50.5.617]
12 **Berry N**, Basha J, Varma N, Varma S, Prasad KK, Vaiphei K, Dhaka N, Sinha SK, Kochhar R. Anemia in celiac disease is multifactorial in etiology: A prospective study from India. *JGH Open* 2018; **2**: 196-200 [PMID: 30483589 DOI: 10.1002/jgh3.12073]
13 **Hosnut FO**, Canan O, Özcay F, Özbek N. Awareness of glucose-6 phosphate-dehydrogenase deficiency in celiac disease. *Acta Paediatr* 2010; **99**: 786-788 [PMID: 20064138 DOI: 10.1111/j.1651]
14 **Dahele A**, Ghosh S. Vitamin B12 deficiency in untreated celiac disease. *Am J Gastroenterol* 2001; **96**: 745-750 [PMID: 11280545 DOI: 10.1111/j.1572-0241.2001.03616.x]
15 **Dickey W**. Low serum vitamin B12 is common in coeliac disease and is not due to autoimmune gastritis. *Eur J Gastroenterol Hepatol* 2002; **14**: 425-427 [PMID: 11943958 DOI: 10.1097/00042737-200204000-00016]
16 **Bodé S**, Gudmand-Høyer E. Symptoms and haematologic features in consecutive adult coeliac patients. *Scand J Gastroenterol* 1996; **31**: 54-60 [PMID: 8927941 DOI: 10.3109/00365529609031627]
17 **Wierdsma NJ**, van Bokhorst-de van der Schueren MA, Berkenpas M, Mulder CJ, van Bodegraven AA. Vitamin and mineral deficiencies are highly prevalent in newly diagnosed celiac disease patients. *Nutrients* 2013; **5**: 3975-3992 [PMID: 24084055 DOI: 10.3390/nu5103975]
18 **McGowan KE**, Lyon ME, Butzner JD. Celiac disease and IgA deficiency: complications of serological testing approaches encountered in the clinic. *Clin Chem* 2008; **54**: 1203-1209 [PMID: 18487281 DOI: 10.1373/clinchem.2008.103606]
19 **Wolf J**, Petroff D, Richter T, Auth MKH, Uhlig HH, Laass MW, Lauenstein P, Krahl A, Händel N, de Laffolie J, Hauer AC, Kehler T, Flemming G, Schmidt F, Rodrigues A, Hasenclever D, Mothes T. Validation of Antibody-Based Strategies for Diagnosis of Pediatric Celiac Disease Without Biopsy. *Gastroenterology* 2017; **153**: 410-419.e17 [PMID: 28461188 DOI: 10.1053/j.gastro.2017.04.023]
20 **Sugai E**, Hwang HJ, Vázquez H, Smecuol E, Niveloni S, Mazure R, Mauriño E, Aeschlimann P, Binder W, Aeschlimann D, Bai JC. New serology assays can detect gluten sensitivity among enteropathy patients seronegative for anti-tissue transglutaminase. *Clin Chem* 2010; **56**: 661-665 [PMID: 20022983 DOI: 10.1373/clinchem.2009.129668]
21 **Boskovica A**, Kitica L, Prokica D and Stankovica I. Pediatric typical vs. atypical celiac disease: Correlation of duodenal histology with tissue transglutaminase levels. *International Journal of Clinical Pediatrics* 2012; **1**: 109-114 [DOI:

10.4021/ijcp57w]

22 **Hawamdeh H**, Al-Zoubi B, Al Sharqi Y, Qasrawi A, Abdelaziz Y and Barbar M. Association of tissue transglutaminase antibody titer with duodenal histological changes in children with Celiac disease. *Gastroenterology research and practice* 2016; **2016**: 6718590 [PMID: 27867394 DOI: 10.1155/2016/6718590]

23 **Donaldson MR**, Firth SD, Wimpee H, Leiferman KM, Zone JJ, Horsley W, O'Gorman MA, Jackson WD, Neuhausen SL, Hull CM, Book LS. Correlation of duodenal histology with tissue transglutaminase and endomysial antibody levels in pediatric celiac disease. *Clin Gastroenterol Hepatol* 2007; **5**: 567-573 [PMID: 17428743 DOI: 10.1016/j.cgh.2007.01.003]

24 **Dore MP**, Pes GM, Dettori I, Villanacci V, Manca A and Realdi G. Clinical and genetic profile of patients with seronegative coeliac disease: The natural history and response to gluten-free diet. *BMJ Open Gastro* 2017; **4**: 1-8 [DOI: 10.1136/bmjgast-2017-]

25 **Brar P**, Kwon GY, Egbuna II, Holleran S, Ramakrishnan R, Bhagat G, Green PH. Lack of correlation of degree of villous atrophy with severity of clinical presentation of coeliac disease. *Dig Liver Dis* 2007; **39**: 26-9; discussion 30 [PMID: 16982222 DOI: 10.1016/j.dld.2006.07.014]

26 **Abu-Zekry M**, Kryszak D, Diab M, Catassi C, Fasano A. Prevalence of Celiac Disease in Egyptian Children Disputes the East-West Agriculture-dependent Spread of the Disease. *J Pediatr Gastroenterol Nutr* 2008; **47**: 136-140 [PMID: 18664863 DOI: 10.1097/MPG.0b013e31815ce5d1]

World Journal of Clinical Pediatrics

Submit a Manuscript: https://www.f6publishing.com

World J Clin Pediatr 2022 May 9; 11(3): 307-320

DOI: 10.5409/wjcp.v11.i3.307

ISSN 2219-2808 (online)

SCIENTOMETRICS

Global research production in neonatal abstinence syndrome: A bibliometric analysis

Sa'ed H Zyoud, Samah W Al-Jabi, Moyad Jamal Shahwan, Ammar Abdulrahman Jairoun

Specialty type: Substance abuse

Provenance and peer review: Invited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): 0
Grade B (Very good): 0
Grade C (Good): C
Grade D (Fair): 0
Grade E (Poor): 0

P-Reviewer: Dong Y, China

Received: March 19, 2021
Peer-review started: March 19, 2021
First decision: May 6, 2021
Revised: May 21, 2021
Accepted: March 16, 2022
Article in press: March 16, 2022
Published online: May 9, 2022

Sa'ed H Zyoud, Samah W Al-Jabi, Department of Clinical and Community Pharmacy, College of Medicine and Health Sciences, An-Najah National University, Nablus 44839, Palestine

Sa'ed H Zyoud, Poison Control and Drug Information Center, College of Medicine and Health Sciences, An-Najah National University, Nablus 44839, Palestine

Sa'ed H Zyoud, Clinical Research Centre, An-Najah National University Hospital, Nablus 44839, Palestine

Moyad Jamal Shahwan, Clinical Sciences, Ajman University, Ajman 2758, United Arab Emirates

Moyad Jamal Shahwan, Centre of Medical and Bio-allied Health Sciences Research, Ajman University, Ajman 2758, United Arab Emirates

Ammar Abdulrahman Jairoun, Department of Health and Safety, Dubai Municipality, Dubai 67, United Arab Emirates

Corresponding author: Sa'ed H Zyoud, PhD, Associate Professor, Department of Clinical and Community Pharmacy, College of Medicine and Health Sciences, An-Najah National University, Academic Street, Nablus 44839, Palestine. saedzyoud@yahoo.com

Abstract

BACKGROUND

Recently, neonatal abstinence syndrome (NAS) emerged as a significant global concern with a dramatic increase in healthcare expenditures. The incidence of the NAS has increased notably in the past decade and emergence as a global public health problem.

AIM

To evaluate the development and trend of global NAS research from 1958 to 2019 by bibliometric analysis.

METHODS

Analyzed aspects included publication output per year, language, document types, journals, countries/territories, h-index, authors, and top research priorities. The VOSviewer was used to determine the top research priorities, and trends, and to present bibliometric networks concerning various dimensions, such as co-authorship, authors, and countries.

RESULTS

A total of 1738 articles were retrieved in the Scopus database from 1958 to 2019. It was found that the great majority of the total NAS documents (n = 1295) were original articles followed by reviews (n = 268) and letters (n = 48). The most productive countries in the NAS field were the United States (n = 833), Canada (n = 112), the United Kingdom (n = 111), and Germany (n = 77). Treatment and hospital outcomes in NAS, evidence-based nurse-driven interventions for the care of newborns with NAS, and a systematic reviews and network meta-analysis for therapeutic approaches of NAS were found in recent years (after 2010), compared with terms such as pathophysiology, mechanisms of NAS, and signs and symptoms in the early years.

CONCLUSION

Treatment and pediatric outcomes and the effectiveness of pharmacological treatment may be frontiers in the NAS field, and continued efforts from researchers are needed in those topics.

Key Words: Neonatal abstinence syndrome; Bibliometric; Scopus; VOSviewer; Visualization

Core Tip: This bibliometric study extracts data on Neonatal abstinence syndrome (NAS) research at a global level, aiming to provide the top-cited articles and top research priorities in NAS and to determine the most prolific countries, journals, and authors. This would enable scientists and clinicians interested in the NAS field to identify the most prevalent topics that have been used for increasing our understanding of NAS and provide a basis for future research. Treatment and pediatric outcomes and the effectiveness of pharmacological treatment may be frontiers in the NAS field, and continued efforts from researchers are needed on these topics.

Citation: Zyoud SH, Al-Jabi SW, Shahwan MJ, Jairoun AA. Global research production in neonatal abstinence syndrome: A bibliometric analysis. *World J Clin Pediatr* 2022; 11(3): 307-320
URL: https://www.wjgnet.com/2219-2808/full/v11/i3/307.htm
DOI: https://dx.doi.org/10.5409/wjcp.v11.i3.307

INTRODUCTION

Neonatal abstinence syndrome (NAS) is a group of signs and symptoms that occur due to the sudden discontinuation of infants shortly after the birth of certain substances that were abused or used during pregnancy[1,2]. NAS can present with a broad range of signs and symptoms including restlessness, agitation, feeding intolerance, gastrointestinal disturbances, hypertonia, tremors, seizures, and respiratory distress[2-6].

Recently, NAS emerged as a significant global concern with a dramatic increase in healthcare expenditures[1,7-9]. The incidence of NAS has increased notably in the past decade and emergence as a global public health problem. The reported rise in antidepressant therapy during pregnancy, or the use of narcotic analgesics for pain relief in pregnant women, or the illicit use of opioids such as heroin and oxycodone[10] may play an important role in this aspect. The most clinically significant interventions in NAS management are appropriate to nonpharmacologic interventions[6,11] including promoting breastfeeding of infants when not contraindicated, rooming-in, positioning of the infant, bed type, and non-insertive acupuncture. Opioids such as morphine or methadone or buprenorphine are usually the first-line agent to treat the symptoms of withdrawal when pharmacological treatment is indicated. Although phenobarbital has been recognized as a second-line agent to be used in infants when opioids fail, clonidine may be used as a nonopioid adjunctive NAS therapy with minimal adverse effects and reduced treatment time[5,6,12,13].

Although several bibliometric analyses have been carried out on several topics related to substance abuse such as illicit drug addiction[14], cocaine intoxication[15], substance use disorders[16], drug and alcohol[17], and drug abuse and dependence[18-23], an extensive literature search did not reveal any bibliometric analysis on NAS. Therefore, this bibliometric study extracts data on NAS research at a global level, aiming to provide the top-cited articles and top research priorities in NAS and to determine the most prolific countries, journals, and authors. This would enable scientists and clinicians interested in the NAS field to identify the most prevalent topics that have been used for increasing our understanding of NAS and provide a basis for future research.

MATERIALS AND METHODS

Database used

To achieve the objectives of the current bibliometric study, we performed a generalized search using the database of Scopus (Elsevier's citation database). The search was performed on July 17, 2020. Because the first publication related to NAS was published in 1958, the timespan was set from 1958 to 2019. The final year (2020) was omitted from the study as, at the time of data collection, certain publications from that year might not have been indexed in databases and this year's data does not represent a complete year of publication in the field.

Search strategy

The search was thus conducted using the following search string: (TITLE-ABS (neonat*) OR TITLE-ABS (newborn) OR TITLE-ABS (birth) OR TITLE-ABS (infant) AND TITLE-ABS ("Abstinence Syndrome") OR TITLE-ABS ("Abstinence Symptom*") OR TITLE-ABS ("Substance Withdrawal") OR TITLE-ABS ("narcotic syndrome") OR TITLE-ABS ("narcotic Symptom*") OR TITLE-ABS ("Withdrawal Symptom*") OR TITLE-ABS ("Withdrawal Syndrome") OR TITLE-ABS ("drug Withdrawal") OR TITLE-ABS ("Passive Addiction") OR TITLE-ABS("opioid withdrawal") OR TITLE-ABS ("opioid syndrome") AND EXCLUDE (DOCTYPE) AND [EXCLUDE (PUBYEAR, 2020). Neonatal abstinence syndrome-related terms were selected for this string based on those identified in previous literature reviews[6,10,24-27]. The search strategy for the terms related to NAS was restricted to Title/Abstract to achieve greater accuracy in the results because many reported publications were not related to NAS (*i.e.*, false-positive data) if applied to other search fields such as keywords. The use of title/abstract search is recommended in bibliometric studies[15,28,29] in contrast to the title-abstract-keywords search query because it substantially increases specificity with minimum loss of sensitivity. The main explanation for the generation of false-positive results by keyword search is that Scopus considers keywords such as "Medline keywords", "EMTREE medical terms" and "EMTRE drug terms" as author and indexed keywords. In the absence of false-positive and false-negative findings, the method adopted in the current study was validated[30]. No language restrictions were applied. Thus, both English and non-English documents were used.

Data analysis and visualization

The data was organized and analyzed using Microsoft Office Excel 2010. Descriptive statistics were used for data analysis, using frequencies and percentages. The downloaded document included the title, abstract, publication date, journal information, authors, country, collaboration patterns, and citations as indicators for quantity and qualitative analysis. These indicators were identified according to previous bibliometric literature[31-33]. The VOSviewer version 1.6.14[34], a software package for analyzing and visualizing large bibliographic datasets, was used for content analysis to determine the top research priority topics, and trends, and to present bibliometric networks concerning various dimensions, such as co-authorship, authors, and countries. To map the network of terms co-occurrence in the title and abstract countries, collaboration co-authorship was extracted from downloaded bibliometric records. In terms with an occurrence frequency no less than 20 authors and countries who had at least five publications were chosen for visualisation. The number of publications related to a certain word is measured by the size of circles in VOSviewer maps and the distance between the two terms means the number of cooccurrences of the terms. Moreover, words that are similar to each other or have a certain color are more likely to deal with the same topic[34]. In addition, the terms co-occurrences were analyzed to distinguish topics used by the authors' overtime. Using the "link strength" indicator extracted from visualization maps, an international research collaboration among active countries was evaluated. The strength of the link is a measure of the strength of cooperation between any two countries in this field. The higher value of link strength means the thickness of the connecting line, which is considered the stronger research collaboration between certain countries in this field[34].

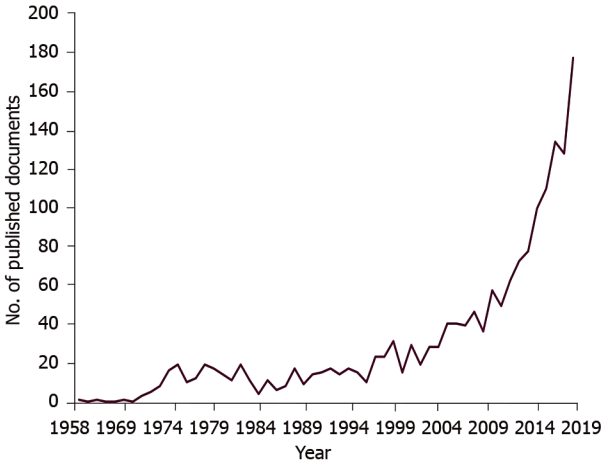
RESULTS

A total of 1738 articles were retrieved in the Scopus database from 1958 to 2019. It was found that the great majority of the total NAS documents (1295; 74.51%) were original articles followed by reviews (268; 15.42%) and letters (48; 2.76%). The yearly publication number of articles increased rapidly from 1958 to 2019 (Figure 1). Yearly articles increased from 2 in 1958 to 40 in 2007 and then to 177 in 2019. Twenty-three languages of publication were identified in the 1,738 articles retrieved. The four predominant languages were English (n = 1489; 85.67%), followed by German (n = 66; 3.80%), Spanish (n = 39; 2.24%), and French (n = 38; 2.13%).

There is a total of 111 countries/areas that make great contribution research publications in neonatal abstinence syndrome. Table 1 presents the 10 most productive countries in the NAS concerning total publications, h-index, as well as the collaboration pattern. The most productive countries in NAS field were the United States (n = 833; 47.93%), Canada (n = 112; 6.44%), the United Kingdom (n = 111; 6.39%)

Table 1 The top 10 countries contributed to publications in neonatal abstinence syndrome research (1958 to 2019)

SCR	Country	Number of documents (%)	h-index	No. of collaborated countries
1st	United States	833 (47.93)	71	38
2nd	Canada	112 (6.44)	29	14
3rd	United Kingdom	111 (6.39)	29	25
4th	Germany	77 (4.43)	17	23
5th	Italy	65 (3.74)	15	21
6th	Australia	63 (3.62)	26	7
7th	France	62 (3.57)	20	23
8th	Spain	59 (3.39)	18	22
9th	Austria	52 (2.99)	19	19
10th	the Netherlands	45 (2.59)	16	24



DOI: 10.5409/wjcp.v11i3.307

Figure 1 Annual number of articles published in neonatal abstinence syndrome.

and Germany (n = 77; 4.43%). The h-index of the total retrieved articles was 87. Among the most productive countries, the United States achieved the highest h-index value with 71, followed by the United Kingdom with 29, and Canada with 29. A network visualization map for country collaboration is shown in Figure 2 and demonstrates that the United States is the most important collaboration country. Of the 111 countries, 33 had at least five publications; the largest set of connected countries consists of 28 countries in 8 clusters. For research collaboration, the strongest was in the United States (total link strength = 111), followed by the United Kingdom (total link strength = 72), and Netherlands (total link strength = 70). For most countries, the link strength was less than 20, suggesting insufficient international research collaboration in this field[34].

Table 2 shows the 11 journals with more than 17 published papers with their impact factors. The journals publishing most papers are *Pediatrics* (n = 43), *Journal of Pediatrics* (n = 34), and *Journal of Perinatology* (n = 33).

Among the top contributive authors (Table 3), Jones Hendrée (42 publications) from the University of North Carolina at Chapel Hill (United States) was ranked first, followed by Fischer, Gabriele (29 publications) from the Medical University Vienna (Austria). Of note, 8 and 3 authors are from the United States and Austria, respectively, suggesting an important contributing role to these countries. A network visualization map for the authors' collaboration is shown in Figure 3. Of the 5305 authors, 118 had at least five publications; the largest set of connected authors consists of 70 authors in 11 clusters.

In VOSviewer, co-occurrence analysis for the title and abstract contents was used to produce the term co-occurrence network of NAS studies (Figure 4). In Figure 4, it can be seen that the top research priorities of NAS studies form five clusters, and the terms in the same cluster show superior connection in each of the research topics. These five clusters were as follows: Cluster 1 (blue) involved terms related to "treatment and hospital outcomes in NAS" such as "hospitalization, length, stay, hospital stay, discharge, neonate outcome"; Cluster 2 (yellow) involved terms related to "evidence-based nurse-

Table 2 The top 11 most productive journals on neonatal abstinence syndrome research from 1958 to 2019

SCR[1]	Journal	Frequency (%)	IF[2]
1st	*Pediatrics*	43 (2.47)	5.359
2nd	*Journal of Pediatrics*	34 (1.96)	3.700
3rd	*Journal of Perinatology*	33 (1.90)	1.967
4th	*Drug and Alcohol Dependence*	31 (1.78)	3.951
5th	*Addiction*	27 (1.55)	6.343
6th	*Advances in Neonatal Care*	23 (1.32)	1.405
6th	*American Journal of Obstetrics and Gynecology*	23 (1.32)	6.502
8th	*Acta Paediatrica*	22 (1.27)	2.111
9th	*Archives of Disease in Childhood Fetal and Neonatal Edition*	19 (1.09)	5.436
10th	*Journal of Addiction Medicine*	18 (1.04)	3.014
10th	*Pediatric Research*	18 (1.04)	2.747

[1]If some journals receive the same ranking number, a gap is left in the next ranking numbers.
[2]Impact factors based on Journal Citation Reports 2019 adapted from Clarivate Analytics which was published in 2020.
IF: Impact factors.

Table 3 The first twelve authors by record count in neonatal abstinence syndrome research

SCR[1]	Author name	Country	Number of documents (%)
1st	Jones HE	United States	42 (2.42)
2nd	Fischer G	Austria	29 (1.67)
3rd	Jansson LM	United States	28 (1.61)
3rd	Patrick SW	United States	28 (1.61)
5th	Finnegan LP	United States	24 (1.38)
5th	Kaltenbach K	United States	24 (1.38)
5th	Wachman EM	United States	24 (1.38)
8th	Davis JM	United States	17 (0.98)
9th	Jagsch R	Austria	14 (0.81)
10th	Huestis MA	United States	12 (0.69)
10th	Koren G	Israel	12 (0.69)
10th	Raith W	Austria	12 (0.69)

[1]If some authors receive the same ranking number, a gap is left in the next ranking numbers.

driven interventions for the care of newborns with NAS" such as "nurse, intervention, guideline, barrier, challenge"; Cluster 3 (red) involved terms related to "pathophysiology and mechanisms of NAS" such as "brain, onset, alteration, activity, administration, tolerance, analgesia, sedation, animal, rat"; Cluster 4 (purple) involved terms related to "systematic review and network meta-analysis for therapeutic approaches of NAS" such as "systematic review, meta-analysis, Medline, trial, search"; and Cluster 5 (green) involved terms related to "signs and symptoms" such as "jitteriness in neonates, meconium, irritability, prematurity". In the analysis of term co-occurrence (Figure 5), we also identified terms in the titles and abstracts related to NAS over time. Treatment and hospital outcomes in NAS, evidence-based nurse-driven interventions for the care of newborns with NAS, and a systematic reviews and network meta-analysis for therapeutic approaches of NAS were found in recent years (after 2010), compared with terms such as pathophysiology, mechanisms of NAS, and signs and symptoms in the early years (before 2010).

The top 20 cited publications in the field of NAS ranked by the total number of citations are shown in Table 4. The highest cited publication in the top 20 was cited 529 times and the lowest cited article 177

Table 4 The 20 Most-cited articles in neonatal abstinence syndrome based on the citation count

SCR	Ref.	Title	Year	Source title	Cited by
1st	Patrick *et al*[1]	Neonatal abstinence syndrome and associated health care expenditures: United States, 2000-2009	2012	*JAMA - Journal of the American Medical Association*	529
2nd	Jones *et al*[43]	Neonatal abstinence syndrome after methadone or buprenorphine exposure	2010	*New England Journal of Medicine*	526
3rd	Hudak *et al*[40]	Neonatal drug withdrawal	2012	*Pediatrics*	443
4th	Finnegan *et al*[38]	Neonatal abstinence syndrome: assessment and management	1975	*Addictive diseases*	435
5th	Sanz *et al*[49]	Selective serotonin reuptake inhibitors in pregnant women and neonatal withdrawal syndrome: A database analysis	2005	*Lancet*	318
6th	Hughes *et al*[41]	Nicotine withdrawal *vs* other drug withdrawal syndromes: similarities and dissimilarities	1994	*Addiction*	253
7th	Patrick *et al*[9]	Increasing incidence and geographic distribution of neonatal abstinence syndrome: United States 2009 to 2012	2015	*Journal of Perinatology*	251
8th	Levinson-Castiel *et al*[45]	Neonatal abstinence syndrome after in utero exposure to selective serotonin reuptake inhibitors in term infants	2006	*Archives of Pediatrics and Adolescent Medicine*	238
9th	Costei *et al*[37]	Perinatal outcome following third trimester exposure to paroxetine	2002	*Archives of Pediatrics and Adolescent Medicine*	233
10th	Kocherlakota[2]	Neonatal abstinence syndrome	2014	*Pediatrics*	219
11th	Tolia *et al*[50]	Increasing incidence of the neonatal abstinence syndrome in United States neonatal ICUs	2015	*New England Journal of Medicine*	213
12th	American Academy of Pediatrics Committee on Drugs[36]	Neonatal drug withdrawal	1998	*Pediatrics*	212
13th	ACOG Committee on Health Care for Underserved Women and American Society of Addiction Medicine[35]	Committee opinion No. 524: Opioid abuse, dependence, and addiction in pregnancy	2012	*Obstetrics and Gynecology*	208
13th	Jones *et al*[42]	Buprenorphine *vs* methadone in the treatment of pregnant opioid-dependent patients: Effects on the neonatal abstinence syndrome	2005	*Drug and Alcohol Dependence*	208
15th	Wisner *et al*[51]	Pharmacologic treatment of depression during pregnancy	1999	*Journal of the American Medical Association*	200
16th	Zajecka *et al*[52]	Discontinuation symptoms after treatment with serotonin reuptake inhibitors: A literature review	1997	*Journal of Clinical Psychiatry*	197
17th	Nau *et al*[46]	Valproic acid and its metabolites: Placental transfer, neonatal pharmacokinetics, transfer *via* mother's milk and clinical status in neonates of epileptic mothers	1981	*Journal of Pharmacology and Experimental Therapeutics*	197
18th	Ryan *et al*[48]	Cocaine abuse in pregnancy: Effects on the fetus and newbornF	1987	*Neurotoxicology and Teratology*	181
19th	Lejeune *et al*[44]	Prospective multicenter observational study of 260 infants born to 259 opiate-dependent mothers on methadone or high-dose buprenophine substitution	2006	*Drug and Alcohol Dependence*	180
20th	Hadeed and Siegel[39]	Maternal cocaine use during pregnancy: Effect on the newborn infant	1989	*Pediatrics*	177
21st	Nordeng *et al*[47]	Neonatal withdrawal syndrome after in utero exposure to selective serotonin reuptake inhibitors	2001	*Acta Paediatrica,*	175

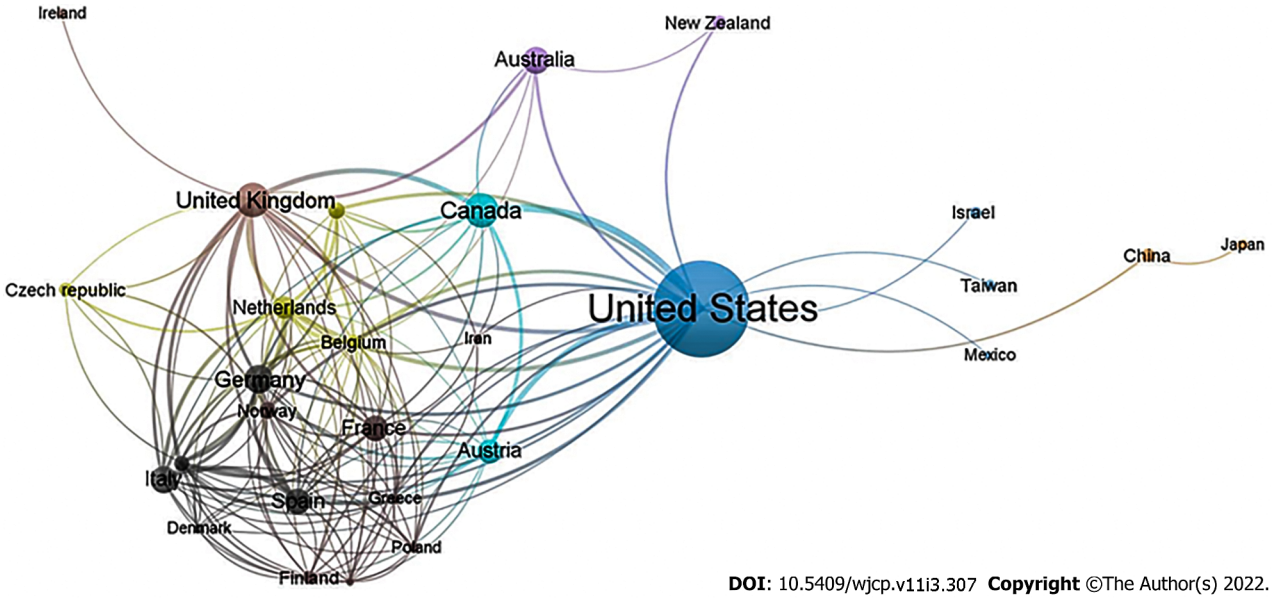
times[1,2,9,35-52]. Table 5 includes the most frequently encountered agents related to NAS literature. "Methadone" (n = 629) was the most commonly occurred in NAS literature, followed by "morphine" (n = 378), "buprenorphine" (n = 313), and "phenobarbital" (n = 275).

DISCUSSION

This study is set out with the aim of investigating the current situation of NAS research at a global level by analysing the related literature bibliometrically. In 2015, member states of the United Nations had

Table 5 List of most frequent drugs occurrences in neonatal abstinence syndrome literature

Drug	Number of publications
Methadone	629
Morphine	378
Buprenorphine	313
Phenobarbital	275
Diamorphine	212
Heroine	138
Cocaine	138
Clonidine	130
Diazepam	124
Alcohol	80
Cannabis	79
Chlorpromazine	76
Naloxone	62
Fentanyl	56



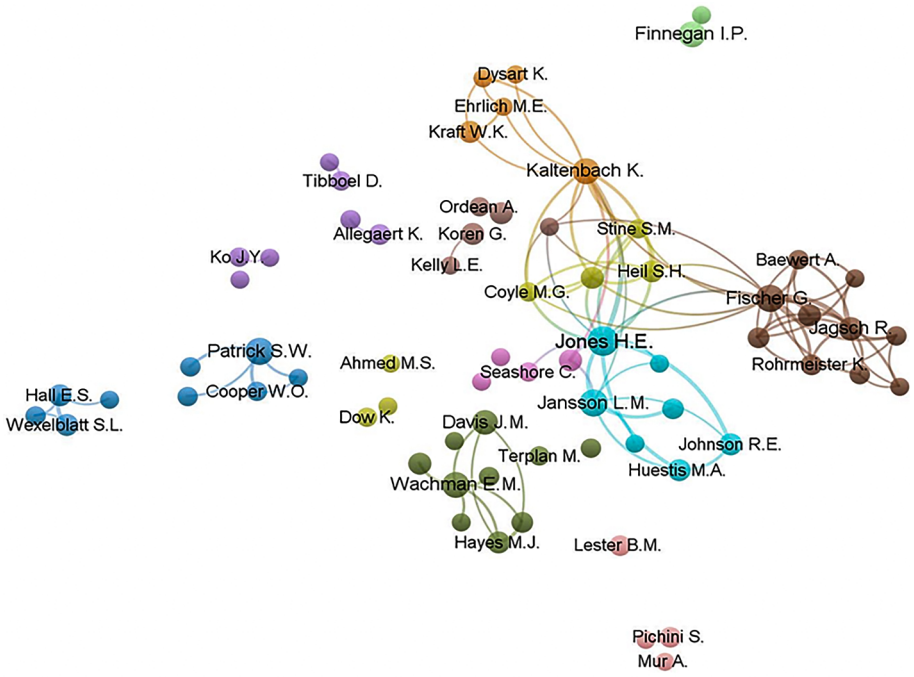
DOI: 10.5409/wjcp.v11i3.307

Figure 2 Network visualization map for countries collaboration among most productive countries. Of the 111 countries, 33 had at least five publications; the largest set of connected countries consists of 28 countries in 8 clusters.

signed and adopted Sustainable Development Goals (SDGs) to be achieved in 2030[53]. The third goal is dedicated to health and well-being. The fifth target of the third goal in the SDGs promotes the prevention and treatment of substance use disorders. Furthermore, the second target of the third goal promotes the health of the newborn and children by minimizing preventable deaths[54]. The current study will endorse the attainment of 2030 goals by shedding light on an important problem related to maternal and newborn health within the context of substance use disorders.

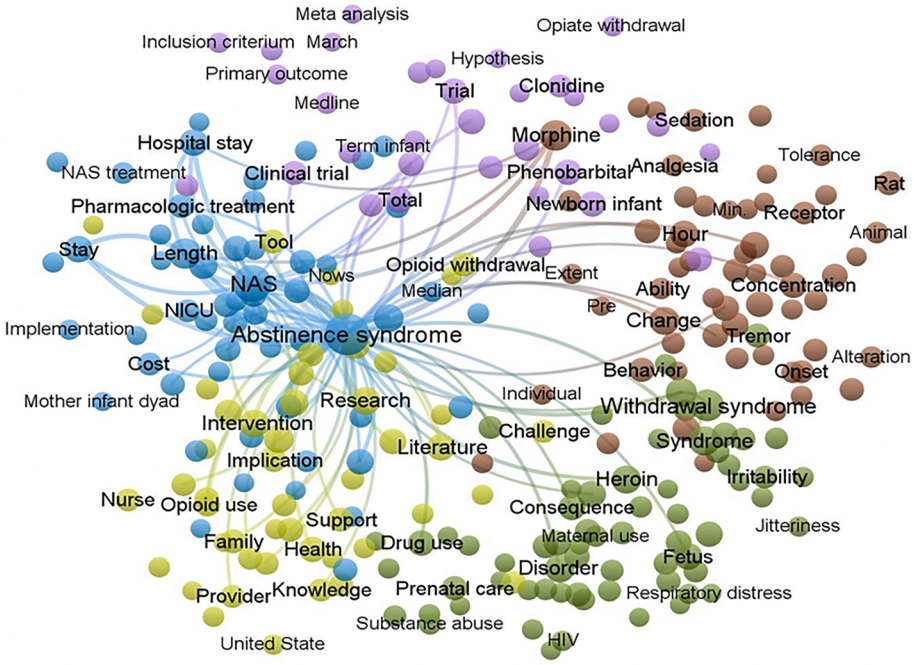
The current study is novel in describing the characteristics of research publications related to NAS across time, *via* bibliometric analysis, and determining the top research priorities in this field during six decades (1958-2019). Advances in the knowledge of NAS by determining the top research priorities for this complex health issue will help to improve future research for maternal and neonatal care.

Overall, the current study demonstrated an increase in the number of publications involving NAS over the period 1958–2019. In another way, the total number of publications related to NAS increased more than twofold from 437 before 2000 to 971 during the last decade (2010–2019). This was possible because of different explanations. First, research concerning substance abuse, with a focus on prevention



DOI: 10.5409/wjcp.v11i3.307

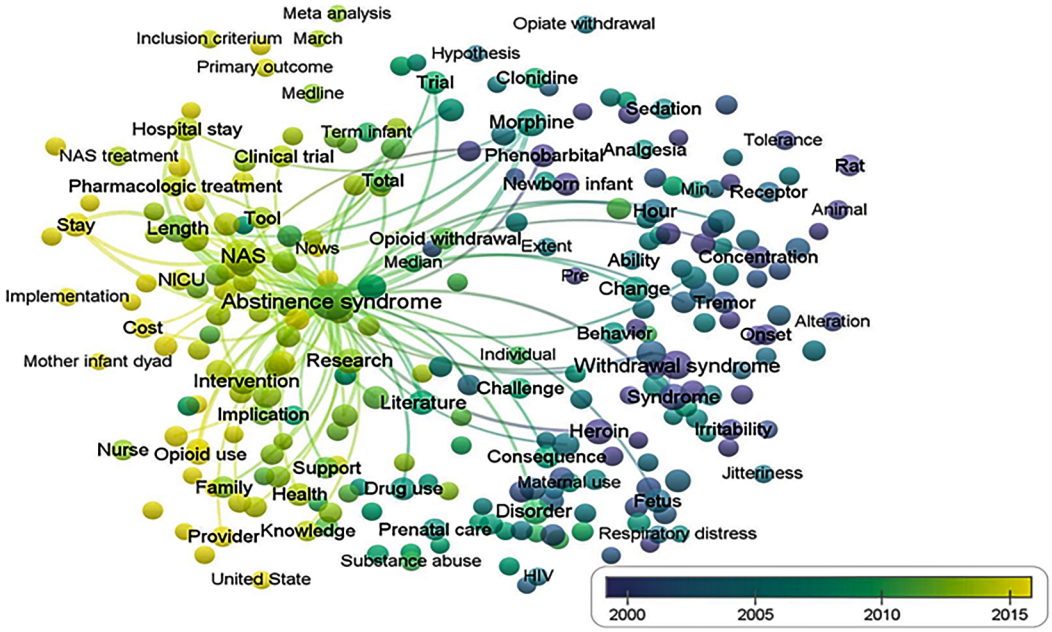
Figure 3 Co-authorship network among most productive authors with the threshold of minimum 5 publications. Of the 5305 authors, 118 had at least five publications; the largest set of connected authors consists of 70 authors in 11 clusters.



DOI: 10.5409/wjcp.v11i3.307

Figure 4 Terms co-occurrence network of neonatal abstinence syndrome studies. Of the 27233 terms, 436 terms occurred at least 20 times. For each of the 436 terms, a relevance score was calculated and used to select the 60% most relevant terms. The largest set of connected terms consists of 262 terms in five clusters.

and policy issues has become a rapidly emerging area in medical sciences and is recently reaching maturity. Second, this progress can be attributed largely to the trend of increasing maternal opiates and illicit drug use across the world. Third, the development of neonatal opioid withdrawal scale to measure opioid withdrawal signs and symptoms in the neonate. Fourth, the rapid growth of the global economy with the development of information technology has contributed to the progress of research to keep up this increasing trend.



DOI: 10.5409/wjcp.v11i3.307

Figure 5 Distribution of terms according to their time of appearance. The blue colored terms mean early appearance and yellow colored terms appeared later.

In this study, the United States was found to be the leader in NAS research. This result may be explained by the fact that the United States is the most prolific country for research in general by most bibliometric studies[31,55-57]. Moreover, the United States is one of the countries that attributed largely to the trend of increasing maternal cocaine, illicit drugs, and opiate use, and subsequently escalating numbers of deaths[58]. Additionally, the United States may be leading because of its size and economic strength and has a large research system including United States institutions, individual colleges, and hospitals[59]. Seven newborns were diagnosed with NAS for every 1,000 hospital stays for newborns, according to 2016 statistics[60]. This is around one baby in the United States diagnosed with NAS every 19 minutes, or almost 80 newborns diagnosed every day[60].

Clearly, the most commonly used keyword in NAS literature was "methadone," followed by "morphine", "buprenorphine", and "phenobarbital". It is interesting to note that three studies from the top 20 cited publications in the field of NAS were evaluating the efficacy and safety of methadone *vs* buprenorphine therapy for treating opioid-dependent pregnant patients[42-44].

The most cited publication in the field of NAS was published in 2012 in *JAMA - Journal of the American Medical Association,* conducted by Patrick *et al*[1], where the authors found an increase in maternal opiate use and incidence of NAS in the United States. The authors analyzed information on 7.4 million discharges from 4121 hospitals in 44 states, to measure the epidemiology and economic damage associated with NAS over the past decade. The authors reported that the number of mothers using opiates rose from 1.19 to 5.63 per 1000 hospital births per year between 2000 and 2009, and that it is estimated that aggregate hospital costs for NAS cases, adjusted for inflation, rose from $190 million to $720 million between 2000 and 2009. The second most cited publication was published in 2010 in *New England Journal of Medicine*, conducted by Jones *et al*[43] resulting from the collaboration between several countries (United States, Canada, and Austria). This study considered the use of buprenorphine as the first-line treatment option instead of methadone for the treatment of opioid dependency during pregnancy. Methadone therapy for heroin addiction began in New York City in 1964[61], and then became the standard therapy for treating opioid-dependent pregnant patients in both the developed and developing worlds[62]. Methadone therapy led to several adverse pregnancy events during withdrawal[63]. After that, marked progress has been made in the area of buprenorphine research as an alternative treatment for opioid dependence[43] which gives relative superiority for buprenorphine to be associated with a lower risk of NAS severity[64]. This finding also accords with our observations, which showed that top research priorities including treatment and pediatric outcomes, and the effectiveness of pharmacological treatment were found in recent years. Furthermore, top research priorities in the field of NAS are consistent with the findings highlighted in the most highly cited publications, which provide substantial and valuable findings that open the door for new areas of research investigation.

Despite the importance of this topic, there remains a paucity of evidence on several issues related to NAS[65-68]. The most significant knowledge gaps in NAS are the long-term outcomes and the international differences between treatments of drug-using mother/infant dyads. What happens to the children

afterward? How have they looked after? Some of the medications used to treat NAS (*e.g.*, methadone) are not sanctioned in countries outside the United States. Opioid-exposed infants are still at significantly higher risk of dying, of being abused, and of sliding towards an unpalatable life trajectory after discharge from the hospital. Further work is needed to highlight this missing information and note the urgent need to prioritize research and clinical care towards improving and ameliorating the impact of maternal drug use. More broadly, the emphasis on the need to conduct more research into pharmacological treatment neglects other aspects of care for infants, including rooming-in, breastfeeding, *etc.* The medications that are used to treat NAS are not innocuous, therefore, research is also needed to avoid pharmacological treatment, rather than to see which treatment is most effective in discharging the infants out of the hospital faster.

Strengths and limitations

This bibliometric study is the first comprehensive investigation to explore the distribution trends and top research priorities in the field of NAS. Additionally, another strength of the current study including a large literature database (*i.e.*, Scopus), benefits from a higher coverage than other databases[69,70], spanning multiple years of analysis to identify relevant NAS literature. Several limitations matching those observed in earlier bibliometric studies[31,71,72] should be noted. The main limitation of this study was the use of the Scopus database which expects that most perspectives of the publications in the field of NAS indexed in this database were analysed. Additionally, the current study used a comprehensive list of keywords based on those identified in previous literature reviews[6,10,24-27]; however, there is a possible slight chance that some keywords have been missed which may lead to false-negative results.

CONCLUSION

In conclusion, this bibliometric review defined the scientific research output in the field of NAS using bibliometric methods over the past 60 years, including publication numbers, countries, organizations, journals, top research priorities, and emerging trends. The findings from this study make several contributions to the current literature. First, this study confirmed the increase in the number of publications involving NAS over the period 1958–2019. Second, the United States, Canada, and the United Kingdom had the leading position in global research productivity in this field. Third, it was found that the comparative studies related to the safety and efficacy of methadone and buprenorphine in NAS were the mainstay of the top-cited studies. In the last treatment and pediatric outcomes, and the effectiveness of pharmacological treatment may be frontiers in the NAS field, and continued efforts from researchers are needed on these topics. This bibliometric study offers an objective and quantitative summary of the progress of research in the NAS field, which can serve as a significant guide and entry point for more scientific research. This information can be used to develop targeted interventions aimed to improve international cooperation between organizations and countries by applying useful initiatives and policies.

ARTICLE HIGHLIGHTS

Research background

Neonatal abstinence syndrome (NAS) has recently become a major global issue, resulting in a substantial rise in healthcare costs. The NAS has become a global public health epidemic in the last decade, with a rise in incidence.

Research motivation

Despite the fact that bibliometric studies have been conducted on a variety of topics related to substance abuse, such as illegal drug addiction, a thorough search of the literature revealed no bibliometric research on NAS.

Research objectives

Bibliometric analysis was used to assess the evolution and pattern of the global NAS research from 1958 to 2019.

Research methods

Yearly publication production, language, document types, journals, countries/territories, h-index, authors, and top research priorities were among the indicators examined. The VOSviewer was used to assess the top research priorities and patterns, as well as to present bibliometric networks on a variety of dimensions, including co-authorship, authors, and countries.

Research results

The current study is novel in that it uses bibliometric analysis to describe the characteristics of research publications relevant to NAS over time and determine the top research priorities in this field over six decades (1958-2019). Advances in NAS awareness will help to enhance future maternal and neonatal care research by identifying the top research priorities for this complex health problem.

Research conclusions

Treatment and pediatric outcome, as well as the efficacy of pharmacological treatment, may be frontiers in the NAS area, and researchers must continue to work on these topics.

Research perspectives

This will allow scientists and clinicians interested in the field of NAS to recognise the most common topics that have been used to improve our understanding of the disease and serve as a foundation for future study.

FOOTNOTES

Author contributions: Zyoud SH designed the study, collected the data, analyzed the data, made major contributions to the manuscript's existing literature search and interpretation, and drafted the manuscript; Al-Jabi SW, Jairoun AA, and Shahwan WM were involved in interpretation of the data, and made revisions to the initial draft; all authors provided a critical review and approved the final manuscript before submission.

Conflict-of-interest statement: The authors have no financial disclosures or conflicts of interest to declare.

PRISMA 2009 Checklist statement: The authors have read the PRISMA 2009 Checklist, and the manuscript was prepared and revised according to the PRISMA 2009 Checklist.

Country/Territory of origin: Palestine

ORCID number: Sa'ed H Zyoud 0000-0002-7369-2058; Samah W Al-Jabi 0000-0002-4414-9427; Moyad Jamal Shahwan 0000-0001-8367-4841; Ammar Abdulrahman Jairoun 0000-0002-4471-0878.

S-Editor: Ma YJ
L-Editor: A
P-Editor: Ma YJ

REFERENCES

1 **Patrick SW**, Schumacher RE, Benneyworth BD, Krans EE, McAllister JM, Davis MM. Neonatal abstinence syndrome and associated health care expenditures: United States, 2000-2009. *JAMA* 2012; **307**: 1934-1940 [PMID: 22546608 DOI: 10.1001/jama.2012.3951]
2 **Kocherlakota P**. Neonatal abstinence syndrome. *Pediatrics* 2014; **134**: e547-e561 [PMID: 25070299 DOI: 10.1542/peds.2013-3524]
3 **Sanlorenzo LA**, Stark AR, Patrick SW. Neonatal abstinence syndrome: an update. *Curr Opin Pediatr* 2018; **30**: 182-186 [PMID: 29346142 DOI: 10.1097/MOP.0000000000000589]
4 **Grossman M**, Seashore C, Holmes AV. Neonatal Abstinence Syndrome Management: A Review of Recent Evidence. *Rev Recent Clin Trials* 2017; **12**: 226-232 [PMID: 28814260 DOI: 10.2174/1574887112666170816144818]
5 **Raffaeli G**, Cavallaro G, Allegaert K, Wildschut ED, Fumagalli M, Agosti M, Tibboel D, Mosca F. Neonatal Abstinence Syndrome: Update on Diagnostic and Therapeutic Strategies. *Pharmacotherapy* 2017; **37**: 814-823 [PMID: 28519244 DOI: 10.1002/phar.1954]
6 **Wachman EM**, Schiff DM, Silverstein M. Neonatal Abstinence Syndrome: Advances in Diagnosis and Treatment. *JAMA* 2018; **319**: 1362-1374 [PMID: 29614184 DOI: 10.1001/jama.2018.2640]
7 **Corr TE**, Hollenbeak CS. The economic burden of neonatal abstinence syndrome in the United States. *Addiction* 2017; **112**: 1590-1599 [PMID: 28612362 DOI: 10.1111/add.13842]
8 **Okoroh EM**, Gee RE, Jiang B, McNeil MB, Hardy-Decuir BA, Zapata AL. Neonatal Abstinence Syndrome: Trend and Expenditure in Louisiana Medicaid, 2003-2013. *Matern Child Health J* 2017; **21**: 1479-1487 [PMID: 28168591 DOI: 10.1007/s10995-017-2268-1]
9 **Patrick SW**, Davis MM, Lehmann CU, Cooper WO. Increasing incidence and geographic distribution of neonatal

abstinence syndrome: United States 2009 to 2012. *J Perinatol* 2015; **35**: 650-655 [PMID: 25927272 DOI: 10.1038/jp.2015.36]

10 **McQueen K**, Murphy-Oikonen J. Neonatal Abstinence Syndrome. *N Engl J Med* 2016; **375**: 2468-2479 [PMID: 28002715 DOI: 10.1056/NEJMra1600879]

11 **Bagley SM**, Wachman EM, Holland E, Brogly SB. Review of the assessment and management of neonatal abstinence syndrome. *Addict Sci Clin Pract* 2014; **9**: 19 [PMID: 25199822 DOI: 10.1186/1940-0640-9-19]

12 **Streetz VN**, Gildon BL, Thompson DF. Role of Clonidine in Neonatal Abstinence Syndrome: A Systematic Review. *Ann Pharmacother* 2016; **50**: 301-310 [PMID: 26783353 DOI: 10.1177/1060028015626438]

13 **McPherson C**. Pharmacotherapy for Neonatal Abstinence Syndrome: Choosing the Right Opioid or No Opioid at All. *Neonatal Netw* 2016; **35**: 314-320 [PMID: 27636696 DOI: 10.1891/0730-0832.35.5.314]

14 **Khalili M**, Rahimi-Movaghar A, Shadloo B, Mojtabai R, Mann K, Amin-Esmaeili M. Global Scientific Production on Illicit Drug Addiction: A Two-Decade Analysis. *Eur Addict Res* 2018; **24**: 60-70 [PMID: 29627821 DOI: 10.1159/000487590]

15 **Zyoud SH**, Waring WS, Al-Jabi SW, Sweileh WM. Global cocaine intoxication research trends during 1975-2015: a bibliometric analysis of Web of Science publications. *Subst Abuse Treat Prev Policy* 2017; **12**: 6 [PMID: 28153037 DOI: 10.1186/s13011-017-0090-9]

16 **González-Alcaide G**, Calafat A, Becoña E, Thijs B, Glänzel W. Co-Citation Analysis of Articles Published in Substance Abuse Journals: Intellectual Structure and Research Fields (2001-2012). *J Stud Alcohol Drugs* 2016; **77**: 710-722 [PMID: 27588529 DOI: 10.15288/jsad.2016.77.710]

17 **Clifford A**, Shakeshaft A. A bibliometric review of drug and alcohol research focused on Indigenous peoples of Australia, New Zealand, Canada and the United States. *Drug Alcohol Rev* 2017; **36**: 509-522 [PMID: 28334457 DOI: 10.1111/dar.12510]

18 **Bramness JG**, Henriksen B, Person O, Mann K. A bibliometric analysis of European versus USA research in the field of addiction. Research on alcohol, narcotics, prescription drug abuse, tobacco and steroids 2001-2011. *Eur Addict Res* 2014; **20**: 16-22 [PMID: 23921359 DOI: 10.1159/000348260]

19 **Condon TP**. Reflecting on 30 years of research. A look at how NIDA has advanced the research, prevention, and treatment of drug abuse and addiction. *Behav Healthc* 2006; **26**: 14, 16 [PMID: 16736912]

20 **Rawson RA**, Woody G, Kresina TF, Gust S. The globalization of addiction research: capacity-building mechanisms and selected examples. *Harv Rev Psychiatry* 2015; **23**: 147-156 [PMID: 25747927 DOI: 10.1097/HRP.0000000000000067]

21 **Blobaum PM**. Mapping the literature of addictions treatment. *J Med Libr Assoc* 2013; **101**: 101-109 [PMID: 23646025 DOI: 10.3163/1536-5050.101.2.005]

22 **Rahimi-Movaghar A**, Amin-Esmaeili M, Safarcherati A, Sarami H, Rafiey H. A Scientometric Study of Iranian Scientific Productions in the Field of Substance Use and Addiction Research in the Years 2008 to 2012. *Addict Health* 2015; **7**: 99-108 [PMID: 26885346]

23 **Valderrama Zurián JC**, Aleixander R, Castellano M. Citation count analysis in addiction (2001). *Addiction* 2004; **99**: 387-388 [PMID: 14982553 DOI: 10.1111/j.1360-0443.2004.00655.x]

24 **Cleary BJ**, Donnelly J, Strawbridge J, Gallagher PJ, Fahey T, Clarke M, Murphy DJ. Methadone dose and neonatal abstinence syndrome-systematic review and meta-analysis. *Addiction* 2010; **105**: 2071-2084 [PMID: 20840198 DOI: 10.1111/j.1360-0443.2010.03120.x]

25 **MacMillan KDL**, Rendon CP, Verma K, Riblet N, Washer DB, Volpe Holmes A. Association of Rooming-in With Outcomes for Neonatal Abstinence Syndrome: A Systematic Review and Meta-analysis. *JAMA Pediatr* 2018; **172**: 345-351 [PMID: 29404599 DOI: 10.1001/jamapediatrics.2017.5195]

26 **Murphy-Oikonen J**, McQueen K. Outpatient pharmacologic weaning for neonatal abstinence syndrome: a systematic review. *Prim Health Care Res Dev* 2018; **20**: e76 [PMID: 29739484 DOI: 10.1017/S1463423618000270]

27 **Rees P**, Stilwell PA, Bolton C, Akillioglu M, Carter B, Gale C, Sutcliffe A. Childhood Health and Educational Outcomes After Neonatal Abstinence Syndrome: A Systematic Review and Meta-analysis. *J Pediatr* 2020; **226**: 149-156.e16 [PMID: 32659230 DOI: 10.1016/j.jpeds.2020.07.013]

28 **Olisah C**, Okoh OO, Okoh AI. A bibliometric analysis of investigations of polybrominated diphenyl ethers (PBDEs) in biological and environmental matrices from 1992 - 2018. *Heliyon* 2018; **4**: e00964 [PMID: 30533544 DOI: 10.1016/j.heliyon.2018.e00964]

29 **Ekundayo TC**, Okoh AI. A global bibliometric analysis of Plesiomonas-related research (1990 - 2017). *PLoS One* 2018; **13**: e0207655 [PMID: 30496198 DOI: 10.1371/journal.pone.0207655]

30 **Sweileh WM**. Bibliometric analysis of global scientific literature on vaccine hesitancy in peer-reviewed journals (1990-2019). *BMC Public Health* 2020; **20**: 1252 [PMID: 32807154 DOI: 10.1186/s12889-020-09368-z]

31 **Zyoud SH**, Waring WS, Al-Jabi SW, Sweileh WM. Bibliometric profile of global scientific research on digoxin toxicity (1849-2015). *Drug Chem Toxicol* 2020; **43**: 553-559 [PMID: 30239237 DOI: 10.1080/01480545.2018.1518453]

32 **Zhang TS**, Qin HL, Wang T, Li HT, Li H, Xia SH, Xiang XH. Bibliometric analysis of top 100 cited articles in nonalcoholic fatty liver disease research. *World J Hepatol* 2016; **8**: 1478-1488 [PMID: 27957247 DOI: 10.4254/wjh.v8.i33.1478]

33 **Wang CY**, Zhou SC, Li XW, Li BH, Zhang JJ, Ge Z, Zhang Q, Hu JH. Bibliometric analysis of randomized controlled trials of colorectal cancer over the last decade. *World J Clin Cases* 2020; **8**: 3021-3030 [PMID: 32775383 DOI: 10.12998/wjcc.v8.i14.3021]

34 **van Eck NJ**, Waltman L. Software survey: VOSviewer, a computer program for bibliometric mapping. *Scientometrics* 2010; **84**: 523-538 [PMID: 20585380 DOI: 10.1007/s11192-009-0146-3]

35 **ACOG Committee on Health Care for Underserved Women**; American Society of Addiction Medicine. ACOG Committee Opinion No. 524: Opioid abuse, dependence, and addiction in pregnancy. *Obstet Gynecol* 2012; **119**: 1070-1076 [PMID: 22525931 DOI: 10.1097/AOG.0b013e318256496e]

36 **Neonatal drug withdrawal**. American Academy of Pediatrics Committee on Drugs. *Pediatrics* 1998; **101**: 1079-1088 [PMID: 9614425]

37 **Costei AM**, Kozer E, Ho T, Ito S, Koren G. Perinatal outcome following third trimester exposure to paroxetine. *Arch Pediatr Adolesc Med* 2002; **156**: 1129-1132 [PMID: 12413342 DOI: 10.1001/archpedi.156.11.1129]

38 **Finnegan LP**, Connaughton JF Jr, Kron RE, Emich JP. Neonatal abstinence syndrome: assessment and management. *Addict Dis* 1975; **2**: 141-158 [PMID: 1163358]

39 **Hadeed AJ**, Siegel SR. Maternal cocaine use during pregnancy: effect on the newborn infant. *Pediatrics* 1989; **84**: 205-210 [PMID: 2748245]

40 **Hudak ML**, Tan RC; COMMITTEE ON DRUGS; COMMITTEE ON FETUS AND NEWBORN; American Academy of Pediatrics. Neonatal drug withdrawal. *Pediatrics* 2012; **129**: e540-e560 [PMID: 22291123 DOI: 10.1542/peds.2011-3212]

41 **Hughes JR**, Higgins ST, Bickel WK. Nicotine withdrawal versus other drug withdrawal syndromes: similarities and dissimilarities. *Addiction* 1994; **89**: 1461-1470 [PMID: 7841857 DOI: 10.1111/j.1360-0443.1994.tb03744.x]

42 **Jones HE**, Johnson RE, Jasinski DR, O'Grady KE, Chisholm CA, Choo RE, Crocetti M, Dudas R, Harrow C, Huestis MA, Jansson LM, Lantz M, Lester BM, Milio L. Buprenorphine versus methadone in the treatment of pregnant opioid-dependent patients: effects on the neonatal abstinence syndrome. *Drug Alcohol Depend* 2005; **79**: 1-10 [PMID: 15943939 DOI: 10.1016/j.drugalcdep.2004.11.013]

43 **Jones HE**, Kaltenbach K, Heil SH, Stine SM, Coyle MG, Arria AM, O'Grady KE, Selby P, Martin PR, Fischer G. Neonatal abstinence syndrome after methadone or buprenorphine exposure. *N Engl J Med* 2010; **363**: 2320-2331 [PMID: 21142534 DOI: 10.1056/NEJMoa1005359]

44 **Lejeune C**, Simmat-Durand L, Gourarier L, Aubisson S; Groupe d'Etudes Grossesse et Addictions (GEGA). Prospective multicenter observational study of 260 infants born to 259 opiate-dependent mothers on methadone or high-dose buprenophine substitution. *Drug Alcohol Depend* 2006; **82**: 250-257 [PMID: 16257138 DOI: 10.1016/j.drugalcdep.2005.10.001]

45 **Levinson-Castiel R**, Merlob P, Linder N, Sirota L, Klinger G. Neonatal abstinence syndrome after in utero exposure to selective serotonin reuptake inhibitors in term infants. *Arch Pediatr Adolesc Med* 2006; **160**: 173-176 [PMID: 16461873 DOI: 10.1001/archpedi.160.2.173]

46 **Nau H**, Rating D, Koch S, Häuser I, Helge H. Valproic acid and its metabolites: placental transfer, neonatal pharmacokinetics, transfer *via* mother's milk and clinical status in neonates of epileptic mothers. *J Pharmacol Exp Ther* 1981; **219**: 768-777 [PMID: 6795343]

47 **Nordeng H**, Lindemann R, Perminov KV, Reikvam A. Neonatal withdrawal syndrome after in utero exposure to selective serotonin reuptake inhibitors. *Acta Paediatr* 2001; **90**: 288-291 [PMID: 11332169 DOI: 10.1111/j.1651-2227.2001.tb00306.x]

48 **Ryan L**, Ehrlich S, Finnegan L. Cocaine abuse in pregnancy: effects on the fetus and newborn. *Neurotoxicol Teratol* 1987; **9**: 295-299 [PMID: 3683347 DOI: 10.1016/0892-0362(87)90018-3]

49 **Sanz EJ**, De-las-Cuevas C, Kiuru A, Bate A, Edwards R. Selective serotonin reuptake inhibitors in pregnant women and neonatal withdrawal syndrome: a database analysis. *Lancet* 2005; **365**: 482-487 [PMID: 15705457 DOI: 10.1016/S0140-6736(05)17865-9]

50 **Tolia VN**, Patrick SW, Bennett MM, Murthy K, Sousa J, Smith PB, Clark RH, Spitzer AR. Increasing incidence of the neonatal abstinence syndrome in U.S. neonatal ICUs. *N Engl J Med* 2015; **372**: 2118-2126 [PMID: 25913111 DOI: 10.1056/NEJMsa1500439]

51 **Wisner KL**, Gelenberg AJ, Leonard H, Zarin D, Frank E. Pharmacologic treatment of depression during pregnancy. *JAMA* 1999; **282**: 1264-1269 [PMID: 10517430 DOI: 10.1001/jama.282.13.1264]

52 **Zajecka J**, Tracy KA, Mitchell S. Discontinuation symptoms after treatment with serotonin reuptake inhibitors: a literature review. *J Clin Psychiatry* 1997; **58**: 291-297 [PMID: 9269249 DOI: 10.4088/jcp.v58n0702]

53 **United Nations**. Sustainable development goals. 2015. Vailable from: https://www.un.org/sustainabledevelopment/

54 **Sweileh WM**. Bibliometric analysis of scientific publications on "sustainable development goals" with emphasis on "good health and well-being" goal (2015-2019). *Global Health* 2020; **16**: 68 [PMID: 32723366 DOI: 10.1186/s12992-020-00602-2]

55 **Sweileh WM**, Al-Jabi SW, Zyoud SH, Sawalha AF. Outdoor air pollution and respiratory health: a bibliometric analysis of publications in peer-reviewed journals (1900 - 2017). *Multidiscip Respir Med* 2018; **13**: 15 [PMID: 29881545 DOI: 10.1186/s40248-018-0128-5]

56 **Zyoud SH**. Investigating global trends in paraquat intoxication research from 1962 to 2015 using bibliometric analysis. *Am J Ind Med* 2018; **61**: 462-470 [PMID: 29537078 DOI: 10.1002/ajim.22835]

57 **González-Alcaide G**, Peris J, Ramos JM. Areas of research and clinical approaches to the study of liver abscess. *World J Gastroenterol* 2017; **23**: 357-365 [PMID: 28127209 DOI: 10.3748/wjg.v23.i2.357]

58 **Volkow ND**. Medications for opioid use disorder: bridging the gap in care. *Lancet* 2018; **391**: 285-287 [PMID: 29150199 DOI: 10.1016/S0140-6736(17)32893-3]

59 **Chen RC**, Chu D, Chiang CH, Chou CT. Bibliometric analysis of ultrasound research trends over the period of 1991 to 2006. *J Clin Ultrasound* 2009; **37**: 319-323 [PMID: 19455690 DOI: 10.1002/jcu.20596]

60 **Centers for Disease Control and Prevention**. Data and Statistics About Opioid Use During Pregnancy. 2020. Available from: https://www.cdc.gov/pregnancy/opioids/data.html

61 **Joseph H**, Stancliff S, Langrod J. Methadone maintenance treatment (MMT): a review of historical and clinical issues. *Mt Sinai J Med* 2000; **67**: 347-364 [PMID: 11064485 DOI: 10.1159/000054256]

62 **Whelan PJ**, Remski K. Buprenorphine vs methadone treatment: A review of evidence in both developed and developing worlds. *J Neurosci Rural Pract* 2012; **3**: 45-50 [PMID: 22346191 DOI: 10.4103/0976-3147.91934]

63 **Kuschel CA**, Austerberry L, Cornwell M, Couch R, Rowley RS. Can methadone concentrations predict the severity of withdrawal in infants at risk of neonatal abstinence syndrome? *Arch Dis Child Fetal Neonatal Ed* 2004; **89**: F390-F393 [PMID: 15321955 DOI: 10.1136/adc.2003.036863]

64 **Siu A**, Robinson CA. Neonatal abstinence syndrome: essentials for the practitioner. *J Pediatr Pharmacol Ther* 2014; **19**: 147-155 [PMID: 25309144 DOI: 10.5863/1551-6776-19.3.147]

65 **Uebel H**, Wright IM, Burns L, Hilder L, Bajuk B, Breen C, Abdel-Latif ME, Feller JM, Falconer J, Clews S, Eastwood J,

Oei JL. Reasons for Rehospitalization in Children Who Had Neonatal Abstinence Syndrome. *Pediatrics* 2015; **136**: e811-e820 [PMID: 26371197 DOI: 10.1542/peds.2014-2767]

66 **Uebel H**, Wright IM, Burns L, Hilder L, Bajuk B, Breen C, Abdel-Latif ME, Falconer J, Clews S, Ward M, Eastwood J, Oei JL. Characteristics and causes of death in children with neonatal abstinence syndrome. *J Paediatr Child Health* 2020; **56**: 1933-1940 [PMID: 32815631 DOI: 10.1111/jpc.15091]

67 **Uebel H**, Wright IM, Melhuish E, Eastwood J, Oei JL. Prenatal Opioid Exposure Research: Shortcomings and Solutions. *J Addict Med* 2020; **14**: 355-356 [PMID: 31855920 DOI: 10.1097/ADM.0000000000000590]

68 **Oei JL**, Wouldes T. Will Simplifying the Finnegan Neonatal Abstinence Scoring Tool Improve Outcomes for Infants With Opioid Exposure? *JAMA Netw Open* 2020; **3**: e202271 [PMID: 32267511 DOI: 10.1001/jamanetworkopen.2020.2271]

69 **de Moya-Anegón F**, Chinchilla-Rodríguez Z, Vargas-Quesada B, Corera-Álvarez E, Muñoz-Fernández FJ, González-Molina A, Herrero-Solana V. Coverage analysis of Scopus: A journal metric approach. *Scientometrics* 2007; **73**: 53-78 [DOI: 10.1007/s11192-007-1681-4]

70 **Mongeon P**, Paul-Hus A. The journal coverage of Web of Science and Scopus: a comparative analysis. *Scientometrics* 2016; **106**: 213-228 [DOI: 10.1007/s11192-015-1765-5]

71 **Yue YY**, Fan XY, Zhang Q, Lu YP, Wu S, Wang S, Yu M, Cui CW, Sun ZR. Bibliometric analysis of subject trends and knowledge structures of gut microbiota. *World J Clin Cases* 2020; **8**: 2817-2832 [PMID: 32742991 DOI: 10.12998/wjcc.v8.i13.2817]

72 **Brennan C**, Laubscher M, Maqungo S, Graham SM. Bibliometric analysis of research on the effects of human immunodeficiency virus in orthopaedic and trauma surgery. *World J Orthop* 2021; **12**: 169-177 [PMID: 33816143 DOI: 10.5312/wjo.v12.i3.169]

Published by **Baishideng Publishing Group Inc**
7041 Koll Center Parkway, Suite 160, Pleasanton, CA 94566, USA
Telephone: +1-925-3991568
E-mail: bpgoffice@wjgnet.com
Help Desk: https://www.f6publishing.com/helpdesk
https://www.wjgnet.com